

MICROFLUIDIC ANALYSER FOR pH IN WATER AND WASTEWATER

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EXTENDED ABSTRACT

The Microfluidic Analyser for pH and Chemical Oxygen Demand (MApCOD) project is the latest stage in the development of a microfluidic platform for autonomous monitoring of environmental water quality [1]. This project focusses on developing and integrating colorimetric methods for pH and chemical oxygen demand (COD) in order to provide low cost, autonomous monitoring systems for these important water quality parameters.

pH measures the acidity or basicity of water. Most aquatic animals are adapted to a range of 6.5–8.0 (close to neutral, pH 7.0). Low pH can also allow toxic substances such as ammonia to become more available for uptake by aquatic plants and animals, greatly increasing their effective toxicity. pH is commonly measured in industrial and municipal wastewaters as well as in monitoring of drinking water, of surface waters such as rivers and lakes, and in many industrial processes.

The autonomous analyser platform which has been previously developed and field-tested for phosphate monitoring applications [2] utilises a combination of:

- microfluidic technology;
- colorimetric reagent chemistry;
- LED and photodiode-based optical detection systems; and
- wireless communications.

In this project pH is measured using a combination of pH indicators, optimised to give a colorimetric response over the pH range 4.0–10.0, which covers the range of pH values commonly encountered in monitoring of surface waters, drinking water and many wastewaters. Dual LEDs and a photodiode are used to measure light absorbance at appropriate wavelengths (430 and 570 nm). The responses of the two pH indicators are complementary, allowing a colorimetric response to be obtained over the pH range of interest.

Keywords: Microfluidics, water quality, environmental monitoring, pH

1. INTRODUCTION

pH is an important and widely used measurement in water quality monitoring. pH measures the acidity or basicity of water in terms of hydronium ion concentration, as defined by Sorensen (1909).

$$\text{pH} = -\log_{10} [\text{H}_3\text{O}^+] \quad (1)$$

Most aquatic organisms are adapted to a relatively range of pH 6.5–8.2, close to neutral (pH 7.0) and many organisms are sensitive to small changes in pH. Low pH can also allow toxic substances such as ammonia to become more available for uptake by aquatic plants and animals, greatly increasing their effective toxicity. pH is measured in a wide range of water quality monitoring applications, including drinking water monitoring, monitoring of freshwater (rivers, lakes and groundwater) and monitoring of transitional and marine waters. pH is also routinely measured in swimming pool and aquaria, industrial and municipal wastewaters, and in numerous industrial processes, including the food and drink industries, and pharmaceuticals.

Glass electrodes are the most widely used pH measurement system. A standard glass pH electrode system consists of a pH sensitive glass measurement electrode and a reference electrode in a potassium chloride (KCl) gel conducting buffer solution. The electrodes are usually housed in a combination electrode which is connected to an electronic meter with a signal amplifier and temperature compensation. The meter displays the pH reading, which may be uploaded to a computer or controller. The reference electrode has a stable potential which is independent of the measuring solution and must be calibrated outside the system in a reference solution. Glass pH electrodes can operate over a temperature range of 0–90°C, and over the full pH range of 0–14. Properly maintained and operated, they provide accurate and precise results. However, they also have a number of limitations which can be particularly significant in long term monitoring applications. The response of the glass electrode is temperature dependent, requiring accurate temperature measurement and compensation. In addition, the signal generated by the glass electrode can drift, or lose accuracy, over time due to a number of factors including fouling and sensor instability. Accurate pH measurement therefore requires either a periodic recalibration procedure or sensor replacement, creating a significant challenge to long term monitoring applications. Other potential disadvantages of glass pH electrodes include physical fragility, leakage of the reference electrode buffer into the sample solution, poor response in low ionic strength solutions, high background noise, and low signal-to-noise ratio. While glass electrodes are relatively inexpensive, servicing, calibration and validation requirements may compromise accuracy as well as increase the cost of operation (Sensorn, 2008).

The glass electrode, in various instrument formats, predominates in water pH monitoring applications. Laboratory pH meters which are used for analysis of manual samples represent the current benchmark for pH measurement; however these are relatively expensive and require careful calibration. This form of monitoring is also dependent on the establishment and implementation of correct procedures for sample collection, transport, storage and analysis, deviation from which can introduce significant error to the measurement. The manpower requirements for manual sample collection also involve significant cost and limit the frequency and geographic density of monitoring which is practicable. The same costs apply to *in situ* monitoring using portable pH meters, while online and *in situ* pH probes are subject to inaccuracies due to instrument drift and fouling, as mentioned above.

Our solution is based on colorimetric sensing using pH indicators in a microfluidic manifold. The advantages of this approach include:

- The small volumes of sample and reagent required.
- Calibration procedures based on standard buffer solutions can be easily automated.
- Optical detection takes place within a microfluidic chip which can be protected from fouling effects by a combination of sample filtration and cleaning procedures.

This work is a further development of the autonomous analyser platform which has been previously developed and field-tested for phosphate monitoring applications (Cleary *et al*, 2008; 2012) utilises a combination of microfluidic technology; colorimetric reagent chemistry; low cost optical sensing based on LED (light emitting diode) and photodiode-based detectors, and wireless communications. The pH analyser which has been developed uses a combination of colorimetric pH indicators, optimised to give a response over the pH range 4.0–10.0, which covers the range of pH values commonly encountered in monitoring of environmental waters, drinking water and many wastewaters. Dual LEDs and a photodiode are used to measure light absorbance at appropriate wavelengths (430 and 570 nm). The responses of the two pH indicators are complementary, allowing a colorimetric response to be obtained over the pH range of interest.

2. COLORIMETRIC SENSING STRATEGY

Colorimetric pH indicators offer a number of advantages for colorimetric detection in microfluidic systems, including their commercial availability at low cost, well established colorimetric response to changes in pH, and their intense colour at low concentrations, which provides significant absorbance signals within the short optical pathlengths which are available in microfluidic systems. However as pH indicators generally exhibit a colour change over a relatively narrow range (typically 1.5–2 pH units), a single indicator cannot provide an adequate response over the full pH range of interest (pH 4–10) for the intended application. The colorimetric sensing strategy used in the pH analyser therefore uses a mixed reagent consisting of two pH indicators. The response of the mixed reagent was initially examined using UV-vis spectrophotometry, and the relative proportion of the two pH indicators was then optimised by measuring the response obtained in a microfluidic chip using the LED and photodiode-based optical detector. In addition to increasing the pH range which could be measured using pH indicators, the data obtained using the dual indicator and dual LED detection strategy could be combined to provide a linear response over the pH range of interest. This linear response facilitates calculation of the pH value based on the light intensity values obtained from the photodiode, and also provides a basis for an integrated calibration protocol based on measurement of the pH indicator reagent mixed with buffer solutions of known pH.

3. PROTOTYPE pH ANALYSER DESIGN

Figure 1 is a schematic illustration of the pH analyser, showing the basic design and operation of the system. The system can be divided into three major elements, each of which contains a number of individual components. The fluidic system consists of the sample intake and filtering unit; storage for the colorimetric indicator reagent solution and pH buffer solutions; pumping system which controls the transport and mixing of the sample, reagent and pH buffer solutions; microfluidic detector chip; and waste storage, as well as the various valves and tubing required. The optical detection system consists of two LED light sources with a common photodiode detector, which enables an absorbance reading to be carried out on the sample/reagent mixture. The control and data layer consists of a microcontroller (MSP430, Texas Instruments) which controls the operation of the pumping system and optical detector, a micro-SD card (COM-08163, Sparkfun Electronics) for data storage; and a Wixel (WRL-10665, Sparkfun Electronics) for wireless

communication using 2.4 GHz Radio. Power is provided by a 12V, 4Ah lead acid battery for autonomous operation, or by a mains power adapter for online operation.

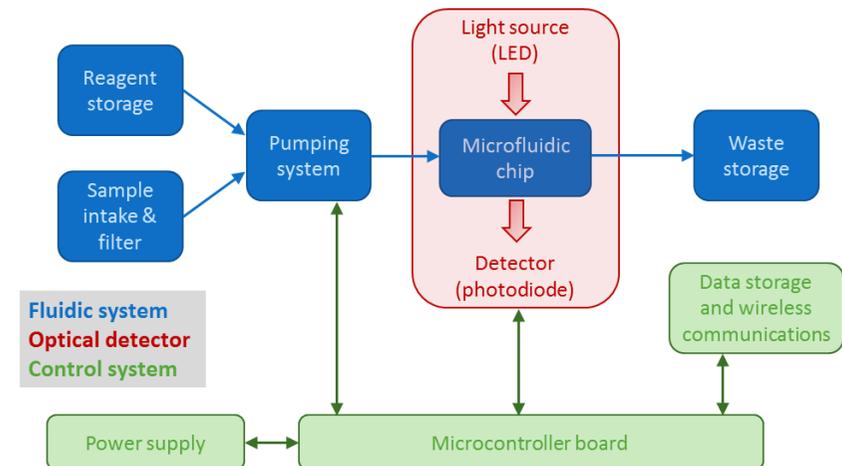


Figure 1. Schematic of the pH analyser.

Figure 2(a) shows a schematic illustration of the optical detection components. The sample is mixed with the dual-indicator colorimetric reagent via a T-junction and then pumped into the detector chip, which is fabricated from UV-bonded layers of PMMA (poly methyl-methacrylate) into which a 1mm channel has been formed by micro-milling. One side of the channel is adjacent to a mirrored surface. Two LEDs (with peak emission intensities of 430 nm and 570 nm) are oriented so that the light emitted is directed across the microfluidic channel, and reflected by the mirrored surface back across the channel to the photodiode, which converts the light intensity into a corresponding ADC value on a scale of 0–2048. This configuration has two advantages. Firstly, the optical pathlength through the sample is effectively doubled, compared to the corresponding pathlength in the commonly-used “across-channel” configuration shown in Figure 2(b). This means that the absorbance signal is increased in accordance with Beer’s law (Equation 2) without needing to increase the physical pathlength. Beer’s law states that:

$$A = \epsilon cL \quad (2)$$

where A is absorbance, ϵ is the extinction coefficient of the absorbing species, c is concentration, and L is pathlength. This means that more sensitive detection can be carried out without increasing the sample volume required. Minimising the sample volume, and thereby also minimising the pumping power requirements, is important in terms of enabling long battery life during deployments of an autonomous sensing system. An additional advantage of the configuration in Figure 2(a) is that the same photodiode is used to detect the signal from each of the LEDs, which emit alternately. Therefore, while photodiode output is subject to variation depending on temperature fluctuations and long-term drift, the same variations will apply to each of the optical channels. This configuration is therefore preferable to using separate photodiodes to detect light emitted by each LED.

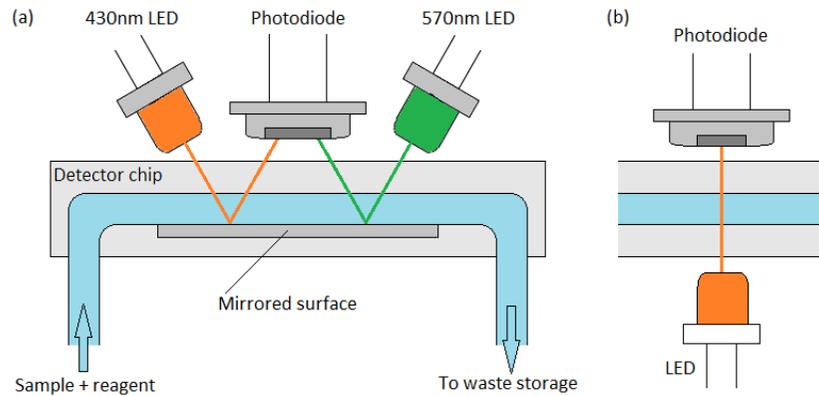


Figure 2. Schematic of the optical detection system.

The pumping system is a key component of the fluidic system, and consists of an array of 4 syringe pumps (Figure 3) which deliver the sample, colorimetric reagent and 2 pH buffer solutions (pH 4.0 and pH 7.0) to the mixing channel and detector chip.



Figure 3. The syringe pump array used for fluid control in the pH analyser.

The syringe pumps array features include robust and simple construction, low cost syringes (1 mL volume) and check valves, and stepper motors which drive the syringe plungers and allow precise control of the volume of fluid delivered. The integrated pH analyser is shown in Figure 4. The analyser was calibrated using by using a number of pH buffer solutions as sample. The analyser mixed each buffer in a 1:1 ratio with the colorimetric reagent, and an optical measurement was taken using the dual-LED and photodiode detector. The raw data, in the form of ADC readings, was transmitted to a laptop computer using the Wixel wireless communication module and processed using Microsoft Excel.

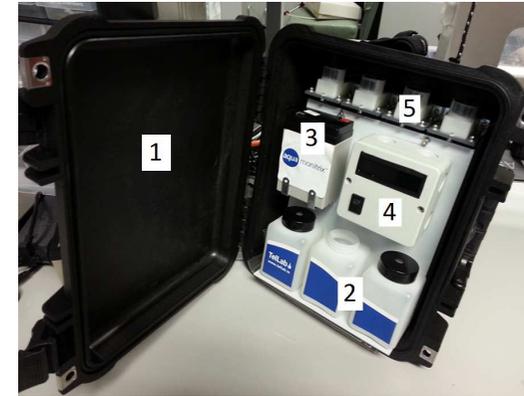


Figure 4. Integrated prototype pH analyser. Visible components include (1) robust and waterproof housing; (2) reagent and buffer storage containers; (3) 12V battery; (4) optical detection enclosure; (5) motors for syringe pump array.

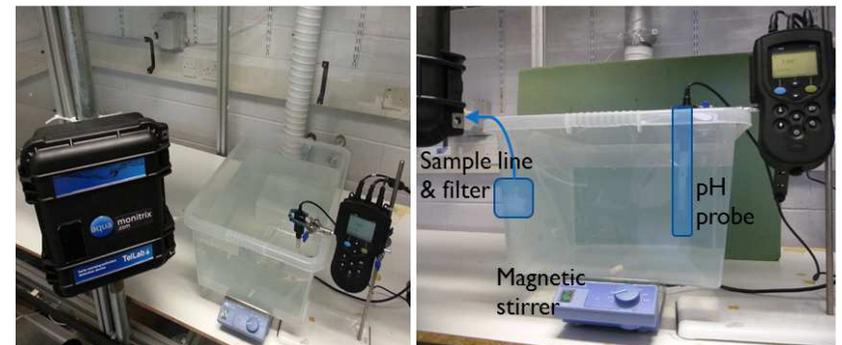


Figure 5. Experimental setup for validation testing of the prototype pH analyser.

Figure 5 shows the experimental setup which was used for validation testing of the pH analyser. A river water sample from the Tolka River (Dublin, Ireland) was placed in a plastic tank (25 L capacity) and agitated using a magnetic stirrer. The pH analyser prototype was mounted adjacent to the tank and sample was drawn into the system via an external line with a membrane filter (polyethersulfone membrane, 25 mm diameter, 0.45 μm pore size, Sigma-Aldrich Ireland) at the intake point. The pH in the tank was periodically adjusted using pH buffer solutions from commercial sources (Sigma-Aldrich Ireland; TE Laboratories, Ireland). Sampling was performed at 45 minute intervals and a pH probe (HQ-40D, Hach-Lange Ireland) was immersed in the tank and used to monitor the pH in the tank for validation purposes.

3. RESULTS

Figures 6 and 7 illustrate the responses obtained by mixing buffer solutions with the dual-indicator colorimetric reagent. Figure 6(a) shows the colour generated over a range of pH, while Figure 6(b) shows the corresponding UV-vis spectra (VWR 1600 PC, VWR International). The absorption peak at 430 nm exhibits a decrease in intensity with

increasing pH, while the peak at 570 nm exhibits an increase in intensity as pH increases. The opposing trend in the absorbance at each wavelength means that the peak absorbances can be used in combination to allow more sensitive detection than would be achievable using the signal from a single peak. Figure 7(a) shows the absorbance readings obtained using the prototype optical detector. The 430 nm LED shows a relatively static response over much of the pH range with major changes only occurring at the upper end of the pH range examined. The 570 nm LED exhibits absorbance change over most of the pH range, and while the response is static over the range pH 8–9, this coincides with the high-response range of the 430 nm LED. When the absorbance ratio is calculated and plotted as a function of pH, a linear response is obtained over a broad range of pH as shown in Figure 7(b).

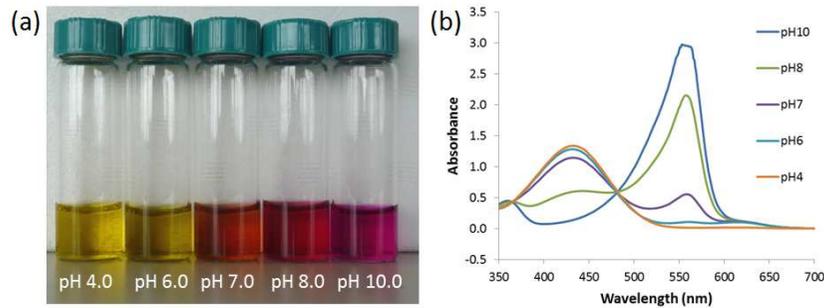


Figure 6. (a) colour generated by the mixed-indicator reagent mixed with a range of pH buffer solutions from pH 4.0–pH 10.0. (b) UV-vis spectra for the same solutions.

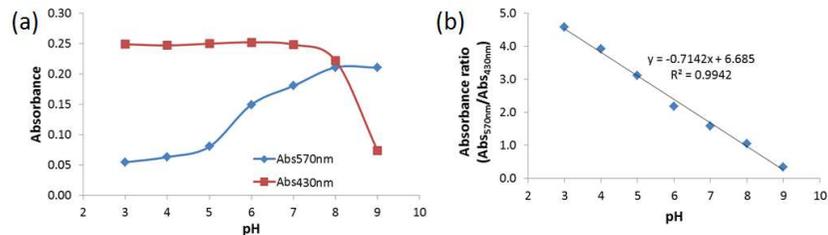


Figure 7. (a) Absorbance readings for the 430 nm and 570 nm LEDs over the pH range 3.0–9.0. Linear response obtained using the ratio of absorbances (Abs_{570nm}/Abs_{430nm}).

Figure 8 shows data obtained from the tank test using a river water sample matrix whose pH was adjusted using various pH buffer solutions. A number of step changes were performed over the duration of the experiment. The pH analyser output closely matched the sample pH values, which were established using the Hach-Lange pH probe, however after each step change the analyser required 2–3 measurements to reach agreement (to within 0.1 pH units) with the reference values for the sample. Table 1 summarises the values obtained after the initial 2 data points after each step change were discarded, and this data shows that good agreement between the reference values and the pH analyser was obtained. Subsequent experiments have shown that flushing the detector cell with an increased volume of sample prior to each assay gives improved adjustment to step changes, such that analyser output reaches agreement with reference values on the first measurement after each step change.

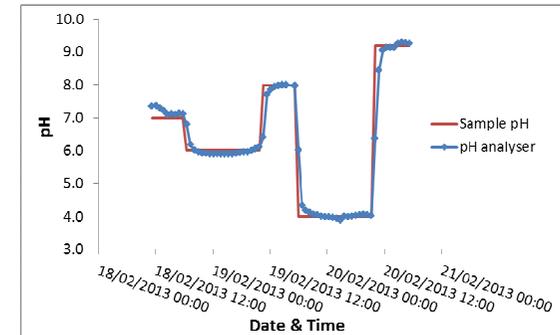


Figure 8. Dynamic response of the pH analyser to varying pH in a river water sample matrix.

Sample pH	pH analyser response		
	Average	Standard deviation	Number of samples
7.00	7.02	0.02	6
6.00	5.96	0.08	18
8.00	7.94	0.11	6
4.00	4.03	0.07	18
9.20	9.19	0.09	8

Table 1. Summary of pH analyser output for test performed in river water sample matrix. All values in pH units.

4. SUMMARY AND CONCLUSIONS

A compact and rugged system for monitoring pH in aqueous samples has been developed and assessed. The system utilises a colorimetric reagent based on a mixture of two pH indicators to generate an optical response over the pH range of interest (pH 4.0–10.0). A dual LED and photodiode-based optical detector is used to perform an absorbance measurement at two wavelengths, 430 nm and 570 nm, corresponding to the two main absorption peaks of the indicator reagent. Calibration using a range of pH buffer solutions has shown that a linear response can be obtained by calculating the ratio of absorbance at the two wavelengths. Tank-based testing using a river water matrix has shown that the analyser responds well to step changes in pH. Current focus is on optimising the performance of the system and carrying out field-based testing of the analyser system.

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