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A prototype industrial sensing system for phosphorus based on micro system technology

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Progress in the development of a miniaturised microfluidic instrument for monitoring phosphorus in natural waters and wastewater is presented. The yellow colorimetric method for phosphate analysis has been transferred to a microfluidic chip configuration This simple method employs one reagent mixed in a 1 : 1 ratio with a sample to produce a yellow colour absorbing strongly below 400 nm. A stopped flow approach is used which, together with the very rapid kinetics and simple reagent stream, enables a very uncomplicated microfluidic manifold design to be adopted. The working wavelength is 380 nm to coincide with the peak output of a recently developed UV-LED narrow bandwidth light source. The limit of detection for the yellow method is 0.2 ppm with a dynamic linear range from 0–50 ppm possible. The reaction time at room temperature is less than 3 min, which means that up to 20 samples per hour can be analysed.

Introduction

Phosphorus is a nutrient required by all organisms. Inorganic phosphorus is found predominantly in the form of phosphates with orthophosphate being the most stable form. Wastewater effluent, food residues, detergents, fertilisers, animal waste, industrial discharge and drinking water treatment all contribute significantly to phosphate levels in natural waters.[1]

Spectrophotometric methods, based on colorimetric detection have been widely applied for the determination of orthophosphate. Almost all wet chemical assays of phosphate are based on the well-known molybdenum blue method, which is ideal for visual titrations, because of the deep blue coloured complex formed. It is also suitable for colorimetric measurements based on tungsten filament sources because the molybdenum blue complex absorbs in the 650–700 nm region of the visible spectrum. However this approach will not transfer easily to a miniaturised format for several important reasons. Ascorbic acid is used in the reduction step of the formation of the molybdenum blue complex. In the presence of ascorbic acid the reagent tends to form a finely dispersed precipitate and the ascorbic acid itself is only stable for a restricted time period. This makes the blue method very unattractive not only because the precipitate could rapidly block the narrow microfluidic channels, but also from the point of view of meeting our target of one-year autonomous functioning for future miniaturised environmental monitoring instruments.

However, the yellow method (molybdovanadophosphoric acid method) has recently become an attractive alternative. This colorimetric technique involves the formation of a heteropoly acid, yellow vanadomolybdophosphoric acid, whereby ammonium molybdate, NH_4VO_3, is reacted with ammonium metavanadate, $(NH_4)_6Mo_7O_{24}.7H_2O$, under acidic conditions.[2] The combined reagent and sample containing orthophosphate react to form the above-mentioned heteropoly acid, $(NH_4)_3PO_4NH_4VO_3.16MoO_3$, resulting in a distinct yellow colour arising from strong absorbance below 400 nm. The reagent has so far not exhibited any signs of precipitating and it is very stable. A batch of this reagent can be continuously used, over extended periods of time, over 1 year with no significant loss in performance.

Flow injection analysis (FIA) is a popular approach for automating many analytical methods, and there are many examples utilising FIA for the determination of phosphates.[3–5] FIA has also been used for phosphate determination in real sample matrices, like those associated with wastewater treatment plants.[6–10] In these systems, mixing of samples and reagents occurs on-line, typically by means of coiled reaction manifolds or special mixing chambers. Total flow rates are typically 1–10 mL min^{-1}.

However, in microfluidic systems, laminar flow dominates and diffusion is the only available mixing option, unless a miniature mixing chamber is specifically introduced. Miniaturisation augments a reduction in reagent consumption and also increases sample throughput.[11] For example, mass transport by diffusion occurs 100 times faster in a microfluidic device, which has dimensions 10-fold smaller than a conventional bench FIA instrument.

FIA was an early target for miniaturisation, with a surge of interest in applications for microfluidic devices.[12,13] Doku and Haswell were particularly active in this area developing a micro flow injection manifold for the determination of phosphate.[14] This μFIA system was based on the molybdenum blue method employing electroosmotic flow to injection of the samples and to mobilise the reagents.[15] Glass has been particularly favoured in the development of capillary electrophoresis (CE) systems.[16] Polymer-based microfluidic systems are in the early stages of development and are particularly attractive because plastics are relatively cheap and easy to fabricate for rapid prototyping.[17]

In this paper the development of a method and the optimisation of a microfluidic system for the analysis of phosphate is presented. In using such a system the total flow rate has been reduced by around 1000 times from those employed in conventional FIA systems, resulting in a massive decrease in reagent consumption and waste generation.

Experimental

Reagents

All chemicals were of analytical-reagent grade and the solutions were prepared with double deionised water. Ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, ammonium metavanadate, (NH_4VO_3), and potassium dihydrogen phosphate, KH_2PO_4, were obtained from FLUKA Chemicals. Hydrochloric acid (37% wt. in water) was obtained from Aldrich.

Milli-q water was used throughout the analysis. It was taken from the Millipore Milli-Ro Plus 30 system. The water purification system includes two purification steps to produce distilled quality water.

Microsystem apparatus

The system is comprised of a Harvard PHD 2000 syringe pump (Antec Leyden BV, The Netherlands) fitted with a syringe holder (Danfoss A/S, Nordborg, Denmark) securing the borosilicate glass 3.3 (DURAN®) syringes in position (Innovative Labor Systeme, Mittelstasse 37, Germany). The syringes are connected to the microchip and holder, by 1/8″ PEEK tubing. Valves labelled **(v1)** and **(v2)** are positioned between the syringes and the microchip, controlling the flow from the reservoirs for refilling, and from the syringes for injecting. **(v1)** controls the sample flow and **(v2)** the reagent. The reservoirs labelled **(r1)** and **(r2)** are connected to the valves **(v1)** and **(v2)** and contain the sample and the reagent respectively. The third reservoir, **(r3)**, is for the waste collection from the chip, after it passes through the waste channel. The microchip holder is designed to facilitate fluidic interconnections in silicon chips with flow channels. The novel concept encompasses wedging the microchip between two rigid plates. The top layer is made of perspex, and is fastened with 4 locator screws for alignment. The chip is aligned to holes in the bottom layer, which connect to the syringe pump *via* PEEK tubing. Chips are typically 23 × 23 mm in size. On the underside of the microchips interconnection holes are spaced 4.6 mm apart in an array. These align with holes of similar dimension in the bottom layer of the holder. The holder orifices are sunken to accommodate O-rings, which provide leak-free interconnects to the external PEEK tubing.[18]

The Ocean Optics S2000 spectrometer (Ocean Optics Inc., Florida, USA) with $OOIBase_{32}$ software was chosen because of it's portability and good sensitivity. Another important component is the UV-LED (NSHU-550E model, Nichia Corporation, Tokushima, Japan). It is embedded in the top perspex lid of the microchip holder. The hole in the perspex layer is positioned directly above the optical cuvette hole. The UV-LED is then aligned in the hole, where it is secured a fixed distance of 3 mm from the surface of the microchip. With the aid of the spectrometer software a spectral integration time of 300 ms was chosen for analysis at the working wavelength of 380 nm. An electrical cable connects the UV-LED to its power supply, which is shown as **(f1)** in Fig. 1. The portable spectrometer is connected to the microchip *via* an optical fibre **(f2)**, which is fitted to the bottom layer of the microchip holder at the point **X**.

Microchip design

The chip was fabricated using the dry reactive ion etching (DRIE) technique resulting in a three-layered wafer of Pyrex–silicon–Pyrex.[19] The 400 μm thick Si layer is protectively coated by deposition of silicon nitride to the surface by a low-pressure chemical vapour process (LPCVD) to combat corrosion. And an advanced silicon etching (ASE) process is used to etch the optical cuvette. After which, all three layers of the chip must be correctly aligned to ensure interconnection of the fluidic components. When the wafer is anodically bonded, it is known as a wafer stack. Anodic bonding is desirable for protecting the chip from the working environment.

In Fig. 2 the actual chip shown is only 4 mm in length and is one of two designs on a larger silicon chip of 23 mm length. The chip contains several integrated components including sample, a, and reagent, b, inlets, a short, narrow, reaction channel, an optical cuvette of 400 μm path length and an outlet to waste, c. On the chip the inlet channels are 200 μm deep and 60 μm wide. The outlet channel is the same depth, but wider (200 μm) to stop air bubbles becoming trapped near the optical cuvette.[20]

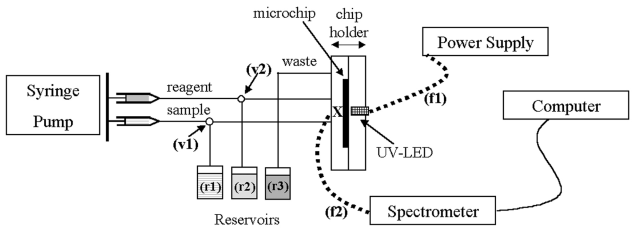

Fig. 1 Schematic of microsystem and all it's components.[18]

Experimental procedure

Firstly a reference baseline was measured with Milli-q water at a total flow-rate of 10 μL min^{-1} for 1 min. For the purpose of this analysis a stopped flow method was employed. Both the sample and the reagent were passed through the chip simultaneously at a total flow rate of 10μL min^{-1} for 1 min. When the flow was stopped diffusional mixing took place rapidly because of the narrow width of the short, mixing channel and the two solutions then reacted to form the yellow heteropoly complex along the channel and over the optical cuvette. The reaction was complete within ~180 s at room temperature, when a steady state signal was observed. This stable signal is known as the plug maximum. The flow was then restarted and at a rate of 10 μL min^{-1} laminar flow again dominated and the whole process was repeated in triplicate. Averages for the baseline and the plug maximum were calculated (n = 30 data points). The absorbance was calculated by subtracting the averaged water baseline from the averaged plug maximum.

Initially the challenge presented with using the yellow method was the fact that the yellow complex absorbed strongly below 400 nm. Although conventional light sources, such as the tungsten–halogen (Model LS-1, Ocean Optics Inc.) and the halogen–deuterium (Model DH2000, Avantes, Soerense Z and 4a, NL-6961 LL Eerbeek, The Netherlands) lamps, emit light in the lower UV range these broadband sources have poor intensity where the yellow complex absorbs and both also require 12VDC power supplies. A GaN blue-LED (Nichia corporation, Tokushima, Japan) was briefly used, but the narrow band-width of operation, λ_{max} at 470 nm, of the LED meant that again the light intensity below 400 nm was not sufficient. Fig. 3 shows a normalised plot of the light output for

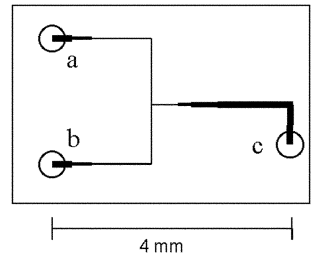

Fig. 2 Microfluidic chip layout.

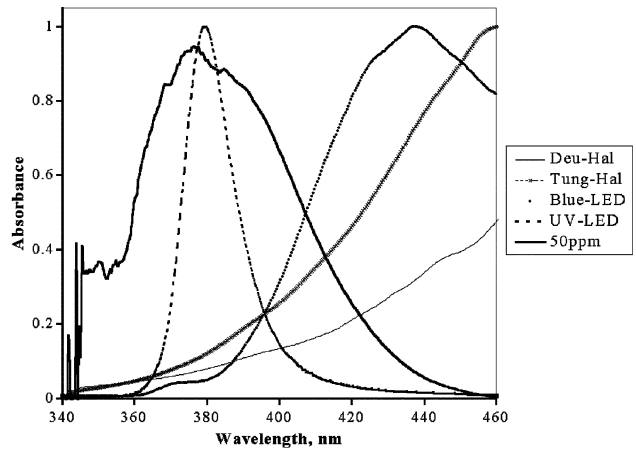

Fig. 3 Normalised plot showing the UV-LED as the most suitable light source for measuring the absorbance of the yellow complex.

a range of sources compared with the absorbance of the yellow complex. Clearly it can be seen that the UV-LED with a λ_{max} at 375 nm is the most favourable choice. This is not only because it has a strong intensity in the wavelength range of interest, but becasuse it requires less power than other light sources and it is the most compact device.

Results of calibration study

Five standard PO^{3-}_4 solutions were prepared by dilution of 50 mg L^{-1} phosphorus (P) (stock solution 219.5 mg KH_2PO_4 dissolved in 1L water containing 10 mL 4 M H_2SO_4). Calibration studies were carried out with a bench UV-VIS spectrometer, model MCS 501 (Karl Zeiss Ltd., Hertfordshire, UK) in a 1 cm quartz cuvette. A second calibration was carried out in the microfluidic set up *via* the stopped flow method as described. For both calibrations triplicate measurements of each standard of the calibration over the concentration range 0–50 mg L^{-1} PO^{3-}_4–P were made at 380 nm.

The path length of the optical cuvette in the microfluidic chip is 400 μm, so in order to compare the chip-based and the bench calibration, the microscale absorbances for each concentration on the calibration were normalised to equivalent (1 cm cuvette) values. In Fig. 4 a graph of both the macro- and microscale calibration is plotted.

The colorimetric chemistry of the method is simple and reproducible. Error bars are included (n = 5 replicates), but are masked by the point symbols. This is due to the fine reproducibility of the repeat measurements (% RSD < 3% for every point). The slope of the line, m, was calculated as 0.0606 ± 0.0033 mg L^{-1} and 0.0617 ± 0.0001 mg L^{-1} for both the macro- and microscale respectively. (The microscale slope was again normalised to enable comparison with the macroscale result). The slopes are comparable considering the calibrations were performed on two different instruments with different path lengths and different spectrometers. R^2 values were also calculated as 0.9965 ± 0.0013 and 0.9941 ± 0.0018 for the macro- and microscale respectively. From Fig. 3 it is clear that the calibrations are highly comparable over the entire range, which shows that the yellow method can be adapted from a bench measurement and be applied in a microsystem exhibiting the same results.

The limit of detection of the yellow method in the microsystem set up described here was shown to be 0.2 ± 1 × 10^{-5} mg L^{-1} (% RSD 2.2792%). The kinetics of the yellow method at room temperature is swift. The reaction reaches completion in ~ 3 min, which indicates that the sample throughput is in the region of 20 samples per hour. The kinetics of the method have been extensively examined over the temperature range 20–45 °C in the microfluidic manifold and will be the subject of a further paper.

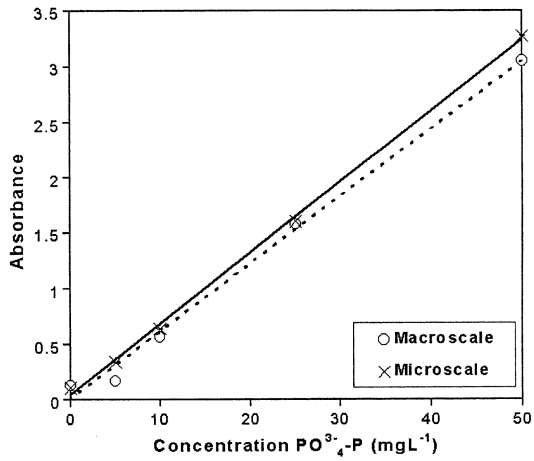

Fig. 4 Comparison linear plots of the macro- and microscale calibrations over the concentration range 0–50 mg L^{-1} of PO^{3-}_4–P at a working wavelength of 380 nm.

Conclusions

The development of a remote, compact, *in-situ* micro-device is the next major stepping stone in the realisation of our goal of the development of an extended environmental 'digital nervous system'. For phosphorus clearly it can be seen that some of the issues have been resolved including the chemistry of the method, long-term reagent stability, and the availability of a low power UV-LED light source. The method has also been successfully applied measuring phosphorus in a local source at specific sampling points along its course, which will be reported soon. Also progress in the solution of related issues such as a low power pumping system, fabrication of chips based on polymer materials, a portable sampling unit and a self-cleaning membrane filter will be the subject of further papers.

The outcome of developing such a device will be reduced costs, efficient energy usage, lower consumption of reagents (1 μL min^{-1} flow rate ≈ 500 ml per year reagent consumption using continuous pumping) coupled with less waste production, compact design, reliable analytical data and higher sample throughput.

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