

NEXT GENERATION AUTONOMOUS CHEMICAL SENSORS FOR ENVIRONMENTAL MONITORING

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Microfluidic technology has great potential as a solution to the increasing demand for environmental monitoring, by producing autonomous chemical sensing platforms at a price level that creates a significant impact on the existing market. The development of sensing platforms for ammonium, nitrate and nitrite in water and wastewater using colorimetric techniques are being investigated in this paper. Our approach has been to combine microfluidic technology with colorimetric chemical assays; low cost LED/photodiode-based optical detection systems; and wireless communications, developing low cost systems which can be deployed for extended periods.

The objectives of this research are:

(i) to develop and optimise colorimetric detection methods for nitrate, nitrite and ammonia, and (ii) to integrate polymeric actuator valves into the microfluidic chip, significantly driving down the overall cost of the platform for a fully integrated, multi-target 'matchbox' analyser ready for field deployment.

The first section of this paper examines the colorimetric study of nitrite using the Griess test, whereby an autonomous nitrite analyser has been developed for a detection system. The work described herein presents the basis of a highly sensitive, low cost, simple colorimetric technique that can be integrated into a field deployable platform.

Secondly, a simplified colorimetric technique for nitrate has also been established and optimised using chromotropic acid in the presence of concentrated sulphuric acid. The method shows great promise as a linear range was achieved from 0-80 mg/L nitrate. The kinetics, reproducibility, limit of detection and reagent stability was investigated. A blind test using real samples was performed and results showed excellent agreement to ion chromatography. The chromotropic method for nitrate determination has been demonstrated to be a direct, simple technique proving that it is an ideal method for an autonomous system. It is also shown to be possible to reduce the concentration of the sulphuric acid used in the assay, hence reducing risk factors and component cost while maximising the lifetime of the system.

A colorimetric method for the determination of ammonium has been also investigated also. The reagent cocktail included a variation on the Berthelot method which employed salicylic acid instead of phenol, thereby eliminating a toxic and unstable reagent component. The intense colour generated has been detected at a wavelength of 630nm.

Results for the direct determination of nitrite and ammonia achieved also suggest that these may be suitable for integration into a similar field deployable platform to that of a phosphate monitoring platform which was previously developed. Results from recent deployments of the phosphate platform in situ at Broadmeadow Water Estuary, Co. Dublin, Ireland, are also presented.

KEYWORDS: Microfluidics, water quality, environmental monitoring, nutrients, chemical analysers.

1. INTRODUCTION

This paper focuses on the detection methods of nutrients in water for microfluidic autonomous sensing for environmental monitoring. The objective of this research is to create a self-sustaining platform, focussing on critical factors like minimisation of device size and minimisation of the reagent consumption. This will allow for the regular automated monitoring of the environment in remote locations. Developing this kind of environmental monitoring system will require a multidisciplinary approach involving electronics, wireless communications, environmental science, engineering and materials science with microfluidics playing a key role.

The ultimate goal is that the quality of the environment would be monitored in real time and at remote locations with systems capable of detecting multiple target analytes. Subsequently, the data generated could then be provided to all bodies of interest be it monitoring agencies or the general public. Presently, the challenges facing this ideal of environmental monitoring include the prohibitive cost of these platforms and the inability to “deploy and forget” due to limited reagent stability and automated platform maintenance requirements (Diamond *et al.*, 2011).

There is a major demand for inexpensive, robust, automated environmental monitoring platforms with an ethos of “deploy and forget”. Most legislation breaches are in relation to water quality (European Commission 2013) indicating the need for an automated water chemical sensor to replace the traditional method of manually taking samples from site and transporting them to a lab to be analysed, which proves to be time consuming and requires skilled personnel. An automated water monitoring system, with microfluidics being the key enabling factor, will allow for the ultimate goal of a robust functioning platform that is capable of performing analysis with prolonged deployment times and a cost of less than €200. This reduction in component cost will be due to the use of biomimetic materials such as soft polymer actuators to control liquid flow replacing conventional pumps and valves (Diamond *et al.*, 2010).

An autonomous, microfluidics-based monitoring system for phosphate has been developed and successfully deployed in wastewater monitoring and environmental monitoring applications (Cleary *et al.*, 2008). This system is based on colorimetric reagent based analysis, an approach which can be adapted to the monitoring of numerous water quality parameters, including other nutrient species. This first-generation platform provides a basis for current research focussing on the development of a next-generation biomimetic platform.

Water quality with a focus on nutrient levels like phosphate, have been studied extensively. For this research, study is focused on nitrite and nitrates. Nutrients like phosphates and nitrates are of on-going concern (European Commission 2010) and legislative control (Dangerous Substances Directive (67/548/EEC), Nitrate Directive (91/676/EEC)) of these species shows the importance of these pollutants on the environment and health. The recommended levels set by the EU for nitrate in groundwater, according to the Nitrates Directive, is 50 mg/L for nitrate and 0.5 mg/L for nitrite.

2. OPTIMISATION OF CHEMISTRY FOR INTEGRATION INTO AUTONOMOUS ANALYTICAL SENSING PLATFORM.

Incorporating the colorimetric chemistries investigated for nitrate, nitrite and ammonia with microfluidic technology will lead to autonomous analysing platforms for the

determination of nutrients in water, similar to the phosphate platform developed previously.

Following an initial laboratory based calibration; the phosphate analyser was placed *in situ* at Broadmeadow Water Estuary, Co. Dublin for the period 22nd Feb 2012 - 2nd March 2012. This site was known to have high nutrient levels present due mostly to inputs from industry, agriculture and a wastewater treatment plant situated close by (Environmental Protection Agency 2010). The analyser was employed to take a sample reading at 20 minute intervals. The sensor performed 350 autonomous measurements and 14 manual samples were collected for lab analysis and validation. The autonomous measurements were analysed and compared to the manual samples, obtained using a Hach-Lange DR 890 portable colorimeter are in good correlation with those reported by the phosphate analyser.

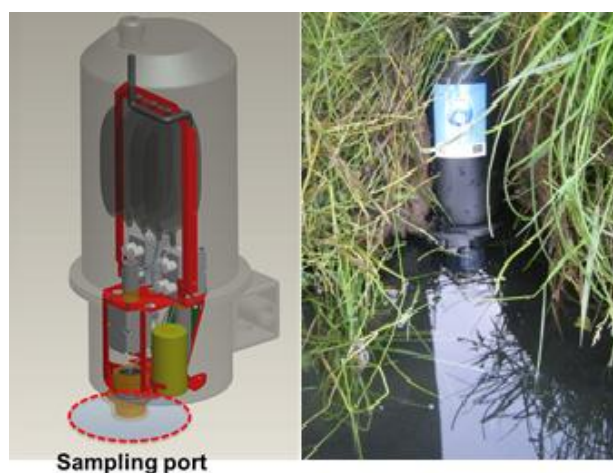


Figure 1. Phosphate analyser and system placed in situ in Broadmeadow Estuary.

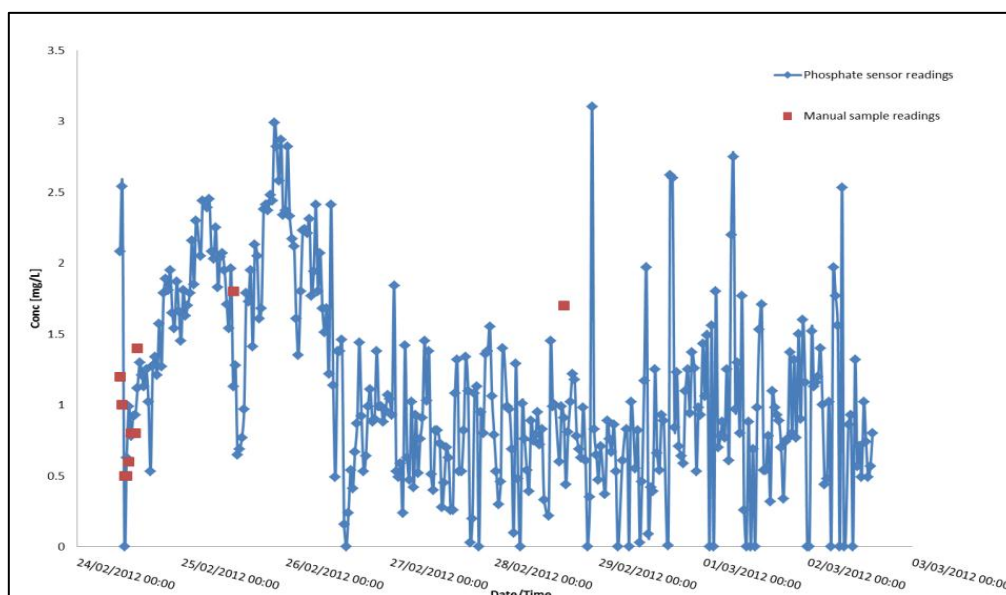


Figure 2. Data from the phosphate analyser and manual calibration samples (red) during the trial commencing 22Feb2012- 2March2012.

3. DETERMINATION OF NITRITE (NO₂⁻)

Testing of the reagent chemistry for the detection of nitrite was performed employing the Griess reagent method, based on the formation of an azo dye which absorbs strongly at 540 nm, the intensity of which is directly related to the nitrite concentration (O'Toole *et al.*, 2007).

The Griess reagent was prepared by adding 10 g of sulfanilimide, 0.25 g N-(1-Naphthyl) ethylenediaminedihydrochloride (NEDD) and 12.5 ml concentrated 37.0% hydrochloric acid, diluted to a final volume of 500 ml with deionised water. A sodium nitrite stock solution was prepared, from which a series of standards were generated.

Testing of the Griess nitrite test was performed using the bench top nitrite detection system shown in Figure 3.

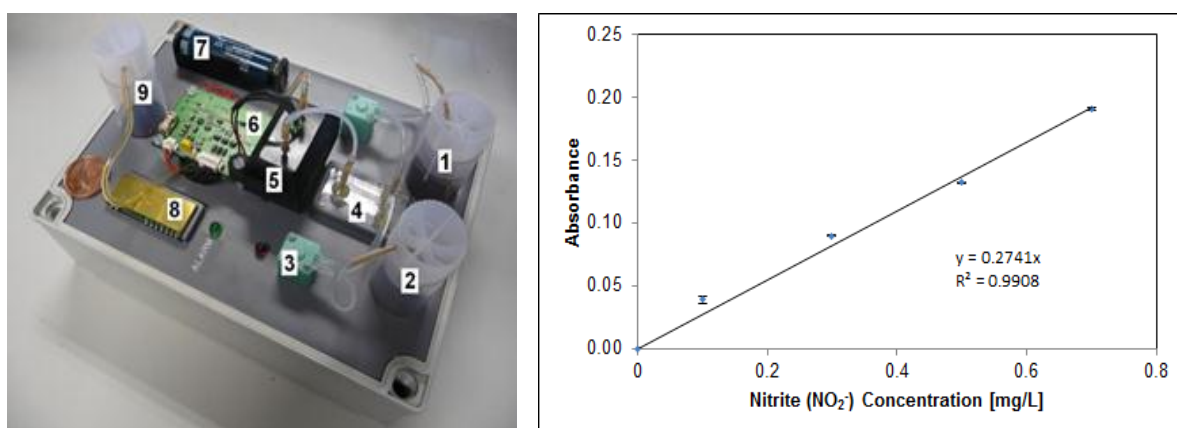


Figure 3. Left: Benchtop nitrite detection system. 1) Reagent container 2) Sample container 3) Micro-pump 4) Mixing chip 5) Detector 6) Control board 7) Battery 8) Easy-Radio 9) Waste container. **Figure 4.** Right: Prototype calibration of nitrite standards 0-0.7 mg/L.

The system comprises of two micro-pumps, a microfluidic mixing chip, and an optical detection system involving a 540 nm light emitting diode (LED) and photodiode detector. The two micro-pumps transport reagent and sample into the mixing chip where the mixture is allowed to react for 3 minutes before the LED and photodiode are used to take an absorbance measurement in the detection chip. The absorbance measured by the LED and photodiode is directly proportional to the concentration of nitrite present in sample. The system was tested using nitrite standards from 0-0.7 mg/L which are presented in Figure 4.

Following on from the results achieved with the nitrite prototype a blind test consisting of 8 samples was performed and coordinated with an ISO accredited laboratory (T.E. Laboratories, Tullow, Ireland). Table 1 presents the good correlation between the prototype and results achieved using ion chromatography.

Sample Reference/Type	Sample ID	Sample Concentration 17/02/2012 by IC	Sample Concentration 21/02/2012 by HACH (LL)	Sample Concentration 21/02/2012 by IC	DCU results: Nitrite Prototype 22/02/12
100413	A	<0.2	0.016	<0.2	0.22
100413 Spiked with 1mg/l NO ₂	A*	0.98	1.020	0.92	1.12
100343	B	<0.2	0.008	<0.2	0.14
100343 Spiked with 1mg/l NO ₂	B*	0.94	1.006	0.85	0.89
100291	C	<0.2	0.012	<0.2	0.25
100291 Spiked with 1mg/l NO ₂	C*	0.96	1.002	0.92	1.25
1mg/l NO ₂ standard	D	0.89	0.870	0.77	1.08
2mg/l NO ₂ standard	D*	1.71	1.865	1.72	1.73

Table 1. Results of various methods used for the analysis of 8 blind samples.

The data in table 1 confirms that the low cost, simple, colorimetric technique for the determination of nitrite using the Griess Reagent is highly sensitive. The method proves to be very feasible to integrate into a field deployable platform. The nitrite prototype has proven to be of great potential due to its excellent sensitivity and the short timescale it takes to reach steady state signal for the samples under laboratory conditions.

4. DETERMINATION OF NITRATE

Direct determination of nitrate was investigated by a spectrophotometric method based on chromotropic acid (Ryan, Estefan, and Rashid 2001). A yellow colour is developed when nitrate is treated with chromotropic acid in the presence of concentrated sulphuric acid and the resulting absorbance measured at a wavelength of 430 nm. The chromotropic reagent (C₁₀H₆Na₂O₈S₂·2H₂O) was prepared by dissolving 0.184 g of chromotropic acid in 100 ml of 98% sulphuric acid. A nitrate stock standard solution (100 mg/L) was prepared using potassium nitrate.

A calibration was performed over the concentration range of 0–80 mg/L nitrate using a set of standards in a 2:1 sample to reagent ratio using a VWR UV-1600PC spectrophotometer measuring at 430 nm as shown in Figure 5. The kinetics was then investigated showing that the colour formation increased rapidly until approximately 3 minutes after which the rate of increase ceased. Consequently, this was the time permitted for colour formation for further studies involving the chromotropic method for the determination of nitrate.

A blind test using 8 different samples from various sources were analysed in conjunction with an ISO accredited lab (T.E Labs Ireland). The chromotropic acid method was utilised here in DCU and also in T.E labs to compare results. These results were then validated using ion chromatography and are presented in Figure 6.

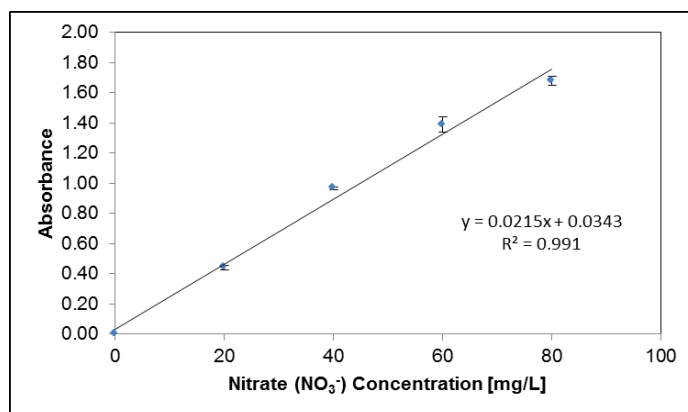


Figure 5. Calibration of nitrate standards ranging from 0-80 mg/L.

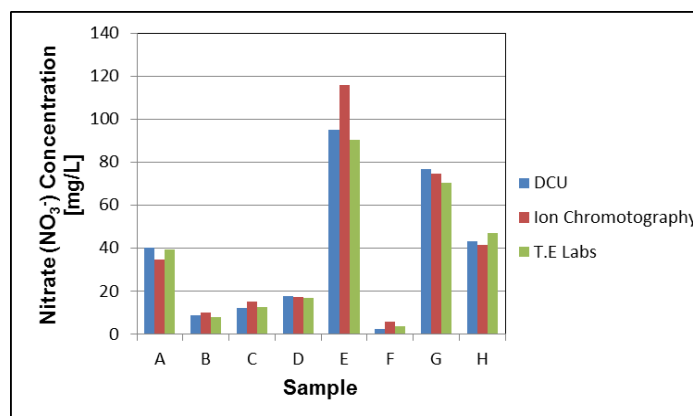


Figure 6. Comparison of results achieved for nitrate concentration of 8 samples, DCU and T.E labs using the chromotropic method and compared to that of ion chromatography.

Based on the successful validation between the chromotropic method and the reference method used (ion chromatography), the design and construction of a generation 1 prototype system for the determination of nitrate has begun. The work represented here in the study for the colorimetric determination of nitrate has demonstrated that it is possible to detect nitrate using a simple colorimetric method. Other methods including the cadmium reduction method require a more complex analytical system. One issue that has been highlighted concerns the use of 96% concentrated sulphuric acid required in the chromotropic acid reagent which must be added to the samples. This could be problematic if the method is to be employed in a field deployable platform, as it would require the use of highly resistant materials resulting in a high component cost for the system.

It was observed that if the concentration of sulphuric acid present in the reagent was below 80%, no colour change occurred when sample was added to reagent. Subsequently, it was concluded that for this reaction to be successful a certain amount of energy is required, which is obtained by the exothermic reaction produced due to the addition of the acid to the aqueous solution as seen with 96% sulphuric acid. It was therefore anticipated that, when using lower concentration of sulphuric acid, by introducing a heating step to the method a colour change may be observed.

For this purpose while decreasing the sulphuric acid from 96% to 50% and keeping the chromotropic acid concentration and nitrate concentration (50 mg/L nitrate) constant, the

complex was heated at 90° C. There was a significant colour formation after 50 minutes of heating. However, this is not an ideal response time for a deployable system due to the high power input required for heating over this period of time.

In order to improve this response time, 64% sulphuric acid, plus chromotropic acid and nitrate sample at 50 mg/L, was also investigated by heating at 90°C. Strong colour formation was found after 20 minutes. 60% sulphuric acid allows for cheaper materials to be used such as PVC and polypropylene, thus significantly lowering the component cost.

5. DETERMINATION OF AMMONIA

The colorimetric determination of ammonia in water was achieved using a variation of the Berthelot method (Daridon *et al.*, 2001). The altered reagent cocktail employed salicylic acid instead of phenol which has been shown to be stable for at least 12 months, thereby eliminating a toxic and relatively unstable reagent component. The intense colour generated in the presence for ammonia is easily detected at a wavelength of 630nm.

The colorimetric determination of ammonium was achieved by preparing an ammonium stock solution (100 mg/L) using ammonium chloride. 0.382 g of NH_4Cl was then dissolved in 1 L of Milli-Q water. Ammonium working standards were prepared by dilution of this stock solution.

The reagents were prepared as follows:

Reagent 1: 30 g/L Potassium sodium tartrate, 12 g/L sodium hydroxide, 4 g/L EDTA.

Reagent 2: 180 g/L Sodium salicylate, 1.5 g/L sodium nitroprusside.

Reagent 3: 667 g/L Sodium hypochlorite, 13% active chlorine, 2 g/L sodium hydroxide.

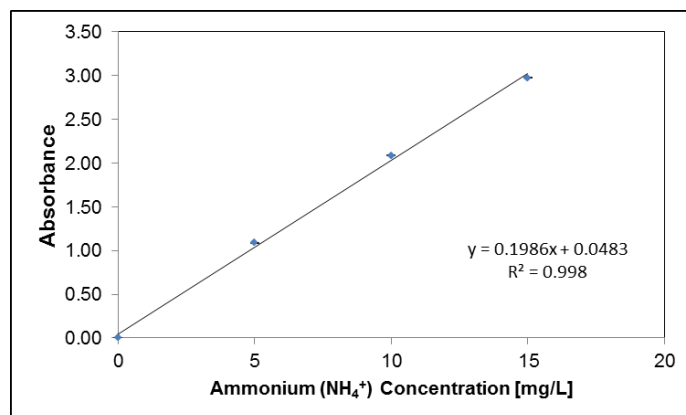


Figure 7. Calibration curve showing ammonium standards from 0-15mg/L.

The method was tested using solutions from 0-15 mg/L ammonium and results are shown in Figure 7. As a result of this agreeable calibration curve, the construction of a generation 1 prototype system has begun as this method proves to be a suitable method for integration into field deployable sensing platform.

6. CONCLUSION

In this study, the aim was to assess the chemistry for the detection of nitrite, nitrate and ammonia in water with the intention of integrating these methods into field deployable platforms. The determination of nitrite using the Griess method colorimetric technique has

been successful and could form the basis of a method to be employed in a field instrument.

The colorimetric chromotropic acid method used for the determination of nitrate has shown success in producing a simple, direct technique suitable for integrating into a working sensing platform. It was also proven that it is possible to reduce the concentration of the sulphuric acid for the determination of nitrate, reducing risk factors and cost while maximising the lifetime of the system.

For the determination of ammonia, the variation of the Berthelot method has yielded reliable, reproducible results showing that a substitute for phenol using sodium salicylate is feasible, achieving a safer method which is suitable for *in-situ* environmental monitoring.

A further objective is to integrate polymer actuator valves into the microfluidic platform, which will significantly drive down the overall cost of the platform. This will lead to a biomimetic approach for liquid movement through microfluidic chips; both electrochemical and photochemical actuation mechanisms will be investigated. The soft-polymer actuators are based on conducting polymers such as polypyrrole, arranged in a laminated structure that flexes (due to differential expansion/contraction) when the applied potential is switched between oxidising and reducing voltages.

The main aims are to maximise the lifetime of these actuators through the use of ionic liquid electrolytes and optimisation of the pumping system (actuator stress/strain). This will be a significant step towards the development of a fully integrated 'matchbox' analyser for field deployment, with a price capability of significant impact on the existing market.

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