Portable Integrated Micro-fluidic Platform for the Monitoring and Detection of Nitrite

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Abstract

A wireless, portable integrated micro-fluidic platform is proposed and applied for the determination of nitrite anions in water. The colourimetric analysis of nitrite is based on the Griess reagent, and the colour intensity of nitrite Griess reagent complex is detected using a low cost Paired Emitter Detector Diode technique. The micro-fluidic device employed a photoswitchable micro-valve, controlled by white light and generated using a light emitting diode. This low-cost and low-power detector exhibited very low limits of detection (34 ± 0.1 μg L^{-1}). Freshwater samples were analysed and the results were compared to those generated with a conventional UV-Vis spectrophotometer showing good agreement. The suitability of the analyser for the precise and continuous measurement of samples was established. In addition, its small size and low weight offered the advantage of portability, while its data logging capabilities allowed for independent nitrite monitoring. Moreover, integration of a wireless communication device allowed for the instrument to be controlled and results to be downloaded remotely.

Keywords

Biomimetic micro-valve; Low-power micro-analytical platform; photoswitchable actuators; LED detector; Nitrite determination; micro-fluidic device.

1 Introduction

Environmental monitoring has grown substantially in recent years in response to increasing concerns over the contamination of natural, industrial, and urban areas with potentially harmful chemical agents. Environmental monitoring by governing bodies more often takes place on a manual basis, and transportation of the samples to centralised facilities equipped with sophisticated instrumentation and analysed by highly trained personnel.[1] Advantages of employing this strategy include high precision and accuracy of the measurements, however because of the expense involved in maintaining these facilities there are inherent restrictions in terms of spatial and temporal sampling.[2, 3]

Characterisation of the nutrients distribution within water bodies is critically important because, depending upon their concentrations, theyhave the potential to greatly disrupt the balance of an aquatic ecosystem. Natural and man-made environmental events can result in dramatic changes in nutrient concentrations, both in time and space.The determination of nitrite (NO_2^-) levels in oceans, rivers, and drinking water is of importance for environmental monitoring agencies since nitrite is both a nutrient and excretion product of phytoplankton and is important in the global nitrogen and carbon cycles with concomitant effects on climate.[5] The over-use of nitrogenous chemicals within inorganic fertilisers, combined with the more general mismanagement of natural resources, have caused significant perturbation of both local and global nitrogen cycles.[6, 7] The high solubility and mobility of the nitrite ion within soil and water has significantly contributed to the eutrophication of lakes and more recently coastal outfalls. This, in turn, results in the generation of algal blooms that wreak havoc with local ecological systems.[8] These problems have been widely recognised, many analytical methods have been proposed for the determination of nitrite, [9, 10] however, by far, spectrophotometric methods are the most popular,[11] due to the excellent limits of detection, dynamic range, and cost efficiency. Together, these characteristics form the basis of a viable and economical relevant option for the production of low-cost miniaturised sensors suitable for on-site analysis. In particular, the spectrophotometric assay based on the Griess reagent has been very popular due to its high stability and sensitivity.[12] Early work in developing platforms for colourimetric sensing based on the Griess reaction was carried out by Greenway *et al.*[13] The system utilised electro-osmotic flow

(EOF) for pumping and an external optical component for absorbance measurements achieving a limit of detection (LOD) of 0.2 mM. Later, Sieben *et al.*[5] have integrated a low cost optical illumination and detection method with a simple micro-fluidic system for nitrite detection using the Griess reaction with a LOD of 14 nM. Although there are commercial available systems capable of to measuring nitrite concentrations, their large physical size *(e.g.* 60 x 14 mm) and power consumption (typically greater than 100 W)[4] limit their practical use.[REFSSS!!!!!] Furthermore, due to the reactivity of the nitrite samples, deterioration can rapidly occur, and therefore a strong motivation exists for the development of on-site measurement systems.[REF]

. Prompt *in situ* analysis, without human intervention, considerably reduce sample contamination possibilities, improve rates of sample throughput, and increase observational endurance. Quite importantly, prompt analysis creates opportunities for adaptive sampling of dynamic chemical gradients, either autonomously (*e.g.*, through sensor control of sampling platforms) or through human-directed responses (*e.g.*, surface-tethered control of depth). *In situ* water analysis generally provides, as well, substantial reductions in overall measurement costs. Chemical sensors generate a route towards a better understanding of natural processes governing chemical species behaviour. Technology has always played an important role within the chemical sensor research community, from characterisation via high-end instrumentation through to miniaturised, low cost, low power consuming devices capable of detection on a similar basis.

An important aspect of environmental analysis is the detection by optical methods. Unlike contact based sensors *e.g.* via electrochemical means, optical detection offers several advantages as such, the possible removal of a reference element, insensitivity to electrical interferences and the possibility of remote sensing. As a result, many optical based environmental sensing systems have been reported including: light dependant resistors,[14] photodiodes,[15] phototransistors,[16, 17] imaging devices (cameras/scanners),[18, 19] however, the energy demands, reliability, and complexity of the sensor are usually very significant limiting factors.[20] Paired emitter detector diode (PEDD) device allows overcoming these limitations because it possesses many advantages over other standardised methods (*e.g.* photodiode approach) such as low cost, high resolution, increased sensitivity, ease of implementation, and a relatively low power demand. Recently it was shown that light emitting diodes (LEDs) based systems can be used for applications where high sensitivity is an absolute requirement.[21]

In the recent years, advances in micro-fluidic techniques for environmental applications have brought wide opportunities for improving water quality monitoring.[ref] However, the development of fully integrated micro-fluidic devices capable of performing complex functions requires the integration of micro-valves with an appropriate performance, as they are essential tools for the control and manipulation of flows in micro-channels.[22] The issue of liquid handling is specific to analytical measurements, and it is the key limiting factor inhibiting chemo-/biosensor network deployments for applications involving liquid-phase measurements such as water quality monitoring. Stimuli responsive materials like photoswitchable gels can facilitate very low energy liquid movement within micro-fluidic devices. By using ionogels, define ionogel, the physical robustness of the photoswitchable materials is achieved. In contrast to the conventional spiropyran/p(NIPAAm) hydrogels, which are susceptible to dry up, ionogels are protected from drying and cracking because of the negligible vapour pressure of the ionic liquid (IL) at room temperature. These polymer actuators can be integrated into micro-fluidic devices, providing a route to 'biomimetic' micro-fluidic systems that are inherently low power, and with functions potentially more reliable when performed in micro-channels than when in equivalent conventional micro-engineered devices.[2] Coupled with low-power wireless communications, the availability of such advanced functions could greatly assist the realisation of analytical devices capable of operation in remote locations.

In this paper, we report, for the first time, the design, fabrication and testing of a wireless, portable, integrated micro-fluidic platform (PIMP) for point-of-care monitoring and quantitative determination of nitrite in freshwater samples. The mniaturised gold-standard Griess assay is implemented for detecting nitrite within a poly-(methylmethacrylate) (PMMA) micro-fluidic device. The platform integrates both the fluid processing and the optical detection, enabling monitoring of the kinetics of the Griess reaction and the detection of nutrient levels within the system. For fluid control, the micro-fluidic device contains a biomimetic photo-switchable micro-valve, as a proof of principle, based on a phosphonium ionogel functionalised with spiropyran chromophores. The micro-valve is actuated by a localised light generated by a simple light emitting diode. In addition, the nitrite concentration is determined by a highly sensitive, low cost wireless paired emitter detector diode detection method, ensuring an inexpensive fabrication and functioning of the whole platform.

2 Experimental

2.1. Chemicals and Reagents

All solutions were prepared from analytical grade chemicals and deionised water from a Millipore Milli-Q Q-GARD® 1 water purification system. Stock solutions were prepared freshly prior use and stored in dark environment at room temperature for no longer than one week. The Griess reagent was prepared following previously published procedures.[23] Phosphoric acid (H_3PO_4), sulfanilic acid, *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED) and sodium nitrite ($NaNO_2$) were purchased from Sigma-Aldrich®, Ireland and used without further purification. Nitrite standard solutions were diluted from a 200 mg L^{-1} $NaNO_2$ stock solution to the appropriate concentrations.

For ionogel micro-valve preparation *N*-isopropylacrylamide, *N,N'*-methylene-bis(acrylamide) (MBAAm), 2,2-5 dimethoxy-2-phenyl acetophenone (DMPA) were used and purchased from Sigma-Aldrich®, Ireland. 1′, 3′, 3′-Trimethyl-6-hydroxyspiro(2H-1-benzopyran-2, 2′-indoline) (Acros Organics, Geel, Belgium), 3-(Trimethoxysilyl) propyl methacrylate was purchased from Sigma-Aldrich®, Ireland. Trihexyltetradecyl-phosphonium dicyanoamide [$P_{6,6,6,14}$][dca] was obtained as compliments of Cytec® Industries, Ontario, Canada. Further purification was carried out as follows: 10 mL of IL decolourised by redissolution in 30 mL of acetone followed by treatment with activated charcoal (Darco-G60, Aldrich) at 40 °C overnight. Carbon was removed by filtration through alumina (acidic, Brockmann I, Aldrich) and the solvent removed under vacuum at 60 °C for 24 h at 0.1 Torr. A liquid prepolymer mixture was prepared by dissolving the NIPAAm monomer (7.0 mmol), the MBAAm (0.07 mmol), acrylated spirobenzopyran monomer (0.75 mmol), and the photo-initiator DMPA (0.35 mmol) into the ionic liquid (5.0 mmol).

2.2. Micro-fluidic Device Fabrication

The micro-fluidic device presented here consisted of a multi-layer structure made of poly(methyl methacrylate) and pressure-sensitive adhesive (PSA) sheets. Using a laser ablation system-excimer/CO_2 laser (Optec LaserMicromachining Systems, Belgium) reservoirs and

micro-channels were machined into the PMMA (Radionics, Ireland) along with a 50 μm and an 86 μm thick double-sided, pressure-sensitive adhesive layers (PSA, AR X and AR8890, respectively, Adhesives Research, Ireland). Once the appropriate pieces had been designed and machined, they were aligned and bonded using a thermal roller laminator (Titan-110, GBC Films, USA).

The ionogel micro-valves were photopolymerised *in situ* in a circular reservoir (500 μm radius)) V_{IL} = XXX nL) for 25 min using a UV irradiation source (λ = 365 nm) placed 8 cm from the solution (UV intensity 10 mW cm^{-2}). When polymerisation was complete, the resulting ionogels were rinsed with deionised water to remove any un-polymerised monomer and excess of ionic liquid. The photopatterned ionogel micro-valves were dried at room temperature for 24 h. Finally, the top PMMA layer was bonded to the micro-fluidic. Fig. 1A shows the micro-fluidic device fabrication procedure. After assembly the upper part of the micro-channel (Y – branches) was then filled with 1 mM HCl aqueous solution and kept for 2 h at 21 °C for swelling of the pSPNIPAAm ionogels (closing of the micro-valve).

The micro-fluidic device consisted of a small structure of 20 mm × 30 mm dimension, as shown in Fig. 1B. Round inlets for water sample (radius 2.25 mm) and for Griess reagent (radius 250 μm) are placed at the top of the Y-shaped channel. The junction of the collecting channels with integrated micro-fluidic micro-valve was followed by the mixing part of the channel which had a total length of 10 mm length and 1 mm wide. The detection chamber (radius 2.4 mm) was followed by a 1 mm width channel to the outlet/waste area, which was connected with the back pressure system.

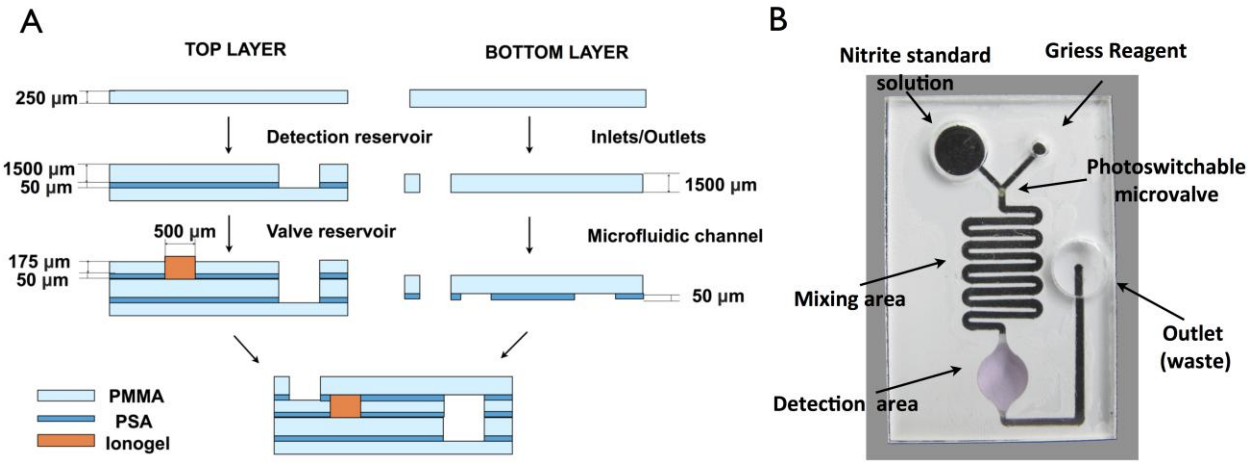

Fig. 1. A) Schematic representation of the micro-fluidic device fabrication procedure. B) Picture of the micro-fluidic device fabricated in PMMA : PSA polymer by CO_2 laser ablation.

2.3. PIMP fabrication

The design of the control, communications, and detection system followed from establishing the dimensions obtained from the fabricated micro-fluidic device. A cradle was designed virtually using 3D CAD software (Pro Engineer 4.0) to hold and restrain the micro-fluidic device during envisaged operational conditions. Fig. 2 presents the cradle consisting of three brackets to constrain the LEDs, which were position to reflect the locations of the micro-fluidic micro-valve in addition to the detection area for successful operation, see Fig. 1. The design was manufactured using a rapid prototyping system (Dimension SST 768) and printed in the colour black to reduce external lighting interferences and/or internal reflections.

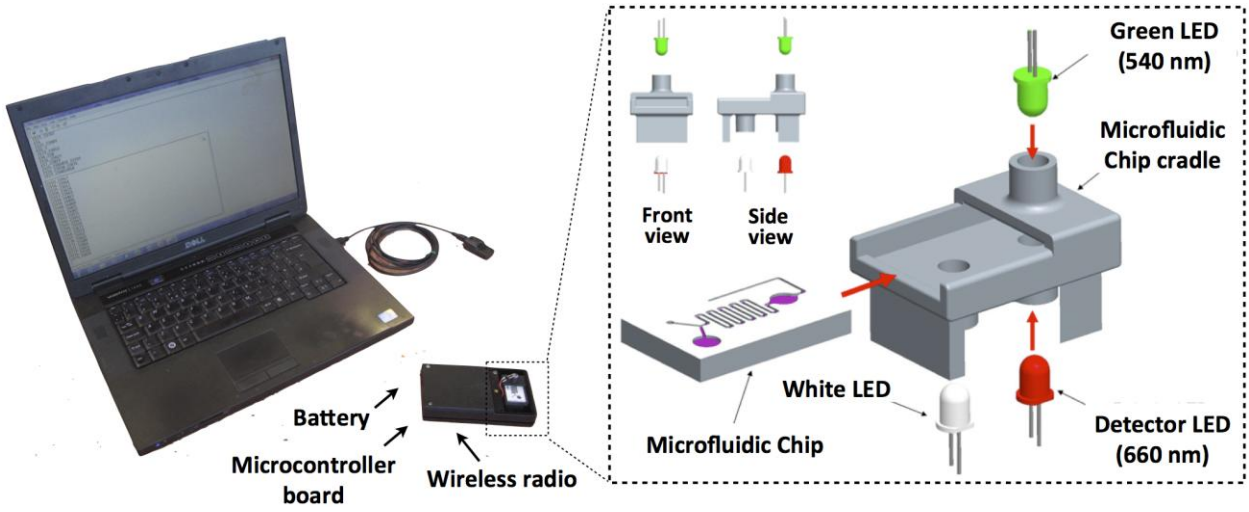

Fig. 2. Computer with the wireless, portable, integrated micro-fluidic platform (left) and CAD assembly model showing the micro-fluidic cradle (right), micro-fluidic device along with the emitter (white/green) and detector LED placements. Side and front views are also provided (right side, top left).

Figure 3 presents three diagrams describing the control and communication sub systems responsible for operational conditions. A microcontroller board (with an MSP430 F449, Texas

Instruments, at its core) was designed, created, and programmed in order to accept user input(s), actuate the white/red LEDs, and to quantify the transmitted light through the reagent/sample mix (Fig. 3a). A 3.7 V lithium-ion battery (Panasonic PAL2) with a low form factor provided power to the portable unit and was regulated to a constant 3.3 V via an on-board, low noise, voltage regulator (LP2985, Texas Instruments), sourced from Farnell, Ireland. The 3.3 V source also supplied power to the white and green LEDs, which was important for maintaining constant illumination conditions.

Communications between the operator and the microcontroller was achieved through two wireless radio transceivers (EZ-Radio ER900TRS, LPRS) with one placed on the base station (connected to a PC/Laptop through a FDTI UB232R USB interface) (Fig. 3b) and the other connected to the microcontroller *via* UART protocol. Users/operators executed pre-programmed subroutines on the microcontroller *via* a command line interface, which was enabled through a terminal program on a PC/Laptop; Hyperterminal was used for this purpose. In addition, data harvested by the detector was wirelessly streamed to the PC/Laptop in real time where it was continuously saved to file for future analysis. A time stamp (in seconds) accompanied each data point and was ensured *via* a real time clock supplied by a 32 KHz crystal (C-001R, Epson Toyocom).

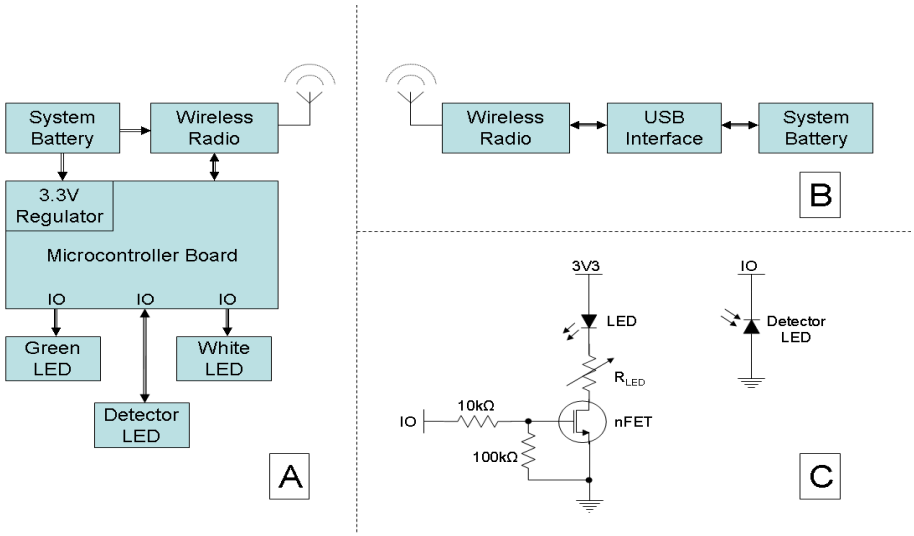

Fig. 3. Diagram showing the inner workings of the device. (A) Block diagram representation of the wireless analyser (B) Block diagram of the base station. (C) Schematic of

the components involved to actuate the power to the emitter LEDs (left) and connection of the detector LED (right).

Colourimetric detection was achieved through the use of the aforementioned LEDs, i.e. green (emitter, 540 nm, Radionics, Ireland) and red (detector, 660 nm, Radionics, Ireland), arranged in absorbance/transmission mode as Fig. 2 illustrates. Light generated by the emitter was partly absorbed by the reagent/sample mixture; the amount of which depended upon the concentration of nitrite within the sample at the time. The transmitted light falling upon the detector LED generated an electrical signal proportional to the concentration of the nitrite within the sample, which followed the PEDD arrangement reported by Lau *et al*.[24] This involved charging the inherent capacitance of the LED by setting the cathode (negative terminal) to 3.3 V (until fully charged) while fixing the anode to ground (0 V). As LEDs are essentially P-N junction diodes, they are sensitive to light in the same way as photodiodes.[25] As a result, the time with which the detector LED takes to discharge was relative to the amount of light falling onto it. In this way, a software counter within the microcontroller was used to count the number of times the signal was above the I/O port's logic threshold over a fixed duration. A time delay was implemented between each increment of the counter. This was determined experimentally by introducing the desired maximum and minimum concentrations into the micro-fluidic device in order to optimise the resolution for a 16-bit software counter *i.e.* in the range of 0 to 65535.

2.3. Measurement protocol

For conditioning the micro-valve, 1mM HCl solution was introduced to the micro-fluidic channel to immerse the micro-valve and the ionogel was allowed to swell for 2 h in dark environment. Next, after the actuation of the micro-valve using the white light LED (Radionics, Ireland), nitrite detection was carried out employing the Griess reaction method based on the conversion of sulfanilic acid to a diazonium salt by reaction with nitrite in acidic conditions[26]; then,the diazonium salt couples to *N*-(1-naphthyl)ethylenediamine (NED) forming an coloured azo dye. The intensity of the colour of the dye was directly related to nitrite concentration and is spectrophotometrically quantified/detected based on its absorbance. The sample to reagent ratio adopted throughout all the experiments was 3 : 0.2 v/v.[23] A 34.5 μL nitrite standard

solution/water sample and 2.3 µL Griess reagent were introduced into the storage reservoirs of the micro-fluidic device, inlets (Fig. 1b). The liquids were moved and allowed to mix through the serpentine reaction micro-channel towards the detection chamber (0.03 µL min^{-1}), where the readout took place, using 25 mbar back pressure from a vacuum pipe connected to the micro-fluidic device *via* the outlet. After the detection chamber was filled with mixed fluids, see Fig. S1, the intensity of the coloured solution was determined using the PEDD system of the portable device. The sampling rate under this protocol was set at 1 Hz.

For comparison, the calibration curve using UV-Vis spectrometer was carried out using a nitrite standard solution/water sample and the Griess reagent pipetted into the cuvette. The development of the nitrite Griess reagent complex colour intensity was monitored, range 0.0 - 1.2 mg L^{-1} NO_2^-, using a Perkin-Elmer Lambda 900 spectrophotometer by taking an absorbance measurement at a λ_{max} of 540 nm for 40 minutes at 21 ^{o}°C. The experiment was carried out in triplicate.

3. Results and Discussion

3.1. Volume Phase Transition of the Photo-switchable Ionogel Micro-valve

The volume phase transition of several pSPNIPAAm ionogels for micro-fluidic valves applications were presented by Benito-Lopez *et al.*[ref Fernando] in our laboratories, as valves for micro-fluidic applications but those valves were never applied in a real functional devices.Here we demonstrate that $[P_{6,6,6,14}]$[dca] based ionogels can be used for micro-valve applications in the PIMP. Figure XX shows the volume phase transition of a pSPNIPAAm ionogel disc induced by white light irradiation. The prepared ionogels reached the height of ~ XX +50 % of XX µm after swelling fot 2 h in HCl (1 mM) solution, which was XX µm after photo-polymerisation, therefore an increase of ~ 57 % from its initial dimensions is enough to block accurately the microfluidic channel. Before white light irradiation, the pSPNIPAAm swollen ionogel had a strong yellow colour due to the protonated open-ring form of the spirobenzopyran chromophore ($MC\text{-}H^+$). White light irradiation induced isomerisation to the

closed-ring form (SP), the yellow colour faded, which was followed by dehydration of the polymer and shrinkage of the pSPNIPAAm ionogel. The ionogel is capable to shrink an approximately 40 % of its initial swollen after white light irradiation at 21 °C.

The dimension of the photo-switchable micro-valve is a crucial parameter to consider in order to achieve a reliable operation of the system. Therefore, ionogel micro-valves of different dimensions (height) were examined. Circular reservoirs with depths ranging from 200 to 300 μm with the same 500 μm diameter were fabricated in the bottomlayer of the micro-fluidic device (Figure 1A), and then filled with the ionogel solution. The micro-valves were generated after photopolymerisation with UV light, as described above and their actuation in the micro-fluidic device was investigated. It was found out that ionogels prepared using 300 μm deep reservoirs were too small to block the channel after swelling and the fluids leaked out above the micro-valves before white light irradiation. On the other hand, ionogel micro-valves prepared using 200 μm deep reservoirs were too large in their swollen state, and the micro-valves were not opened even after long exposure to white light irradiation Therefore the ionogels micro-valves employed for the micro-fluidic analytical platform, Fig. 1, have the final dimensions of 1 mm diameter and 225 μm depth in a circular shape, which allowed quick actuation and so fluid manipulation.Fluidic Control in the Micro-fluidic Device

Micro-valve control by local light irradiation was demonstrated using the micro-fluidic device shown in Fig. 2. A nitrite standard solution and the Griess reagent were loaded into the reservoirs and a back pressure was applied in order to move the liquids from the inlets to the detection area. The micro-valve was in the closed (swollen) state, thus blocking the fluids to pass through the microchannels (Fig. 4, left). White light was locally irradiated over the pSPNIPAAm ionogel micro-valve using the white LED (intensity 1 W cm^{-2}). Light induced the shrinkage of the pSPNIPAAm ionogel, as described above, which resulted in the opening of the micro-valve after 30 ± xx s of irradiation (Fig. 4, right). The cracking pressure of the pSPNIPAAm ionogel micro-valve was determined to be 30 ± 3 mbar (n = 3 micro-valves). Over this pressure, the ionogels got deformed and the fluids leaked through the micro-valve.

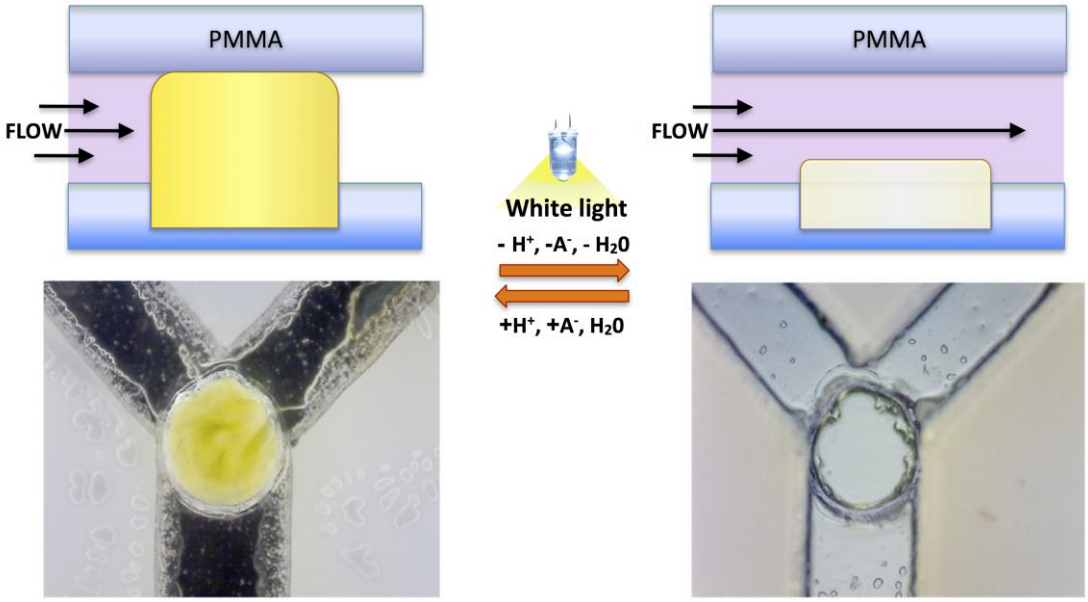

Fig. 4. Schematic (top) and images (bottom) of the photoresponsive micro-valve actuation/ in closed (left) and opened (right) state.

After the opening of the micro-valve both the water sample and Griess reagent were driven towards the serpentine mixing micro-channel. The preliminary experiments showed that the micro-fluidic design ensures efficient mixing of fluids. Fig. S1 shows a photograph demonstrating the effectiveness of the mixing in the micro-fluidic, which occurs after ~ 5 mm (first channel loop). Then the fluids were drawn through the micro-fluidic device serpentine by the back pressure. The complex changes colour according to their respective nitrite concentration and the absorbance of formed pink azo dye can be measured at 540 nm.

3.2. PIMP Characteristics

The micro-fluidic device presented in this study was integrated into a portable analytical platform that incorporated all the features necessary to carry out the actuation of the micro-valve, perform appropriate mixing, detection of the nitrite concentration within a sample, and transmission of harvested data wirelessly to a base station.

3.3.1 Reagent consumption

Typical flow injection analysis (FIA) systems employing the Griess method for nitrite detection consume vast amounts of reagent (approx. 5–20 mL/sample)[19] making the technique unsuitable for on-site analysis. In contrast, PIMP offers a solution to this reagent consumption issue by reducing the amount of reagent required for a single sample analysis to only 2.3 μL of Griess reagent, resulting in significant reduction in costs. The obvious advantages of PIMP system arise not only from lowered reagent consumption, but also smaller sensor size, reduced power requirements, as the size and power challenges are closely associated with macro-sized wet chemical sensors.

3.3.2 Micro-valve actuation

The photo-switchable ionogel was used for the fabrication of the micro-valve since ensures precise control by light irradiation, provides non-contact operation and permits multiple fluids manipulation on the micro-fluidic device. Therefore, optical fluid manipulation creates a novel prospect for highly integrated micro-fluidic devices like PIMP. The intensity of the white light necessary to control the pSPNIPAAm ionogel micro-valve is 1 mW cm^{-2} in our system, which is substantially smaller than previously reported optically controlled nanocomposite hydrogel micro-valves (> 1600 mW cm^{-2})[27] and similar to our previous work.[28] This demonstrates that the actuation behavior of ionogel micro-valves for uses in micro-fluidic devices can be kept low cost, as a simple, off-the-shelf, LED (€0.8) is capable of providing the required light intensity to control the actuation of the micro-valve.

3.3.3 Detection system

As previously stated, the detection system was based on a paired emitter detector diode set-up. The emitter 540 nm LED wavelength was chosen to be compatible with the azo dye absorption spectrum for optimal sensitivity, whereas the 660 nm LED was used as a detector because it was previously demonstrated that an LED is sensitive to all wavelengths of light equal to or shorter than the emission wavelength.[24] Only a single LED was required for the detection of light, in addition to a single I/O channel, which is available as a standard feature on all microcontrollers. When compared to the traditional approaches of implementing a

photodiodes,[refs] using an LED as a detector does not require analogue conditioning circuitry, or a dedicated ADC channel, and it also possess a higher resolution as it implements a software counter (16-bits in this case, but can utilise 32-bits if required) which is greater than a standard 10 or 12 bit ADC channel resolution. In addition, these detectors have shown outstanding properties, from low-power consumption and long lifetime, to extremely low detection limits.[23] Moreover, the variety of LEDs is numerous, inexpensive, and can be obtained off-the-shelf in a broad range of sizes and wavelengths[29] making them a very attractive type of detectors for their implementation in micro analytical platforms for a broad range of analytes.[refs] Furthermore, the modular aspect for considering an alternate target analyte is attractive; for this system one only needs to replace two hardware elements *i.e.* the emitter LED and appropriate colourimetric reagent.

3.3.4 Communications

Integration of wireless communications capability into the platform allowed for acquisition parameters to be controlled remotely and adjusted according to individual needs, in addition to enabling data transfer. The capabilities of the system, combined with its portability and wireless communication, provided the flexibility needed for on-site water analysis.

Many microcontroller devices are equipping wireless modules within mote-based systems and are electing for the 2.4 GHz ISM band.[30] There are good reasons for this, one of which is the cost effective nature of device construction as this band has become increasing popular due to pervasive technologies such as Zigbee, WiFi, 3G, *etc*. However, it was decided to modularise this platform at the design stage to allow for ease of integration into other available networks, if required at future stages. In addition, for use within the environment 900 MHz radio band offers an advantage over the 2.4 GHz model with an inherent capability of communicating around objects such as trees, or the landscape, *etc*., which can attenuate the 2.4 GHz approach.[31]

It was also important to examine the effects of EM effects on the detector. As mentioned previously, the use of an LED as a detector employs a very small amount of charge to function.[24] The advantage of this was a higher sensitivity and low power use therefore attributed to the longevity of the system. However, by equipping the system with a communications module, it

was possible to examine its effects on the detector and to develop a time-delayed strategy for sampling and wireless transmissions. Examining sources of internal interferences is an important aspect for these and other detectors where as external interferences can be minimised via a Faraday cage.

3.3. PIMP Performance

To demonstrate the utility of the portable integrated micro-fluidic platform, the system was applied for the determination of nitrite levels in water samples.. Therefore, the kinetics of the reaction for the formation of the pink azo dye and the determination of nitrite were studied. Each standard solution was analysed in triplicate and the resulting kinetic curves obtained from PIMP are presented in Fig. S2. The normalised discharge time *versus* time curves for the NO_2^- concentration were all exponential, following the equation 1:

$$D_t = a \cdot [1 - e^{(-k \cdot t)}] \qquad \text{eqn. 1}$$

where D_t is the discharge time (μs) at the end of the reaction, a is a scaling factor, k is the first order rate constant (s^{-1}) at time t respectively.

First-order kinetic models were fitted (Microsoft Excel Solver)[32] to each of the curves and the rate constants were calculated over the concentration range 0.0 - 1.2 mg L^{-1} of NO_2^-. The average response and fitted models are presented in Fig. 5A.

It was found that for nitrite concentrations higher than 0.4 mg L^{-1} the data of the kinetic measurements in the t = 0 min is already above 0 μs. The reason behind this phenomenon can be due to the efficient mixing provided by the serpentine micro-channel, which caused the nitrite Griess reagent complex colour formation already in the serpentine, for a fixed flow rate of 0.03 μL min^{-1}. Higher back pressure can be used in order to detect the earlier stage of the reaction as far as the pressure does not reach the cracking pressure of the micro-valve. Once the mixed standard solution and Griess reagent solutions filled the detection chamber, the optical detection was initiated. In Fig. 5 it can be seen that the colour formation increased rapidly until approximately 20 min after which the rate of increase ceased. This was the time allowed for

colour development and the time adopted for the determination of the different nitrite concentration in freshwater samples.

The calibration of the PIMP for nitrite detection was proved to be linear up to 1.2 mg L^{-1} of nitrite (R^2 value 0.98) with a RSD of 1.93 % (n = 3). The normalised discharge times were plotted against the nitrite Griess reagent complex concentration in accordance with the model (Equation 1) and the results are presented in Fig. 5B. The LOD, calculated as the concentration of nitrite which produced an analytical signal three times the standard deviation of a blank, turned out to be 34 ± 0.1 μg L^{-1} of nitrite, and the limit of quantification (LOQ) of portable sensor was found to be 115 ± 3 μg L^{-1}.

As a comparison study, the absorbance of the same nitrite Griess reagent complex concentrations was acquired employing a UV-Vis spectrophotometer. As shown in Fig. S3 the normalised absorbance obtained at a λ_{max} of 540 nm was plotted against nitrite Griess reagent complex concentration resulting in a linear range (R^2 = 0.99) of 0.0 - 1.2 mg L^{-1} with a RSD of 1.57 % (n = 3). A significantly higher LOD, 1.50 ± 0.02 μg L^{-1}, and LOQ, 14.8 ± 0.23 μg L^{-1}, were determined using the UV-Vis spectrohotometer (Table 1). Although this is lower than the detection limits achievable with our wireless portable integrated micro-fluidic platform, the limits were obtained by using much larger samples (36.8 μL in comparison to 1.5 mL; *ca.* 40 times smaller volume). Moreover, the sensitivity of the PIMP could be improved by elongation of path length of the detection chamber, which now is 1.8 mm, in comparison to 1 cm pathlength of optical cuvette for UV-Vis spectrophotometer (PIMP has *ca.* 5.6 times shorter path length). According to World Health Organisation the detection limits achievable by spectrometric standard procedures are reported to be 0.005–0.010 mg L^{-1} for nitrite.[33] The levels obtained by the PIMP remain lower than the allowable limits, and are therefore useful for quantification as well as threshold testing.

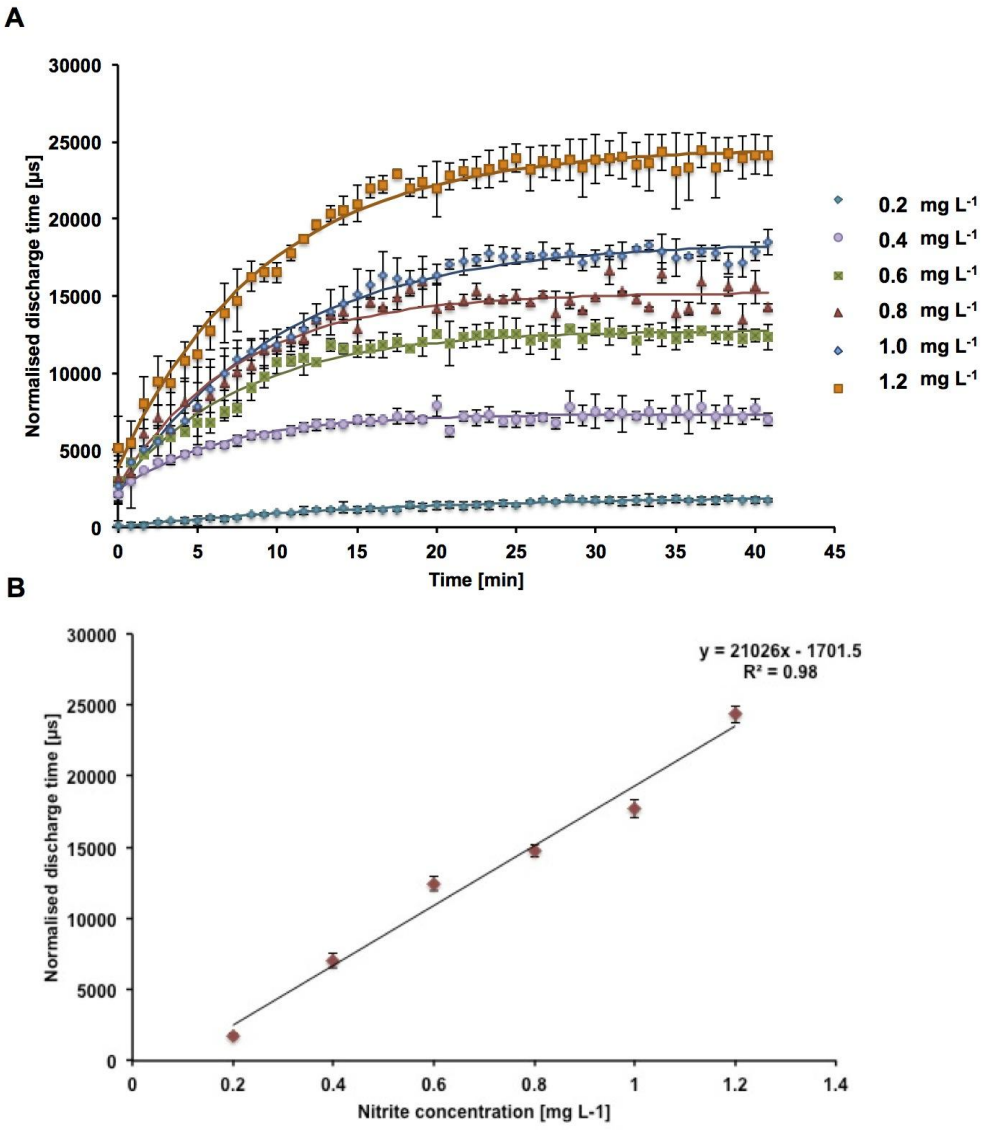

Fig. 5. A) Kinetic study of the colour formation monitored at λ_{max} 540 nm for the nitrite Griess reagent complex formation (n = 3) and B) normalised discharge time *versus* nitrite Griess reagent complex concentration using the PIMP.

Table 1. Comparison of the data obtained for the detection of nitrite Griess reagent complex using both the PIMP and a UV-Vis spectrophotometer (n = 3).

	PIMP (λ_{max} = 540 nm)	**UV-Vis spectrophotometer** (λ_{max} = 540 nm)
R^2	0.98	0.993
LOD	34.0 ± 0.1 μg L^{-1}	1.50 ± 0.02 μg L^{-1}
LOQ	115 ± 3 μg L^{-1}	14.8 ± 0.2 μg L^{-1}
RSD	1.93 %	1.57 %

The usefulness of the proposed portable integrated micro-fluidic platform for the determination of traces of nitrite in freshwater samples was evaluated using our system and UV-Vis spectrophotometer, for control. Table 1 collects the results for nitrite analysis where very good correlation between the bench top instrument and the portable platform was obtained, thus indicating the validity of the proposed platform for direct analysis of nitrite ions.

Table 1. Freshwater samples analysis for the detection of nitrite using the portable sensor and the UV-Vis spectrophotometer (n = 3).

Water sample no.	**UV-Vis spectrophotometer**	**PIMP**
1	0.010 ± 0.001	0.010 ± 0.003
2	0.410 ± 0.004	0.400 ± 0.006
3	0.420 ± 0.003	0.410 ± 0.009
4	0.380 ± 0.025	0.310 ± 0.045

3.4. PIMP Perspectives and Future Work

It has been demonstrated through this study that the use of a light actuated micro-valve can be operated successfully within a micro-fluidic channel and that micro-valve is capable of controlling the flow of the reagent and the sample prior mixing in the micro-channel. Although the overall control of the set-up was essentially manual, the methodology learned from this study can easily be applied to a more automated system to perform independent measurements for industrialised applications or for *in-situ* field measurements within the environment. A critical challenge faced by such environmental systems is the optimisation of its deployment time. Two key aspects affect this, *i.e.* regent supply and power consumption. The micro-fluidic approach addresses the reagent usage through the use of small quantities (micro- to nanoliters). In addition, an advantage of adopting the light actuated micro-valve is the reduction of high power demanding pumps/valves. By implementing the reagent supply at a higher level than the micro-valve, one can allow the gravitational force to encourage the flow along the channel. This offers a lower power demanding system based only on the power requirements of a single white LED.

In terms of photoresponsive micro-valves, their performance limitation may arise from the need to expose the ionogel to the acidic solution in order to induce swelling. PSPNIPAAm ionogel exhibits good response in 1 mM HCl aqueous solution, in which most of the spirobenzopyran chromophores are in the protonated open-ring form. Although the chemistry presented in this work is not affected by the release of protons into the external solution during shrinkage of the micro-valve, this constrain may limit the range of practical applications of the system. In order to handle biological cells and proteins, manipulation of fluids with neutral pH is desired. Therefore for these applications micro-valves with a diaphragm between ionogel and fluid are applicable or improvements to the existing formulations can be made to allow the material to be actuated without additional chemicals. Moreover, to improve the time for micro-valve response, the chemical modification of the chromophore is expected; in addition, micro-valves of smaller dimensions will also enable a faster response.

The capability of this platform can traverse beyond nitrite detection and be applied to a number of different colourimetric sensing targets by replacing the emitter LED to match the maximum absorbance peak of the target reagent to be analysed. Moreover, the platform can be easily integrated into existing environmental platforms.[34]

4. Conclusions

A novel and attractive approach for the *in situ* detection of nitrite, employing the Griess reaction, method was presented. Fluid manipulation was photo-controlled using an pSPNIPAAm ionogel micro-valve actuated by a simple white LED facilitating non-contact and non invasive operation. It is demonstrated that pSPNIPAAm ionogel micro-valves can be an advantageous technique for integrated multifunctional micro-fluidic devices. It is clear that such photo-responsive ionogel micro-valves have the potential to greatly enhance the fabrication and subsequent operation of multifunctional micro-fluidic devices. Moreover, the micro-fluidic device combined with a low-cost optical sensor, PEDD, allows for monitoring the nitrite of water samples in real time. Apart from the low-power detection and the communication system, the integration of a wireless communication device allows for the acquisition of parameters to be controlled remotely and to be adjusted according to individual needs. In addition, the results can be downloaded remotely and displayed in real time. This work describes an important step towards the realisation of a functioning micro-fluidic analytical platform with integrated low-power fluidic control and detection. This approach can open the way to much more reliable, low-cost and low-power approaches to controlling fluidic movement within micro-fluidic manifolds.

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