The Development of Low Cost Autonomous Chemical Sensors for Environmental Monitoring

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Thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

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Declaration

I hereby certify that this material, which I now submit for assessment on the program of study leading to the award of Doctor of Philosophy is entirely my own work, and that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: _____

Deirdre Cogan

ID No: 58106324

Date:

For my parents,

Peter and Bridie

"Weaken your ambition and toughen your resolve."

James Frey, Tao Te Ching

Acknowledgments

Patrick Kavanagh once said that we love most what makes us miserable, “the damned are damned because they enjoyed being damned”. While Paddy was quite dramatic in nature and had a tendency to moan, I find these words somewhat relatable to research. It’s been the most amazing experience where I have grown personally and broadened my knowledge beyond measure.

This is all thanks to Prof. Dermot Diamond, for giving me this wonderful opportunity to work in his group and for his contribution, patience, and support during my PhD. His wisdom, guidance and “not so good sense of humour” has given me some of the best experiences a young researcher could ask for. I will carry these experiences proudly into my future path.

I would also like to sincerely thank John Cleary for contributing his knowledge and experience to my project. His support throughout my PhD was essential in finding my feet and confidence as a researcher, and I will be forever grateful for his input and encouragement.

I have decided not to write a long-list of friends and colleagues for fear of omitting one vital name, and words could never fully convey my gratitude and appreciation. So to all the Adaptive Sensors Group, both past and present, and to everyone I know and have met in the past three and half years, a thank you from the bottom of my heart. Your support and guidance has not only helped me over the finish line, but your friendship has been invaluable to me, and I am thankful for the lifelong friends I have made here.

Finally, I am especially indebted to my family and friends, I consider myself the luckiest girl in the world when it comes to this crowd. They have stood by me and demonstrated incredible patience, understanding

and cooperation through the good and bad times of the last four years. In particular, I must single out my parents, Conor, Tadhg, and of course Bonnie, who I consider to be not only my mentors, but my best friends in life. Thank you for always reminding me that all I can do is my best, and helping me through my battles of worry. You have taught me so much about the true qualities in life like kindness and honesty. Thank you.

List of Publications

Peer-Reviewed Articles

1. Deirdre Cogan, John Cleary, Thomas Phelan, Eoghan Mc Namara, Mark Bowkett and Dermot Diamond, "*Integrated flow analysis platform for the direct detection of nitrate in water using a simplified chromotropic acid method",* DOI: 10.1039/C3AY41098F (Paper) Anal. Methods, 2013, 5, 4798-4804.

2. Deirdre Cogan, John Cleary, Cormac Fay, Aoife Rickard, Kamil Jankowski, Thomas Phelan, Mark Bowkett and Dermot Diamond, *"The development of an autonomous sensing platform for the monitoring of ammonia in water using a simplified Berthelot method",* DOI: 10.1039/C4AY01359J (Paper) Anal. Methods, 2014, 6, 7606-7614.

3. Deirdre Cogan, Cormac Fay, Conor Osborne, John Cleary and Dermot Diamond, "*Development of a low cost microfluidic sensor for the direct determination of nitrate using chromotropic acid in natural waters*", DOI: 10.1039/C5AY01357G (Paper) Anal. Methods, 2015.

Articles

1. Deirdre Cogan, John Cleary, Tom Glennon, Conor O' Quigley, Eoghan Mc Namara and Dermot Diamond, *"Low cost autonomous sensors for water quality monitoring*" International Environmental Technology", Environmental Technology, May- June (2014) 18-20.

Conference Proceedings

1. Deirdre Cogan, John Cleary, Thomas Phelan and Dermot Diamond, *"Next generation autonomous chemical sensors for environmental monitoring",* 13th International Conference on Environmental Science and Technology (CEST 2013), 5-7 September 2013, Athens, Greece.
2. Deirdre Cogan, Fiachra Collins, Kate Meagher, John Cleary, Thomas Phelan and Dermot Diamond, *"Strategies for Realising Long-Term Autonomous Chemical Sensing Devices",* SENSORDEVICES 2013, August 2013, Barcelona, Spain.
3. Deirdre Cogan, John Cleary, Kamil Jankowski, Eoghan Mc Namara and Dermot Diamond and Mark Bowkett, *"Low cost autonomous sensing platforms for the direct determination of nutrients in water",* 7th International Conference on Environmental Science and Technology (ICEST2014), June 9-13 2014, Houston, Texas, USA.
4. John Cleary, Deirdre Cogan, Thomas Phelan and Dermot Diamond, "*Microfluidic Analyser for pH in Water and Wastewater*", 13th International Conference on Environmental Science and Technology (CEST 2013), 5-7 September 2013, Athens, Greece.

Oral Presentations

1. Deirdre Cogan, John Cleary, Thomas Phelan and Dermot Diamond "*Next generation autonomous chemical sensors for environmental monitoring",* ENVIRON 2013, Galway, Ireland.
2. Deirdre Cogan, John Cleary, Thomas Phelan and Dermot Diamond, "*Next generation autonomous chemical sensors for environmental monitoring*", 13th International Conference on

Environmental Science and Technology (CEST 2013), 5-7 September 2013, Athens, Greece.

3. Deirdre Cogan, John Cleary, Thomas Phelan and Dermot Diamond, *"Protecting Our Environment - Minus the Wellies!"* SFI Science Summit 2012, 12-13 November 2012, Athlone, Ireland.
4. Deirdre Cogan, John Cleary, Thomas Phelan and Dermot Diamond, *"Protecting Our Environment - Minus the Wellies!"* Thesis in Three All-Ireland final 2012, 25 October, Dublin, Ireland.
5. John Cleary and Damien Maher and Deirdre Cogan and Dermot Diamond, *"A microfluidic platform for multi-parametric water quality analysis"* In: ICEST 2012 – the 6th International Conference on Environmental Science and Technology 2012, 25-29 June 2012, Houston, USA.
6. Fiachra Collins, Deirdre Cogan and Diamond Dermot, Discussion panel for the Environmental Sensing session during the RBI showcase, 22 Oct 2013, Sao Paulo, Brazil.
7. John Cleary, Mark Bowkett, Deirdre Cogan, Thomas Phelan, Dermot Diamond*, "Real Time Remote Water Pollution Monitoring with Aquamonitrix,"* ArabLab 2013 EnviroLab Seminar, 12 March 2013 Dubai International Convention and Exhibition Centre, Dubai, United Arab Emirates.
8. Deirdre Cogan, John Cleary, Kamil Jankowski, Eoghan Mc Namara and Dermot Diamond and Mark Bowkett, *"Low cost autonomous sensing platforms for the direct determination of nutrients in water",* 7th International Conference on Environmental Science and Technology (ICEST2014), June 9-13 2014, Houston, Texas, USA.
9. Deirdre Cogan, John Cleary, Kamil Jankowski, Thomas Phelan and Dermot Diamond, *"A Microfluidic Colorimetric Analyser for pH",* 7th International Conference on Environmental Science and Technology (ICEST2014), June 9-13 2014, Houston, Texas, USA.

10. Deirdre Cogan, John Cleary, Cormac Fay and Dermot Diamond, *"Low cost autonomous chemical sensors for the direct determination of ammonia and nitrate in water",* Analytical Research Forum 2014, Royal Society of Chemistry, London, UK.
11. Deirdre Cogan, John Cleary, Thomas Phelan and Dermot Diamond *"Development of low cost autonomous chemical sensing platforms for the direct determination of ammonia and nitrate in water",* ENVIRON 2014, Dublin, Ireland.

Demonstrations

1. Demo presentation of phosphate analyser, EPA STRIVE-Research Conference, June 28th 2012, Trinity College Dublin.
2. Demo presentation of phosphate analyser and poster, Smart Ocean and Volvo Ocean Race, 2nd-6th July 2012, Galway City.
3. Demo presentation of phosphate analyser, EuroScience Open Forum, Dublin Convention Centre, 12th July 2012.

Poster presentations

1. Deirdre Cogan and John Cleary and Damien Maher and Dermot Diamond, *"Next Generation Autonomous Chemical Sensors for Environmental Monitoring."* Questor Meeting 29th November 2011, Queen's University, Belfast, UK.
2. Deirdre Cogan, John Cleary, Damien Maher and Dermot Diamond, *"Next Generation Autonomous Chemical Sensors for Environmental Monitoring."* Questor Meeting, 1st and 2nd May, Cranfield University, UK.
3. Deirdre Cogan, John Cleary, Damien Maher and Dermot Diamond, *"Next generation autonomous chemical sensors for environmental monitoring".* Questor Meeting, 29-30 Nov 2011, Belfast , UK.

4. Deirdre Cogan, John Cleary, Damien Maher and Dermot Diamond, "*Next generation autonomous chemical sensors for environmental monitoring".* QUESTOR Meeting 1st and 2nd May, 2012 Cranfield University, 1-2 May 2012, Cranfield University, Cranfield Bedfordshire, UK.
5. Damien Maher, John Cleary, Deirdre Cogan and Dermot Diamond, *"Autonomous nutrient detection for water quality monitoring".* International Water Association, Conference on Water, Climate and Energy, 13-18 May 2012, Convention Centre Dublin, Dublin, Ireland.
6. Deirdre Cogan, John Cleary, Damien Maher and Dermot Diamond, *"Next generation autonomous chemical sensors for environmental monitoring".* 64th Irish Universities Chemistry Research Colloquium 2012, 14-15 June 2012. , University of Limerick, Ireland.
7. Deirdre Cogan, John Cleary, Damien Maher and Dermot Diamond *,"Next generation chemical sensors: detecting nitrate and ammonia in water".* RSC Advances in Sensor Technologies and Applications for Monitoring, 19 June 2013, London, UK.
8. Deirdre Cogan, John Cleary, Damien Maher and Dermot Diamond, "*Next generation autonomous chemical sensors: low cost nutrient detection for water quality monitoring".* QUESTOR/CASE meeting, 12-13 Nov 2013, AFBI Hillsborough, Northern Ireland, UK.
9. Deirdre Cogan, John Cleary, Thomas Phelan, Ernane José Xavier Costa, Rafaella Angelini, Marcelo Morgado and Dermot Diamond, *"Next generation autonomous chemical sensors: low cost nutrient detection for water quality monitoring",* 13th Analitica Latin America, September 24th - 26th, 2013, Sao Paulo, Brazil.
10. Deirdre Cogan, John Cleary, Thomas Phelan and Dermot Diamond "*Autonomous chemical sensors for environmental sensing: nitrate and ammonia detection in water",* Water: the greatest global

challenge, ATWARM Conference, 14-16 May 2013 Dublin City University, Dublin, Ireland.

11. Deirdre Cogan, John Cleary, Thomas Phelan and Dermot Diamond, Water, *"Next Generation Autonomous Chemical Sensors for Environmental Monitoring,"* Wastewater and Environmental Monitoring Conference (WWEM 2012), 7-8 Nov 2012, Telford, UK.
12. Deirdre Cogan, John Cleary, Thomas Phelan and Dermot Diamond, "*Next generation autonomous chemical sensors",* The Technology Touchdown Symposium, 30-31August 2012, Dublin Ireland.
13. Deirdre Cogan, John Cleary and Dermot Diamond, "*Low cost autonomous sensor for water quality analysis,"* Water: The Greatest Global Challenge, 4-5 December 2014, DCU Water Institute, Dublin, Ireland.

Awards

1. CLARITY Thesis in Three, Second Place, *"Protecting the environment- minus the wellies!",* 20 September 2012, Speak Easy Café Bar, Dublin, Ireland
2. SFI CSET Thesis in Three All Ireland National Winner, *"Protecting the environment- minus the wellies!",* 25 October 2012, Smock Alley Theatre, Dublin, Ireland (Part of Innovation Dublin 2012, showcased research from 18 students coming from the 9 SFI funded CSETS (CLARITY, SBI, CNGL, CRANN, Lero, BDI, CTVR, APC and DERI).
3. SWIG Early Career Research International Poster Competition 2012, Second Prize*, "Next generation autonomous chemical sensors for environmental monitoring",* 7 November 2012, Telford, UK (Selected as top 3 entrants and final round of the SWIG Poster Competition was at WWEM, Telford 7th November 2012).

4. Best paper award, Deirdre Cogan, Fiachra Collins, Kate Meagher, John Cleary, Thomas Phelan and Dermot Diamond, *"Strategies for Realising Long-Term Autonomous Chemical Sensing Devices",* SENSORDEVICES 2013 International Conference, August 2013, Barcelona, Spain.
5. Best paper award (second prize), Deirdre Cogan, John Cleary, Kamil Jankowski, Eoghan Mc Namara and Dermot Diamond and Mark Bowkett, *"Low cost autonomous sensing platforms for the direct determination of nutrients in water",* 7th International Conference on Environmental Science and Technology (ICEST2014), June 9-13 2014, Houston, Texas, USA
6. Best presentation award, Deirdre Cogan, John Cleary, Cormac Fay and Dermot Diamond*, "Low cost autonomous chemical sensors for the direct determination of ammonia and nitrate in water",* Analytical Research Forum 2014, Royal Society of Chemistry, London, UK
7. Invent commercialisation award, John Cleary, Thomas Phelan, Deirdre Cogan and Dermot Diamond, *"Development of an autonomous water analysis platform",* DCU, Dublin, Ireland.
8. Nominated by peers for the Faculty of Science and Health Outstanding Graduate Award (DCU)

Aims of the Thesis

This project has the overall objective of developing low-cost analytical platforms for autonomous monitoring of environmental water quality so as to reduce the fabrication cost of the devices by an order of magnitude. The platforms will be capable of monitoring nutrients in waste water and freshwater with detection ranges and detection limits in compliance with EU environmental legislative requirements. The question of how to gather, integrate, analyse and share sensed environmental data is the driving force behind this research and in particular, how to sense the chemistry of our environment and how to realise chemical sensors that are reliable and robust with the ability to function autonomously for periods of weeks to months. This will be achieved by integrating simplified and optimised reagent-based methods within a microfluidic based format that is further integrated into a robust, autonomous analyser platform.

Selected Publications and Author Contribution

This thesis includes three original manuscripts published in peer-reviewed journals and one conference proceedings paper. The core of the thesis is the development of low cost microfluidic based analysers for water quality analysis. The ideas, development and writing up of all manuscripts in the thesis were the principal responsibility of me, the candidate, working within the School of Chemical Sciences under the supervision of Prof. Dermot Diamond. The inclusion of co-authors reflects the fact that part of the work came from active collaborations between researchers and industry and acknowledges the strong team-based research ethos in the Adaptive Sensors Group. In the case of chapters 2 to 4 my contribution to the work involved the following:

Thesis Chapter	Publication title	Publication Status*	Nature and extent of candidate's contribution
2	Integrated flow analysis platform for the direct detection of nitrate in water using a simplified chromotropic acid method	Published, Analytical Methods, 2013 DOI: 10.1039/C3AY41098F	First author, initiation, key ideas, data collection and analysis, manuscript development and writing up.
3	Development of a Low Cost Microfluidic Sensor for the Direct Determination of Nitrate Using Chromotropic Acid	Published, Analytical Methods, 2015 DOI: 10.1039/C4AY01359J	First author, initiation, key ideas, data collection and analysis, manuscript development and writing up.
4	The Development of an Autonomous Sensing Platform for the Monitoring of Ammonia in Water Using a Simplified Berthelot Method	Published, Analytical Methods, 2014 DOI: 10.1039/C3AY41098	First author, initiation, key ideas, data collection and analysis, manuscript development and writing up.

*I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Signed: _____ Date: _____

Deirdre Cogan

Signed: _____ Date: _____

Prof. Dermot Diamond

Signed: _____ Date: _____

Dr. John Cleary

Thesis Outline

A detailed overview of each chapter is given below:

Chapter 1

This chapter gives an overview the common themes apparent within each chapter included in the thesis and sets the following chapters in the context of existing literature. Important topics including: water quality analysis, microfluidics, analytical methods for the determination of nutrients and automated sensors in order to inform the reader on the concepts that will be further used in experimental chapters (chapter 2 to 5). Moreover, this chapter presents the context in which the experimental chapters contribute to the scientific advancement of the research area.

Chapter 2

This chapter is a study on the optimisation and validation of a modified version of the chromotropic acid method for the direct determination of nitrate in water for integration into a sensing platform. The study has demonstrated, for the first time, a direct reagent-based analyser for nitrate, incorporating chromotropic acid and resulting in a quick, inexpensive and simple procedure to measure nitrate in water. The nitrate analyser and modified chromotropic method has displayed excellent correlation to ion chromatography. This work has been done in collaboration with engineers in the Adaptive Sensors Group, in particular Thomas Phelan, who developed the PEDD and wireless communication system.

Chapter 3

This chapter is a study on the integration of the chromotropic method for the determination of nitrate in natural waters into a microfluidic based autonomous analyser. The chapter highlights the importance of materials used for environmental sensors and demonstrates a microfluidic chip that

withstands the acidic nature of the reagent, resulting in a reliable, quick, inexpensive and simple procedure to measure nitrate *in situ*. This work has been done in collaboration with engineers in the Adaptive Sensors Group, in particular Cormac Fay and Dave Boyle, who helped fabricate the microfluidic chip the LED/PD detection and communication system.

Chapter 4

This chapter is a study on the integration of a modified Berthelot method for the direct determination of ammonia in water into an autonomous sensing platform resulting in a reliable, quick, inexpensive and simple procedure to measure ammonia in water. The modified Berthelot method has displayed excellent correlation to ion chromatography. This work has been done in collaboration with engineers in the Adaptive Sensors Group, in particular Cormac Fay and Kamil Jankowski who helped fabricate the microfluidic chip the LED/PD detection and communication system.

Chapter 5

This chapter presents the conclusions of the thesis, summarises the work carried out and gives an overview on the impact that this thesis could have on the development of low-cost, colorimetric sensors. Additionally, this chapter outlines the challenges and opportunities lying ahead, along with some progressive developments that should take place in the near future.

Contents

List of Abbreviations

ACS	American Chemical Society
ADC	Analog to digital converter
CAD	Computer aided design
CCD	Charged-coupled device
COC	Cyclic Olefin Polymer
COP	Cyclic Olefin Co-polymer
EC	European Commission
EPA	Environmental Protection Agency
EU	European Union
FIA	Flow injection analysis
FEP	Fluorinated ethylene propylene
GC-MS	Gas chromatography – mass spectrometry
GPS	Global Positioning System
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
ICT	Information and communications technology
IL	Ionic liquid
IR	Infrared
ISUS	In Situ Ultraviolet Spectrometer
LED	Light-emitting diode
LOC	Lab-on-a-chip
NaOH	Sodium hydroxide
ORP	Oxidation-Reduction-Potential
PC	Polycarbonate
PCB	Printed circuit board
PDMS	Polydimethylsiloxane
PEDD	Paired emitter detector diode
PMMA	Poly(methylmethacyylate)
PSA	Pressure-sensitive adhesive
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene fluoride

R^2	Coefficient of correlation
RSD	Relative standard deviation
SP	Spiropyran
T_g	Glass transition temperature
μTas	Micro total analysis system
UV	Ultraviolet
Vis	Visible
WHO	World Health Organisation
WNS	Wireless network systems
WMO	World Meteorological Organisation

Abstract

The Development of Low Cost Autonomous Chemical Sensors for Environmental Monitoring
Deirdre Cogan

Our ability to effectively monitor the aquatic environment is essential due to the increasing pressure on the environment from pollution, global climate change and the fact that water is an increasingly scarce natural resource. Nutrients such as nitrate and ammonia are essential for ecosystems but surplus levels of nutrients entering water bodies are a serious pollutant, causing eutrophication, contaminating drinking water and killing ecosystems which can cost nations up to $2.2 billion per year. There is therefore a growing need for low cost, remote sensing systems which can be deployed in sufficiently large numbers to ensure that data on key water quality parameters is readily available.

This project has the overall objective of developing low cost analytical platform for autonomous monitoring of environmental water quality. This will be achieved by further development of existing monitoring platforms developed at DCU combined with modified chemical methods in order to reduce the fabrication cost of the devices by an order of magnitude.

Through this strategy, a microfluidic sensing platform for the direct determination of nitrate in water using chromotropic acid has been developed. The chromotropic acid method has been modified to facilitate its implementation into an autonomous platform, resulting in a quick and simple procedure to measure nitrate. The device incorporates a low cost, highly sensitive detection with excellent correlation to the standard method, ion chromatography. Ultimately, this system provides a base in terms of monitoring waters for nitrate levels *in situ* in a rapid, simple and inexpensive manner.

For the determination of ammonia, a simplified variation of the Berthelot method has been integrated into an autonomous sensing platform for reliable, reproducible results showing excellent correlation with ion chromatography.

Chapter 1

Introduction

"Those who dwell, as scientists or laymen, among the beauties and mysteries of the earth, are never alone or weary of life."

Rachel Carson

1.1 The grand challenge

Environmental concern has filtered through the scientific community and landed itself firmly into the everyday lives of today's society. Media broadcasts, along with many of the latest blockbuster films such as *The Day after Tomorrow, Wall-E, Oblivion, Avatar,* share a common theme of man versus nature, with man playing the role of the protagonist villain. Regardless of the extent to which these films adhere to scientific evidence, the fact that this subject is given such attention shows that this issue is of mainstream concern today, making protecting the quality of our environment top of our priorities list.

In order to effectively protect the environment, we must have a clear understanding of the environment. Our ability to effectively monitor the environment at remote locations is essential due to the increasing pressure on the environment from pollution, global climate change and the world's rapidly increasing population. Globally, water is an essential resource for living systems, industrial processes, agricultural production and domestic use, where its quality and availability influences the entire human activity from economics to health. In a period where we are faced with the crucial challenge of providing safe drinking water for almost 9 billion people by 2040, alternative measures must be considered and pursued to provide a solution to this challenge.[1, 2]

In 2012, the World Health Organisation (WHO) released the "UN-Water Global Analysis and Assessment of Sanitation and Drinking-Water" stating that the main objective is to monitor the input required to extend and sustain water, sanitation and hygiene systems.[3] There is a prevailing global necessity for the identification of disruptive water quality monitoring systems or remote sensing platforms to warn of early contamination, which can be deployed *in situ,* in sufficiently large numbers to ensure that data on key water quality parameters is readily

available. Subsequently, the data generated could then be provided to all bodies of interest, be it monitoring agencies, local authorities or the general public, providing the much needed information to allow the development and implementation of effective policies.

In recent years, sensor networks have been the centre of much attention driven largely by the accessibility of wireless communication networks, the influence and capabilities of mobile phone platforms and the interest of large ICT, networks and mobile phone companies. The modern world is now accustomed to instant access to information and data on web-repositories such as news or weather updates. Recent events involving catastrophic environmental disasters such as the 2011 earthquake which struck Japan and the more recent Typhoon Haiyan which hit the Philippines in 2013 and is regarded as the most catastrophic typhoon ever recorded, have highlighted the need for access to real-time environmental data. This data could lead to early warning signs of events while potentially minimising loss of life and increasing health and safety measures.

Environmental chemical sensors with microfluidics playing a key role have great potential as a solution to the increasing demand for environmental monitoring.[4] The advantages of microfluidics-based *in situ* sensing systems are discussed in detail in section 1.4 but can be summarised as:

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

Nonetheless, realisation of this vision for widely distributed environmental sensor networks will require the application of ingenious and creative solutions to sensor development, engineering, platform design and systems integration. To achieve this, the question of how to efficiently gather, analyse and distribute data must be addressed. Furthermore, there are particular issues around the realisation of reliable and robust chemical sensors that can be used for long-term deployments.

Presently, the main challenge facing this ideal of *in situ* environmental monitoring is the multi-disciplinary approach that is needed involving electronics, environmental science, engineering and material science, along with fundamental challenges around surfaces, fouling processes, and fluidics which is described in further detail throughout this chapter. The cost of current sensing platforms and the inability to "deploy and forget" due to limited long term stability and automated platform maintenance requirements are also a major issue that inhibits this model.[5] In a 2010 editorial, Royce Murray, the former editor of the ACS journal '*Analytical Chemistry*' called this the 'grand challenge' for analytical scientists.[6] Given his wide knowledge of the discipline that brings together a tremendous exposure to the emerging capabilities of analytical science, and an appreciation of the needs of society, his words are particularly informed and relevant;

"The "Grand Challenge" posed for analytical chemistry is to develop a capability for sampling and monitoring air, water, and soil much more extensively and frequently than is now possible. Such goals will require improvements in sampling methodology and in techniques for remote measurements, as well as approaches that greatly lower per-sample and per-measurement costs."[6]

Bearing this in mind, the objective of this research is to produce a reliable and robust platform at a price level that creates a significant impact on the existing market. We hope this will lead to the development of compact autonomous instruments for *in situ* continuous monitoring of remote locations with long deployable lifetimes (at least 3 months unattended use).

1.2. Water quality monitoring

Monitoring the quality of our waters influences many areas of society including public health and the general economy due to the industrial and agricultural processes it affects. In 2012, the Environmental Protection Agency (EPA) stressed the crucial links between the environment and the green economy with health, food production, climate change, well-being and national economic recovery.[7] Taking this into consideration, almost 900 million people in the world do not have access to safe drinking water.[8] This outstanding number showcases the need to effectively monitor water supplies to enable early warning detection of contamination and point sources of pollution to the environment.[9]

Water quality assessments are based on a range of parameters including chemical (oxygen, alkalinity, nitrogen, phosphorus), physical (pH, temperature, conductivity) and certain biological taxa. Environmental water quality monitoring can also include the determination of toxins and heavy metals or hydrocarbons. In Ireland, enforcement of environmental law and monitoring, analysing and reporting on the environment is the responsibility of the EPA.[10] The four major purposes for conducting water monitoring procedures are listed below:

1. Monitor and characterise the quality of rivers, lakes and identify changes or trends in water quality over time.
2. Promoting compliance and enforcing regulations.

3. Design and coordinate specific pollution prevention or remediation programs.
4. Respond to emergencies, such as spills and floods.

The EU Water Framework Directive (WFD 2000/60/EC)[11] including the EU nitrates directive (91/676/EEC) are introducing more stringent monitoring of aquatic environments, in particular the nitrates action programme.[12] Although sensor technology is rapidly evolving and some parameters can be measured online (such as pH, conductivity and dissolved oxygen), most of the water analysis and monitoring of nutrients such as nitrate and ammonia is based on *in situ* manual sampling followed by laboratory analysis using sophisticated standard laboratory instruments.[13] This results in the infrequent monitoring of water, as it is time consuming, expensive, non-scalable and requires skilled personnel.[14]This is highlighted in a study carried out by Looney *et al.* who describe how the Department of Energy in the Savannah River, South Carolina, USA, require approximately 40,000 manual groundwater samples per year costing from $100 - $1000 per sample for off-site analysis.[15] Wilson *et al.* explain how 80% of the total cost of clean-up and site classifications is owed to sampling and laboratory analysis alone.[16]

This inability to effectively monitor our water systems at a high frequency and at sufficient temporal densities is the leading challenge facing water quality monitoring today. Current sampling of our oceans may only be achieved on a yearly basis due to the harsh environments and long permeation times to mid-ocean locations while fresh water and coastal sampling for chemical species may only be accomplished at monthly intervals.[17-20] These sampling rates cause concern as this could give rise to inadequate conclusions on the water quality of certain water bodies, as it fails to take into consideration dominant seasonal or daily processes as well as certain weather events such as heavy rainfall or storms.[21, 22] Reliable long-term observations of the environment are

imperative to understanding trends and changeability through either anthropogenic or natural phenomena. This loss of vital data is evident in Figure 1.1, when nitrate measurments were made off the central coast of California at hourly intervals through the use of an optical nitrate sensor, and through manual samples collected near the coast at approximately 21 day intervals. The annual cycle captured by the sensor depicts a series of events throughout the year showing concentrations of nitrate as high as 25μM. This cycle of events is clearly seen in the hourly data using the optical sensor, but is not easily resolved in the manual monthly sampling data.[23] Long-term intensive measurement programmes are difficult to sustain but recent technological breakthroughs may hold the key to address these issues as *in situ* sensors will allow for higher frequency data that cannot be achieved with manual sampling.

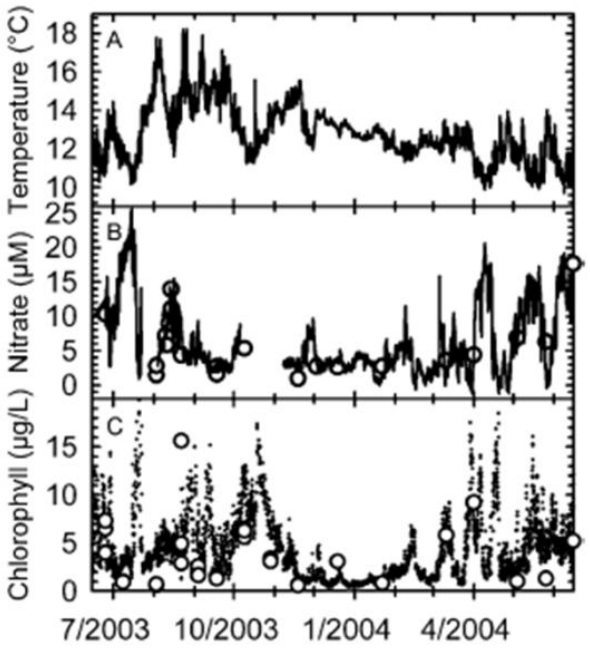

Figure 1.1. Measurements of temperature (A), nitrate (B) and chlorophyll (C) made at hourly intervals over the period from July 2003 – April 2004, offshore in Monterey Bay, California, US. The open circles depict nitrate and chlorophyll measurments achieved by monthly manual sampling and subsequent laboratory analysis. [24]

Autonomous environmental sensors that are low cost (price maximum of €5,000) with relatively long service intervals (3 months or ideally longer) will offer a scalable model, measuring much more frequently at higher geographical densities that will allow for new information on natural processes and hence open the way to a deeper understanding of the dynamics of environmental processes.[25, 26]

1.2.1 Chemical sensors networks

Monitoring of aquatic environments and biogeochemical cycles and their impacts on the environment and climate change will require dense networks of chemical sensors that can operate globally within oceans, lakes, rivers and the atmosphere. Chemical sensor networks have helped add to our understanding of the effects driven by storms,[27] eddies,[28] El Niño oscillations,[29] tidal patterns[30] and the melting of ice caps.[31-33] The best example of an effective chemical analysis sensor network is the system of stations that collect atmospheric measurments of carbon dioxide and oxygen, pioneered by the work of the late Charles David Keeling, who began real time measurments of CO_2 at Mauna Loa in Hawaii.[34, 35] Keeling's work has contributed significantly in raising awareness of global chemical cycles. It is the most influential environmental data set to this date and the continued measurements of atmospheric O_2 combined with atmospheric CO_2 concentrations, have shown to be imperative in constraining global carbon budgets.[36, 37]

While there are no direct examples of dense networks operating globally today, the Argo project particularly demonstrates the potential for the development of networks that sense chemical properties. The project, established in 2000, is comprised of a series of floating platforms which vary their depth through inflation of a bladder. The floats spend most of their lives drifting at depths where they are stabilised by being neutrally

buoyant at the "parking depth" pressure by having a density equal to the ambient pressure and a compressibility that is less than that of sea water.[38, 39] During ascent to the surface, the float measures oceanic variables, transmits the data to orbiting satellite communication networks while on the surface, and subsequently descends back to their parking depth to begin the process again. Although convenient to gather data without human intervention, these floats are limited in their capabilities due to power constrictions (limited by the on-board battery capacity) and their maximum payload (limited by the buoyancy change). Each float is also quite expensive ranging from ca. $15,000. This cost effectively doubles when project is operating due to maintenance and data handling costs. There are currently ca. 3,800 (July 2015) of these platforms deployed and to maintain the array, 800 floats are needed each year making the total cost 800 x $30,000 = $24 million per annum. However, this platform cost does not include the sensor, which would significantly increase the overall cost. [40,38]

1.2.2 Nutrient monitoring

Nutrients like nitrate (NO_3^-), ammonia (NH_3) and phosphate (PO_4^{3-}) are required by all organisms to exist and for the production of food, fibre, and livestock feed. The effect of industrialisation and population growth in most countries has led to a surplus of these nutrients in aquatic systems, in particular phosphorus and nitrogen to estuaries, coastal water and freshwaters through domestic and municipal wastewater, industrial discharges and agricultural sources. This can result in eutrophication, defined by Nixon as “an increase in the rate of supply of organic matter to an ecosystem,”[41] and the affiliated production of undesirable effects.[42] These undesirable effects from nutrient enrichment include high concentrations of algal blooms, oxygen deficiencies or changes in benthic zones and death to biota.[43] The economic costs of these

effects cost the US approximately $2.2 billion per year.[44, 45] Climate change is expected to increase the inputs of these nutrients to estuaries, which may lead to further eutrophication in coastal waters.[46]

Consequently, nutrient pollution is one of the most problematic environmental challenges and it is difficult to implement effective mitigation remedies, without an accurate picture of the concentration dynamics and distribution.[47] Tracking nutrients like nitrate in various waters is therefore an issue of the highest relevance, and is the subject of an international global challenge set by the US-based Alliance for Coastal Technologies.[48] As mentioned previously, current measurements of nitrate are mainly based on manual sampling followed by expensive laboratory analysis using standard laboratory techniques. This 2015 international challenge presents an opportunity to enable groups to show-case low-cost, autonomous prototype monitoring systems for nitrate and phosphate under standardised testing conditions.

Nutrient sensors that have a purchase price of <$5000 purchase price, and meet the specifications laid out in Table 1.1 are in high demand by agencies, utilities, watershed managers, and researchers across the globe. According to a survey carried out in the US by the Association of Clean Water Administrators, state agencies across 28 states envision purchasing up to 750 nitrate sensors, and a further 2500 nitrate sensors for communities.[42]

Table 1.1. Target criteria for nutrient sensor challenge.[48]

Criteria	Nitrate (± nitrite)	Orthophosphate	Weights
Accuracy	± 5 % or 0.01 mg/L – N (at upper range from reference value)	± 5 % or 0.005 mg/L – P (at upper range from reference value)	20%
Precision	± 5 % or 0.01 mg/L – N (at upper range from reference value)	± 5 % or 0.005 mg/L – P (at upper range from reference value)	15%
Range	0.005 – 60 mg/L - N	0.005 – 2 mg/L - P	15%
Deployment	3 months (15 min sampling intervals)		25%
Cost	< $5,000		25%

The main drive of this initiative is to enable informed decisions to effectively reduce excess nutrients, and to capture the full complexity of how nutrients exist in space and time within an ecosystem. This challenge reiterates the need for low-cost reliable sensors for measuring nutrients *in situ* as current methods for measuring nutrient loads cannot meet this growing demand.

1.3 *In situ* chemical sensors

There are three main types of field deployable water quality instruments available; electrochemical instruments like ion-selective electrodes, wet chemical analysers based on flow analysis approaches, and direct spectroscopic analysers including UV-vis systems. Typical environmental parameters of major interest and corresponding measurement techniques are listed in Table 1.2. Despite the inherent attractiveness of distributed environmental sensing, penetration of autonomous chemical sensing technologies into sensor networks remains disappointingly low in comparison to physical sensing. This is strikingly illustrated by statistics from the Argo project, as currently there are ca. 3,843 floats feeding temperature, salinity and pressure (depth) data to the network. In terms

of chemical species however, there are currently only 14 sites monitoring pH, and 51 monitoring nitrate (Figure 1.2), presumably due to the technical drawbacks of commercially available wet chemical sensors which can be prohibitive and often entail regular maintenance over time.[40]

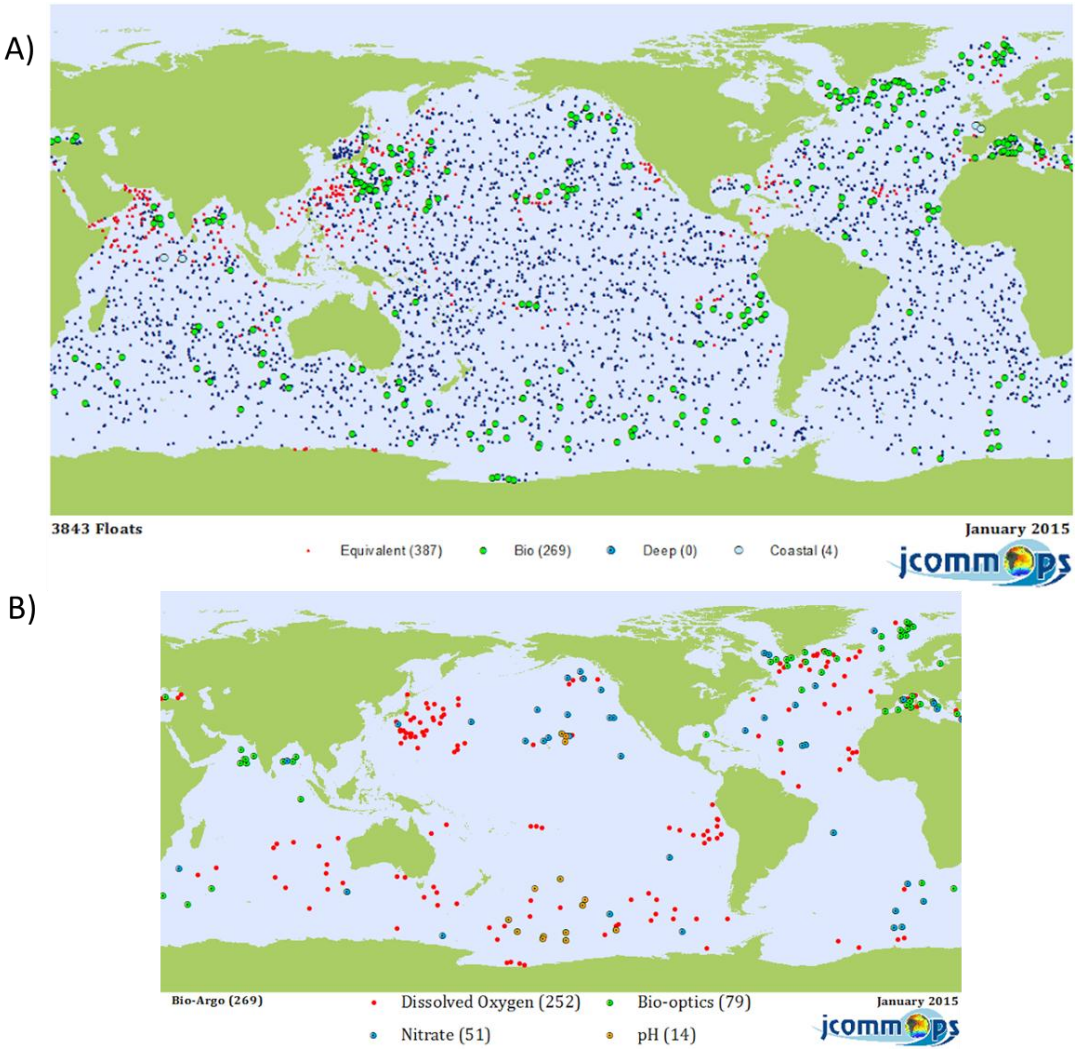

Figure 1.2. Data from the Argo project web site showing the striking number of sensor floats (A) and chem/bio parameters (B).[40]

Table 1.2. Typical list of standard sensing technologies for water quality parameters.[49]

Water Quality Parameter	Sensing Technology	Parameter Applicability
Nitrogen-Ammonia	Colorimetric (phenate/Berthelot reaction), Ammonia-selective electrode	Naturally occurring form of nitrogen in the nitrogen cycle. Dissolved ammonia gas is toxic to aquatic life at concentrations as low as 0.04 milligram per liter (mg/L). EC Drinking Water Directive (98/83/EC) states an ammonia limit value of 0.3 mg/L NH_4^+ and the Quality of Salmonid Waters Regulations (S.I. no. 293 of 1988) stating a total ammonia limit of <1 mg/L NH_4^+.
Chlorine-Chloride	Colorimetric (mercuric thiocyanate reaction); Potentiometric	Indicator of salinity. Associated with a secondary maximum contaminant level (MCL) of 250 mg/L in drinking water.
Conductance	Conductivity cell	Ability of water to carry an electrical current. Strong indicator of dissolved salts. Serves as a surrogate for total dissolved solids.
Dissolved Oxygen	Membrane electrode	Concentration of oxygen dissolved in water can serve as an indicator of chemical and biochemical activity in water.
Nitrogen – Nitrate	Direct ultraviolet spectrophotometric screening, Nitrate electrode, Colorimetric (cadmium reduction or hydrazine reduction followed by analysis with Griess reagent)	Essential nutrient for plants and animals. Nitrate is the most soluble form of nitrogen. Causes health problems in humans. Nitrate levels are regulated through the EC Groundwater Directive (2006/118/EC) and the Drinking Water Directive (98/ 83/EC) which both state an upper nitrate limit of 50 mg/L NO_3^-.
Oxidation-Reduction Potential (ORP)	ORP electrode	Indicator of dissolved oxidizing and reducing agents (metal salts, chlorine, sulfite ion). ORP values above 700 millivolts (mV) kill unwanted organisms in drinking water. A ground water incursion may lower ORP by increasing chlorine demand. Chlorination of drinking water

		produces an ORP background of ~700 millivolts
pH	pH electrode	Indicator of hydrogen ion activity (acidity or alkalinity) of water. Most chemical and biochemical processes are pH dependent. Carbon dioxide/bicarbonate/ carbonate and ammonia/ammonium equilibria are pH dependent. pH of drinking water is well established and controlled. A change of more than 0.5 pH unit indicates a problem.
Phosphorus-Phosphates (Orthophosphate)	Colorimetric (Vanadomolybdo phosphoric Acid, stannous chloride, ascorbic acid)	Naturally occurring but can indicate sewage, runoff from agriculture. Build-up causes imbalance in the nutrient process and eutrophication.
Temperature	Thermistor	A measurement indicator of how hot or cold the water is. DO and specific conductance change with temperature.
Turbidity	Optical sensor; Nephelometric	Indicator of suspended matter and microscopic organisms. Pathogens are more likely to be present in highly turbid waters.

To gather data for oceanography studies, low powered sensors are generally fixed on buoys allowing for real time measurements of temperature, current flow and speed.[50] Other methods for data collection involve deployments with a vehicle, including towing sensors behind scientific vessels. Although this allows for real time data from relatively sophisticated sensors, these deployments are rather expensive. For example the annual running cost of the four Natural Environmental Research Council (NERC) scientist research vessels is approximately $13.5m per annum, or $9k per day per vessel.[51]

A recent review on power sources for remote sensors by Dewan *et* al., comments on how chemical sensors are making little headway into practical adoption for autonomous field based instruments.[5] The reason being that there are few chemical sensors available that are sufficiently robust for continual and wide-spread sensor networks.[52] The major

analytical challenge in measuring chemical analytes in natural waters is the complex background matrices along with the low concentrations of analyte that must be detected. In the laboratory setting, these challenges can be solved through the use of sophisticated instrumentation e.g. gas chromatography- mass spectrometry (GC-MS), high performance liquid chromatography (HPLC), organic extraction-graphite furnace atomic absorption spectrophotometry, and isotope dilution-high-resolution inductively coupled plasma mass spectrometry (ICP-MS).[53,17]

Some institutes have adopted these sophisticated instruments into portable, deployable platforms for *in situ* measurements. Volpe *et al.* demonstrated that ICP-MS could be used for the continuous monitoring of isotopes and trace elements such as barium and molybdenum during a sea deployment. An attractive aspect of this study was the high sensitivity achieved (nmol kg^{-1}), however, signal intensity for internal standards dropped to 60% of initial intensity due to the build-up of salt deposits in the instrument during the sea trial, indicating the need for routine maintenance.[54] Nonetheless, little work has been done since then to adopt ICP-MS for *in situ* monitoring. More recently, ongoing innovations targeting the decrease in cost, size, and complexity of HPLC and HPLC-MS instrumentation raise the possibility that such approaches may become portable and widespread in usage, migrating from the traditional laboratory settings. A study by Welch *et al.* demonstrated that the dependence of these techniques on organic solvents currently limits such deployments. As a result, this study showed that ethanol-based mobile phases for HPLC suggest a greener and more sustainable alternative to petrochemical-derived eluents such as acetonitrile. Although this study showcases household items as mobile phases (rum, vodka, vinegar) for low cost, green alternatives to organic solvents, reduced chromatographic performance was obtained in some cases, indicating that modern HPLC column instrumentation and technology is still necessary. The overall impression is that this approach

to remote environmental monitoring is still at the early development stage.[55] Additionally, a recent study carried out by Elkin has demonstrated an autonomous and field deployable ion chromatography system for on-site analysis of common anions in sub to low micromolar range (Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}). The system was capable of operating for 4 weeks while collecting and analysing stream water at 15-min intervals. It was estimated that 100 ml of eluent allowed the instrument to make ca. 6000 injections. However, the filter back-pressure limited the auto-sampler function to 1900 injections. The reproducibility of the system was below 1% relative standard deviation over a 14-day period. A limit of detection (LOD) for nitrate and phosphate was measured at 0.16 mg/L and 0.55 mg/L respectively. However, pre-treatment of samples was critical to ensuring the chromatographic reproducibility of the instrument as highly charged organic acids, like humics and fulvics, can bind permanently to the analytical column, degrading its capacity. Consequently, trap columns, filter voids and pre-concentration steps were necessary to extend the column lifetime. The main limitation of this system was the high-energy consumption of the eluent pump (maximum power consumption of 62 Wh with a current draw of 5.2 A). This significantly impacted on the size of the instrument itself (ca. 13 kg) due to battery size and therefore mains power is probably needed in order to carry out continuous monitoring in the field.[56] Consequently, in order to obtain a reliable deployment for extended periods of times, simpler and more robust methods for chemical analysis must be implemented.[32]

The most attractive chemical sensors involve the direct interaction with the sensor and analyte such as electrochemical sensors, as these avoid any chemical reagents and greatly simplify the analytical procedure.[57, 58] Ion-selective electrodes for nitrate have been reported with <1 mg/L limit of detection and a linear range of 0.62 - 600 mg/L.[59] Although ISEs are in development with considerably improved sensitivity,[60, 61] in practice they can be complex to use, and may require masking of interfering ions, such

as Cl for NO_3 and K for ammonium (NH_4), through adding complexing reagents or membranes. Ion selective electrodes are not widely used for autonomous environmental monitoring due to essential manual re-calibrations at regular intervals (few hours), however next generation ion-selective electrode membranes might be successful for longer periods without necessary calibrations.[62,63, 64] A nitrate electrode using N,N,N-triallyeucine betaine chloride immobilized in a polymer membrane has been developed that demonstrates long-term stability over months. Scholefield *et al.* autonomously measured diurnal variations of nitrate concentration in stream water over a 2-month period using this approach which was validated using a 90 hr discrete sampling approach.[65]

Direct optical sensors are becoming increasingly popular due to recent progress in optoelectronics. Analytes such as nitrate and sulphide can be measured directly using ultraviolet absorption spectra and do not require chemical manipulations or reagents.[66-68] Reagentless techniques can offer advantages of real-time field measurements over long intervals (months) as reagent and waste bags do not need replacing. Finch *et al.* and Clayson described direct UV analysers for the determination of nitrate with good agreement with a segmented flow analyser.[69, 70] A study by Johnson *et al.* described a high resolution *in situ* ultraviolet spectrophotometer (ISUS) (ca. 1 sec) for detecting nitrate in marine waters with a LOD of 0.93 mg/L nitrate. The analyser was in good agreement with a flow analyser, achieving a correlation co-efficient of 0.97. However nitrate concentrations were 10% lower than the flow analyser at a depth of 400 m due to low temperatures found at this depth. Consequently, optical nitrate measurements required a correction for the impacts of temperature on the instrument performance. Therefore, a correction scheme was put in place where UV nitrate measurments were increased by the same fraction that the UV salinity underestimates the correct value. When this correction factor was applied, results were equivalent to those achieved from the flow analyser reference data.[24]

While this approach offers many advantages for simple fluidic designs, this approach possesses a number of limitations. Substantial power is required for these optical sensors with some systems requiring 5 - 10 W for continuous monitoring.[23] The UV absorption at 220 nm enables direct determination of nitrate, however as dissolved organic matter also absorbs at this wavelength, interferences can be a major area of concern. As dissolved organic matter also absorbs at 275 nm while nitrate does not, a second measurement can be made in this region to correct the nitrate value for these interferents.[71] An *in situ* UV spectrophotometer for measuring nitrate in the North Sea was explored by Frank *et* al.[72] Although good correlations were achieved when compared to wet chemical analysis, discrepancies were found between reference data and *in situ* data derived from the UV spectra due to the technical limits of the sensor. The authors concluded that optical methods may provide a solution where wet chemical analysers cannot be used due to technical difficulties or insufficient temporal resolution. More recently, Johnson *et al.* have made great advances in sensing nitrate in oceans through the integration of ISUS sensors with Teledyne Webb Research's Autonomous Profiling Explorer (APEX) profiling floats. Again the sensors were relatively high power when operating continuously (7.5 W), but the instrument had the ability to operate on low-power mode, where nitrate measurments required a few seconds, using only 45 J of energy per measurement. This allowed for 60 nitrate observations on each profile to 1000 m. Lithium batteries allowed for over 300 vertical profiles from a depth of 1000 m to be made, which results in a deployment lifetime of four years at a 5-day cycle time. This integration of ISUS nitrate sensors into APEX profiling floats has resulted in the production of unparalleled data of nutrient cycling in the ocean.[68] However, the availability and cost of UV light sources that emit to 270 nm and below, in particular UV-LEDs, can be prohibitive for rapid prototyping of these platforms. Although, recent step change advances in manufacturing processes are allowing for more cost-effective, reliable UV-LEDs.

The main limitation of direct sensing, however, is the loss of sensitivity over time and the tendency of these instruments to drift which therefore requires regular system calibrations in order to maintain accuracy.[66] Biofouling can also have a radical effect on ISUS sensors and electrochemical sensor surfaces which are directly exposed to environmental samples, with challenges occurring within days of exposure to sample, especially in waters of high biological productivity.[73] This can cause blockages in pumps and optical flow cells or windows, causing artefacts in the measured chemical concentrations. Consequently, fouling mitigation has been the subject of much research in the last decade[74, 75] but with little fundamental impact.

Through continued efforts however, a variety of sensors have been successfully deployed *in situ* for periods of months. Sensors for dissolved gases are amongst the most used chemical sensors in the aquatic environment.[32] Oxygen sensors are the most popular chemical sensor currently on the field, and mostly incorporate a membrane based amperometric electrode or Clark electrode.[76-78] They are widely used on conductivity, temperature and depth sampler packages that are lowered from ships and measure the vertical distribution of temperature, salinity and oxygen. However biofouling remains an issue, requiring weekly cleaning and monthly calibrations.[79]

The use of floats for marine monitoring has greatly improved the biofouling problem as sensors spend the majority of their time in depths below the eutrophic zone where microbial life is minimal.[80] Nonetheless, ion-selective electrodes have not been widely integrated into autonomous observing systems as they remain subject to drift due to temperature or pressure fluctuations. Figure 1.3 shows the growth of biofilms on an ion selective electrode over a 7-day period. It is apparent that biofilm growth can be detected within one day of exposure to the sample[73] concluding that electrochemical sensors need to be enclosed in less hostile

environments in order to achieve successful long-term deployments and to maintain reliable analytical data.

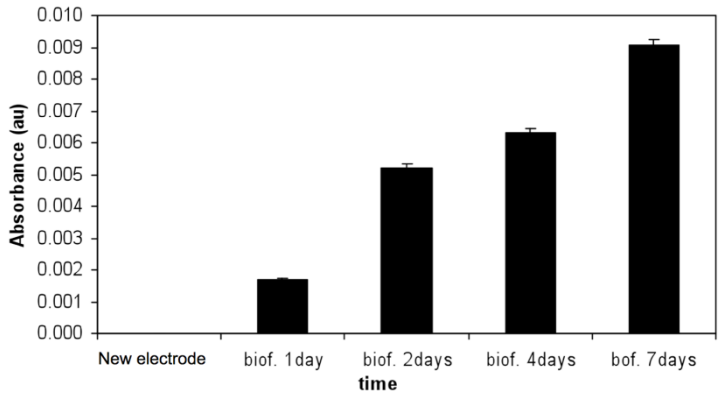

Figure. 1.3. Growth of biofilm on ion-selective electrode over a 1 week period (error bars represent standard deviation $n = 3$).[73]

1.4 Microfluidics

The development of a rapid, reliable, robust microscale system that could offer an alternative to the issues and challenges raised above would be truly revolutionary in terms of the impact on existing market. It is not sufficient or cost effective to use the majority of systems currently on the market that are based on macro-scale fluidics. Challenges associated with these systems include dead volume within the system, consumption of power (10 – 150 W) and the high volume of sample and reagents required (ml per sample) leading to an increase in waste production.[66, 81] However, the miniaturisation of analytical devices through the advent of microfluidics is an important development for future applications. Microfluidic devices are defined as miniaturised reaction vessels fabricated by using methods of microtechnology and precision engineering, that employ three-dimensional structures (microchannels) with diameters typically in the range of 10-500 µm, which are used to

manipulate reagent solutions.[82] Microfluidics provide many advantages such as;

- The small sample sizes used, minimise reagent consumption and waste generation.
- The minimisation of fluid volumes reduces the power requirements associated with fluid control/movement, allowing for long deployable lifetimes.
- The small size of the microfluidic manifold facilitates the development of compact and portable analytical systems.
- Fast analysis times result from performing chemical analysis on the μm scale, where diffusion-based mixing can be an efficient process, allowing high frequency measurements.
- Low-cost sensing devices can be developed by combining microfluidic systems with simple, low cost detectors.

The ability to manipulate fluids on such a small scale has allowed for the evolution of Lab on Chip (LOC) techniques enabling the analysis of small volumes of sample.[83] The LOC should emulate the functionality of laboratory instrument while placed *in situ,* and ideally should be standalone with sample inlet, waste output and data communications as the only external connections, resulting in a Micro Total Analysis System (μTAS).[84] Sensor systems capable of fully investigating the role and impacts of nutrients in the environment will rely heavily on the μTAS ethos as the future of remote sensing platforms means low power, simplicity and robustness. The use of microfluidics not only offers advantages such as the small volumes of fluid used and therefore a decrease in cost, but the low thermal mass and large surface area to volume ratio of small components facilitates rapid heat transfer, enabling quick temperature changes and precise temperature control. In exothermic reactions, this feature can help to eliminate the build-up of heat or "hot spots" that could otherwise lead to undesired side reactions or even explosions.[85] At the small length scales of microfluidic devices,

diffusive mixing is fast, often increasing the speed and accuracy of reactions. Furthermore, a single microfluidic device can perform all the fluidic handling operations, saving time, reducing risk of sample loss and contamination, and significantly decreasing the size and weight of a system, eliminating the need for bulky, expensive laboratory equipment. Additionally, the operation of the microfluidic device can be fully automated, thus increasing throughput, improving ease of use, repeatability and reducing the element of human error. Automation is especially useful in applications requiring remote operation, such as devices performing continuous monitoring of chemical or environmental processes in inaccessible locations.[4]

George Whitesides (Harvard), one of the foremost thinkers in analytical science, and a recognised world leader in microfluidics /lab-on-a-chip (LOC) research stated in 2010;

"Many people, myself included, expected that the ability to manipulate fluid streams, in microchannels, easily, would result in a proliferation of commercial LoC systems, and that we would see applications of these devices proliferating throughout science. In fact, it has not (yet) happened."[86]

He went on to articulate that the coming decade would be one in which the science of microfluidics would come to fruition through a multitude of devices that solve real problems. In the environmental sector, the world market for sensors incorporating microfluidics is expected to grow 23% per annum from 2012 to 2016, to ca. €4 billion[87], driven mainly by the implementation of a battery of environmental legislation in Europe and the USA. France and Germany are the largest and most attractive markets, with the United Kingdom having very high potential but lagging behind.[88] In 2010, the global water market was valued at $515 billion and this is expected to grow to $1 trillion by 2020.[89] In Europe, the

water sector has a turnover of €100 billion per annum, incorporating 9,000 small and medium enterprises (SMEs) and 600,000 direct jobs in water services, accounting for one third of the entire world market.[90] An estimated 54,000 surface water monitoring stations and 24,000 surveillance monitoring are currently in operation in Europe, with an additional 51,000 groundwater monitoring stations. On a global scale, there is an estimated 250,000 water utility companies in operation, 84% under public ownership while the remaining number are privately owned.[91] These numbers illustrate the major economic potential within the water sector. Moreover, the critical issue of water resource scarcity, where global water requirements are expected to grow from 4,500 billion m^3 today to 6,900 billion m^3 by 2030[89], contributes to the significant market for low cost monitoring platforms.

1.4.1 Microfluidics for aquatic environmental monitoring

Thermoplastics are the most popular and suitable substrate materials for microfluidic devices compared to the more conventional microfluidic materials such as silicon or glass. Thermoplastics are a class of synthetic polymers which are soft in behaviour when exposed above their characteristic glass transition temperature (T_g), while returning to their original chemical state upon cooling. They can be easily fabricated and modified using direct machining techniques. Microfluidic devices are generally made by etching or moulding microchannels and subsequently covered using another layer. Silicon micromachining techniques have proven very popular since the growth of the semiconductor industry in the 1950s. However, alternative approaches were explored to result in faster, less expensive approaches to microfluidic machining, such as milling,[92] injection moulding,[93] hot embossing,[94] and laser ablation.[95] Methods for bonding of these substrates together include adhesive

bonding,[96] thermal fusion bonding,[97] surface treatment and modification[98] and solvent bonding.[99]

For colorimetric nutrient sensors, microfluidic substrates must be optically transparent and must not degrade significantly over time. Polydimethylsiloxane (PDMS) is often used for microfluidics chips and valves as it easily fabricated and bound, along with with polymethylmethacrylate (PMMA), polycarbonate (PC), and cyclic olefin polymers (COP) or copolymers (COC).[100] However, care must be taken to ensure the substrate is compatible with sensing functions and reagents for instance, polycarbonate which, although is optically transparent when initially manufactured, turns a yellowish colour after long term exposure to UV light.[101] As a result PDMS, PMMA and more recently COC and COP are frequently chosen for microfluidic applications in autonomous water quality sensors due to their low cost, bio-compatibility, and excellent optical properties that are transparent to light from UV to IR.

A great example of *in situ* microfluidic devices for water quality monitoring is the work carried out by the National Oceanographic Centre Southampton, UK. The devices for monitoring phosphate and nitrate which are discussed in greater detail in section 1.5, incorporated a tinted PMMA microfluidic chip to reduce the amount of light reaching the detector coming from ambient sources or stray light from the LEDs that did not pass through the analyte. The chip was fabricated from milled layers of PMMA and bound using solvent vapour. Stray light from the emitter passes through a greater thickness of the tinted substrate (typically the length of the cell) and is preferentially absorbed. This results in a very specific configuration over the length of the absorption or detection cell, ultimately improving sensitivity, signal/noise (S/N) ratios, baseline noise and limit of detections when used as an absorption cell compared to clear substrates. This is strikingly apparent when an improvement in the limit of detection for phosphate determination (52

nM) was two orders of magnitude below that found in current portable systems based on the vanadomolybdate method.[102] This approach also achieved an LOD of 20 nM with a colorimetric iron assay and an LOD of 0.22 milli-absorption units with a pH assay. [103] Rerolle *et al.* also employed this tinted PMMA approach to develop a microfluidic pH sensor deployed *in situ* for a period of more than a month during a shipboard deployment in European shelf waters, using less than 30 mL of indicator over that period, featuring a short term precision of 0.001 pH ($n = 20$) and an accuracy within the range of a certified Tris buffer (0.004 pH).[104]

In most cases PMMA is the chosen material for prototype microfluidic applications, but for market considerations, PMMA is limited in its range as it is easily dissolved in organic solvents and the optimum operating temperature is ca. 50°C.[105] Chemical resistivity may also be an issue as colorimetric reagents often tend to be of a strongly acidic or alkaline nature (in order to drive the reactions required to generate the analytical chromophore in the presence of the sample), and in some cases, PDMS and PMMA do not offer sufficient chemical resistance to the aggressive nature of these reagents. Water sensors, unlike other sensor technologies, are also exposed to chemically aggressive environments, making the roll out of novel technologies a major challenge. Extreme chemical reagents that may cause corrosion generally require a glass material such as glass or quartz. However, they are not suitable for mass-production as glass chips can be difficult and costly to manufacture. Similar issues apply to chemically resistant fluoropolymers such as Teflons like polytetrafluoroethylene (PTFE) and fluorinated ethylene-propylene (FEP). As aggressive surface treatments are required to sufficiently bond layers.[106]

Polymers such as COP and COC which are made up of hydrocarbons containing oxygen and nitrogen are now more favourable due to their low-cost, excellent optical properties, low oxygen permeability and

resistance to many chemical agents including acids, bases and polar solvents.[107] Surprisingly to date, there are few reports of complete COP/COC microfluidic devices.[108] These surfaces have low specific energy and tend to be hydrophobic which can lead to difficulties in bonding these substrates.[98] By enhancing the surface energy of these substrates bonding can be improved, through processes such as thermal cycling,[109],[110] solvent vapour,[100] and atmospheric plasmas which are explained in greater detail in chapter 3.[111]

COC and COP, thanks to their increasing ease of fabrication and manufacturing, will be increasingly integrated into environmental sensing platforms, compared to traditional substrates used in microfluidic chips, and could significantly advance the development of reliable and robust microfluidic chips.

1.4.2 Optical and colorimetric sensors coupled with microfluidics

An optical sensor converts light energy into electronic signals which in turn, can be transmitted and analysed by a processer unit. A variance in input signal/light energy will result in a change in the electrical output. Optical sensors exhibit many advantages over traditional sensing methods such as good sensitivity, large dynamic range, and multiplexing capabilities.[112] Light emitting diodes (LEDs) have shown great potential to perform optical measurements within microfluidic sensing devices by providing high brightness and high efficiency that can interrogate small sample volumes (ca. 10 nL - >100 μL) and low analyte concentrations.[113] Many studies have explored the use of LED sources and photodetector technologies to produce inexpensive, compact and low power detectors for incorporating colorimetric methods into remotely deployable systems.[114] Most optical detection systems that employ LEDs as a light source, often use a charge coupled device (CCD),[115] a light wave multimeter[116] or a

photodiode (PD).[117] To date, photodiodes are the most commonly used detectors coupled with LEDs for photometry. Dasgupta *et al.* have contributed greatly to the advancements of LEDs as a light source coupled with a photodiode as the detector for analytical measurments.[118-120] The stability and reliability of LEDs have led to many applications for environmental analysis. Hauser *et al.* first reported the use of a blue LED as a spectroscopic source coupled with a photodiode as a detector and was use for the analysis of Cr, Mn, Zn, Fe and Cl and compared to molecular absorption spectroscopy.[121]

Much of the work carried out by Diamond *and co-workers* and Mowlem *and co-workers* have successfully implemented LED/PD detectors in field and process analysers and are mentioned in greater detail in section 1.5.[122-124] A study by Shimazaki *et al.* developed a portable colorimeter with an LED light source and a dual photodiode detector which was successfully deployed on-site for the detection of nitrite ions in river water and results were comparable to that of conventional spectrophotometer with a comparable precision of approximately 1%. The platform had a linear range from 0 – 200 ng/ml N with a limit of detection of 3.7 ng/ml N.[125]

Photodiodes are versatile, with a rapid response and wide linear range, normally three of four orders of magnitude better than phototransistors, and are used in many analytical platforms such as flow injection analysis[126, 127], separation systems[128] and probe photometers.[129] But LEDs can also be employed as a photo-detector as first explored by Mims III *et al.*[130] By combining a reversed biased LED with an operational amplifier used to measure the photocurrent, the LED functioned as a photodiode, and this was used measure to sunlight intensity. The innovative use of an LED as both light source and detector (paired emitter detector diode, PEDD) for chemical analysis was then explored by Lau *et al.*[131] Instead of measuring the photocurrent directly like Mims III

et al. [130] a simpler timer circuit was used to measure the time taken for the photocurrent generated by the emitter LED to discharge the detector LED from 5 V (logic 1) to 1.7 V (logic 0) to give a direct digital output and an external ADC was therefore not necessary. This approach achieved excellent sensitivity when compared to the conventional LED/PD set up.[132] A discharge profile of an LED with a λ_{max} of 610 nm was obtained using a Fluke Scopmeter® (Fluke Corporation, WA, USA). The LED was charged up to 5 V for 500 µs and subsequently switched to discharge mode. This time taken to for the capacitor voltage to drop to 1.7 V is ca. 632 µs, shown in Figure 1.4. A voltage comparator was used to investigate the capacitive voltage compared to a preset threshold. Comparisons are made at fast time intervals and the number of values for which the voltage is greater than the set value is integrated over a fixed time interval (e.g. 100 µs). This integrated number is inversely proportional to the light density (and hence the photo-discharge current).

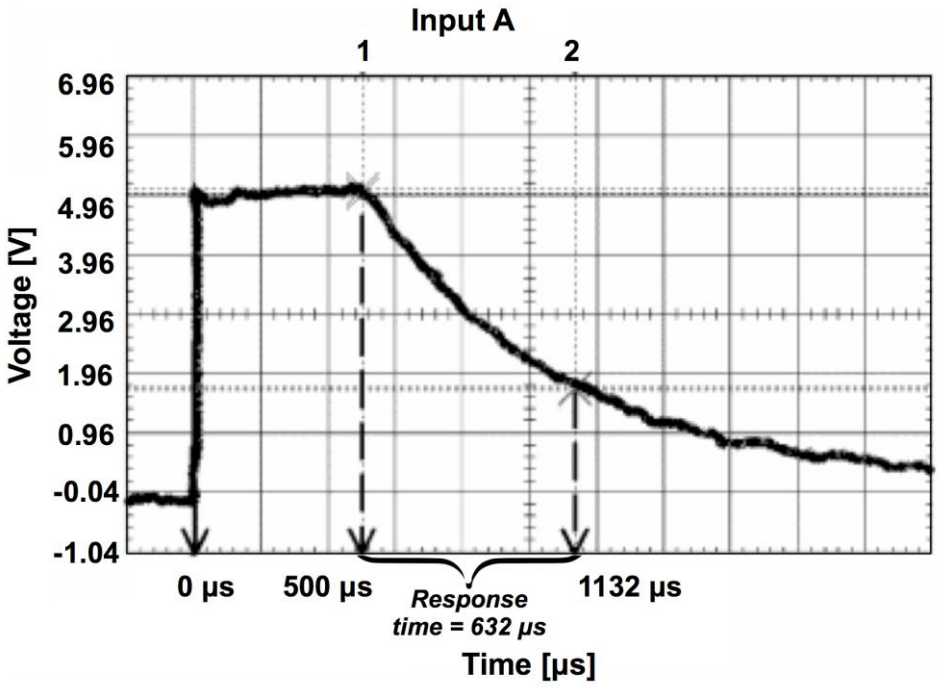

Figure 1.4. Typical discharge curve for an LED charged up to 5 V and then discharged to a threshold of 1.7 V under artificial lighting.[133]

O'Toole *et al.* applied a paired emitter-detector diode for the detection of nitrite using the Griess reagent in a flow injection analysis system. The emitter and detector LED had a λ_{max} of 530 nm and 623 nm respectively. The PEDD flow device achieved a linear range of 0.2 – 100 μM with an LOD of 70 nM NO_2. The PEDD was determined to be reproducible with a relative standard deviation of 2.5% for the detection of 2 μM nitrite Griess reagent complex (n = 10). The PEDD flow device offers advantages of reproducibility, extremely low power consumption, no requirement for an A/D converter or operational amplifier and the sensor can be operated from a 9 V battery. The authors mention this platform can be applied to an autonomous remote sensing system for the monitoring of nitrite levels *in situ*.[134]

A definite advantage of using the PEDD optical detection system in comparison with LED photodiode systems is that the LED–LED combination is much more cost-effective due to components costs (35 US cents per sensor) and the reduced cost of the signal transduction electronics.[135] Additional benefits to the PEDD device is the size, low power consumption (can operate in microwatt range), high sensitivity, broad spectral response range (247 to > 900 nm) and excellent S/N ratio.

1.5 Colorimetric based chemical sensors for nutrient monitoring

Colorimetric assays for the determination of nutrients like phosphate[136], nitrate,[137] nitrite[138] and ammonia[139] are the most commonly used analytical methods due to their relative ease of use, and ability to measure a wide range of concentrations. Table 1.3 contains a list of the most widely used colorimetric methods with relevant features.

Table 1.3. Colorimetric methods for the determination of various nutrients.

Nutrient	Reagent	Operating pH	Sample: Reagent	Measurement Wavelength	Optical Range	Limit of Detection	Reference
Nitrite	Griess	pH 1 – 2	1:1	525 nm	50 nM – 10 µM (0.0023 – 0.46 mg/L NO_2^-)	14 nM (0.0006 mg/L NO_2^-)	Sieben et al.[124]
Nitrate	Griess following a reduction step mainly using a cadmium column	NA	1:1* *Buffer is added to nitrate sample prior to reduction step and further reagents may be necessary to recover reduction efficiency	525 nm	Up to 350 µM (21.7 mg/L NO_3^-)	0.025 µM (0.0016 mg/L NO_3^-)	Margeson et al,[122, 140]
Ammonia	Berthelot/ phenate	pH 11	2:1:1	630 nm	0.04 mM – 1.4 mM (0.6 – 25 mg/L NH_4^+)	7.09 µM (0.13 mg/L NH_4^+)	Sequeira *et al.*[141]
Phosphate	"yellow method" ammonium molybdate, ammonium metavanadate, hydrochloric acid	pH 2	1:1	380 nm	Up to 0.53 mM (50 mg/L PO_4^{3-} - P)	1.05 µM (0.1 mg/L PO_4^{3-})	Bowden *et al.* [142]
Phosphate	"blue method" ammonium molybdate, ascorbic acid, antinomy	pH 2	1:1	715 / 900 nm	Up to 0.02 mM (2 mg/L PO_4^{3-} - P)	0.5 nM (0.05 mg/L PO_4^{3-})	Murphy and Riley[143]

The Beer-Lambert law shown in equation 1.1, forms the basis of light absorbance measurements on solutions within the UV-visible and IR-region,[144] where A is the absorbance of the sample, ε is the molar absorptivity or molar extinction coefficient of the compound ($Lmol^{-1}$ cm^{-1}), b is the path length of the sample and c is the concentration ($molL^{-1}$). The absorbance can be determined using I_0 as the intensity of the light before reaching the sample and I as the intensity of the light after passing through the sample (equation 1.2), which has been reduced to due to absorbance in the sample (reduction also due to reflection losses at the cell and scattering at dispersed particles will constitute an error and must be minimised).

$$A = \varepsilon bc \qquad \text{(Equation 1.1)}$$

$$A = log_{10}\left(\frac{I_0}{I}\right) \qquad \text{(Equation 1.2)}$$

Hence, for an absorbing species in solution, if the Beer-Lambert law is obeyed, the absorbance at a particular wavelength is directly proportional to the concentration. This equation indicates the factors that will affect the sensitivity and resolution of colorimetric methods when integrated into field deployable platforms.

Perhaps the biggest challenge in chemical sensor research is deciphering the best approach to employ within the sensing platform. Dutt *et al.* examines the determination of nitrite by means of spectroscopic techniques, electrochemical techniques and chromatography. The study concludes that although the chromatographic methodology is precise in its results it faces many challenges when encountering complex samples. Therefore spectroscopic assay techniques prevail as the most viable option for integration into an autonomous sensing platform.[145] Operating platforms incorporating a simpler design that can perform analysis

without subject to drift with high sensitivity are scarce among most chemical analysers and are generally based on flow injection analysis (FIA).

Wet chemical sensors are based on processes to accomplish reliable analysis of a sample, based on standard spectrophotometric methods originally established for the laboratory. This generally involves the mixing of a sample with a reagent, resulting in a change in the optical properties, by means of a colour change, if the analyte of interest is present. This colour change is then detected by measuring the absorbance of the resultant sample which can then be compared to the absorbance of a blank or standards to generate a quantitative value of the analyte in question. The choice of optical detection with this approach is dependent upon the specific chemistry of the nutrient, reagents and the physical properties of the product. Field deployable measurements mainly include dissolved nutrient species like nitrate, nitrite, ammonia and phosphate as well as total nitrogen and phosphorus. Many standard measurement techniques for detecting nutrients like nitrate and phosphate have been integrated for field deployment. Generally, the nutrients are reduced by chemical or photo-reduction, followed by colour formation (often using azo dyes or molybdate blue dyes) and analysis at a particular wavelength.[146] Hardware components include pumps to control the transport and mixing of sample and reagents using tubing, valves, and a manifold for mixing and detection. Reagent and sample are generally pumped into a mixing chamber, and this resultant complex is pumped through a fixed length optical cell, the length of which gives the absorbance length determining the concentration range over which useful measurements can be made according to equation 1.1.

Wet chemistry analysers are capable of detecting the greatest range of N and P species. UV-vis and ISEs are limited in the number of detectable analytes but offer the greatest potential for fast measurements (with

some sampling frequencies down to < 20 s). However, wet chemical analysers detect nutrient concentrations down to approximately 0.002 mg/L while few deployable ISEs offer detection limits less than 0.1 mg/L.[147] Colorimetric chemistries probably offer the most reliable and dependable methods for the detection of nutrients in water. These include the Berthelot method for determination of ammonia and the Griess test for the determination of nitrite.[145, 148] Be that as it may, these well tried and tested laboratory methods are making little headway into the market for autonomous field based instruments. Cost is undoubtedly a major factor, as reagent based analysers are often expensive, mainly due to the need to incorporate expensive fluidic handling components like pumps and valves, which can comprise 60% or more of the total component cost. [149, 150]

Standard colorimetric techniques have been adopted for continuous flow analysers of nutrients including the cadmium reduction method for nitrate and the molybdate method for phosphate.[151, 152] However the chemical reagents used within these methods may deteriorate over time, therefore auto-calibration may be necessary to improve accuracy and data quality. A unique and exciting prospect of these platforms is the capability of self-calibration by incorporating a blank solution and a solution of known concentration within the system.[67, 153] A study by Chapin *et al.* demonstrates that this approach can achieve a years' worth of data when measuring iron in hydrothermal vents at depths of over 2000 m.[154]

Diamond and co-workers presented a low cost chemical sensor for long-term monitoring of phosphate levels based on the "yellow method" that incorporates sampling, reagent and waste storage, detection, and wireless communications into a compact and portable device shown in Figure 1.5.[142] A yellow colour is formed when a sample containing phosphate is mixed with the reagent containing ammonium molybdate and ammonium

metavanadate under acidic conditions, which absorbs strongly at 370 nm. The first-generation prototype (Figure 1.5(A)) for this system consisted of bottles for storing the reagent, calibration solutions and cleaner, a sample port for collecting the water sample to be analysed, and an array of solenoid pumps for pumping the required liquids through the microfluidic chip. The microfluidic chip allowed mixing of the reagent and sample, and also presented the reacted sample to a photodiode and LED for an absorbance measurement. The analysed sample was then pumped to the waste storage container. All of the fluid handling and analytical components were controlled by a microcontroller which also performed the data acquisition and stored the data in a flash memory unit. A GSM modem was used to communicate the data via the SMS protocol to a laptop computer. The first generation phosphate analyser was validated in the laboratory[155] and during deployments in wastewater[156] and estuarine water.[157]

A substantial redesign of the phosphate analyser was later carried out in collaboration with an industry partner in order to reduce the component cost of the system (to approx. €200 per unit) and improve manufacturability while maintaining or improving the analytical performance. The major design alterations and improvements implemented during this process were described in detail by Cleary *et al.*[158] and include:

- More compact, cylindrical design for better portability and deployability.
- Improved external mounting system.
- Flexible bags for storage of colorimetric reagent, calibration solutions and waste.
- Low-cost dual channel peristaltic pumps.
- Redesign of microfluidic chip.

- Bubble detection and removal protocol.
- Implementation of ZigBee radio based communications.
- Redesign of sample intake/filter module to incorporate antifouling measures

The mixing and detection took place in a custom-built PMMA microfluidic chip where the absorbance of the compound formed was measured using a 370 nm light emitting diode (LED) and a photodiode. The sensor was placed *in situ* at Broadmeadow Water Estuary Co. Dublin, Ireland for a 62-hour deployment in which 124 autonomous measurements were made. For validation purposes, manual samples were collected followed by laboratory analysis using a HACH-Lange DR890 portable colorimeter. Phosphate concentrations measured by the sensor were in good agreement with the samples analysed by the colorimeter, achieving a correlation coefficient of 0.9706. Following the success of the initial field trial, the sensor was then placed *in situ* at an outflow tank in Osberstown wastewater treatment plant, Co. Kildare, Ireland for a deployment period of one month. A ZigBee radio communicator was used to transmit the data generated to a network gateway and local database on site. This allowed for instant access to data and the ability to remotely control the sampling rate of the system and results are shown in Figure 1.6.[159] Although the design of the analyser is based on the phosphate chemistry, the system can be adapted to fit colorimetric reagents and detectors for other target analytes. This work demonstrates that by combining microfluidic technologies with colorimetric chemistry, light emitting diode (LED) based optical detection systems and wireless communications, reliable low cost monitoring systems can be developed.

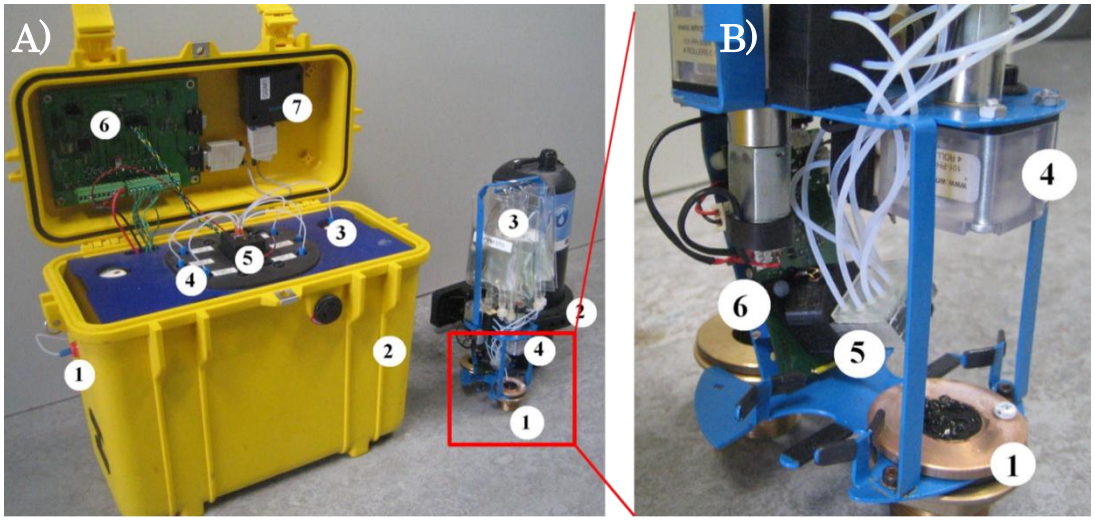

Figure 1.5. (A) First and **(B)** second generation phosphate systems designed by Diamond et al. (1) Sample inlet; (2) IP68 Enclosure; (3) Reagent storage; (4) Pumps; (5) Microfluidic detection system; (6) Control board; (7) Communications.[159]

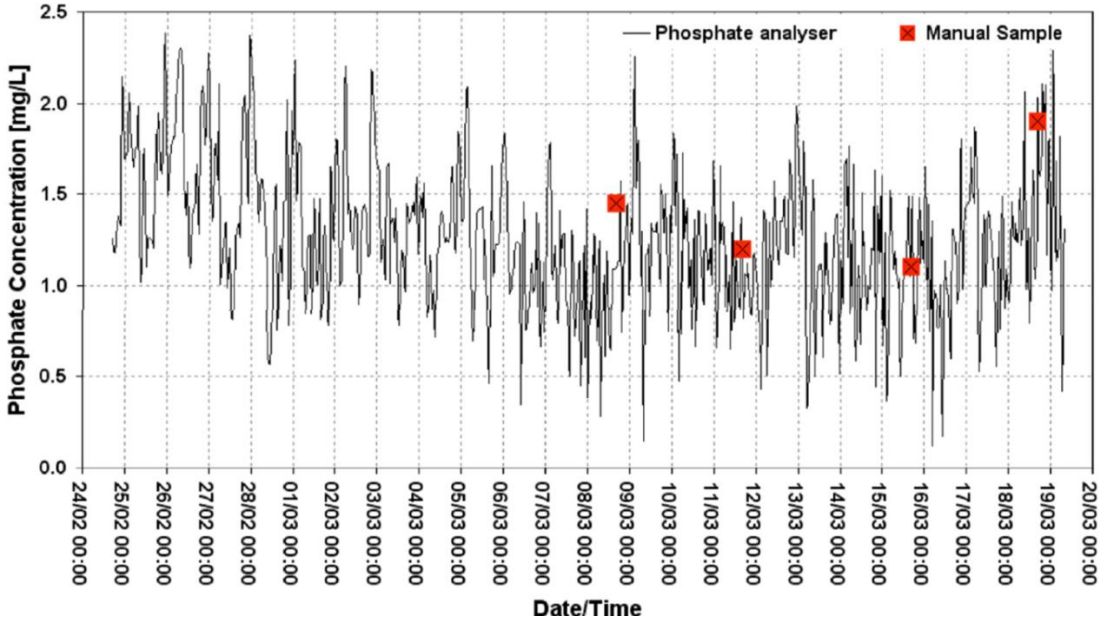

Figure 1.6. Data from the DCU phosphate analyser and manual calibrations samples represented as red squares, over the period 24/02/2011 – 20/03/2011, the regular fluctuations arise from the switching of local feeder pumping stations. [159]

The colorimetric determination of other analytes such as nitrate however, can prove a more difficult task due to the relatively intricate procedures involved, probability of interferences present and the limited detection ranges associated with various methods. Many techniques have been developed and studied for the determination of nitrate including methods involving ultraviolet spectrophotometric screening, nitrate electrodes as mentioned previously, but the majority of spectrophotometric methods based on colorimetric methods for determination of nitrate in natural waters require its reduction to the more reactive nitrite followed by colorimetric detection. The most commonly used reduction methods use cadmium[160], hydrazine[161], zinc[162] and nitrate reductase. [163] An early application of the detection of nitrate by reduction to nitrite using a cadmium column is described by Greenway *et al.* through the use of an electro-osmotic flow in a microchip. A reductor frit was employed to reduce nitrate to nitrite followed by analysis of the complex using the Griess test. The findings demonstrated that nitrate and nitrite could be sequentially investigated by flow injection analysis on the same device and was capable of measuring nitrate between 0.5 and 20 μM with a correlation coefficient of 0.985. The relative standard deviation (RSD) at 5μM NO_3^- was 8.3% (n = 6) and the limit of detection was calculated at 0.51 μM (0.026 μg/ml NO_3^-). The method however produces shortcomings in terms of its reproducibility compared to conventional FIA systems due to mixing challenges.[164]

Reduction of nitrate through the use of hydrazine was investigated by Kempers *et al.*[165] The purpose of this study was to investigate the reduction mechanism of nitrate by hydrazine sulphate. The homogenous catalysed reduction compared well with the heterogeneous catalysed cadmium reduction column, obtaining a linear range of 0.7 nM to 70 nM and a limit of detection of 0.3 nM NO_3^- with a RSD of 0.7%. However, while these approaches may yield reliable data, there are drawbacks. To maintain satisfactory analytical results using a cadmium column,

substantially skilled personnel are needed, and a more complex system than is ideal is required.[166] To ensure a low-cost automated, reliable platform, the colorimetric method should ideally be comprised of one reagent, mixed in a 1:1 sample to reagent ratio.[155] Moreover, cadmium is highly toxic in aquatic systems due and disposal can prove difficult and expensive.

In an effort to reduce the hazardous nature of this method by avoiding the cadmium metal, Schnetger *et al.* developed a method to determine nitrate in marine waters using vanadium (III) chloride as a reduction step. This method allowed for cost-effective analysis of nitrate in freshwater, brackish water and seawater, with nitrate reduction efficiency of 100 ± 3% (n = 12), limit of detection of 0.4 μM and a linear range up to 80 μM. The authors stated that the method had been adopted to a fully automated discrete analyser using a loop flow analyser technique. However, a long reaction time was necessary for colour formation, 10 - 20 h at room temperature, or 60 min at 45°C, which is not ideally suited for an autonomous field deployable platform as this will give rise to low sample throughputs and a heating requirement introduces a complex additional step to the procedure.[167]

Beaton *et al.* presented a microfluidic colorimetric sensor applied *in situ* for the determination of nitrite at Southampton Dockhead for a deployment period of 57 hours where 375 measurements were made, describing the transition from the laboratory setting to the field. This study is as a good example of an *in situ* microfluidic based sensor. The work achieved focused initially on the detection of nitrite using the Griess reagent. In this method, the anion reacts with sulfonyl amide and the resulting product with N-naphthyl-ethylenediamine (NED), resulting in an azo compound detected at 543 nm. The reaction took place in a microfluidic chip incorporating a 150 x 150 μm channel where mixing of the reagent and sample took place, with a subsequent 0.5 cm long

detection cell shown in Figure 1.7. A light emitting diode at a wavelength of 525 nm was used as the light source, where the authors reported a limit of detection of 15 nM.[122]

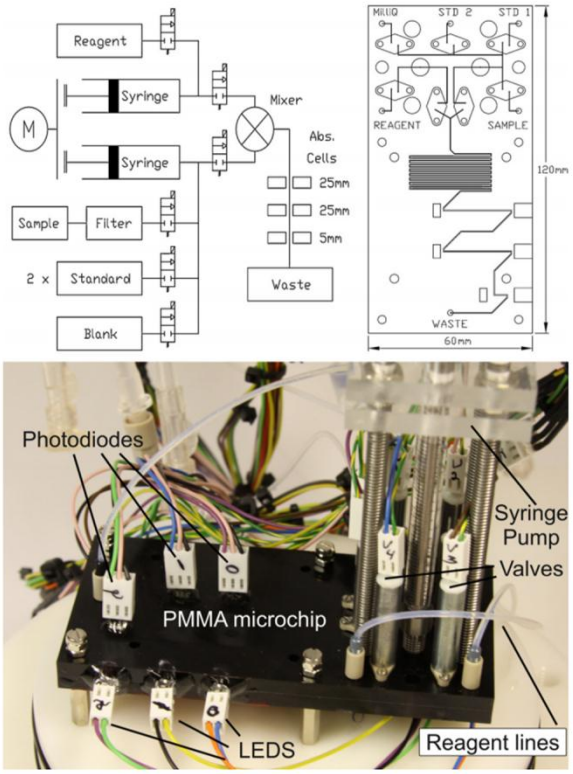

Figure 1.7. (Top) System fluidic diagram and chip schematic, depicting channels 150 µm wide and 300 µm deep, and an optical detection cell of 300 µm wide and 300 µm deep. (Bottom) Nitrite PMMA microfluidic device. [122]

Following the success of this deployment, nitrate detection was also incorporated onto the platform. The detection cell was reduced to 0.25 mm, as samples would reach concentrations above 30 mM. For nitrite detection, the sample was filtered at the input and pumped to a reference cell where sample was mixed with Griess reagent. For nitrate detection, the sample was filtered at the input and passed through a cadmium column for reduction to nitrite, followed by a similar process as nitrite. The device was then placed in an estuary for a period of 27 days (Figure 1.8). During this time storage bags were replaced. Although the study successfully demonstrates a, standalone, automated nitrite and nitrate

sensor with low power and reagent consumption, the authors stated the need for further optimization, including a possible valve that would allow Griess reagent to be returned to the storage bag on all but the final stroke of each flush cycle, and the use of multiple parallel stopped flow to increase sampling rate, and the possibility to integrate the cadmium tube into the microfluidic chip.[168]

This approach for determining nitrate is an indirect method, and requires measurement of background nitrite followed by a second measurement of total nitrate (after reduction to nitrite) and nitrite. The nitrate concentration is then obtained by subtraction. The reduction step adds complexity to the fluidic system; e.g. valves for diverting flow through the reduction column as well as the addition of buffers and column washing.

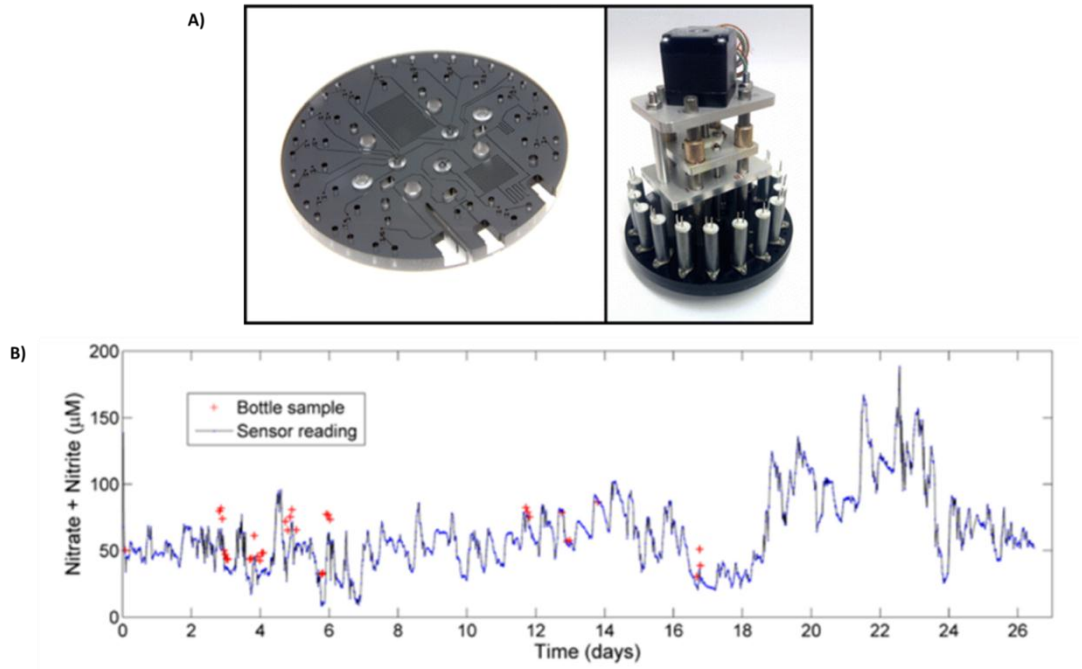

Figure 1.8. (A) Nitrate microfluidic chip and sensing platform (B) 26-day estuarine deployment measurements of total (nitrate + nitrite) data and grab reference samples represented with red crosses. [168]

It is self-evident that the best approach to take with reagent based analysers is to keep the fluidic design as simple as possible as this will improve the overall reliability of the system and reduce cost. An ideal example of this is a simple "T" design incorporating one channel for reagent, and the other for sample or standards. This requires simplification of previously developed colorimetric methods, which are usually multi-stage, but are often not reported in the literature.

Alternative colorimetric methods used for the direct detection of nitrate which do not incorporate a reduction step include the chromotropic acid method and resorcinol method.[169, 170] Although a recent study by Bulgariu *et al.*[171] has been carried out describing the chromotropic acid method, no single study exists describing the integration of the method into a sensing platform which is discussed in further detail in Chapter 2. This may be due to the intricate steps involved with the method. Batten *et al.* describes the successful determination for nitrate using chromotropic acid where a solution of chromotropic acid was prepared in a sulphuric acid medium. A yellow colour was observed and the absorbance was measured at 400 nm on a spectrophotometer. However the method initially prompts challenges due to its complexity in the heating and cooling steps.[172] Afghan *et al.* performed a similar study but investigating the effect of concentrated sulphuric acid, temperature, time of colour development and reagent concentration.[173]

A recent study by Zhang *et al.* reported on the direct analysis of nitrate using resorcinol. The method appeared relatively simple to implement although there were a number of steps involved in the procedure including the addition of concentrated sulphuric acid, a waiting period of 30 min, followed by a water bath at room temperature for 5 min, and the addition of deionised water.[174] This resulted in a maximum absorption at 505 nm, and a detection limit of 0.5 μM with a linear relationship up to 400 μM, and results obtained were in good agreement when compared to the cadmium reduction method.

Both the chromotropic acid and resorcinol method involve the presence of sulphuric acid in order to form nitric acid in the sample. This may be an issue as the strongly acidic environment drastically constrains the materials that can be used to store and transport the reagent around the fluidic system. The acid plays an important role within the reaction and is discussed in greater detail in chapter 2. Both chromotropic acid and resorcinol attach to the nitronium ion forming an unstable intermediate due to the breaking of the electron delocalization of the benzene ring. An interesting aspect to note here is that the breaking of this delocalization requires energy (i.e. heating) for the reaction to occur. It was observed however, that the exothermic reaction caused by the dilution of the concentrated sulphuric acid by the sample should provide an ample amount of heat to allow the reaction to occur at a sufficient rate.[175] Yet in their approach to the resorcinol method Zhang *et al.* required a heated water bath to aid sufficient colour formation. In contrast to this Bulgariu *et al.* did not use a heating step in their study on the direct determination of nitrate using chromotropic acid and a sufficient colour was formed under 10 minutes. In keeping with the ethos that simple colorimetric methods aid the integration and reliability of autonomous sensing platforms, it can be concluded that the chromotropic acid approach would be more suitable than the resorcinol method for integration into a working platform, and hence there is major scope to explore this.

1.5.1 Current commercial systems

Although there is a considerable amount of literature on chemical sensors for the analysis of dissolved chemical species, much of this work is focused on the development of prototype sensors while the current market possesses a limited number of sufficiently robust sensors capable of being deployed for extended periods of time. There are five main commercial suppliers providing *in situ* wet chemical analysers focused

mainly for continuous wastewater treatment monitoring and these are represented in Table 1.4 along with the instrument related costs.[147]

Wet chemical analysers tend to be more expensive with prices in the range of €10,000 - €100,000, but vary significantly for the instruments considered in Table 1.4 and depend on which measurement capabilities are chosen. The wet chemical instruments incur additional costs due to chemical reagents, preparation and waste disposal. Additional costs may also be necessary if supplementary hardware is needed like microprocessor controllers, data loggers, or recommended components like on-board cleaning units.

Table 1.4. Specification for high frequency *in situ* wet chemical analysers. [147]

Provider/ Equipment	**YSI™/ YSI96000**	**Systea™/ Micromac C**	**Ecotech™/ FIA NUT100**	**EnviroTech™/ AutoLAB/MicroLab**	**FIALab™/ SIA**
Parameters	NO_3-N	TN, NO_3-N, NO_2-N, NH_4, N, TP, PO_4-P	PO_4-P	NO_3-N, NH_4-N, PO_4-P	NO_3-N, NO_3-N, NH_4-N, PO_4-P
*Cost/unit**	€20 000	€67 500	€12 800	€33 000	€71 000
Minimum Temporal Resolution	30 min	15 min (single channel), 38 min (multi-channel), 60 min for TN and TP using multi-channel	30 s (1/30 Hz)	15 min (single channel), 60 min (multi-channel)	15 min
Detection Limits (mg/L)	NO_3-N: 0.005-2	TN, NO_3-N: 0.002 NO_2-N: 0.002 NH_4-N: 0.002 TP, PO_4-P: 0.001	PO_4-P: 0.002-0.120	NO_3-N: 0.002 – 13 NH_4-N: 0.002-4 PO_4-P: 0.002-1.5	NO_3-N: 0.003, NO_2-N: 0.001, NH_4-N:0.010, PO_4-P: 0.003
Ambient operating conditions	1-45 °C	NO_3-N, NH_4-N, PO_4-P: 4-40 °C TN, TP: 10-30°C	n.a.	n.a.	n.a.
Accuracy	± 5% of range	± 3% of range	± 2% of range	±2% of range	±2% of range
Telemetry	Yes	Yes	Yes	Yes	Yes
Warranty	1 year	2 years	1 year	1 years	1 year
Weight	18.2 kg	36 kg	n.a.	25 kg	n.a.

* Indicative price of instrument only, 2010.

1.6 Biomimetic actuators- next generation systems for water quality analysis

In microfluidic based instruments, valves play an important role in the control and manipulation of the liquid flow. Autonomous analysers built by Diamond *et al.* and their resulting component cost is shown in Figure 1.9. Through the use of microfluidics (Gen 2) and 'future' platforms based on all-polymer pumps and valves developed through fundamental materials science research, the cost of these systems can be brought down considerably by focusing on reducing the fluidic components cost. [176, 177]

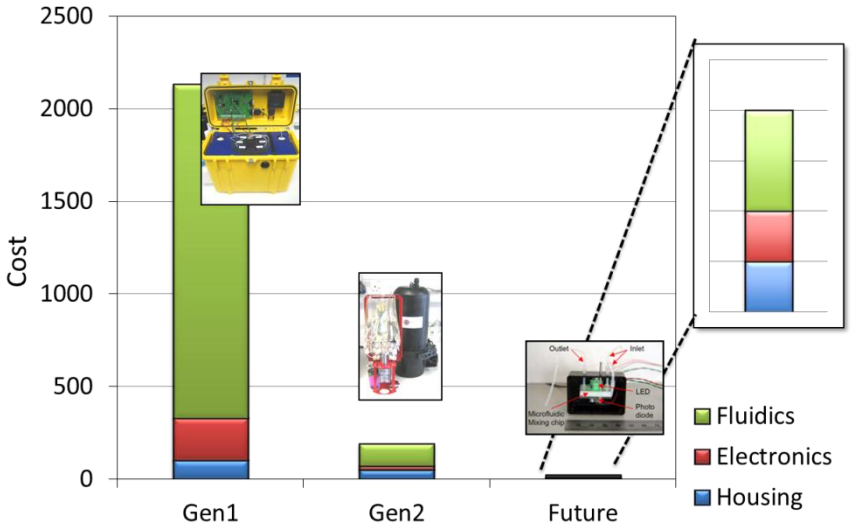

Fig. 1.9. Component cost analysis of autonomous chemical sensing platforms illustrating fluidic handling (pumps and valves) as the largest contribution to cost falling less than 50% through the integration of microfluidics (Gen 2) and on all-polymer pumps and valves developed through fundamental materials science research (future). [150]

An area of interest that can provide solutions to the mentioned problems for truly revolutionary analytical systems are polymer actuator valves.

These valves can be fully integrated into microfluidic chips and used to control liquid movement thus replacing the conventional valves that are the fundamental limiting factor in existing platforms, in terms of price and miniaturisation. Novel multifunctional materials based on polymer gels (in particular ionogels) have been used as light-actuated valve structures in micro-fluidic platforms.[178] Through variation of the composition of the ionogels, the micro-valves can be opened or closed in order to control liquid flow within the micro-channel. Benito-Lopez *et al.* presents the fabrication, characterisation and optimisation of different ionogels as photo-actuated valves and the integration of these into a microfluidic manifold.[179] Although many different forms of microvalves have been demonstrated, it is important to note that none exist on a working sensing platform fit for field deployment and this is discussed in further detail in chapter 6.[150] Notwithstanding this issue, replacing pumps and valves with stimuli- responsive polymers presents itself as a tipping point in terms of scale of deployments for water quality monitoring.

1.7 Conclusion

The over-arching challenge is to develop *in situ* chemical sensors which are sufficiently inexpensive and robust to be deployed at the temporal and geographic densities required to deliver reliable data on key environmental parameters such as nutrients. There is no doubt that there is a need for low-cost reliable monitoring systems capable of measuring nutrients *in situ* for long periods of time as current systems do not meet the operational requirements and cost constraints.

Through the use of a multidisciplinary approach with microfluidics and simplified colorimetric chemistries at the forefront, progress can be made to truly revolutionise environmental monitoring. This thesis presents the

various chemical methods and techniques that could be used in an effort to simplify and effectively lower the cost of these platforms while maintaining satisfactory analytical results.

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Chapter 2

Integrated flow analysis platform for the direct detection of nitrate in water using a simplified chromotropic acid method

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Aims and objectives

Chapter 2 describes the optimisation, validation and integration of a modified version of the chromotropic method for the direct determination of nitrate in water, into a flow analysis system to allow monitoring of nitrate in wastewater and freshwater with a detection range and limit of detection in agreement with EU legislative compliances.

Abstract

This work describes the first use of a direct nitrate analyser using chromotropic acid. A simplified chromotropic acid method eliminating several steps previously associated with this method is employed in the platform. In a sulphuric acid medium, chromotropic acid reacts with nitrate ions and produces a characteristic yellow colour associated with an absorbance band in the visible region (λmax = 430 nm). The modified method allows for nitrate determination over the linear range 0.9 – 80 mg/L nitrate with a limit of detection of 0.73 µg/L nitrate. Validation was achieved by analysing water samples from various sources including groundwater, trade effluent and drinking water by the modified method and by ion chromatography. The method was implemented on a flow analysis platform incorporating a paired emitter-detector diode (PEDD) as the optical detector. An excellent correlation coefficient of 0.993 was obtained between the modified method and ion chromatography. The modified chromotropic acid method represents a rapid, simple, low cost technique for the direct determination of nitrate in water.

2.1 Introduction

The implementation of the Water Framework Directive in 2000 provides a major incentive for the improvement of water quality in Europe as all European Commission members must achieve "good water status" by 2015, and represents a significant driver towards increased monitoring of the quality of our water systems.[1] The introduction of Horizon 2020 in 2014 will also provide significant actions in environmental monitoring.[2] There is therefore a growing need for low cost, reliable sensing systems which can be deployed in sufficiently large numbers to ensure that data on key water quality parameters is readily available. This would allow

local authorities and other stakeholders to make well-informed decisions on the management and protection of our environmental waters.

Autonomous environmental sensors have great potential as a solution to the increasing demand for environmental monitoring.[3] By combining microfluidic technologies with colorimetric chemistry, light emitting diode (LED) based optical detection systems and wireless communications, low cost monitoring systems can be developed which are capable of being deployed for extended periods of time. Sensors for nutrients such as nitrate in water and wastewater are particularly important. Nitrate based fertilisers are applied to promote plant growth, but surplus nitrate entering water bodies is a serious pollutant, causing eutrophication.

Nitrate can also affect health directly, for example through methemoglobinemia, a process by which increased levels of methemoglobin are produced in the blood, resulting in the disruption of haemoglobin and preventing adequate oxygen from reaching the body tissue.[4] Nitrate levels in various types of water are therefore regulated as per the European Commission's Groundwater Directive (2006/118/EC) which states a nitrate limit value of 50 mg/L and the Drinking Water Directive (98/ 83/EC) also stating a nitrate limit of 50 mg/L. Consequently the demand for inexpensive and robust systems for monitoring nitrate has risen sharply in recent years.[5, 6]

Despite the inherent attractiveness of distributed environmental sensing, paying particular attention to nitrate, penetration of autonomous chemical sensing technologies into sensor networks remains disappointingly low in comparison to physical sensing. This is strikingly illustrated by statistics from the Argo project, which was established in 2000 to provide distributed sensor information related to the global

marine environment.[7] Currently, there are 3,558 floats feeding temperature, salinity and pressure (depth) data to the network. In terms of chemical species however, there are only 2 sites monitoring pH, and 27 monitoring nitrate. Of the latter, all are employing the direct optical measurement of nitrate (despite the limited selectivity and rather poor sensitivity of the method) in preference to much more selective and sensitive reagent or separation-based techniques.

Furthermore, monitoring for nutrients such as nitrate in our waters is predominantly based on *in situ* manual sampling followed by laboratory analysis using standard laboratory instrumental and/or wet chemical methods. This results in the infrequent monitoring of water at a fewer number of locations than is desirable, as it is time consuming, expensive, non-scalable and requires skilled personnel. Determination of nitrate can prove difficult at times due to the relatively intricate procedures involved, probability of interferences present and the limited detection ranges associated with various methods. Many techniques have been developed and studied for the determination of nitrate including methods involving ultraviolet spectrophotometric screening, nitrate electrodes, cadmium reduction, titanous chloride and hydrazine reduction.[8] Why are well tried and tested laboratory methods for nitrate making so little headway into practical adoption for autonomous field based instruments? Cost is undoubtedly a major factor, as reagent based analysers are often up to €15,000 per unit (Microlab Ltd. autonomous phosphate analyser retails at ca. €25,000 per unit, including vat, in Ireland; autonomous instruments from YSI, Abb and Hach are similarly priced), mainly due to the need to incorporate expensive fluidic handling components, like pumps and valves, which can comprise approximately 60% of the total component cost. [9, 10]

Therefore, in order to drive down the cost of ownership of these devices, it is important to keep the fluidic handling requirement as simple as possible, as complex, multistage methods are correspondingly difficult and expensive to implement as well as being less reliable in long-term deployments due to high purchase and maintenance costs. Bearing this in mind, it is not surprising that direct UV-spectroscopy is the current method of choice, despite its analytical performance issues and relatively high cost. This method proves difficult to implement into a microfluidic platform as LEDs in the low UV range (ca. 220 nm) are still in development while LEDs in the range from 240 nm – 300 nm cost approximately US$200 with relatively limited operating lifetimes. Recently, the Griess method has been investigated in autonomous microfluidic environments with some success, but the method is inherently complex due to the need to incorporate a reduction step (usually via a cadmium reduction column), and estimation of the nitrate concentration through a differential 2-stage measurement.[11] Notwithstanding these issues, the Griess method remains popular, and a number of recent articles describe its implementation through various fluidic platforms.[12] Concisely, there is no method available that meets the operational requirements and cost constraints at this present time for the direct determination of nitrate.

The purpose of this study therefore, is to investigate a simple, reliable and rapid method for the direct determination of nitrate without the use of columns or expensive materials, allowing the integration of a method into a simple deployable platform for the autonomous monitoring of nitrate. For that reason, a colorimetric technique for nitrate based on the use of chromotropic acid has been examined and optimised.

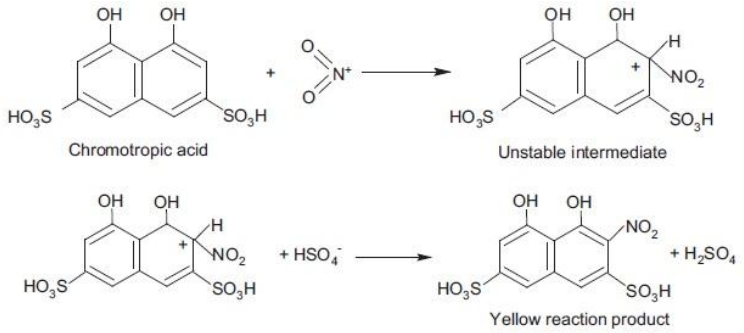

Figure 2.1. Mechanism of nitrate detection employing the chromotropic reaction method.

The method was first described in1966 by West *et al.* followed by a study performed by Clarke *et al.* for the determination of nitrate in soil.[13,14] Although a recent study by Bulgariu *et al.* has been carried out describing this method, no single study exists describing the integration of the method into a sensing platform.[15] This paper describes the modification of this method to produce a simple, rapid technique for the determination of nitrate with a wider linear range, lower limit of detection and faster reaction time, allowing for its incorporation into an autonomous monitoring system.

In a sulphuric acid medium, chromotropic acid reacts with nitrate ions to produce a characteristic yellow colour (λ_{max} = 430 nm), via the mechanism shown in Figure 2.1. In concentrated sulphuric acid, nitrate ions are converted into nitronium ions which react with the chromotropic acid to form an intermediate which is relatively unstable at high temperatures (ca. 130 °C). This energy is generated by the exothermic reaction produced by addition of the aqueous solution to the sulphuric acid. The proton is then removed and recombines with the hydrogen sulphate ion leading to the reestablishment of the electron delocalisation in the aromatic ring. The exact structure of the product formed as a result of this reaction is still the subject of debate.[15]

The first reported methods using chromotropic acid as a technique to determine nitrate included complex procedures such as cooling and heating steps, with added reagents for increased sensitivity, see Figure 2.2. [16] This study however has eliminated this complexity, reducing the method to a one-step process based on a 1:1 reagent to sample ratio, which is ideally suited for an automated sensing platform for nitrate.

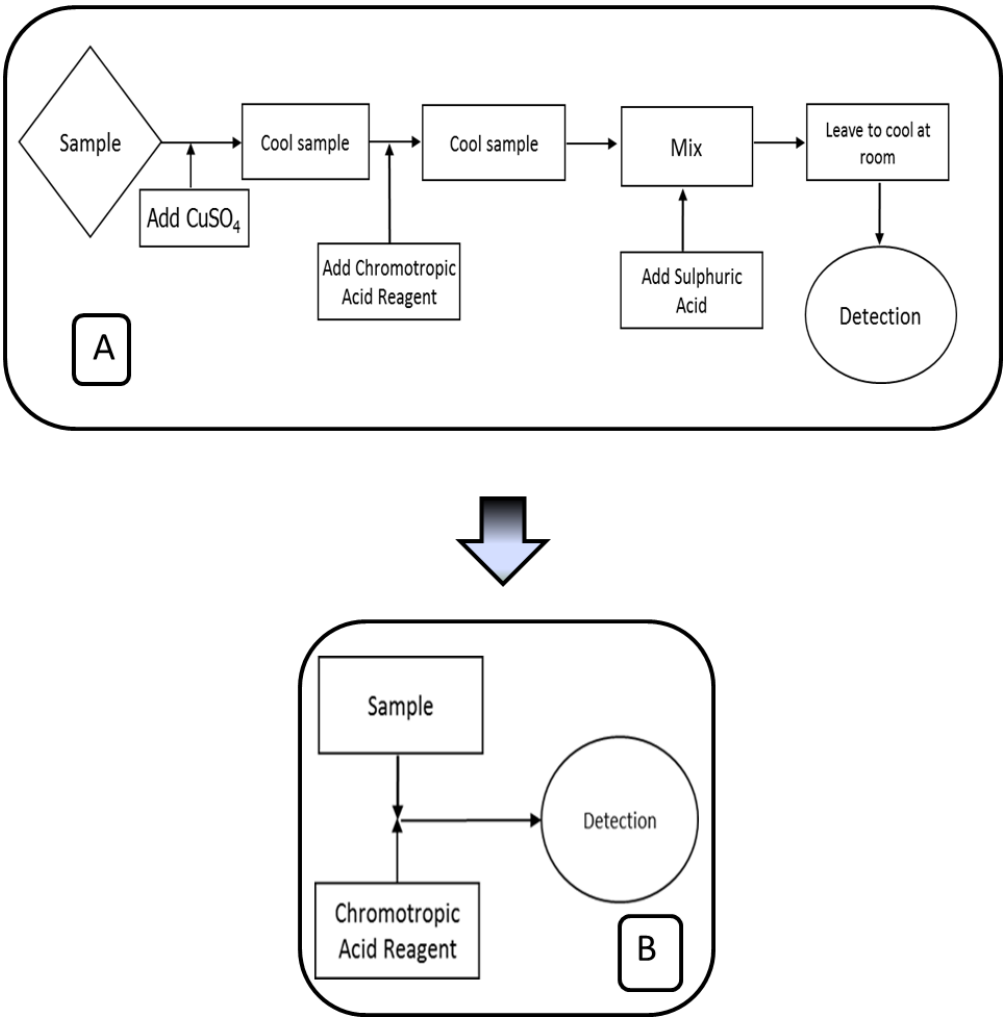

Figure 2.2.(A) The conventional chromotropic acid method for direct determination of nitrate, showing the various stages involved in generating the coloured complex; **(B)** The simplified chromotropic acid method.

In this chapter, a novel automated detection system is introduced incorporating the modified chromotropic method for the direct determination of nitrate to produce a low-cost, wireless optical sensor based on a paired emitter-detector diode (PEDD) as a photometric detector system. Concisely, the PEDD system employs an emitter and detector LED with λ_{max} of 430 nm and 630 nm respectively. The benefits of using the PEDD system as a detection source are its low cost and high sensitivity.[17] The PEDD system has previously been used for a range of chemical detection applications including sensing of pH, phosphate, nitrite and carbon dioxide.[18-21] This inexpensive, miniature, power efficient detection system achieves exceptional sensitivity while also being remarkably simple to fabricate, making it an ideal detector for coupling with this method.

2.2 Experimental

2.2.1 Colorimetric reagent

The modified method established involves dissolving 0.0294 g of chromotropic acid ($C_{10}H_6Na_2O_8S_2.2H_2O$, Sigma-Aldrich, Ireland) in 100 ml concentrated (96% v/v) sulphuric acid (H_2SO_4, BDH Laboratory Supplies, UK). When stored under coloured glass and protected from light the reagent is stable for at least one month.

2.2.2 Deionised water and standards

All solutions were made up using analytical grade chemicals. Deionised water from a Millipore Milli-Q water purification system was used throughout the analysis. Stock standards were freshly prepared weekly. Nitrate stock standard solution (100 mg/L NO_3^-) was prepared from

potassium nitrate (KNO_3, Sigma- Aldrich, Ireland) that was pre-dried for 1 hour at 110 °C.

2.2.3 Measurement procedure

The colorimetric reagent was added in a 1:1 v/v reagent to sample ratio and the solution was allowed to stand for at least 5 minutes in the dark for colour development. The absorbance of the solution was measured at a λmax of 430nm. Spectrophotometric measurements were performed using a VWR UV-1600PC spectrophotometer. Measurements were recorded using M.Wave Professional software (VWR, Ireland).

2.2.4 Fabrication of PEDD optical detector and measurement procedure using nitrate analyser

The autonomous nitrate analyser manifold consisted of two peristaltic pumps, mixing junction, and a sensing channel in which the absorbance from the complex formed was measured using a PEDD system with a Wixel microcontroller (Pololu Corporation, USA). The PEDD detector device contains two LEDs with one acting as the light source and the other acting as a detector as described previously. The LEDs were then placed into a PEDD flow cell generated by a 3D printer (Dimension SST 768) ensuring accurate alignment of the two LEDs.[17]

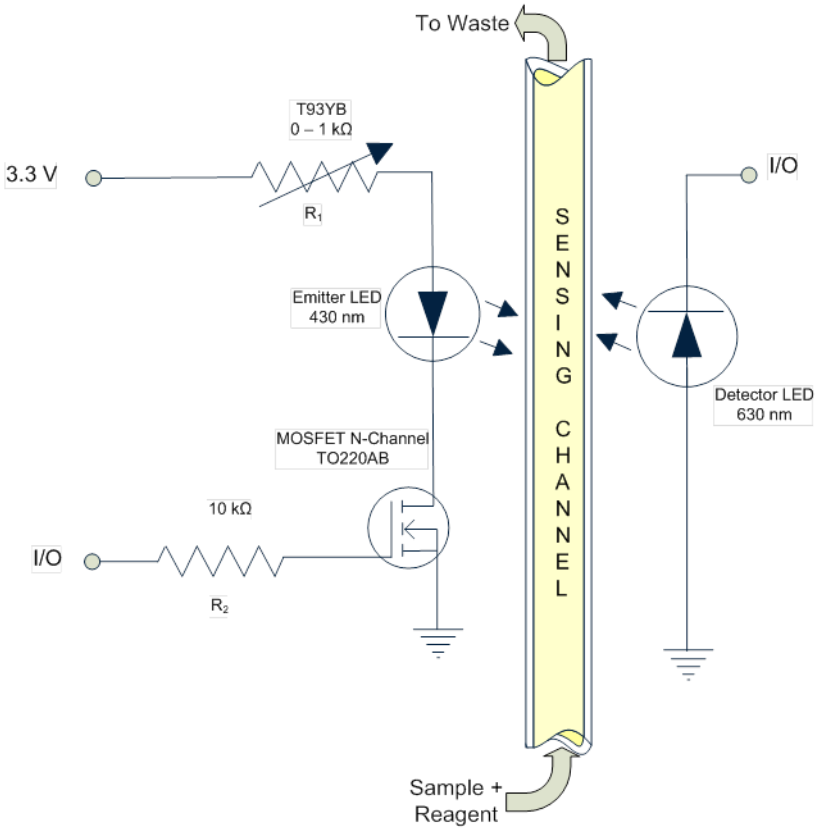

Figure 2.3. Schematic of the PEDD detection system.

The fabrication of the PEDD cell was described in detail by O'Toole et al. and is illustrated in Figure 2.3.[17] Briefly, a regulated voltage source was used to power the emitter LED. A 430 nm LED (Radionics Ltd, Ireland) was used with a λ_{max} of 430 nm and an emission bandwidth at $^1/_2\lambda_{max}$ of 60 nm (Kingbright Specifications) which overlaps the absorbance spectrum of the nitrate—chromotropic acid complex. A variable resistor altered the LED light intensity while a transistor switched the LED on. The detector LED, connected in reverse bias (acting as a capacitor) to the microcontroller switchable input/output (I/O) pin, was supplied with 3.3 V for 100 μs and then switched to input mode. The detector LED generates a photocurrent related to the intensity of the light arriving from the emitter LED through the sample, and discharges this (3.3V) stored voltage. The more light absorbed by the sample, the slower the rate of discharge. The micro-controller then measures the time taken for

the detector LED to discharge, in other words, the time taken to discharge from an initial value of 3.3 V (logic 1) to a preset value of 1.7 V (logic 0). This discharge time is referred to as the PEDD count.

The nitrate analyser manifold (Figure 2.4) consists of two micro peristaltic pumps (Series 100, Williamson Manufacturing Company Ltd) employing Santoprene™ tubing of 3.2mm bore (Radionics Ltd, Ireland). The pumps deliver the sample and reagent via Tygon® medical grade tubing (formula 2075 I.D 1.6mm, Sigma Aldrich, Ireland) to the mixing junction. The coloured complex formed passes through the PEDD system in a modified glass flow cell (Brand Ltd Cat. No. 7477 15) at a combined flow rate of 0.8 ml/min and an optical path length of 2.5 mm.

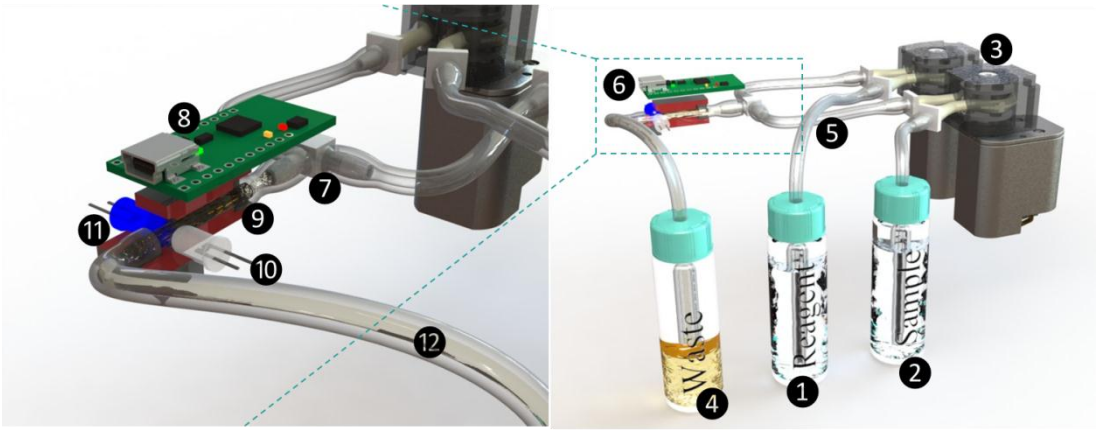

Figure 2.4. Nitrate analyser and PEDD detection system. (1) Reagent storage (2) Sample storage (3) Peristaltic micro pumps containing Santropene® tubing (4) Waste storage (5) Tygon® tubing (6) PEDD flow cell (7) Mixing junction (8) Wixel microcontroller wit with breakout board containing wireless serial link and data logger (9) Glass flow cell (10) Detector LED at 630 nm (11) Emitter LED at 430 nm (12) Waste line (Tygon tubing).

The reagent and samples were pumped through the mixing chamber followed by a 15 minute reaction time prior to monitoring of the absorbance by the PEDD detector. This results in a minimum sampling frequency of 30 minutes due to the pumping of reagent and sample (5 minutes respectively) followed by reaction time (15 minutes) and detection (5 minutes). The detector signal was transmitted wirelessly to a laptop via the Wixel's wireless communication function and saved as a text file followed by analysis in Microsoft Excel.

2.2.5 Fabrication of heating device

In order to investigate the effect of heat on the reaction, a heating device was constructed consisting of 6 x 2 W metal film resistors which were placed within a vial holder surrounded by insulation foam. A regulated 12 V voltage supply was used to power the heating device. By choosing a

low resistance of 100 Ohms for each resistor, a high current was produced, resulting in the heating of each resistor.

2.3 Results and discussions

2.3.1 Optimisation of method parameters; calibration

The optimum wavelength was investigated by obtaining the absorbance spectrum of a chromotropic acid and 80 mg/L nitrate complex. The absorbance of the complex is quite broad, with the λ_{max} around 430 nm. This was consistent with literature values, which typically range from ca. 400 – 450 nm.[14, 16, 22] A calibration plot was performed using a set of prepared nitrate standards from 0.9 - 80 mg/L in a 1:1 v/v sample to chromotropic reagent ratio (Figure 2.5A).

The molar extinction coefficient (ε) was estimated to be 1.302×10^3 L mol^{-1} cm^{-1} at 430 nm, with a correlation coefficient of 0.991 and an average RSD (n = 3) of 2.2%. The linear range was also improved to 80 mg/L nitrate compared to previous methods cited with a linear range of 0 – 11 mg/L nitrate.[15]

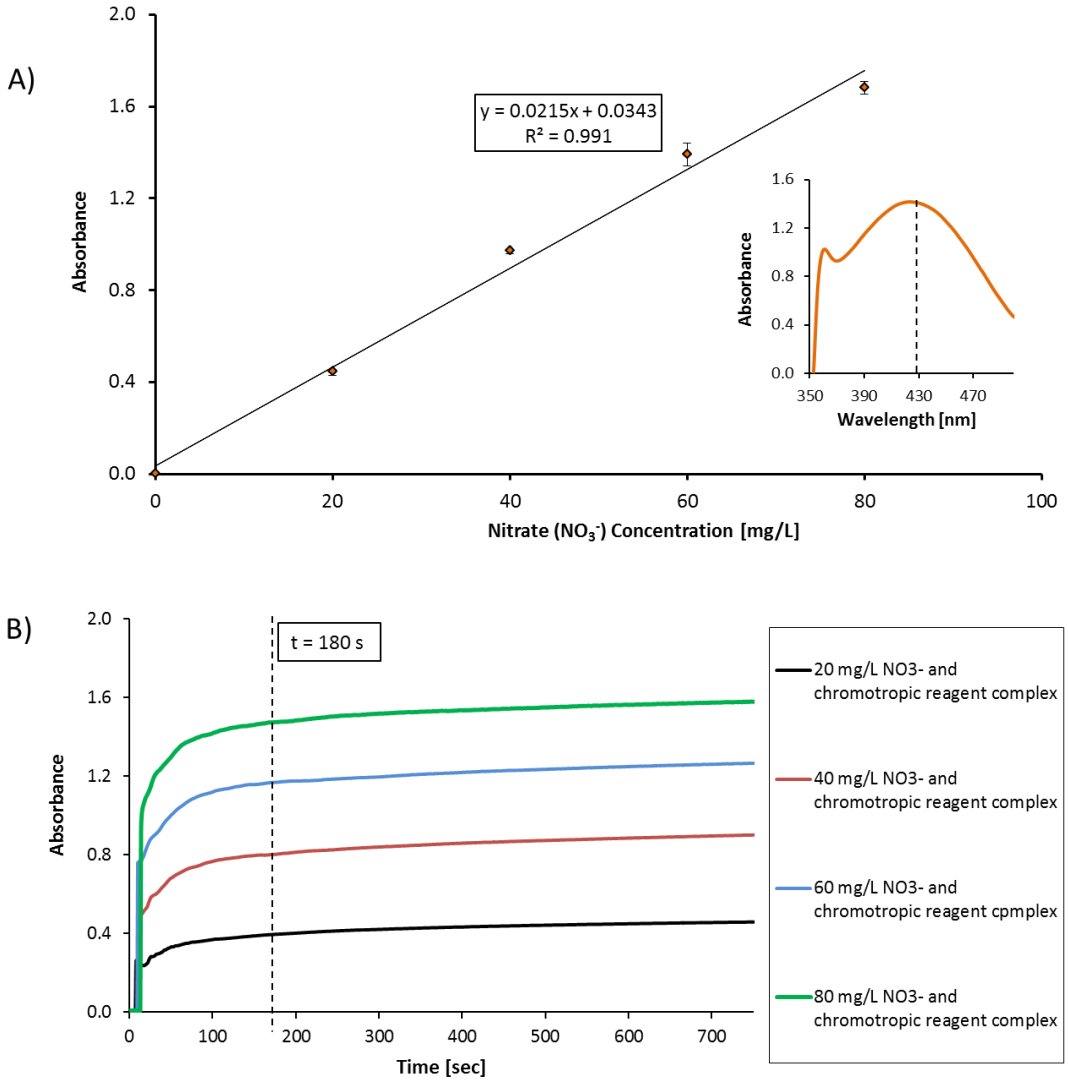

Figure 2.5. (A) Calibration plot of nitrate standards ranging from 0 - 80 mg/L nitrate in the chromotropic acid reagent (1:1 v/v). The error bars represent the standard deviations for n = 3. The absorbance spectrum of the nitrate-chromotropic acid complex using 60 mg/L nitrate illustrating the λ_{max} at ca. 430nm is also displayed.**(B)** Kinetic study of the chromotropic acid-nitrate complex using various concentrations of nitrate [mg/L].

2.3.2 Kinetic study

The rate of development of the nitrate chromotropic reagent complex colour intensity was monitored at a temperature of 23 °C for the detection of 20 – 80 mg/L nitrate. The absorbance at 430 nm was taken every 30 seconds for 900 seconds. The colour formation followed first order kinetics, increasing rapidly until approximately 180 seconds, after which the rate of increase was minimal as shown in Figure 2.5(B). Previously, a reaction time of 1800 seconds was recommended, but the significantly faster reaction time obtained here is an obvious improvement in terms of potential sample throughput in an autonomous integrated system.[15]

2.3.3 Limit of detection

The limit of detection (LOD) was determined by obtaining a calibration graph of low nitrate concentrations from 0.01 mg/L to 0.05 mg/L and the standard deviation (SD) of the baseline of the blank which consisted of the chromotropic acid reagent and 0 mg/L nitrate (n=60 data points, frequency of measurement: 1 data point/sec) figures for which are shown in Table 2.1. The LOD was calculated as the concentration equivalent of 3xSD of the blank solution, giving a value of 0.64 μg/L nitrate, a significant improvement on the previously reported limit of detection of 0.12 mg/L nitrate.[15]

Table 2.1. Absorbance values of blank (0 mg/L nitrate and chromotropic acid reagent) and low nitrate concentrations (0.01 – 0.05 mg/L nitrate and chromotropic acid reagent).

Concentration [mg/L NO_3^-]	Average Absorbance	Standard Deviation
Blank	0.0032	0.00026
0.01	0.0106	0.00049
0.02	0.0228	0.00010
0.03	0.0316	0.00032
0.04	0.0398	0.00081
0.05	0.0552	0.00060

2.3.4 Validation of method

Following optimisation of the measurement parameters, a validation process was implemented using 8 samples from various environmental sources including waste water effluent, surface water, drinking water and standards. It was noted from the literature that only nitrite ions give a positive interference when determining nitrate with the proposed method.[14] As nitrite in the micro molar region is found only in eutrophic environments and nitrite present in surface water is readily oxidised to nitrate, nitrite interferences were not of major concern.[14]

The samples were split, and parallel assays independently performed at the T.E. Laboratory site. The samples were filtered prior to analysis using nylon membrane filters with pore size of 0.45 µm (Acrodisc® syringe filters, Sigma, Ireland). The chromotropic method was used at both sites and the results compared to reference measurements obtained using ion chromatography.[23]

There is an excellent correlation as illustrated in Figure 2.6 between the chromotropic method and ion chromatography concluding that the chromotropic method is ideal for integration into an autonomous analyser for the direct detection of nitrate. The discrepancy observed for sample E (nursing home effluent), with the chromotropic acid method showing a concentration of ca. 90 mg L^{-1} nitrate compared to 115 mg L^{-1} nitrate obtained using ion chromatography, is due to the sample concentration being above the linear range for the chromotropic acid method as described above.

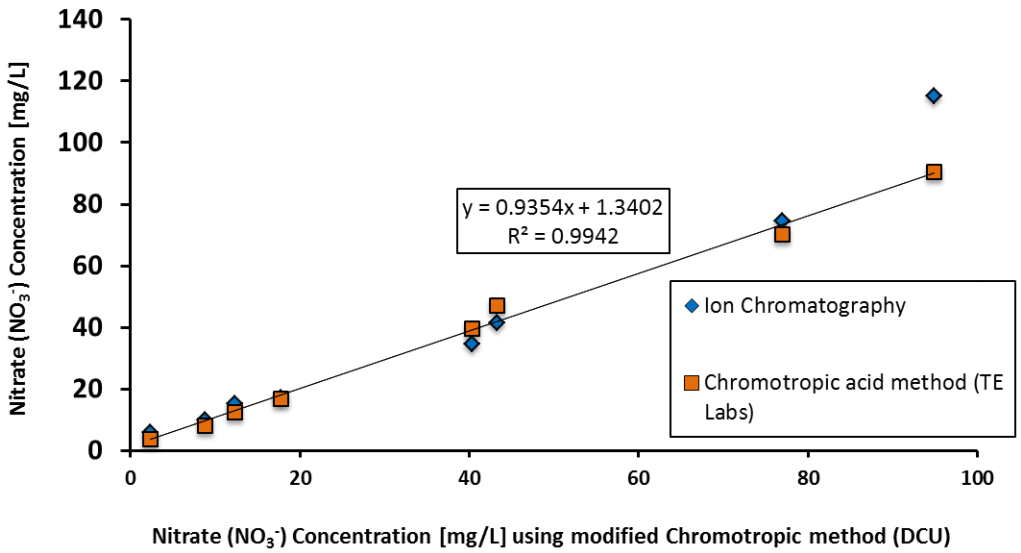

Figure 2.6. Correlation plot of sample concentrations obtained using the chromotropic acid method (DCU) and ion chromatography (TE Labs).

2.3.5 Limitations of method

It should be noted that difficulties may arise when mixing the sample and chromotropic reagent. As mentioned previously the heat energy produced from the exothermic reaction, due to the dilution of the concentrated sulphuric acid, aids the colour formation for the colorimetric detection of nitrate. It was observed at the benchtop level, that

significant discrepancies can be found when varying the mixing and addition techniques of the sample to the acidic reagent. These discrepancies may be due to different mixing behaviour arising from the manual addition of the reagents to the cuvette, which leads to differences in the quantities of heat energy released, which in turn affects the formation of the coloured complex. For instance, if the sample is added rapidly the acid will react with the water more vigorously, generating a higher temperature, and creating an intense colour. On the contrary, if the sample is added more gradually to the reagent, the heat energy is developed over a longer time, resulting in a less intense colour.

Notwithstanding these issues, by implementing the method into an integrated flow analysis platform, these inconsistencies are significantly reduced by consistent pumping of reagent and sample and reproducible mixing behaviour in the flow system.

2.3.6 Integration of method into flow analysis platform

The chromotropic reagent and a range of standards were prepared as per section 2.2.1. Figure 2.7(A) shows the calibration plot obtained using nitrate standards up to 80 mg/L with an average RSD ($n = 3$) of 0.9%.

10 blind samples were provided by T.E. Laboratories Ltd. and analysed with the prototype nitrate analyser platform. In order to provide reference data, the samples were also measured using ion chromatography. The strong correlation between the nitrate analyser and ion chromatography is shown in Figure 2.7(B).

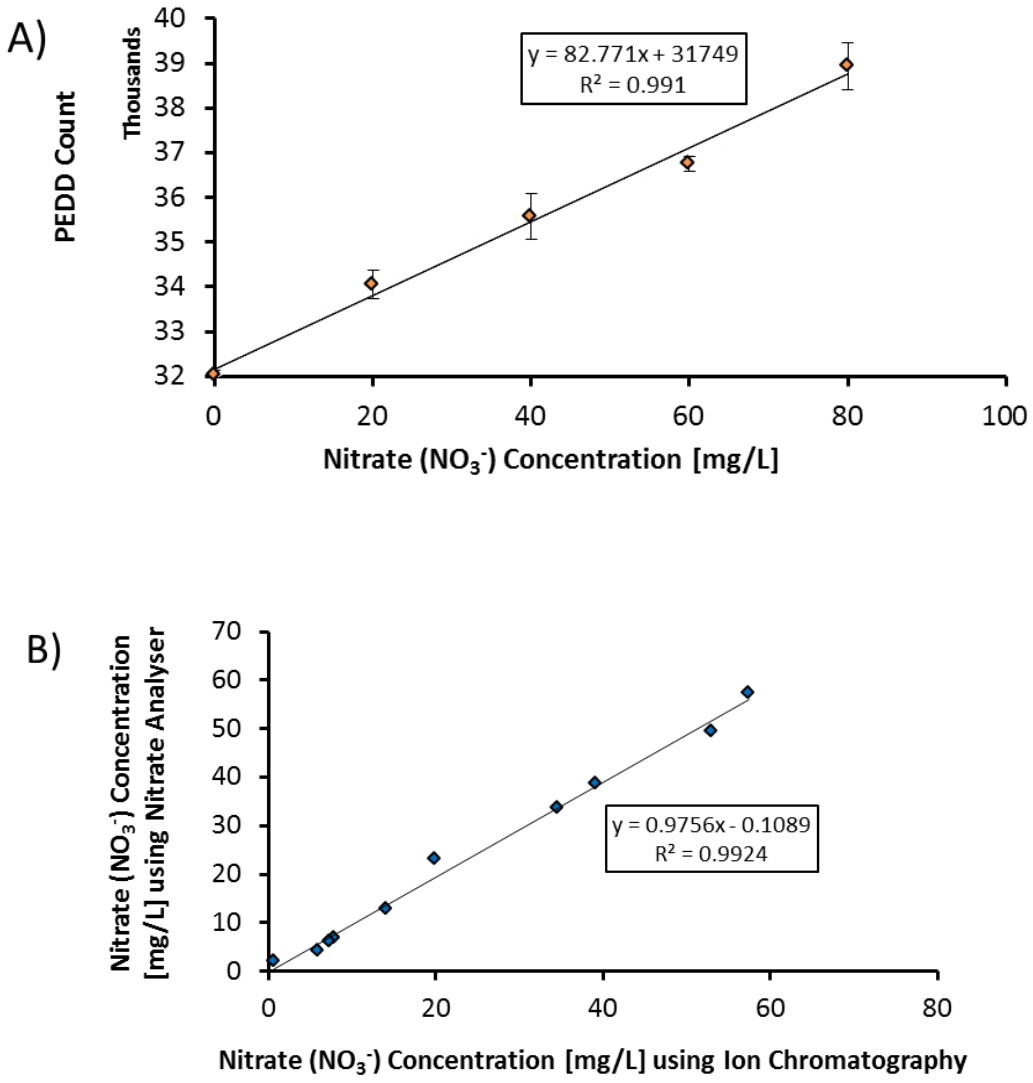

Figure 2.7. (A) Calibration curve using the nitrate analyser from 0 - 80 mg/L nitrate and chromotropic acid reagent complex. The standard deviations as represented as error bars. **(B)** Correlation plot of sample concentration obtained by nitrate analyser and ion chromatography.

2.3.7 Effect of sulphuric acid concentration and heat on nitrate-chromotropic acid complex formation

An area of concern that has been highlighted with this method is the use of 96% sulphuric acid (ca. 18 M) that must be present in the chromotropic acid reagent for effective formation of the nitrate complex. This is an issue as the strongly acidic environment drastically constrains the materials that can be used to store and move the reagent in the overall fluidic system. Therefore it is of interest to reduce the sulphuric acid concentration. The effect of reducing the sulphuric acid concentration was therefore investigated by obtaining an absorbance spectrum of a mixture of 80 mg/L nitrate and the chromotropic acid reagent, while reducing the background concentration of the sulphuric acid from 96% - 80% (v/v).

The results (Figure 2.8) show that lowering the concentration of the sulphuric acid below 96%, leads to a large reduction in the absorbance. However, reducing the acid concentration also reduces the spontaneous heat generated by mixing of the reagent with the sample (i.e. these results were obtained without external heating).

The chromotropic acid reagent was prepared as per section 2.2 with the exception of using 50% sulphuric acid in the reagent. To this, the 80 mg/L nitrate sample was mixed in a 1:1 volume ratio and then heated in the custom built heating device described in section 2.2.5, in a 4 ml capped vial for 25 minutes until a temperature of 130 °C was achieved as measured using a laser infrared heat thermometer (Walyou Ltd). The spectrum presented in Figure 2.8 shows that strong colour formation can be produced despite significantly decreasing the sulphuric acid concentration by introducing external heating. The blank consisted of the chromotropic acid reagent and 0 mg/L nitrate. The absorbance value

obtained using the 50% sulphuric acid reagent is similar to the absorbance value achieved using the 96% sulphuric acid reagent. These preliminary results are significant as the extremely high concentration of sulphuric acid recommended for the method can be avoided, which simplifies the integration of the method into an autonomous flow system, and broadens the range of possible materials that can be used.

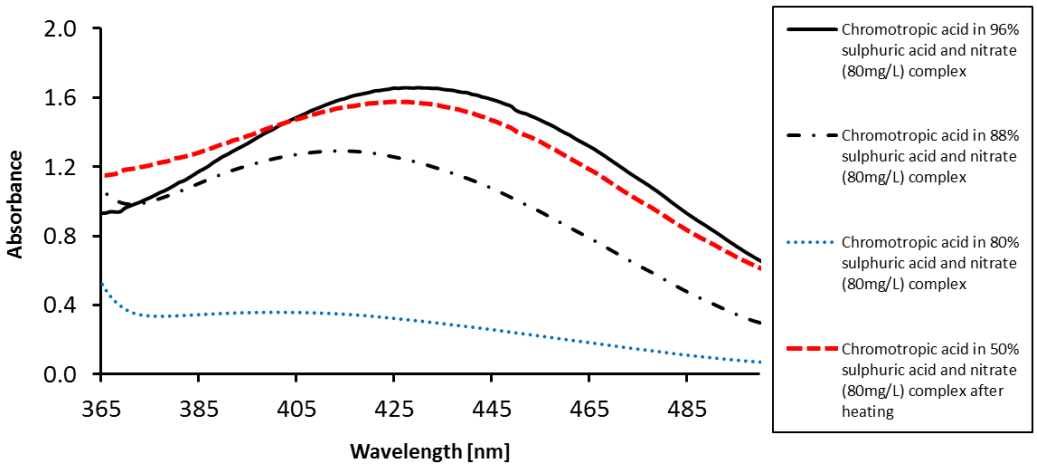

Figure 2.8. Spectra of 80 mg/L nitrate and chromotropic acid with varying concentrations of sulphuric acid within the chromotropic acid reagent.

2.4 Conclusion

An analysis system for the direct determination of nitrate in water using chromotropic acid has been developed. The chromotropic acid method has been modified to facilitate its implementation into a flow analysis platform, resulting in a quick and simple procedure to measure nitrate. The results presented here are the first for a nitrate analyser incorporating the chromotropic acid method and the PEDD detector. This forms the basis of a simple and low cost autonomous detection device which could be used as an alternative to expensive optical detectors for the detection of nitrate in water. This study demonstrates advantages

including the low limit of detection of 0.73 µg/L, the wide linear range of 0.9 – 80 mg/L, and the fast response time of the method. The flow analysis system possesses a sample turnaround of 30 minutes, which may be capable of significant further improvement through further operational optimisation. It was also proved within this study that the sulphuric acid concentration could be halved while maintaining satisfactory analytical results producing a safer and more convenient method. The device incorporates low cost, highly sensitive detection with excellent correlation to the standard method, ion chromatography. Ultimately, this system may provide a base for monitoring waters for nitrate levels *in situ* in a rapid, simple and inexpensive manner.

2.5 Acknowledgments

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Chapter 3

Development of a low cost microfluidic sensor for the direct determination of nitrate using chromotropic acid in natural waters

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Aims and objectives

Chapter 3 reports for the first time the integration of a modified chromotropic method described in chapter 2 for the direct determination of nitrate, to a microfluidic chip configuration within an autonomous field deployable platform that can withstand the acidic nature of the reagent, resulting in a reliable, quick, inexpensive and simple procedure to measure nitrate. The sensor allows for the monitoring of nitrate in wastewater and freshwater with a detection range and limit of detection in agreement with EU legislative compliances.

Abstract

Progress towards the development of a miniaturised microfluidic instrument for the direct measurement of nitrate in natural waters and wastewater using chromotropic acid is presented. For the first time, the chromotropic method for nitrate analysis has been transferred to a microfluidic chip configuration that can withstand the extremely acidic nature of the reagent within a field deployable platform. This simple method employs one reagent mixed in a 1:1 ratio with the sample to produce a yellow colour absorbing strongly at 430 nm. A stopped flow approach is used which, together with the very rapid kinetics and simple reagent stream, enables an uncomplicated microfluidic design and field deployable platform with a sample throughput of 9 samples h^{-1}, limits of detection of 0.70 mg/L NO_3^- and 0.31 mg/L NO_3^- for seawater samples, with a dynamic linear range from 0 – 80 mg/L NO_3^- and long-term reagent stability of up to 6 months. Validation was achieved by analysing split water samples by the analyser and ion chromatography, resulting in an excellent correlation co-efficient of 0.9969. The fully integrated sensing platform consists of a sample inlet with filter, storage units for chromotropic reagent and standards for self-calibration, pumping system which controls the transport and mixing of the sample, a microfluidic mixing and detection chip, and waste storage, all contained within a ruggedized, waterproof housing. The optical detection system consists of a LED light source with a photodiode detector, which enables sensitive detection of the coloured complex formed. The low cost of the platform coupled with integrated wireless communication makes it an ideal platform for *in situ* environmental monitoring.

Keywords Environmental monitoring, microfluidics, COP, nitrate, chromotropic acid, colorimetry

3.1 Introduction

Nitrate is an important nutrient to promote plant growth, but excess of nutrients like nitrate in water bodies promotes excessive growth of algae, *i.e.* eutrophication. Nutrient pollution is considered one of the most difficult environmental challenges as it is difficult to implement effective mitigation remedies.[1] Nitrate levels in various types of water are therefore regulated through the European Commission's Groundwater Directive (2006/118/EC) and the Drinking Water Directive (98/ 83/EC) which both state an upper nitrate limit of 50 mg/L.[2,3] Reliable measurements of nitrate are therefore essential for effective assessment and management of environmental water quality. However, despite this growing demand for real-time, *in situ* monitoring of nitrate, penetration of autonomous chemical sensing technologies into commercial markets remains disappointingly low. The cost and the technical drawbacks of commercially available wet chemical sensors for the determination of nitrate can be prohibitive and often entail regular maintenance over time. This may be due to the difficulties associated with determining nitrate such as the relatively intricate procedures involved, probability of interferences present and the limited detection ranges associated with various methods. Many techniques have been developed and studied for the determination of nitrate *via* on-site measurements, including direct ultraviolet spectrophotometric screening, nitrate-responsive electrodes, and colorimetric techniques. Nonetheless, these methods can involve a number of multistage or complex procedures and can result in a high component cost. A field deployable platform for automated *in situ* nitrate monitoring developed by Beaton *et al.* employed a reduction step through the use of a cadmium column followed by nitrite analysis using the Griess method.[4] Although this method is sensitive and has been employed for field measurements successfully, it is an indirect method, and requires measurement of background nitrite followed by a second measurement of

total nitrate (after reduction to nitrite) and nitrite. The nitrate concentration is then obtained by subtraction. The reduction step adds complexity to the fluidic system; e.g. valves for diverting flow through the reduction column as well as the addition of buffers and column washing. Issues may also arise in the reproducibility of a cadmium column and the unstable nature of the Griess reagent and nitrite standards over time (few weeks).

In the realisation of a fully reliable low-cost platform, it is obviously an advantage to keep the fluidic handling requirement as simple as possible as complex, multistage approaches are correspondingly challenging and expensive to implement due to significant additional component costs, and higher maintenance costs. Therefore in this research we focussed on developing a simple, single stage direct colorimetric method for the determination of nitrate. Direct UV absorbance is an attractive option for *in situ* monitoring of nitrate, as there is no need for fluidics and reagents and was the chosen detection method for 50 platforms in the Argo project, which was established in 2000 to provide distributed sensor information related to the global marine environment.[5] This approach was also explored by Frank *et al.* for *in situ* nitrate monitoring in the North Sea.[6] The authors stated that direct UV may provide a practical solution where wet chemical analysers cannot due to technical difficulties or insufficient temporal resolution. However, interferences arising from dissolved organic matter and surfactants can be problematic and the high cost of technical components are significant issues.[7]

Recently, direct detection of nitrate using a simplified chromotropic acid reagent method was reported by Cogan *et al.*[8] wherein chromotropic acid, in a sulphuric acid medium, was reacted with nitrate ions to produce a characteristic yellow colour associated with an absorbance band in the visible region (λ_{max} = 430 nm). This modified method had a linear range of 0.9 – 80 mg/L nitrate, and a limit of detection of 0.73 mg/L nitrate. An

issue with this method is the use of highly concentrated sulphuric acid (98%) in the chromotropic acid reagent. This strongly acidic medium drastically constrains the types of materials and components that can be used to implement the method in a microfluidic analytical fluidic system. However, despite the highly reactive and corrosive nature of the reagent, we have successfully transferred the method from a conventional flow analysis platform into a microfluidic sensing platform through which we have implemented a fully automated, rapid and sensitive method, with a high sample throughput. The method can be applied to the analysis of nitrate in freshwater and wastewater, and also in marine waters following a system calibration of relevant seawater samples. The analyser shows excellent correlation with ion chromatography and good repeatability as well as a low limit of detection. In this paper, we describe the analytical performance of the method in this configuration, and demonstrate that, through careful choice of key materials and components, a reliable and robust field deployable platform can be realised.

The miniaturisation of analytical devices through the implementation of microfluidics is an important development for automated monitoring platforms, providing many advantages such as;

- The small volumes of sample and reagent required.
- Calibration procedures based on standard buffer solutions can be automated.
- Optical detection takes place within a microfluidic chip which can be protected from fouling effects by a combination of sample filtration and reagent-based fouling deterrents.
- Power efficiency and robustness (e.g. the sensor is not directly exposed to the sample as all detection takes place within the chip).

Polydimethylsiloxane (PDMS) and poly(methyl methacrylate) (PMMA) are often used for forming microfluidic chips as finely featured fluidic

structures such as channels, mixing regions and detector flow cells can be easily produced.[9] However, as colorimetric reagents used for environmental analysis often tend to be of a strongly acidic/alkaline or redox active nature (in order to drive the reactions required to generate the analytical chromophore in the presence of the sample), in some cases, PDMS does not offer sufficient chemical resistance to the drastic nature of these reagents, and this can result in swelling and irreversible chemical alteration of the polymer. PMMA is suitable for use with dilute acid/alkali solutions but is not resistant to more concentrated acids. Methods based on very reactive chemical reagents are therefore usually implemented in fluidic systems based on highly inert materials such as glass or quartz. While these can be successful in small batches, they are difficult and costly to produce, requiring highly toxic etchants to produce the channels, and are not suitable for mass-production. This is also the case for robust fluoropolymers such as Teflons like polytetrafluoethylene (PTFE) and fluorinated ethylene-propylene (FEP). While the physical and chemical properties of these materials offer many advantages such as chemical inertness, thermal stability, and excellent anti-bio-fouling properties, sufficient adhesion and bond strength is hard to accomplish due to its low frictional resistance and therefore aggressive surface treatments are required for surface functionalization for sealing and bonding.[10] Focus has therefore turned to alternative thermoplastics such as cyclic olefin co-polymers (COC) and cyclic olefin polymers (COP). COP is a useful substrate material for the fabrication of microfluidic devices due to its excellent optical properties, low oxygen permeability and resistance to many chemical agents including acids, bases and polar solvents.[11] These surfaces have low surface reactivity and tend to be hydrophobic which can lead to difficulties in sealing and bonding these substrates together.[12] However, their surface reactivity can be enhanced by mechanical interlocking and interdiffusion of chains using techniques such as thermal cycling, solvent vapour treatment, acid and plasma surface activation. It has been well documented that energetic ions,

electrons and UV photons achieve sufficient energy to break bonds on the surface of the substrate which results in the production of highly reactive free radicals which assists in forming the charged surface groups responsible for increasing the overall surface energy in order to improve the wettability between mating surfaces, and improving bond strengths.[13]

In this paper, we present a low-cost, robust sensing platform with a microfluidic reaction manifold and light emitting diode (LED) based optical detection system that can withstand the acidic nature of the chromotropic method in a cost-effective, simple and robust manner by using reliable materials that opens the possibility of long-term *in situ* deployments. We also demonstrate the effectiveness of UV surface treatment and thermal fusion of COP surfaces and microchannels, to produce the first microfluidic chip capable of detecting nitrate directly using chromotropic acid. Considering the highly corrosive nature of the reagent, and the success of the implementation, we also discuss the crucial role that materials and design play in the realisation of reliable, robust long-term deployments of environmental sensors.

3.2 Experimental

3.2.1 Colorimetric reagent

The chromotropic acid reagent was prepared by dissolving 0.0735 g of chromotropic acid ($C_{10}H_6Na_2O_8S_2 \cdot 2H_2O$, Sigma-Aldrich, Ireland) in 250 mL concentrated (98% v/v) sulphuric acid (H_2SO_4, BDH Laboratory Supplies, UK). When protected from light, the reagent is stable for at least 6 months as shown in Figure SIA.1 in Supporting Information A.

3.2.2 Deionised water and standards

All solutions were prepared using analytical grade chemicals. Deionised water (< 18.2 Ω cm^{-1}) from a Millipore Milli-Q water purification system was used throughout the analysis. Nitrate stock standard solution (500 mg/L NO_3^-) was prepared from potassium nitrate (KNO_3, Sigma-Aldrich, Ireland) that was pre-dried for 1 hour at 110 °C. Nitrate standards are stable for several months due to the addition of 0.1% chloroform.[4]

3.2.3 Material compatibility study; generation 1 prototype platform

The chemical compatibility of various elastomers and polymers with sulphuric acid was investigated, by fully immersing the material in the acid (98% H_2SO_4, BDH Laboratory Supplies, UK) contained in a glass vial. Subsequently, a prototype bench-top system was developed to investigate the chemical compatibility of various components and materials with the nitrate-chromotropic acid complex. The prototype consisted of a peristaltic micro pump (Series 100, Williamson Manufacturing Company Ltd.) which incorporated various tubing within the pump head. Reagent and sample were pumped through a Kynar (PVDF) 1.6 mm Y-connecting hose barbed union (11806643, Cole-Parmer, Ireland) and delivered to a modified glass flow cell (Brand Ltd Cat. No.7477 15) at a flow rate of 600 µL/min over a period of 7 months. Initially, the coloured complex formed passed through a paired emitter-detector diode (PEDD) detection system similar to that described in detail by O'Toole *et al.* consisting of two LEDs, one operating as a light source/emitter and the other as a light detector, with λ_{max} of 430 nm and 623 nm respectively.[8,14] Subsequently, an LED/PD detector was used to measure the coloured complex formed. The detector signal was

transmitted wirelessly to a laptop via a Wixel controller (Pololu Corporation, USA) and saved as a text file which was subsequently analysed using Microsoft Excel.

3.2.4 COP microfluidic chip and detector design

Bonding of COP layers together has mainly involved solvent bonding, adhesive printing bonding, surface treatment bonding and thermal fusion bonding.[15] A study by Tsao *et al.* showed that UV/ozone surface treatments could be used for achieving strong low temperature bonding of PMMA and COP microfluidic substrates. When applying pressure to PMMA and COC substrates at a temperature of 90°C, the UV/Ozone treated surfaces displayed bond strengths orders of magnitude more than control units not subjected to this treatment.[12] A recent report by Jackson *et* al. characterised UV activation of microstructures in (COC) followed by thermal fusion bonding for circulating tumour cells analysis.[16] In this study, direct UV exposure to COP was demonstrated as an alternative approach to achieving high bond strengths between the microfluidic system layers.

The function of the microfluidic chip shown in Figure 3.1, is to mix the sample, blank or nitrate standard with the reagent and to present the resulting mixture to the detector which consists of an LED light source with a photodiode (PD) detector. The seven layers were fabricated using a Roland CNC micro-mill (Modela MDX40A) from 2.0 mm thick COP (Zeonex mcs-COP-04, Microfluidic Chip Shop, Netherlands). The chip with dimensions 60 mm x 30 mm x 14 mm contained 6 inlets in total, 3 for reagent followed by a sample, high and low inlet all of 1.7 mm diameter and a 109.73 mm serpentine mixer succeeded by a micro-cuvette with a pathlength of 8 mm. To ensure mixing of the reagent and sample was in 1:1 ratio (which is ideally suited for an automated

platform)[17], channels were of equal length and cross-sectional area of 500 μm x 500 μm. This ensures that the fluidic resistance is equal for all channels leading to the serpentine channel, provided that the solutions to be mixed are injected at equal pressure.

The milled layers were cleaned and degreased using isopropyl alcohol and deionised water. To assemble the chip the mating surfaces were irradiated with UV light (Dymax 5000-PC) (λ < 180 nm) for approximately 15 min. This causes the normally hydrophobic COP surfaces to become hydrophilic which allows for bonding below the glass transition temperature of COP which is 135°C (Figure SIA.2, Supporting Information A). The chip is then assembled using 1.5 mm steel dowel pins at the chip corners to ensure correct alignment. The assembled chip is clamped between two brass plates to apply pressure. The alignment of the plates is checked at the four corners using a vernier callipers to ensure even pressure is applied across the chip faces. The chip is then heated to 110°C for 2 hr.

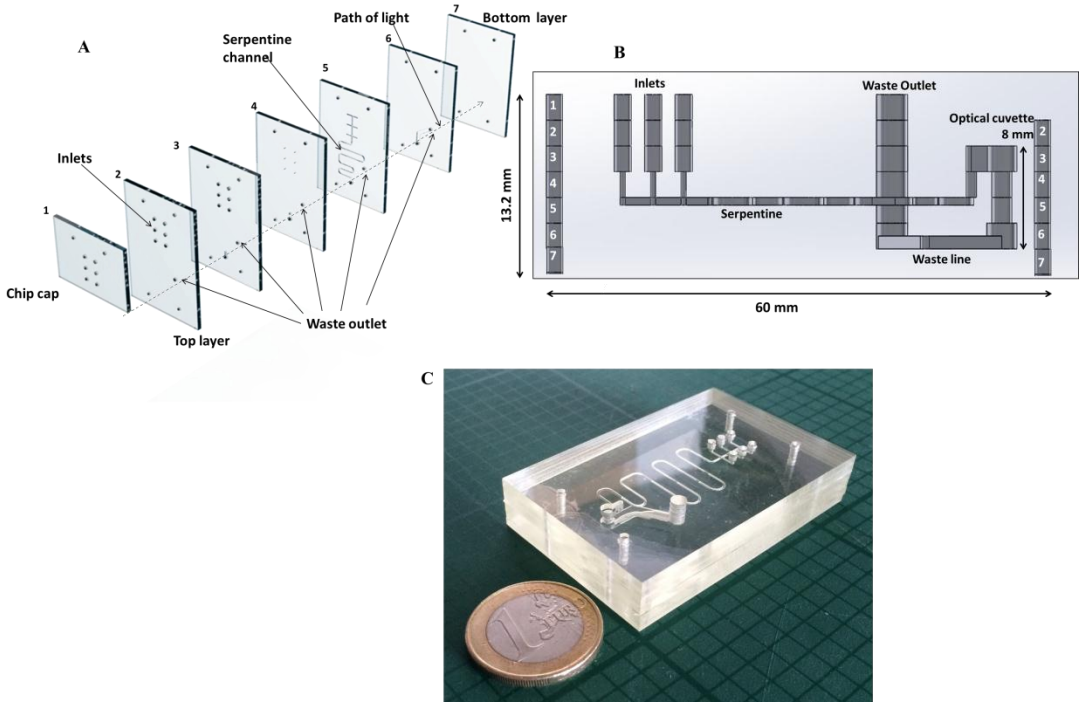

Figure 3.1. (A) Expanded view of microfluidic chip layers **(B)** Side view of fully assembled chip layers showing the micro-cuvette **(C)** fully assembled microfluidic chip.

Once the chip layers were bonded, Teflon® tubing connected to the peristaltic pumps was inserted into the chip input through holes by feeding through a chip cap which holds the tubing securely in place. Epoxy resin adhesive (Loctite 3421 A&B Hysol, Henkel) was applied to the chip cap and the cap pushed into place. The surface around the tube inlet of the chip was previously roughened using sand paper to increase the bond strength. The detection components and chip were placed into the detection cell holder generated by a 3D printer (Dimension SST 768), which incorporated custom designed features for housing the photodiode (epd-440-0/2.5, UV, Roithner) and the 430 nm LED (1045418, Blued, 5 mm, emission bandwidth of 40 nm, Farnell, Ireland)[18] which overlaps the spectrum of the nitrate-chromotropic acid complex as shown in Figure. SIA3. The photodiode detector and LED were aligned on opposite sides of

the chip's optical cuvette allowing for absorbance measurements of the nitrate-chromotropic acid complex (Figure 3.2(A)).

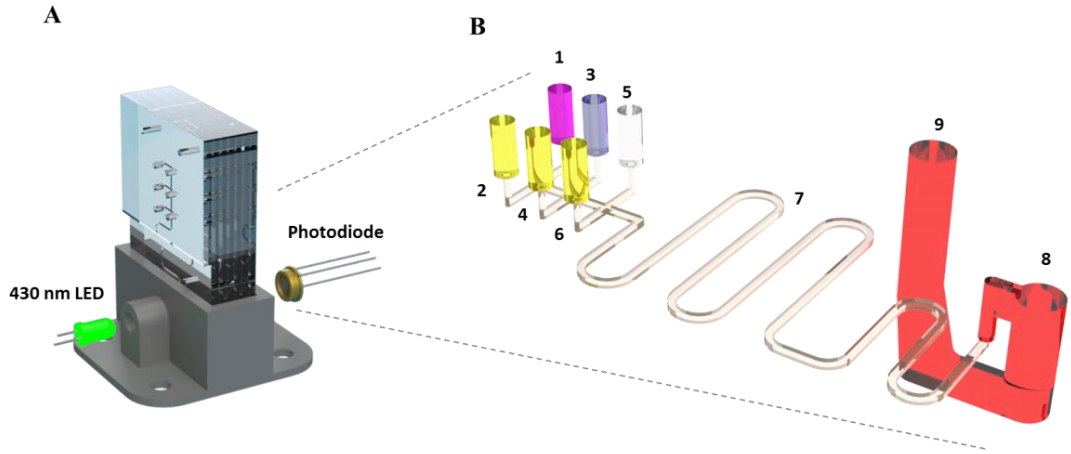

Figure 3.2. (A) Rendered representation of the flow system and LED/PD detection cell with LED and photodiode **(B)** Details of the fluidic system design [1] High standard inlet (1.7 mm diameter) [2] Reagent inlet (1.7 mm diameter) [3] Low standard inlet (1.7 mm diameter) [4] Reagent inlet (1.7 mm diameter) [5] Sample inlet (1.7 mm diameter) [6] Reagent inlet (1.7 mm diameter) [7] Serpentine/mixing channel (500 µm x 500 µm x 109. 74 mm) [8] micro cuvette (3 mm x 8 mm) [9] Waste Outlet (3.1 mm diameter).

3.2.5 Autonomous nitrate analyser

The nitrate analyser shown in Figure 3.3 is a fully integrated system consisting of sampling, detection and communication components. Components included storage units for the chromotropic acid reagent, waste solution and calibration standards, pumping system to control the transport and mixing of the sample, and a microfluidic mixing and detection chip resulting in a low cost, rapid and simple instrument for the measurement of nitrate.

Three miniature double headed peristaltic pumps (102-005-012-016/4, Williamson Ltd.) allowed for the pumping of reagent and sample into the microfluidic chip at a flow rate of 600 µl/min using the same motor to

ensure a 1:1 v/v reagent to sample ratio. Viton® tubing of 1.6 mm internal diameter and 5 mm outer diameter (Williamson Pumps Ltd.) was used within the peristaltic pump. To prevent back-flow within the system, polyvinylidene fluoride (PVDF) miniature check valves with a Viton® diaphragm (11873843, Cole-Parmer, Ireland) were used. Teflon tubing of 0.3 mm inner diameter and 1/16" outer diameter (008T16-30-20, Kinesis Ltd.) was used to introduce the sample and reagent to the chip and Teflon tubing of 1/16" inner diameter, 1/8" outer diameter was used for the waste line. Epoxy resin adhesive (Loctite 3421 A&B Hysol, Henkel) provided leak-free connections between the Viton tubing, the Teflon tubing and the microfluidic chip. By using the epoxy resin adhesive additional components such as Teflon unions, tees or adapters were avoided, effectively lowering the component cost of the platform.

For each sample assay, the instrument measured a 'blank' solution (0 mg/L NO_3^-, deionised water as per section 3.2.2) and a 'high' nitrate standard (80 mg/L NO_3^-). The high standard measurement was performed initially. The reagent and high standard were pumped into the serpentine/mixing channel *via* inlets 1 and 2 as per Figure 3.2.(B). The resultant mixture was delivered to the optical cuvette within the chip for measurement, followed by transfer to the waste storage container *via* outlet 7. The procedure was then followed for the blank (low standard, 0 mg/L NO_3^-) and sample measurements respectively.

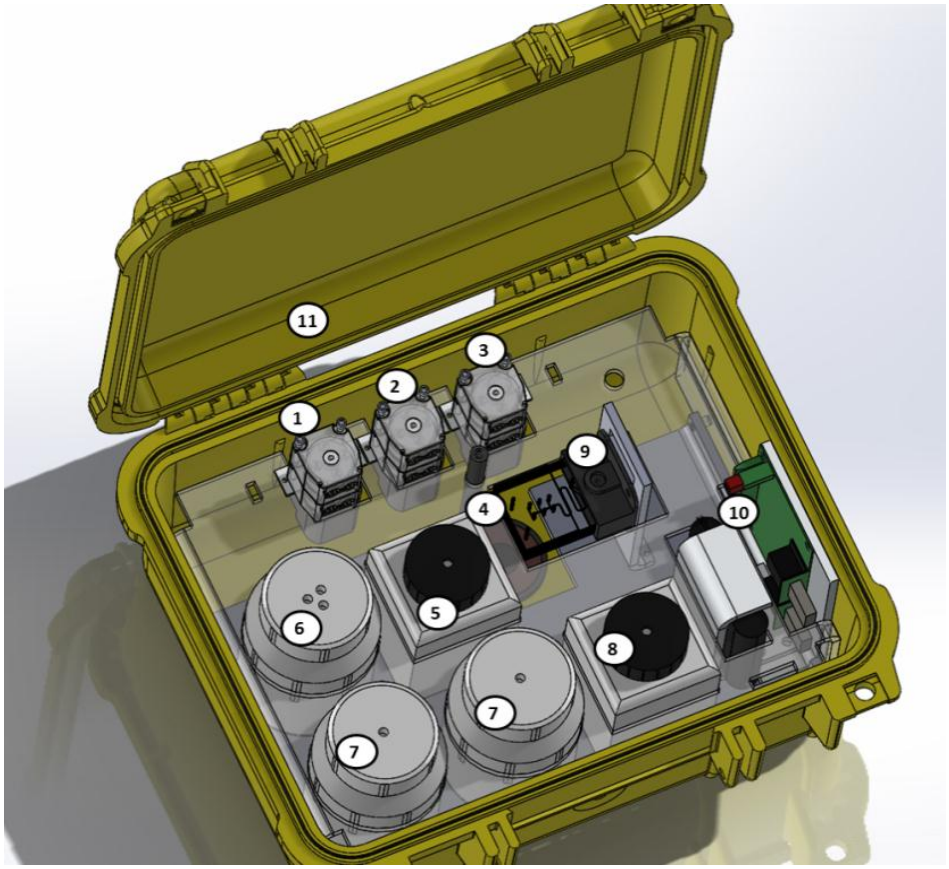

Figure 3.3. Integrated nitrate analyser (Tubing removed for easy viewing); [1] sample pump [2] high standard pump [3] low standard pump [4] sample inlet [5] high standard container [6] reagent container [7] waste containers [8] low standard container [9] microfluidic chip with LED/PD [10] control and communications board with 14 V lithium-ion polymer battery [11] waterproof housing.

As mentioned above, the nitrate analyser implemented a two-point measurement protocol using a 'low' blank solution (0 mg/L NO_3^-) and a 'high' standard solution (80 mg/L NO_3^-), the concentration of which can be varied depending on the particular site/sample in question. The reagent was stored in a Teflon® 250 mL bottle and the waste was stored in 2 x 250 mL Teflon® bottles to facilitate a larger volume of waste due to high and low standard measurements (VWR International Ltd., Ireland). The reagent storage capacity allowed for ca. 200 nitrate measurements. Teflon bottles are not necessary for the high and low standards and therefore standard 250 mL polyethylene bottles (HS25ON, Richmond

CTP Ltd.) were used. The reagent bottle was attached to the tubing of one channel of each peristaltic pump; the tubing of the second channel of the pump was attached to the water sample, low standard or high standard as required.

Development of a field-deployable sensing platform requires a rugged enclosure to ensure the device is fully protected from the environment while still allowing for reliable sampling and communication. Therefore the system was installed into a waterproof and crushproof polycarbonate box (Peli™ Case 1400 Case). The interior dimensions (30 x 22.5 x 13.2 cm) allowed for housing of the peristaltic pumps, microfluidic chip and detection system, reagent and waste storage containers. The sampling inlet consisted of a sampling port with filter (Supor® 25 mm membrane filters, pore size 0.45 mm) holder. The filter holder (Figure 3.4) was designed and manufactured to minimise inlet dead volume while utilising a large portion of the filter surface. The filter membrane support unit shown in Figure 3.4(A), was fabricated from milled 1.5 mm thick PMMA using a Roland CNC micro-mill (Modela MDX40A) and placed onto a brass fitting using epoxy (Loctite 3421 A&B Hysol, Henkel). A knot was tied in the inlet tube and placed in the centre of the mould. Silicone sealant (Silcoset 158, ACC Silicones Ltd.) was poured into the brass fitting and allowed to dry for 48 hours. The filter membrane was placed on the mould and secured in place using a 25 mm diameter o-ring, 25 mm diameter washer and the brass fitting cap shown in Figure 3.4(B). To ensure all the containers, electronics and chip were secure; a holder was designed and fabricated from 3.5 mm thick PMMA using a laser cutter (Epilog Zing Laser), hot wire strip heater (CR Clarke 600) and folding jig (CR Clarke F0600). The transparency of the PMMA allowed easy visual checking for blockage or damage of the fluidics, and the system was designed so that components like bottles/batteries could be simply and safely removed and replaced when necessary.

A microcontroller (MSP430, F449, Texas Instruments) was used to control the operation of the pumping system and optical detector. The data was stored on a flash memory chip. For wireless communications, a GSM module interface powered by a rechargeable 14 V lithium-ion polymer battery (Olimex Ltd.) was used. Data files were in text format and communications employed RS232 data handling protocols.

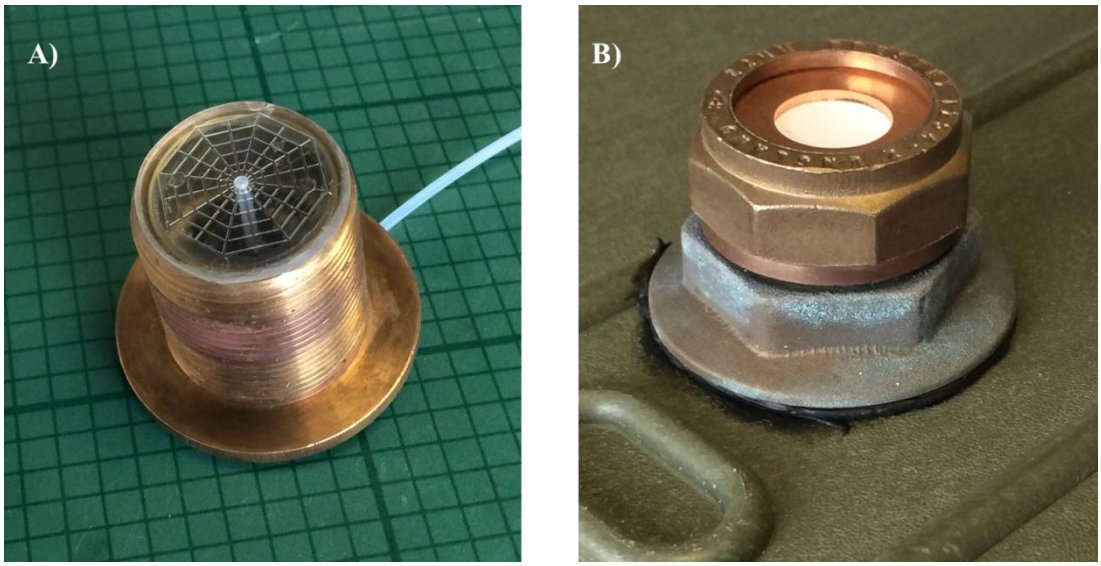

Figure 3.4. (A) Filter holder membrane support unit **(B)** membrane installation.

3.2.6 Analytical Procedure

The analytical protocol involves five main steps:

1. Introduction of the water sample into the analyser for analysis.
2. Pumping of sample and reagent into the microfluidic chip, and on-chip mixing of sample and reagent.
3. Colour formation.
4. LED/PD absorbance measurement.
5. Storage of waste.

Mixing was performed on the chip by pumping the sample, low standard or high standard together with reagent through the serpentine mixer. Both the reagent and sample were pumped through the chip at a flow rate of 600 µL/min. When the chip had been completely filled, the flow was stopped for 5 min (as per section 3.3.2) to allow colour formation to reach a steady-state, and the associated absorbance was measured in the micro cuvette detector. Performing analysis using a stopped flow procedure not only results in a simple flow design but also minimises the consumption of reagent and standards, and generates less waste. The colour of the fluid in the micro-cuvette was then measured using a LED-PD set up. Combining unknown measurements with simultaneous reference measurements using standards enabled automatic compensation for drift and temperature effects on the system detector over time. Each unknown measurement was therefore compared to a blank and an 80 mg/L NO_3^- standard. The unknown sample concentration was estimated using equation 3.1 via a calibration model created previously with standard nitrate solutions (0, 5, 20, 40, 80 mg/L NO_3^-). This approach enabled the overall stability of the calibration model to be checked and adjusted for each sample measurement.

$$NO_{3\,conc} = h_c \frac{log_{10}(s_a/l_a)}{log_{10}(h_a/l_a)} \qquad \text{(Equation 3.1)}$$

Where NO_{3conc} is the nitrate concentration in mg/L, h_c is the high standard concentration in mg/L, s_a is the sample ADC reading, l_a is the low standard ADC reading and h_a is the high standard ADC reading.

3.3 Results and discussion

Wet chemical analysers contain many components that are exposed to a range of hostile environments due to the highly reactive reagent components like hydrochloric acid, phosphoric acid or sodium hydroxide

that are often used. These can adversely affect the useful lifetime of autonomous analysers, which therefore require regular servicing and replacement of components due to degradation.[4, 17, 19, 20] For example, we found that Tygon tubing used within the previous flow analysis platform[8] had a shelf life of less than 1 month of continuous use with the chromotropic acid method, due to the poor chemical compatibility with sulphuric acid which resulted in softening and discolouration (Figure SIA.4). Sulphuric acid is a strong acid, and a highly corrosive and viscous liquid. It is a powerful protonating and oxidising agent and vigorous dehydrating agent. Therefore, an extensive study was implemented over one year into the chemical compatibility of the chromotropic acid reagent and sulphuric acid (as per section 3.2.1) with various materials (see Table 3.1).

Table 3.1. Effect of prolonged exposure (ca. 12 months) of concentrated sulphuric acid on a range of materials commonly used in microfluidics.

Material	*Compatibility*
Polypropylene	Severe effect
Polyurethane	Severe effect
PTFE (Teflon)	Excellent stability
Kynar (PVDF)	Excellent stability
Polyether ether ketone (PEEK)	Severe effect
PVC (Polyvinyl chloride)	Severe effect
PMMA (Poly(methyl methacrylate))	Severe effect
Silicone	Severe effect
Tygon®	Severe effect
Tygon® Fuel (Lubricant) Tubing	Severe effect
Neoprene	Severe effect
Santoprene	Severe effect
Viton®	Excellent stability
COP (cyclic olefin polymer)	Excellent stability

Degradation was observed with many of the materials with sulphuric acid almost instantly (Figure SIA.5), specifically with Polyether ether ketone (PEEK) as shown in Vid.SIA1, and in some cases, contrary to the manufacturer specifications. For example, polypropylene and

polyethylene we screened had specifications that stated excellent or good resistance to 96% sulphuric acid according to the manufacturers.[21, 22] In contrast, Viton® (a brand of synthetic rubber and fluoropolymer elastomer from DuPont Performance Elastomers L.L.C.) showed excellent resistance with no observable degradation effects, corrosion or discoloration when used in the presence of 98% sulphuric acid even when the exposure period was extended to 18 months as shown in Figure SIA.5G. As a result, peristaltic pumps were the preferred option over syringe pumps as Viton® tubing could be used with a peristaltic pump for the safe and reliable movement of fluid throughout the sensing platform. The prototype system described in section 3.2.3 was tested continuously over a period of 7 months in which the chemical compatibility of nitrate chromotropic acid complex with Viton® tubing and the peristaltic pumps were tested. Calibration plots were carried out in April 2014 and November 2014 and are comparable with a slope ratio of 1: 1.02 as shown in Figure 3.5. As a PEDD detection system was used in April and a LED/PD detection system was used in November, the limit of detection (LOD) was calculated for both April and November plots, by multiplying the average standard deviation (sd) of the baseline absorbance of the blank by 3 (repeated $n = 5$ times, each baseline standard deviation taken from 30 baseline data points, frequency of measurement: 1 Hz). When this was applied to the equation of the line, the LOD was found to be 1.48 mg/L NO_3^- (April 2014 using a PEDD detection system) and 1.78 mg/L NO_3^- (November 2014 using a LED/PD detection system), respectively. These results are significant as the high concentration and aggressive nature of the highly concentrated sulphuric acid does not affect the materials in the fluidic system, in particular the Viton® tubing and peristaltic pump making this an ideal set-up for a field deployable platform. Furthermore, the reagents and method (optimised as reported previously)[8] are suitable for use over an extended period of time.

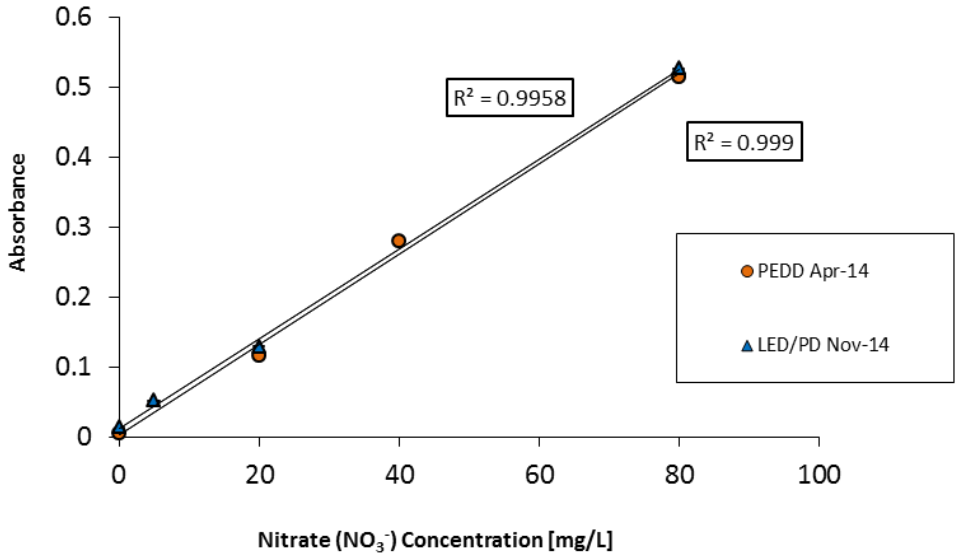

Figure 3.5. Calibration curves using the nitrate prototype analyser from 0 - 80 mg/L NO_3^- and chromotropic acid complex from April 2014 – Nov 2014 (7 months). The standard deviations are represented as error bars ($n = 3$).

COP discs of 2 mm thickness and 120 mm diameter (Zeonex mcs-COP-04, Microfluidic Chip Shop, Netherlands) were tested using 98% concentrated sulphuric acid over a period of 6 months. No degradation or colour formation appeared within this time as evident from Figure SIA.6, indicating that this would be a suitable material for producing microfluidic chips for this method.

3.3.2 Kinetics study

In previous work, it was shown that the simplified chromotropic acid method followed first order kinetics, with the absorbance increasing rapidly until approximately 180 sec after reagent/sample mixing, at which point a steady state was reached.[8] For the same method implemented within the microfluidic chip, the signal stabilised after around 300 sec at room temperature, Figure 3.6. As, with the current fluidic design, it takes 1 min pumping to transport the sample through

the fluidic system and 5 min to achieve the steady state under stopped flow conditions, the minimum measurement time for each measurement is ca. 6.5 min.

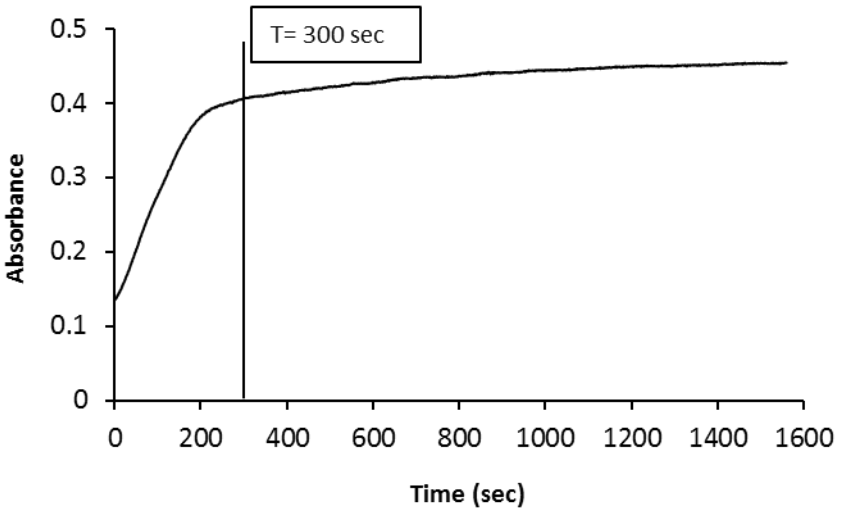

Figure 3.6. Time profile of the dynamics of colour formation obtained with 80 mg/L NO_3^- standard and chromotropic acid reagent at room temperature.

3.3.3 Calibration study

A calibration study was carried out with the microfluidic sensing platform using nitrate standards ranging from 0 – 80 mg/L NO_3^- at a wavelength of 430 nm. Results (Figure 3.7) show a correlation coefficient of 0.9901 with an average RSD of 7.38%. The method gives a linear response to nitrate concentrations up to 80 mg/L NO_3^- after which the absorbance plateaus.

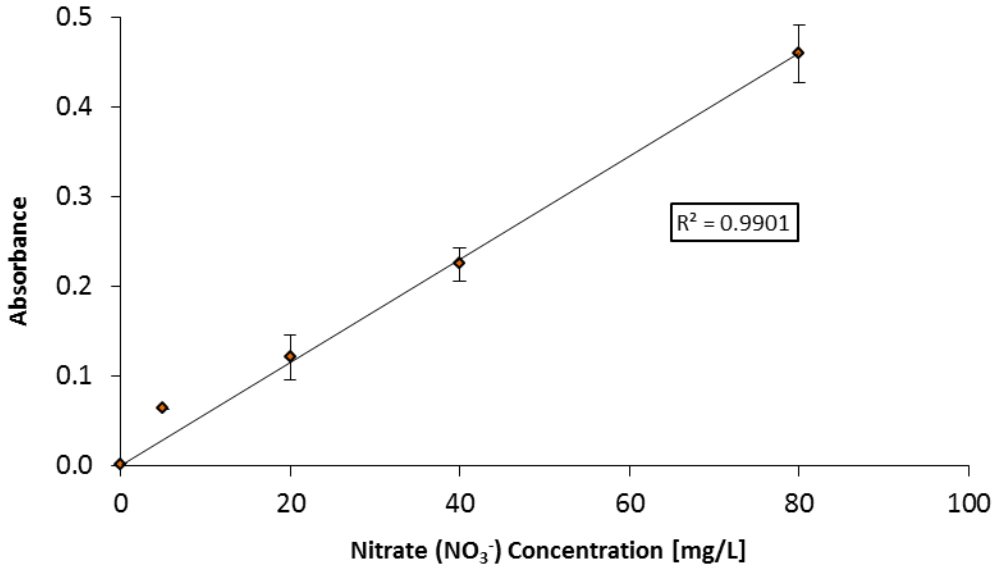

Figure 3.7. Chromotropic acid method calibration curve generated with the microfluidic nitrate analyser, using nitrate standards ranging from 0 – 80 mg/L nitrate. The error bars represent standard deviations for $n = 3$ replicates.

3.3.4 Repeatability and limit of detection

The repeatability of the measurement was determined by stop flow analysis of the blank (0 mg/L NO_3^-) and the high measurement (80 mg/L NO_3^-) seven times. The sample and reagent were passed through the chip simultaneously at a flow rate of 600 µl/min. When the flow stopped diffusional mixing took place and the two solutions then reacted to form the nitrate-chromotropic acid complex. Excellent signal stability and repeatability of the measurement technique is shown in Figure 3.8 with an average RSD of 0.28% for the blank and 0.19 % RSD for the high standard.

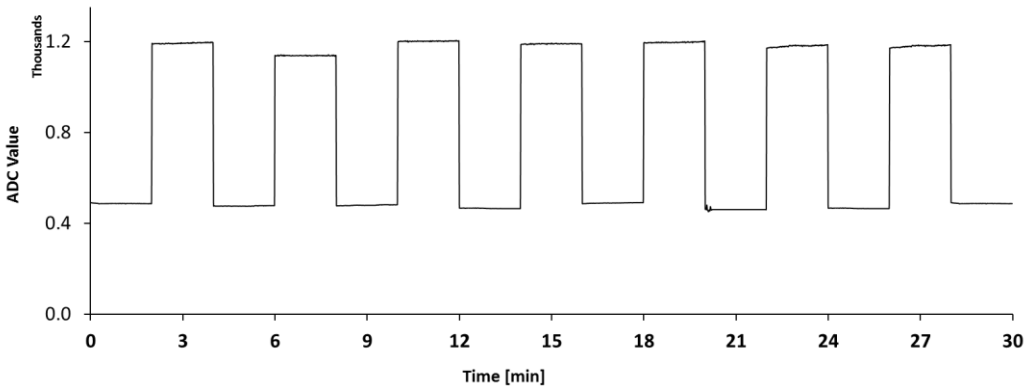

Figure 3.8. Multiple injection plugs of 0 mg/L NO_3^- and 80 mg/L NO_3^- with chromotropic acid reagent under stopped flow conditions.

The limit of detection (LOD) was found by multiplying the standard deviation (sd) of the baseline absorbance by 3 (n = 7, 10 data points per each blank measurement, frequency of measurement: 1 Hz). Using the equation of the line, a LOD of 0.70 mg/L NO_3^-chromotropic acid reagent complex was obtained.

3.3.5 Application to real samples

The analyser was applied to determine the nitrate concentration in nine water samples from various environmental sources including surface water, effluent, seawater and standards. The samples were split, and parallel assays independently performed at the T.E. Laboratory site (Carlow, Ireland). The samples were filtered prior to analysis using the membrane and sample inlet described in section 3.2.5. The results obtained for the nine samples with the autonomous analyser, and a Hach® handheld portable colorimeter using NitraVer® 5 reagent powder pillows (detection range: 1.32 – 133 mg/L NO_3^-), were compared to reference measurements obtained using ion chromatography (IC), see Table 3.2. Overall, the results for IC and the nitrate analyser are in good agreement while the Hach® handheld colorimeter results are generally

higher than both IC and the analyser. Samples #8 and #9 were measured with reference to nitrate standards (0, 10, 40, 80 mg/L NO_3^-) made using artificial seawater free from nitrates (Instant Ocean® Aquarium Systems, Inc.) due to the highly different background matrix which shifts the calibration of the chromotropic acid method slightly (Figure SIA.7). The LOD using seawater standards was found by multiplying the standard deviation (sd) of the baseline absorbance by 3 (n = 7, 10 data points per each blank measurement, frequency of measurement: 1 Hz). Using the equation of the line, a LOD of 0.31 mg/L NO_3^- chromotropic acid regent complex was obtained. Samples #8 and #9 were calculated as <0.31 mg/L NO_3^- and 24.44 mg/L NO_3^-, respectively. These results are in good agreement with those obtained by ion chromatography, indicating that the chromotropic method can be successfully applied to the determination of nitrate in marine waters. Overall, an excellent correlation coefficient of 0.9969 was obtained between the analyser and ion chromatography for samples #1 to #9 (Figure SIA.8).

Table 3.2. Determination of nitrate in nine water samples using the microfluidic nitrate analyser (n = 3) and comparison with ion chromatography reference method and Hach® colorimeter. *These results were obtained using the nitrate analyser calibrated with nitrate standards prepared from nitrate-free artificial seawater (Instant Ocean® Aquarium Systems, Inc.).

Sample reference	*Sample characteristics*	*Ion chromatography (NO_3^- mg/L)*	*Microfluidic Nitrate analyser (NO_3^- mg/L)*	*Hach® colorimeter (NO_3^- mg/L)*
1	Drinking water	10.6	12.29 ± 0.82	14.7
2	Surface water	20.3	19.86 ± 0.73	27.8
3	Effluent	11.1	12.93 ± 0.50	20.6
4	Effluent	<0.5	<0.70 ± 0.95	1.3
5	Surface water	<0.5	<0.70 ± 1.25	5.7
6	Deionised water (Blank)	<0.5	<0.70 ± 1.89	5.5

7	Deionised water (50 mg/L NO_3^- standard)	50	50.47 ± 0.81	63.7
8	Seawater (Blank)	0	<0.31 ± 0.61*	3.07
9	Seawater (25 mg/L NO_3^- standard)	25	24.44 ±0.62*	31.5

3.4 Conclusion

A low-cost, novel portable system for long-term monitoring of nitrate has been developed. This completely autonomous device incorporates sampling, reagent and waste storage, colorimetric detection, wireless communication and a power supply into a complete, miniaturized system. For nitrate detection, the method is simpler than the popular Griess method due to the elimination of the necessity for a reduction step. We have successfully identified materials and components that are chemically resistant to the acidic nature of the reagent, enabling a robust microfluidic chip based platform to be produced. The analyser was applied to real samples including drinking water, freshwater, wastewater and seawater. The results obtained were in excellent agreement with ion chromatography. Table 3.3 summarises the overall analytical figures of merit and system specifications for the microfluidic analyser platform.

Table 3.3 System Specifications

	Matrix	
	Deionised water	**Artificial Seawater**
LOD (NO_3^- mg/L)	0.70	0.31
Linearity (R^2)	0.9901	0.9887
Repeatability RSD % (n =3)	7.38	1.62
Sample throughput	9 sample h^{-1}	
Reagent stability	At least 6 months	
Reagent consumption per sample	1.2 ml	
Component cost	€600	
Size and weight	30 x 22.5 x 13.2 cm, 3.5 kg	

The prototype system has a component cost of ca. €600 as shown in Figure SIA.9, potentially delivering a highly competitive cost compared to commercially available autonomous sensing platforms for nitrate (YSI™/YSI96000 autonomous nitrate analyser retails at an indicative cost of ca. €25000 per unit; similar instruments from EcotechTM/FIA NUT1000, EnviroTechTM/AutoLab and S:CAN spectrolyser typically retail in the range €15000 - €70000).[23,24] The advantages of this device include its simplicity, reduced costs, efficient energy use, low consumption of reagents and less waste production, compact design, acceptable analytical performance and high sample throughput.

3.5 Acknowledgments

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Chapter 4

The development of an autonomous sensing platform for the monitoring of ammonia in water using a simplified Berthelot method

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Aims and objectives

Chapter 4 is a study on the integration of a modified Berthelot method for the direct determination of ammonia in water into an autonomous sensing platform resulting in a reliable, quick, inexpensive and simple procedure to measure ammonia in water. The method should allow for the determination of ammonia in waste water and freshwater in conjunction with EU legislative requirements with a detection limit below ca. <0.3 mg/L NH_4^+.

Abstract

This study demonstrates that by combining a modified version of the Berthelot method with microfluidic technologies and LED based optical detection systems, a low cost monitoring system for detection of ammonia in fresh water and wastewater can be developed. The assay developed is a variation on the Berthelot method, eliminating several steps previously associated with the method to create a nontoxic and simple colorimetric assay. The previous Berthelot method required the addition of three reagents, mixed sequentially with the sample, which complicates the microfluidic system design. With the modified method, comparable results were attained using a single reagent addition step at a 1:1 v/v reagent to sample ratio, which significantly simplifies the fluidic handling requirement for integration into an autonomous sensing platform. The intense colour generated in the presence of ammonia is detected at a wavelength of 660 nm. The method allows for ammonia determination up to 12 mg/L NH_4^+ with a limit of detection of 0.015 mg/L NH_4^+. Validation was achieved by analysing split water samples by the modified method and by ion chromatography, resulting in an excellent correlation coefficient of 0.9954. The method was then implemented into a fully integrated sensing platform consisting of a sample inlet with filter, storage units for the Berthelot reagent and standards for self-calibration, pumping system which controls the transport and mixing of the sample, a microfluidic mixing and detection chip, and waste storage. The optical detection system consists of a LED light source with a photodiode detector, which enables sensitive detection of the coloured complex formed. The robustness and low cost of the microfluidic platform coupled with integrated wireless communications makes it an ideal platform for *in situ* environmental monitoring. This is the first demonstration of a fully functional microfluidic platform employing this modified version of the Berthelot method.

Keywords Microfluidics, water quality, environmental monitoring, ammonia, Berthelot method

4.1 Introduction

Ammonia is naturally present in surface waters and in low concentrations in groundwater as soil particles readily absorbs ammonia. It is formed by the deamination of organic nitrogen compounds and by the hydrolysis of urea. It can be found at concentrations as low as 10 μg ammonia nitrogen/L in natural surface and groundwaters and up to 30 mg/L in wastewaters.[1] It is the preferred nitrogen-containing nutrient for plant growth.[2] However it is also considered one of the most important pollutants in the aquatic environment because of its highly toxic nature. Ammonia can enter the aquatic environment via anthropogenic sources such as municipal effluent discharges and agricultural runoff, as well as natural sources such as nitrogen fixation and animal wastes. Exposure to even low levels of ammonia are toxic to many forms of aquatic life.[3] Ammonia is also a major problem in aquaria as it is the major excretory product from fish and other aquatic life.[4] The toxicity of ammonia is highly dependent on pH and temperature. Ammonia (NH_3) in the un-ionized form is more toxic than the ionized form (NH_4^+). As temperature and pH increase NH_4^+ is converted to NH_3 and therefore the toxicity increases. Ammonium levels in various water types are regulated as per the European Commission's Drinking Water Directive (98/83/EC) which states an ammonia limit value of 0.3 mg/L NH_4^+ and the Quality of Salmonid Waters Regulations (S.I. No. 293 of 1988) stating a total ammonia limit of ≤ 1 mg/L. Consequently, there is major interest in a low cost, reliable system for the continuous monitoring of dissolved ammonia in freshwater, marine and coastal waters, wastewater discharges, and water associated with aquaria and fish farms.

Currently, monitoring for nutrients such as ammonia in these waters is primarily based on manual sampling followed by analysis using standard laboratory methods. This results in the infrequent monitoring of water at a much lower number of locations than is desirable, as it is time consuming, expensive, non-scalable and requires skilled personnel. Our solution is to develop an autonomous water monitoring system, by combining colorimetric detection chemistries and microfluidics with a light emitting diode (LED) based optical detection system to produce a robust analytical platform that is capable of autonomous monitoring over prolonged deployment times, all within a total component cost of ca. €200 or less per unit. Our approach employs microfluidics for reagent and sample handling, and allows up to several thousand assays to be performed in a relatively compact format due to the low volumes of reagent and sample required. Furthermore, through regular automated system calibration, the analytical performance over the deployment period can be validated. Power management optimisation can be employed to sustain the sensing platform over a period of months using only a small rechargeable lead-acid battery.

Despite the enormous activity both into sensor networks and into the development of improved chemical sensors over the years, there has been virtually no penetration of chemical sensing platforms into distributed sensor networks, although the key challenges have been repeatedly emphasised.[5-7] Approaches to water quality monitoring of nutrient levels like ammonia have been the subject of much research for many years as the accurate determination of ammonia is vital in many routine water management systems. The Berthelot method[8] and the Nessler method[9] are the two most popular laboratory methods. However these methods have not been adopted significantly into autonomous field based instruments. Cost is undoubtedly a major factor of this, as reagent based analysers can cost up to €15,000 per unit.[10] For instance, many variations of the Berthelot method incorporate a three-stage reagent addition to

sample process, which increases the unit cost due to the more complex fluidic handling and the performance and reliability issues associated with this increased complexity. Therefore, in order to drive down the cost of ownership of these devices, it is important to keep the fluidic handling requirement as simple as possible, as this is typically the most expensive contribution in the overall component costs.[11]

Accordingly, this research initially focuses on simplifying the Berthelot method for simple, inexpensive and reliable integration into a sensing platform. In this respect, the strategy is similar to previous work on the integration of a simplified chromotropic acid method for the direct determination of nitrate using an autonomous platform[7] by taking a well-established colorimetric method; i.e. simplify complex procedures to a single reagent-to-sample addition stage. Such simplification of the analytical approach reduces the unit cost and the potential for component malfunction, improving reliability during long-term autonomous deployments. However, it is necessary to verify that this step-elimination process does not adversely affect the analytical performance of the system.

In this study the Berthelot method for the direct determination of ammonia was employed due to its high sensitivity at the µg/L level and intense colour development in the visible region at 630 nm with a full width at half maximum of ca. 140 nm[12] and a molar absorption coefficient of 1.23×10^{-4} l mol^{-1} cm^{-1}.[13] In 1859, Berthelot first reported the generation of a green colour when ammonia, phenol and hypochlorite were mixed, and this has formed the basis of the most commonly used colorimetric method for the detection of ammonia. In recent years, sodium salicylate has been recommended in place of phenol[8], due to the high toxicity of phenol[14]. It has also been demonstrated that the salicylate method can be applied when monitoring and analysing freshwater and saline water.[1] In a sodium hydroxide medium, the Berthelot reagent reacts with

ammonium ions (NH_4^+) to produce a characteristic green colour (λ_{max} at 660 nm arising from the use of salicylate rather than phenol) via the mechanism is shown in Figure 4.1. In the presence of hypochlorite, ammonia forms a monochloramine (Equation 4.1), which is converted to 5-aminosalicylate by the salicylate (Equation 4.2). This is oxidised and complexes with salicylate to produce the highly conjugated blue indophenol complex (Equation 4.3). Sodium nitroprusside is the catalyst predominantly used.[8] Horn and Squire demonstrated that the actual complex is an iron (II) nitritopentacyano complex arising from the formation of sodium nitroprusside in sodium hydroxide, ensuring the stability of the monochloramine at high pH, which is essential for the formation of the indophenol chromophore. In the absence of the catalyst, colour formation is very slow. It also increases the rate of formation of the 5-aminosalicylate and provides the necessary oxidation prior to generation of the indophenol chromophore.[15] All experimental results are represented as NH_4^+ but due to the high pH of the reagent in this study, all NH_3/NH_4^+ exists as NH_3.

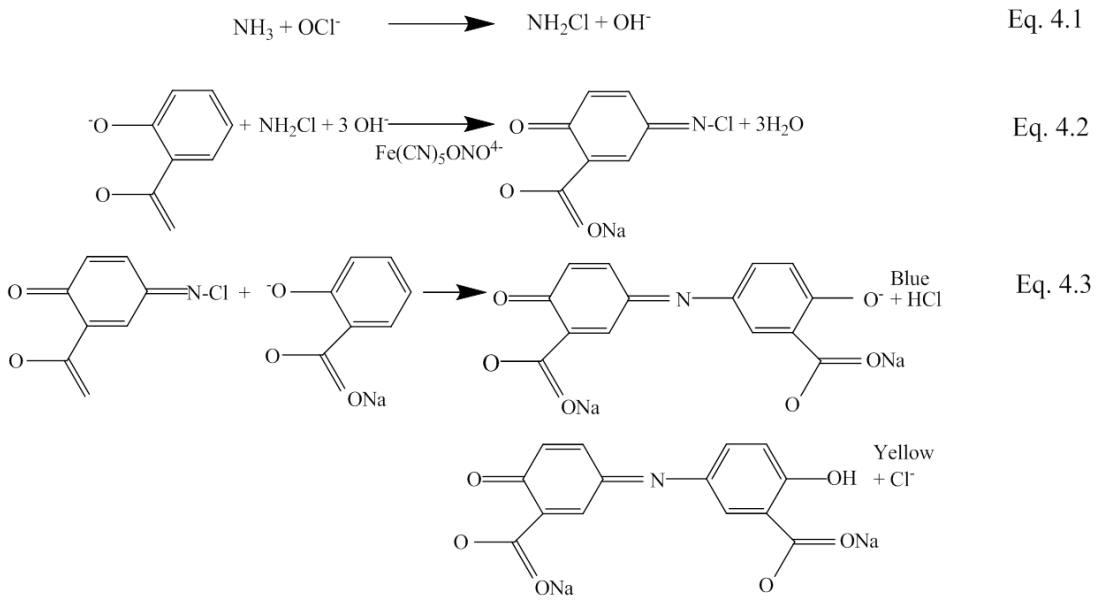

Figure 4.1. Mechanism of ammonia detection employing the Berthelot reaction.

Measurement procedures employed with the Berthelot method differ throughout the literature. Usually, three reagents (mainly incorporating phenol) are used along with a heating step. With these variants of the method, it has been reported that the reagent addition sequence and reaction times are important for reproducibility and bringing the system to a steady state. Daridon *et al.* stated that the optimum order of the reagent addition was reagent 1 (Potassium sodium tartrate, sodium hydroxide, EDTA) + reagent 2 (Phenol, sodium nitroprusside) + reagent 3 (sodium hypochlorite, sodium hydroxide) by means of mixing sample with reagent 1 in an optical cuvette, followed by addition of reagent 2 and waiting 90 seconds for reaction, and finally addition of reagent 3. If needed, the solutions were heated to temperatures up to 45°C to ensure maximum colour development, before additions were made and the cuvette was kept in a thermostated oven. [16-18] A recent study has demonstrated a two-stage reagent reaction sequence for the determination of ammonia in air.[19] Ammonia was transferred to the liquid phase using an ion exchange column and sampling line. The liquid phase then entered the instrument and was analysed spectrophotometrically using the Berthelot method with phenol and hypochlorite reagents. As mentioned above, it is important to keep the fluidic handling as simple as possible and hence our goal in this study was to simplify the Berthelot method to a single reagent addition stage, to allow for easy integration into an autonomous platform while maintaining satisfactory analytical results.

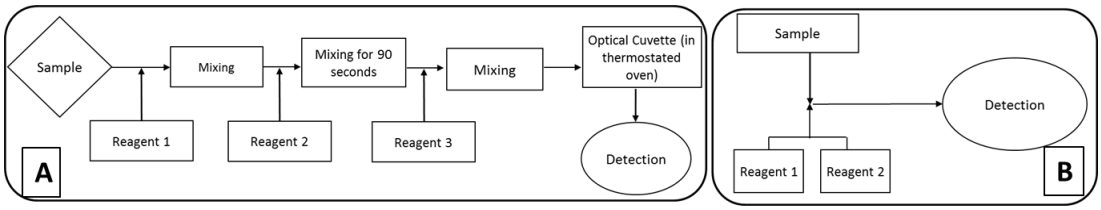

Figure 4.2. (A) Traditional Berthelot method incorporating a multistage process for generating the coloured complex; **(B)** Modified Berthelot method

incorporating a reagent mixing stage prior to sample introduction at a 1:1 v/v reagent to sample ratio.

Therefore, we have further adapted this two-step reagent method for the determination of ammonia in water and employed a reagent pre-mix stage immediately prior to addition to the sample at a 1:1 v/v reagent to sample ratio (Figure 4.2) to allow for easy integration into a low cost, simple sensing platform and increase the deployable lifetime of the system .We have also substituted phenol with sodium salicylate. This is the first demonstration of a fully functional microfluidic sensing system employing this simplified version of the Berthelot method.

4.2 Experimental and fabrication of automated ammonia analyser and optical detection system

4.2.1 Colorimetric reagents

Reagent 1 was prepared by dissolving 6.906 g of sodium salicylate and 0.225 g of sodium nitroprusside in 250 ml of 0.5 mol l^{-1} sodium hydroxide solution.

Reagent 2 was prepared by adding 7.5 ml of sodium hypochlorite (10-15 % available chlorine, used as received, Sigma-Aldrich 425044) to 250 ml of 1.0 mol l^{-1} sodium hydroxide solution. Both reagents were protected from direct sunlight by storing under amber coloured glass.

4.2.2 Deionised water and standards

All solutions were made up using analytical grade chemicals. Deionised water from a Millipore Milli-Q water purification system (18.2 MΩ. cm at 25 °C) was used throughout the analysis. A 100 mg /L ammonium stock

standard was made from dried ammonium chloride; ammonium working standards were freshly prepared weekly from this stock solution by serial dilution.

4.2.3 Instrumentation design and measurement procedure

The ammonia analyser shown in Figure 4.3 (A) is a fully integrated system incorporating a sample inlet with filter (Supor® 25 mm membrane filters, pore size 0.45 μm), storage units for the Berthelot reagent and calibration standards, pumping system to control the transport and mixing of the sample, a microfluidic mixing and detection chip and waste storage resulting in a low cost, rapid and simple instrument for the measurement of ammonia. The sensor implements a two-point calibration protocol using a blank solution (0 mg/L NH_4^+) and a standard solution (is this study 9 mg/L NH_4^+ is used), the concentration of which can vary depending on the particular site/sample in question. Standards are stable for at least 6 months under normal conditions in a sealed container. The optical detection system consists of two 660 nm LEDs (SSL-LX5093SRC/DW, Lumex, Farnell, Ireland) light source with a photodiode detector (OPT101P Texas Instruments, Radionics Ltd, Ireland). The microcontroller (CC2511F32, Texas Instruments) controls the operation of the pumping system and optical detector. The data is stored on a Wixel (WRL10665, Sparkfun Electronics) for wireless communication using 2.4 GHz Radio. Power is provided by a 6V lead acid battery.

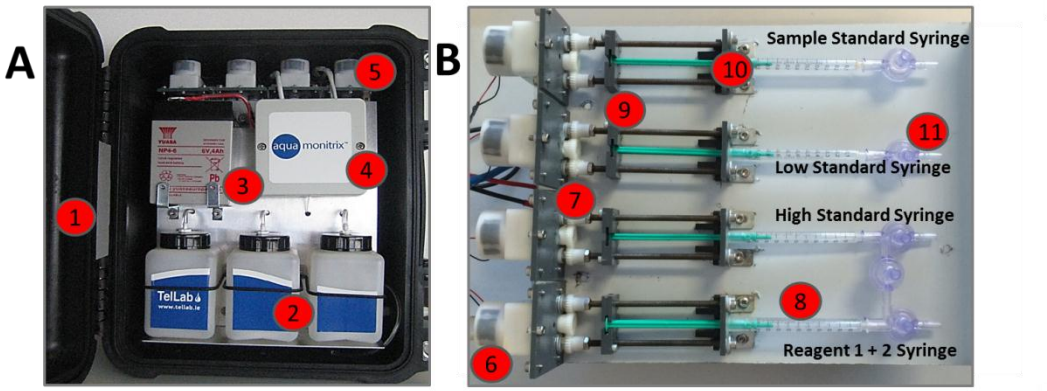

Figure 4.3. (A) Integrated ammonia analyser; [1] Waterproof housing [2] reagent and standard containers [3] 6V battery [4] optical detector [5] syringe pump array. **(B)** The syringe pump array for fluid control; [6] motors for syringe pumps [7] gears [8] syringe [9] syringe holder [10] proximity sensor [11] double check valves.

The mixing of the sample and standards with reagent and detection of the resulting complex takes place within the microfluidic chip. The syringe pump array delivers the reagent and samples to the microfluidic chip. The syringe pump array was designed and built in-house using low cost syringes (1 mL volume, Omnifix-f®, B.Braun), double check valves (Value Plastics, Inc. Part no. DCV118-001) and stepper motors as shown in Figure 4.3 (B). This fluidic system controls the syringe plungers which are used to deliver the sample and reagent via Tygon® tubing (I.D. 2.4 mm, Sigma Aldrich, Ireland) to the microfluidic mixing and detection chip through hose barbs connected to the chip (Build-a-Part 200 Series Barb, Part no. BDMR 210-40, Value Plastics, Inc.) A schematic of the syringe pump array is provided in Figure 4.4. The motor with corresponding gearbox turns the gear which subsequently turns the threaded bars causing the syringe holder to move laterally, pumping the fluid in the process. The syringe holder translates to the direction of the motor. While the infrared LED shines on the photodiode (A), the syringe holder continues to pump until the syringe holder breaks the light gate, signalling that the syringe should be refilled (B). The time taken to fill the syringe was pre-calibrated before implementation. This was

necessary as the reagent syringe holder requires two syringes, therefore, has a heavier load but is accounted for through the pre-calibration process.

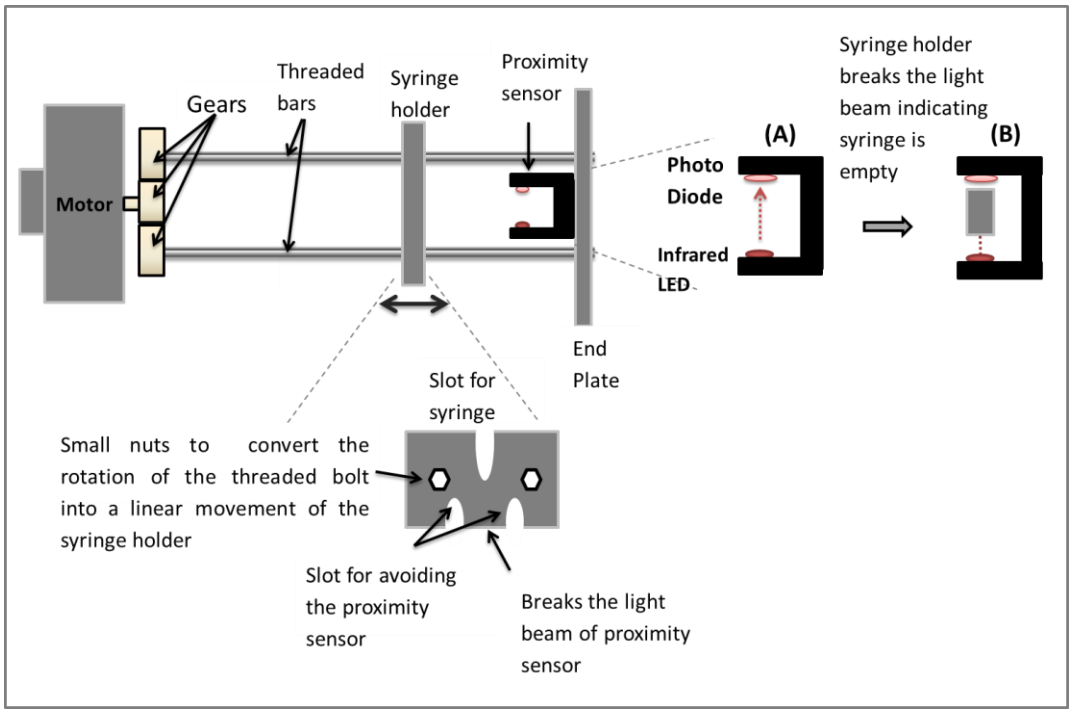

Figure 4.4. Schematic of syringe pump.

To ensure mixing at a 1:1 ratio, all channels from the reagent inlet, reagent mixing channel, sample inlet, sample mixing channel and detector channel are of equal length, cross-sectional area and therefore delivered their respective liquids at an equal flow rate of 0.6 mL min^{-1}. The microfluidic detector chip shown in Figure 4.5 was fabricated from micro-milled PMMA (poly methyl-methacrylate) layers using a DATRON CAT3D-M6 milling machine, to give a 0.5 x 0.5 mm channel. The PMMA layers were cleaned using a mild detergent before being placed under a UV lamp (Dymax 5000- EC, 400 W Power Supply) with at a wavelength of 180 nm for approximately 10 - 12 minutes causing the surface to become hydrophilic. The chip was then assembled using two 1.6 mm steel dowel pins at the chip corners to ensure accurate alignment. To ensure

uniform pressure was applied across the chip during this process, two brass plates were placed above and below the chip. The alignment of the plates was assured at the four corners using vernier callipers. The chip was then heated to 90 °C for 2-3 hours while under pressure to seal the layers. The two LEDs which overlap with the absorbance spectrum of the ammonia-Berthelot complex and the photodiode were placed within the detection cell generated by a 3D printer (Dimension SST 768).

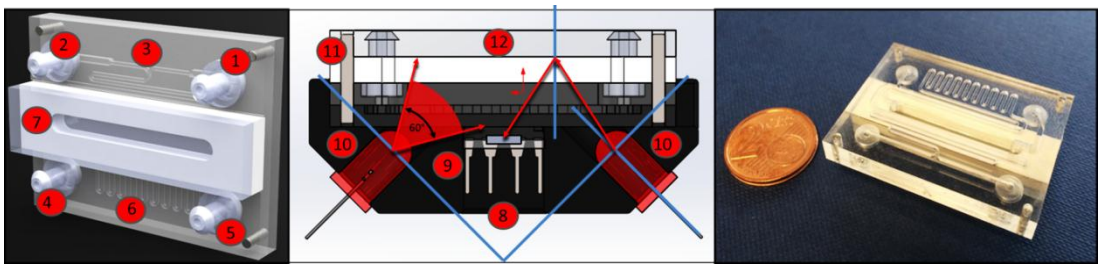

Figure 4.5. Microfluidic chip (40 x 30 mm) with reagent and sample mixing channels and detector channel with mirror (28 x 3 mm). [1] Reagent 1 inlet [2] Reagent 2 inlet [3] Reagent mixing channel [4] Sample inlet [5] Waste Outlet [6] Sample and reagent mixing channel [7] Detection channel with mirror [8] Chip and detection cell [9] Photodiode [10] 660 nm LED [11] Microfluidic chip [12] Mirrored surface

The Berthelot reagent consists of reagent 1 and 2, each of which are stable when stored separately. For each sample assay, a "high measurement" was performed initially. Reagents 1 and 2 were pumped into the reagent mixing channel in a 1:1 v/v ratio using a dual syringe pump via inlets 1 and 2 as per Figure 4.5. The resulting reagent mixture was delivered to the serpentine reagent–sample mixing channel where passive mixing occurred as the high standard was delivered to the chip via inlet 4. The sample to mixed reagent ratio was 1:1 v/v. The resulting mixture was then allowed to react for 1200 seconds within the microfluidic chip at which point two data columns were outputted, the first was the count equivalent to time (seconds) and the second was the reading from the photodiode. This procedure was then followed for the low and sample measurements respectively.

The photodiode generates a small photo current when photons impact on the diode junction region. This small change in current is converted to a change in voltage and amplified using a transimpedance amplifier. The ADC pin of the microcontroller converts the signal into a corresponding ADC value. This detector signal, in the form of ADC readings, was transmitted wirelessly to a laptop computer via the 2.4GHz WIXEL radio communications in text file format.

One side of the detector channel within the chip is parallel to a mirrored surface. The light emitted by the LED is directed across the detector channel, and reflected back by the mirrored surface to the photodiode. The light will reflect within the dispersion angle and the remaining light will be absorbed by the black casing of the detection cell. This results in an increased absorbance signal by increasing the effective pathlength without needing to increase the physical pathlength. Given that the half viewing angle of the emitter LED is 30° (as shown in Figure 4.5), coupled with the known dimensions of the microfluidic chip and holder, the direct path length for the light ray through the solution was calculated as 5.96 mm (ca. 6 mm). In comparison to a directly aligned arrangement (2.5 mm in this case), this represents an increase of the path length by a factor of 2.4. Furthermore, this offers a singular planar arrangement which can be important for packaging purposes. Two LEDs are present within the detection cell to provide sufficient light intensity for sensitive photodiode detection. Another advantage to this set up is that the detection cell is modular and LEDs can be easily replaced to suit colorimetric methods focused on other regions of the spectrum.

4.3 Results and discussion

4.3.1 Optimisation and method parameters

To investigate if both reagents can be mixed to obtain sufficient colour formation, reagents 1 and 2 were mixed and a spectrum was measured immediately after mixing. An absorbance spectrum of 9 mg/L NH_4^+ and the mixed Berthelot reagents was taken at various time intervals.

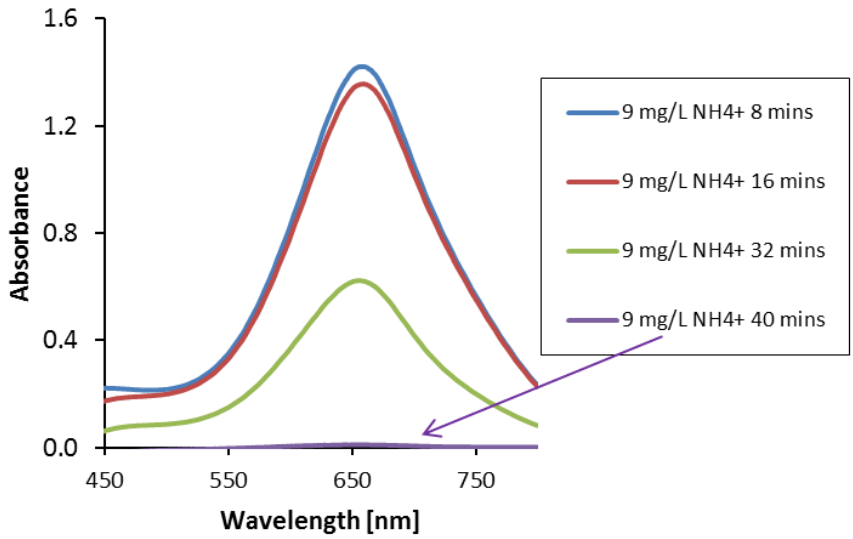

Figure 4.6. Mixed reagent stability over time.

As shown in Figure 4.6, the absorbance decreases significantly within 30 minutes after mixing reagents 1 and 2, to the extent that after 40 minutes, the absorbance is almost zero. It is therefore necessary that reagent 1 and 2 are mixed immediately prior to addition to the sample. The fluidic design within this system has been designed with this constraint in mind.

The optimum wavelength was investigated by obtaining the absorbance spectrum of the modified Berthelot reagent and standards ranging from 3 - 9 mg/L NH_4^+ showing a λ_{max} at 660 nm (arising from the use of salicylate rather than phenol) with a full width at half maximum of 124

nm and a correlation coefficient of 0.9998, molar extinction coefficient (ε) of 6.07 x 10^{-2} L mol^{-1} cm^{-1} and an average relative standard deviation (RSD) (n = 3) of 0.5%. The method gave a linear response to ammonia concentrations up to 12 mg/L NH_4^+ after which the absorbance plateaus. Results suggest that when both reagents are stored separately, they are stable for at least 6 months as an initial calibration plot was compared to a calibration plot 6 months later using the same standards and reagents, yielding R^2 values of 0.9998 and 0.9949 respectively.

4.3.2 Kinetics study

The rate of development of the ammonia Berthelot reagent complex colour intensity was monitored using a UV-vis spectrometer at a temperature of 23 °C for the detection of samples in the range 3 - 9 mg/L NH_4^+. As mentioned above the reaction progresses promptly in the presence of a catalyst, proceeding to the formation of the indophenol, which is the rate determining step. Indophenol formation is a second-order reaction, but as all reagents are added simultaneously and in excess, the formation of 5-aminosalicylate exhibits pseudo first order kinetics. The absorbance at 660 nm was measured every 5 seconds for 3000 seconds. The resulting kinetic curves obtained were modelled using a first order exponential equation:

$$T = a\,(1 - e^{-kt}) + b \qquad \text{(Equation 4.4)}$$

Where T is the time (s) at the end of the reaction, a is the scaling factor, k is the first order rate constant (s^{-1}), t is the time (s) and b is baseline offset. First-order kinetic models were fitted (Microsoft Excel Solver)[20] to each of the data sets and the rate constants were calculated for each concentration ranging from 3 – 9 mg/L NH_4^+. The absorbance (3mg/L NH_4^+ average RSD = 2.67%, 6 mg/L NH_4^+ average RSD = 0.26% and

9mg/L NH_4^+ average RSD = 5.74%) and fitted models (3mg/L NH_4^+ average RSD = 7.19%, 6 mg/L NH_4^+ average RSD = 3.92% and 9mg/L NH_4^+ average RSD = 3.29%) are shown in Figure 4.7 with the corresponding rate constants shown in Table 4.1 showing negligible difference. It can be seen that the colour formation increases rapidly for all concentrations until approximately 1500 seconds, after which the increase in absorbance was reduced.

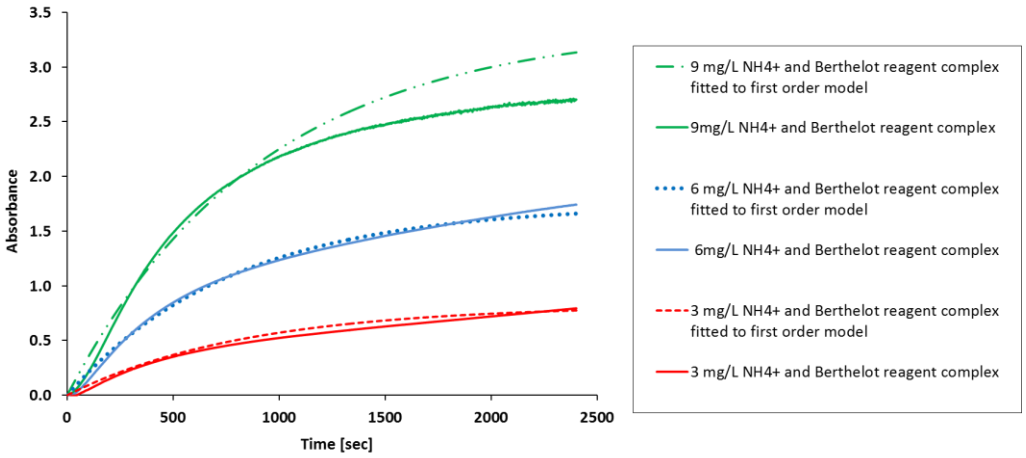

Figure 4.7. Kinetic study of the Berthelot- ammonia complex formation using standards ranging from 3 – 9 mg/L NH_4^+ with corresponding first order model fits (23.0 °C).

Table 4.1. Comparison of average rate constants for colour formation using NH_4^+ samples with the Berthelot method (23.0 ± 0.5 °C; n = 3).

Ammonia Concentration [mg/L]	Average k ($x\ 10^{-3}$; s^{-1})	Standard deviation ($x\ 10^{-4}$; s^{-1})
3	1.21	1.73
6	1.29	1.63
9	1.10	1.62

4.3.3 Limit of detection

The limit of detection (LOD) was found by obtaining signals of multiple reagent blanks (n = 7) and first determining the signal detection limit using equation 4.5:

$$y_{dl} = y_{blank} + 3s \qquad \text{(Equation 4.5)}$$

where y_{dl} is the minimum detectable signal, y_{blank} is the signal from the reagent blanks (containing no analyte) and s is the standard deviation of the blank measurements. The minimum detectable concentration was then obtained from equation 4.6:

$$Detection\ limit = 3s/m \qquad \text{(Equation 4.6)}$$

Where m is the slope of the linear calibration curve obtained using standards ranging from 0.003 mg/L to 3 mg/L NH_4^+. The minimum detectable signal was calculated as 0.0289. The detection limit was then calculated as 0.015 mg/L NH_4^+. Figure 4.8 illustrates the signal obtained from the reagent blank and the minimum detectable signal and also signals from sample concentrations and reagent.

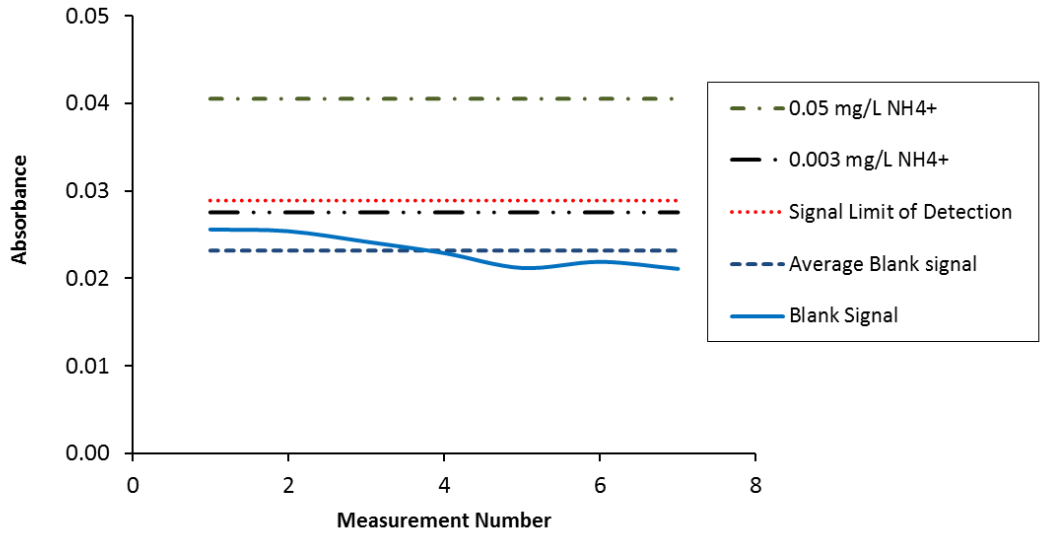

Figure 4.8. Replicate absorbance measurements on blank.

4.3.2 Interferences

It is known that nitrogen compounds and some metals including copper can interfere with the Berthelot reaction.[8, 21] It is therefore important to study these interferences to ensure the autonomous platform can achieve successful analytical results in varying sample matrices. The effect of copper was investigated by obtaining an absorbance reading of the Berthelot reagent and 0.03 mg/L NH_4^+ sample and spiking the sample with various concentrations of copper. This procedure was also carried out for phosphate, iron, chlorine and nitrate (Table 4.2). A significant decrease in the indophenol colour intensity was observed in the presence of copper at a concentration of 10 mg /L copper which is in agreement with the literature.[6] However, this concentration tested is higher than that typically found in natural waters and therefore these interferences would only be significant in very polluted waters[22] as copper is typically present in uncontaminated surface waters and groundwater at low concentrations less than 0.01 mg/L.[23] However, algicides or copper mining/smelting operations may give rise to elevated levels copper [24], in

such samples measures can be performed to remove copper such as treating with EDTA.[25]

Table 4.2. Effect of potential interferents on the signals obtained for 0.03 mg/L NH_4^+ and Berthelot complex (n = 3).

Species	Added Concentration [mg/L]	Apparent relative percentage error [%RE ± SD]
PO_4^{3-}	10 mg/L	$1.52 \pm 3.10 \times 10^{-3}$
Fe(II)	10 mg/L	$0.78 \pm 1.08 \times 10^{-3}$
Cu(II)	10 mg/L	$33.02 \pm 1.3 \times 10^{-3}$
Cl^-	10 mg/L	$0.26 \pm 2.80 \times 10^{-3}$
NO_3^-	10 mg/L	$0.58 \pm 6.66 \times 10^{-4}$

An issue that has raised some concern is the potential for contamination of water samples by atmospheric ammonia.[26, 27] Control standards were prepared and were stored under screw top bottles and paraffin film (Parafilm) so as to prevent any contamination from air and tested against standards that were stored in open air flasks within the laboratory. A third set of standards were prepared and stored under open caps in an enclosed container alongside an open bottle of ammonium sulphate powder to observe any uptake of ammonia that may arise from this. Absorbance values from all ammonia standards and Berthelot complex are presented in Table 4.3 and results suggest that there was possible absorption of ammonia from ambient air in the open capped samples due to the apparent difference in absorbance values from the control standards and the open cap containers. Interestingly, results from a questionnaire by Aminot et al. sent to participants in the Fifth ICES Intercomparison Exercise for nutrients in seawater, stated that many nitrogenous compounds are volatile and found that several laboratories

suggested that vapour through hoods and ventilation ducts from adjoining laboratories can be a major source for air-borne contamination.[17] This indicates that correct storage conditions are essential in achieving accurate results. Therefore all calibration standards and reagents will be enclosed in air tight containers which are not permeable to gases within the system to avoid any possibility of contamination from air.

It has also been well established in the literature that the Berthelot method is significantly sensitive to reagent addition sequence and temperature variations.[16, 28] Searle highlights the need for the addition of phenol to the sample prior to hypochlorite for maximum colour formation.[8] However this study shows that excellent colour formation can be achieved with the reagents pre-mixed immediately prior to use.

Table 4.3. Absorbance values of 0.03 mg/L NH_4^+ and Berthelot complex under different storage conditions (n = 3).

	0.03 mg/L NH_4^+ and Berthelot complex absorbance		
Standard Type	**Average**	**Standard Deviation (n = 3)**	**Apparent relative percentage error [%RE]**
Control (closed cap)	0.034	0.0017	0.0
Open cap	0.029	0.0018	14
Container with ammonia source (open cap)	0.036	0.0020	6.4

The Berthelot method is known to be temperature dependent, and therefore the prototype platform implements a two-point calibration protocol prior to every analytical measurement using a blank solution (0

mg/L NH_4^+) and a standard solution to automatically compensate for such effects. The concentration of the standard solution can be varied depending on the range of ammonia levels in a particular sample/site. The importance of this procedure lies in its ability to automatically compensate for any local variables such as temperature changes as these affect the analytical sample and the calibration measurement in a similar manner, and largely cancel out during the analytical calculation of the sample concentration. In addition to variations in the rate of the Berthelot reaction, but also this compensates for changes in LED output/photodiode response due to temperature fluctuations, possible drift in response of detection system over time, and possible change in sensitivity of detection system over time.

4.3.5 Validation of the modified Berthelot method

Prior to implementing the modified chemistry onto the sensing platform, a validation process was achieved using 6 blind samples from various environmental sources including wastewater effluent, river water and standards. The samples were split and parallel assays independently performed by T.E. Laboratories using ion-chromatography. The samples were filtered prior to analysis using membrane filters (Supor® 25 mm membrane filters, pore size 0.45 μm). The modified Berthelot method was performed using a UV-vis spectrophotometer and compared to the ion chromatography reference measurements as shown in Table 4.4.

An excellent correlation coefficient value of 0.9954 was achieved between the modified Berthelot method and the ion chromatography method suggesting that the former method is suitable for integration into an autonomous analyser for the direct determination of ammonia.

Table 4.4. Absorbance values of 0.03 mg/L NH_4^+ and Berthelot complex under different storage conditions (n = 3).

Sample/ Water Matrix	Modified Berthelot Method NH_4^+[mg/L]	Standard Deviation (n = 3)	Ion Chromatography NH_4^+ [mg/L]
Std. 1 mg/L	0.94	9.90×10^{-04}	1.00
Std. 0.5 mg/L	0.48	1.20×10^{-03}	0.46
Effluent	0.31	5.66×10^{-04}	0.33
Effluent	0.02	7.07×10^{-05}	0.02
River water	0.01	0.00	0.01
Effluent	0.09	1.06×10^{-03}	0.09
River water	0.17	2.83×10^{-03}	0.13

4.3.6 Integration of chemistry into autonomous sensing platform

The modified Berthelot reagents and a range of standards were prepared as described in section 4.2. A calibration plot was obtained using standards up to 9 mg/L NH_4^+ with the microfluidic chip and low cost LED - photodiode detection system within the fully autonomous sensing platform as shown in Figure 4.3 with a correlation coefficient value of 0.9983 and an RSD of 7.0%. Samples of known concentration were then analysed by the sensor and an excellent correlation between the estimated sample concentrations as a function of known sample concentration was obtained of 0.9987 with an average RSD of 7.4%. These results confirm that sufficient mixing of the reagents takes place prior to addition to sample and further confirm that satisfactory analytical results can be achieved with the autonomous platform.

4.4 Conclusion

A sensing platform for the direct determination of ammonia in water has been developed. The main function of this paper is to report the optimisation parameters of a simplified Berthelot to facilitate the integration of the method into an analysis system, resulting in a simple procedure for the real-time measurement of ammonia. The work presented describes analytical merits such as the low limit of detection achieved of 0.015 mg/L NH_4^+ and the excellent correlation achieved with the reference method, ion chromatography. However an area capable of further improvement through further optimisation studies is the sample turnaround of 60 minutes due to the pumping of reagent and sample (10 minutes respectively) followed by reaction time and detection time of (20 minutes). Also further investigation is required for possible interferences especially with regards atmospheric ammonia and exposure time to sample etc.

Future developments will focus initially on improving the resolution of the system for the determination of low ammonia concentrations. Subsequently, the major emphasis is on field deployments with this modified approach for *in situ* environmental monitoring. The priority will be on the real issues related to the analytical approach and sampling within environmental waters and in particular, achieving autonomous operation of the sensor platform over extended periods of time.

4.5 Acknowledgments

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Chapter 5

Summary, conclusions and future perspectives

"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less."

Marie Curie-Sklodowska

5.1 Overall summary and conclusion

Sensor networks along with wireless communications, access to data repositories, cloud-databases and internet of things, constitute an emerging field of research that will affect many aspects of our daily lives such as environmental and health monitoring, infrastructure security, surveillance applications, industrial sensing and traffic control.[1] The question of how to gather, integrate, analyse and share sensed environmental data is the driving force behind this research and in particular, how to sense the chemistry of our environment and how to realise chemical sensors that are reliable and robust with the ability to function autonomously for periods of weeks to months. The cost of ownership of these devices must be as low as possible to achieve the ultimate goal of a widely deployed network of chemical sensors otherwise the use model is not scalable.[2]

Reagent based platforms like the ones mentioned in chapters 3 - 5 are an important step along this road, and this thesis has presented several innovative methods for analysing nutrients in water specifically designed for ease of integration into autonomous monitoring platforms, a list of analytical figures of merit for each method and sensor specification are presented in table 5.1. Furthermore, novel microfluidic approaches were investigated and tested for suitability. Although the focus for the colorimetric methods for ammonia and nitrate has been on easy integration into an autonomous platform, the methods also have great potential as rapid, simple and reliable lab bench methods, and can be applied to many applications such as *in situ* portable appliances or test kits in various formats (paper, solid reagent, blister packs etc.).

It is well known that nitrate can be difficult to monitor in water and wastewater due to the relatively complex procedures needed, and the high probability of interferences along with the limited concentration

ranges associated with many established methods. Therefore in chapters 2 - 3, a relatively unused method for the determination of nitrate was revisited which resulted in a direct, simple, sensitive, rapid approach for monitoring of nitrate in water. The study has demonstrated, for the first time, the integration of the chromotropic acid method into a low-cost, autonomous monitoring platform using a COP microfluidic chip to measure nitrate in natural waters. The microfluidic analyser displayed excellent correlation with ion chromatography when analysing real samples including drinking water, freshwater, wastewater and seawater, through blind tests with our industry partners. The prototype system has a component cost of ca. €600, potentially delivering a highly competitive cost compared to commercially available autonomous sensing platforms, due to its simplicity, low consumption of reagents and waste production, compact design, acceptable analytical performance and high sample throughput. This research also raised awareness of other issues such as the choice of polymer material, the range of microfabrication techniques available and the integration of multiple components into one device.

Research has been previously undertaken to improve the formulation of the Berthelot method for the determination of ammonia in water. The number of reagents used and the toxicity of the method have been addressed in recent years. Therefore chapter 4 describes a study on the integration of a modified Berthelot method for the direct determination of ammonia in water into a microfluidic sensing platform, resulting in a reliable, quick and simple procedure which is less toxic than other variants of the Berthelot method.

The low-cost modular design of these systems offers a flexible and versatile approach to colorimetric analysis. The platforms described in chapter 2 - 4 incorporate easy interchangeable optical detection units, which will allow for most colorimetric reagent based assays since LEDs can be easily replaced to cover the spectra range ca. 247 – 1550 nm. This

is also the case for colorimetric approaches that may use harsh chemical reagents, as it has shown that COP based microfluidic chips have excellent chemical/thermal stability, allowing for integration of a much broader range of colorimetric reagents compared to the conventional materials used up to now.

Table 5.1. Sensor specification and analytical figures of merit

	Analyte	
	Nitrate (NO_3^- mg/L)	**Ammonia (NH_4^+ mg/L)**
Linear range	0.9 - 80	0.01 – 12
LOD	0.70	0.015
Linearity (R^2)	0.9901	0.9983
Repeatability RSD % (n =3)	7.4	7.0
Reagent stability	At least 6 months	At least 12 months
Component cost (€)	600	300
Size and weight	30x 22.5 x 13.2 cm, 3.5 kg	30x 22.5 x 13.2 cm, 2.1 kg

5.2 Possible future applications and limitations

Considering the knowledge gained during this research, there are several strands/research paths which should be further explored:

5.2.1 Extending the autonomous nature of chemical analyser platforms

The long-term deployment (months unattended) of chemical analysers is hindered by the lack of reliable chemical sensing platforms, and while this research is focused on the development of robust prototype sensors, work must be achieved in transcending this research into the real analytical world. Conventional automated flow injection analysis (FIA) systems consume relatively large amounts of reagents and standards[3], making the microfluidic aspect of this thesis relevant to the miniaturisation of these analytical techniques.[4] Through the miniaturisation of these devices and keeping the fluidic design as simple as possible, the overall reliability of the systems can be improved while reducing cost as shown in chapters 3-5, opening the way to the realisation of functional platforms of practical use. The importance of this goal has been highlighted by George Whitesides (2010, Harvard), a recognised leader in microfluidics who states:

"*Microfluidics, to date, has been largely focused on the development of science and technology, and on scientific papers, rather than on the solution of problems.*"[5]

Considering the fully integrated sensor platforms have been successfully designed, optimised and validated in chapters 3-5, the major emphasis will now shift to field deployments with these devices for *in situ* environmental monitoring. The priority will be on the real issues related

to the analytical approach and sampling within environmental waters and in particular, achieving autonomous operation of the sensor platforms over extended periods of time (at least 3 months unattended use). Initially, it would be advantageous to deploy the sensing devices at suitable locations such as a wastewater treatment plant that offers access to on-going parallel measurements for validation purposes while capturing transient changes in nitrate and ammonia levels. Such sites can also offer access to mains power systems and communications if needed. The data retrieval and transmission can be established by GSM or other wireless communications technologies and the data uploaded to a cloud-database. The power requirements will be kept to a minimum by applying "sleep mode" operation of the system between measurements. After validation of this initial set-up, progress can be made for expanding the number of deployed units, and selecting a broader range of locations.

The nitrate and ammonia sensing platforms have great potential for commercialisation considering the validation of the platforms using the gold standard instrumentation in conjunction with an accredited laboratory. However, a key goal for scalability of deployments has to be duration of autonomous operations. Therefore, ways to extend deployment lifetimes should be investigated, for example through the use of solar cells as a local energy generation source. For example, experience gained in a parallel study by Collins *et al.* wherein a solar cell was used to provide sufficient power to charge a battery in an autonomous landfill gas monitoring platform. When integrated, the solar cells immediately began to produce a net positive increase in battery level, even in low lightning conditions, leading to a fully autonomous gas sensing platform, at least in terms of energy requirement.[6] Such developments can be easily integrated into these analyser platforms, although other issues such as reagent consumption will make service visits still necessary, albeit at relatively long intervals (several months, preferably longer).

5.2.2 Integration of biomimetic actuators on microfluidic platform

In order for the cost, reliability and duration of autonomous operations to improve dramatically, research must deliver potentially disruptive technologies. While this research has addressed microfluidics coupled with optical devices in order to deliver significant technical progress, the future will witness truly disruptive advances. The autonomous analysers of the future are likely to increasingly incorporate bio-inspired concepts as the development of fully integrated microfluidics devices is still prohibited by the lack of scalable components for fluid control.[7] Conventional pumps and valves are the fundamental limiting factor in terms of miniaturisation and cost but remain essential to control liquid flow in the micro-channels. Diamond and *co-workers* recently presented a concept based integrated analyser using soft polymer actuator valves to control liquid flow, with an estimated component cost of €20.[8, 9] The scope of this work is to explore polymer actuator valves that could be incorporated into an established microfluidic sensing platform such as the platform described in chapter 4 (work described in chapter 3 would be unsuitable due to the highly acidic nature of the chromotropic reagent).

To produce microvalves using polymers the following requirements must be achieved;

- Minimal flow resistance when valve is open
- Negligible flow in channel when valve is closed (no leakages)
- Tolerance to pressure
- Rapid on/off response time in switching the valve
- Excellent reproducibility
- Easy manufacturing
- Chemical stability/durability
- Thermal stability

- Increase the range of solvents that can be used

Much current research on fully integrated microvalves is focused on electroosmotic, pressure, thermoneumatic and electromagnetic effects.[10] However these approaches require external power to supply the actuation signal and therefore have limited practical applications, in terms of *in situ* monitoring. However, these conventional valves require high power consumption, which is not favourable for autonomous field deployable platforms.

On the other hand, soft polymer actuators appear to be gaining much interest, as in principle require relatively low energy for actuation. They are easy to fabricate and are often used as smart materials due to their ability to change volume in response to stimuli to open or close a channel. For example, hydrogels swell and contract through water movement triggered by alteration of external stimuli. There are some shortcomings associated with this approach however as these valves can be difficult to control precisely due to their actuation speed and limit of pH range as they require an acidic environment, however extended research is continuing in this area.[10]

Stimuli responsive gels are constructed by crosslinking monomers to form macromolecular structures which are commonly porous. These pores can be filled with a solvent such as water, to provide a gel-like network arrangement. The gels themselves are also hydrated; if highly hydrated, the gel takes its swollen form; the lower critical solution temperature (LCST) behaviour leads to the formation of a globular compact structure, expelling the water from the polymer and the pores. Stimuli responsiveness of these materials is based on the existence of two metastable energy states associated with two structure forms. If the energy minima of those two states are well separated then the material is switchable and can be forced to change from one structural arrangement

to the other. These changes can be induced with different stimuli depending on the switchable functional group attached to the gel network *e.g.* thermal, light, pH or electromagnetic stimuli.

An interesting approach to this area of materials science is the combining of ionic liquids within a polymer matrix to produce materials known as ionogels (ILs). ILs have been defined as "a solid interconnected network spreading throughout a liquid phase", the ionogel therefore combines the properties of both its solid and liquid components.[11] Diamond and *co-workers* have carried out extensive research in the area of ionogels and their applications. An interesting aspect is the incorporation of ionogels with the chromophoric molecule- spiropyran (SP) resulting in a photoswitachable material. These ionogels show great promise as they do not require an 'invasive' (direct physical contact) for the stimulus to have an effect. An ideal application for this gel is a microchannel within a microfluidic system as the opening/closing of the valve would be controlled by simply applying light of a suitable wavelength (Figure 5.1).[12]

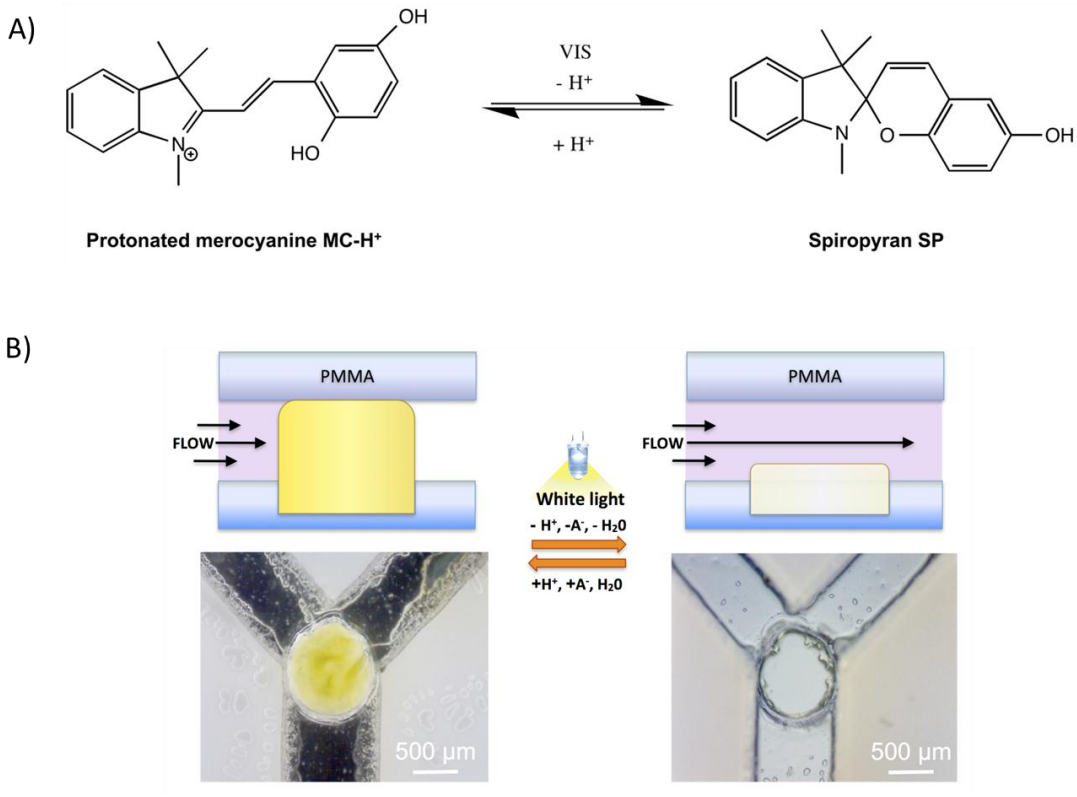

Figure 5.1. A) Photo-isomerisation of the protonated isomer (MC–H^+) into spiropyran (SP). **B)** Schematic of the valve actuation and scheme of the photoswitchable polymer matrix.[12]

Benito-Lopez *et al.* presents the fabrication, characterisation and optimisation of different ionogels as photo-responsive valves. The ionogels based on poly(N-isopropylacrylamide) incorporate benzospiropyran units and phosphonium-based ionic liquids. Each ionogel was photo-polymerised *in situ* in the channels of a PMMA micro-fluidic device, generating a manifold with four different micro-valves. The valves were actuated by simply applying localised white light irradiation. The reported micro-valve gels presented fast response times with some gels showing an actuation response of a few seconds to go to 'open' valve state. However, valve closure required a relatively long time of ca. 30 min and required contact with an acidic solution. Nonetheless, the gels used within this manifold facilitate flexible, low-cost, and low power control of

liquid movement in micro-fluidic devices, while the tailoring of the chemical and physical properties of these ionogels such as acid/base character, response times and reproducibility are the subject of much on-going research.[13,14] The incorporation of these gels as valves into a microfluidic chip as shown in the platform concept (Figure 5.2) will require additional research to overcome current performance limitations.

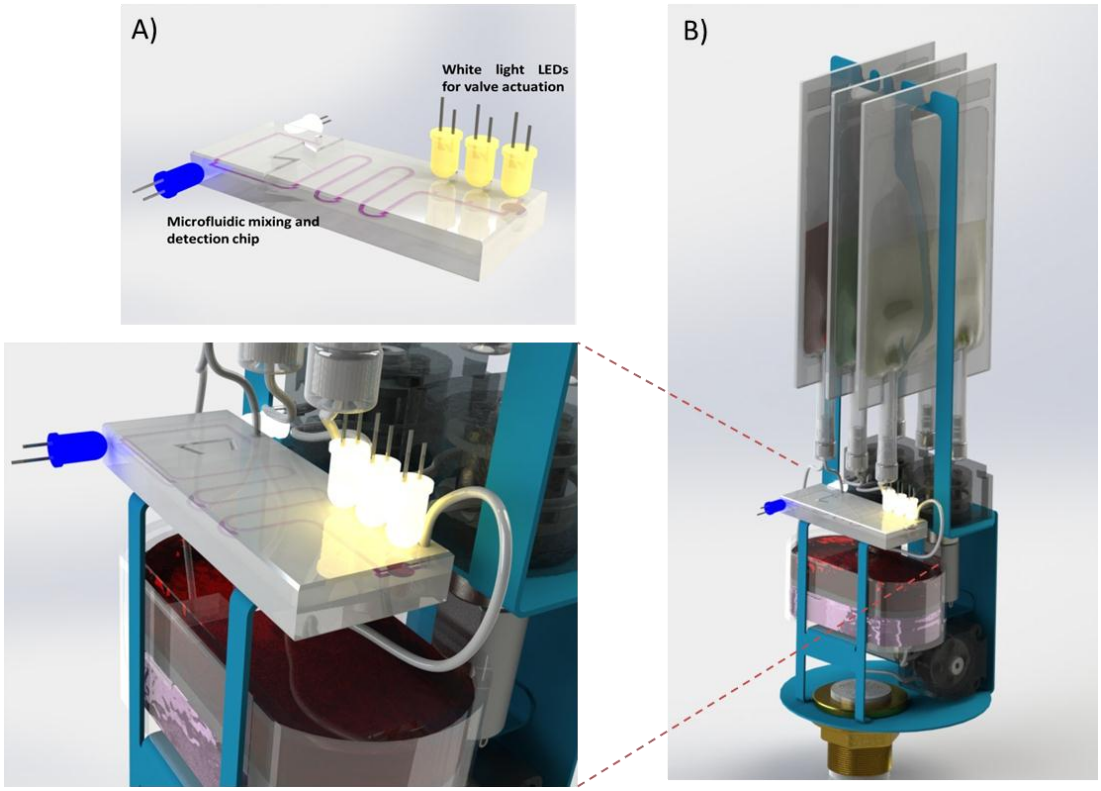

Figure 5.2.(A) Integrated polymer valves within microfluidic chip. **(B)** (Concept) Replacement of conventional valves on previously developed phosphate system[15] with photoswitchable polymer valves to control liquid movement.

5.2.3 Advances in photoactuated gels

As mentioned above, photoresponsive ionogels with pendant spiropyran groups require exposure to external acidic solution (usually millimolar hydrochloric acid (HCl)) to generate the swollen gel prior to photo-triggered contraction. This functional limitation is a major challenge to implement within a microfluidic set up as exposure of the gel to HCl is required to induce re-swelling. However recent studies by Ziolkowski *et al.* have demonstrated that the gel matrix can be copolymerised with acrylic acid, providing an internal source of protons, no longer requiring an external acidic solution.[16] Furthermore, the actuation cycles of these gels in deionised water are repeatable even in non-buffered water (pH ca. 6.5), as protonation throughout the gel does not rely on diffusion of protons from an external acidic solution. In contrast to previous formulations, their photo-induced shrinking ability does not deteriorate after multiple washings in deionised water and repeated switching has been demonstrated over a 2 month period.

The incorporation of these gels within microfluidics devices and the subsequent characterisation of the microvalve performance is currently under investigation. Work by Schiphorst *et al.* have shown these self-protonating gel formulations using spiropyran in a hydrogel network, to achieve a swollen state of the hydrogel in water at neutral pH within microfluidic channels. The valves were photopolymerised *in situ* allowing reversible and repeatable operation, with opening and closing of the valves in minutes (Figure 5.3). The light induced flow variations within the channel were measured over time by illuminating the valve with a blue LED for 1 min followed by switching off the LED for 5 min. During illumination, a flow rate of 7 μL/min was observed after 1 min. When the LED was switched off, the flow decreased to almost zero within 5 min.[17] This approach demonstrates a new way to control liquid flow in microfluidic devices that can be widely employed in chemical analysis

and bio-analysis as platforms could incorporate thousands of valves allowing complex fluidic operations to be fully integrated. Multiple parallel sensor arrays will open the way to sequential activation of short term use sensors (e.g. biosensors) to extend the operational lifetime of the platform

This work towards truly futuristic biomimetic platforms could provide a tipping point in terms of scale of deployments for water quality monitoring as it could greatly enhance the possibility of making reliable chips in bulk volumes and low unit costs.

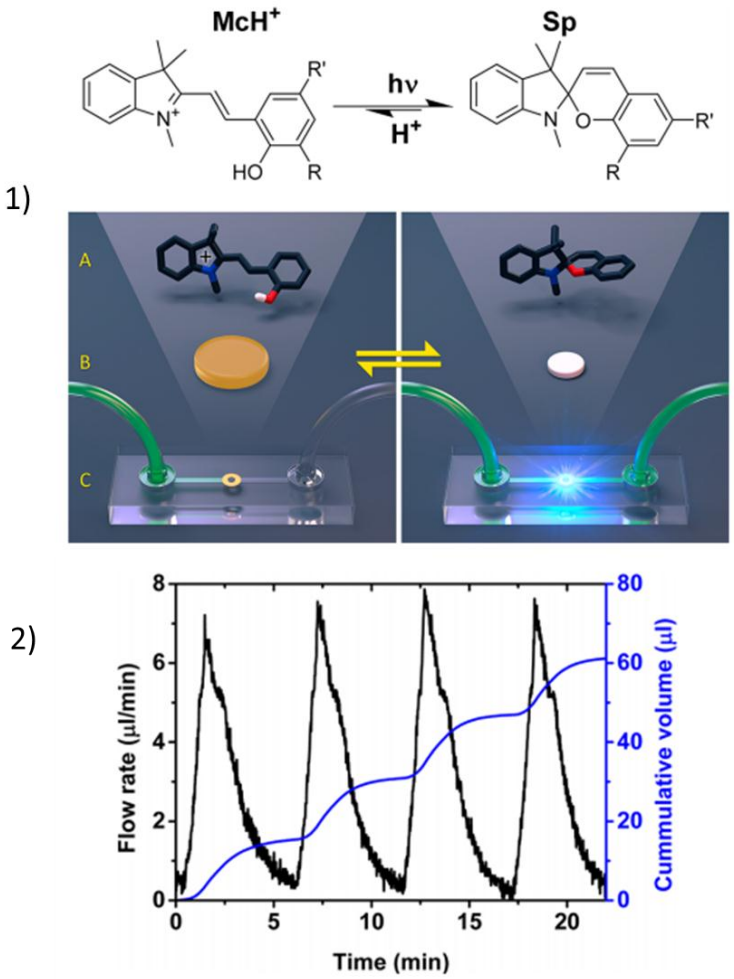

Figure 5.3. [1] Isomerization of a protonated merocyanine (McH+) and the spiropyran (Sp) form (A) with the corresponding effect on the size of a hydrogel by irradiation with light (B), implemented as lightresponsive valve in

microfluidics (C) [2] Flow profile of water in a chip containing a gel valve with spiropyran derivative 1, upon illumination with blue light for 1 min. Reproducible opening and closing is observed, indicated by the flow profile (black) as well as the cumulative flow volume profile (blue).[17]

5.3 References

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Supporting Information A

Development of a low cost microfluidic sensor for the direct determination of nitrate using chromotropic acid in natural waters

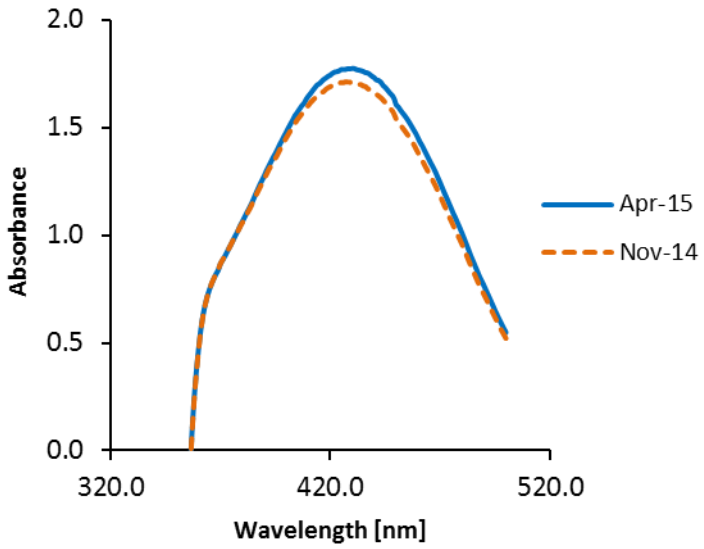

Figure SIA.1.
Spectra of 80 mg/L NO_3^- in the chromotropic acid reagent prepared in November 2014 and using the same reagent again in April 2015.

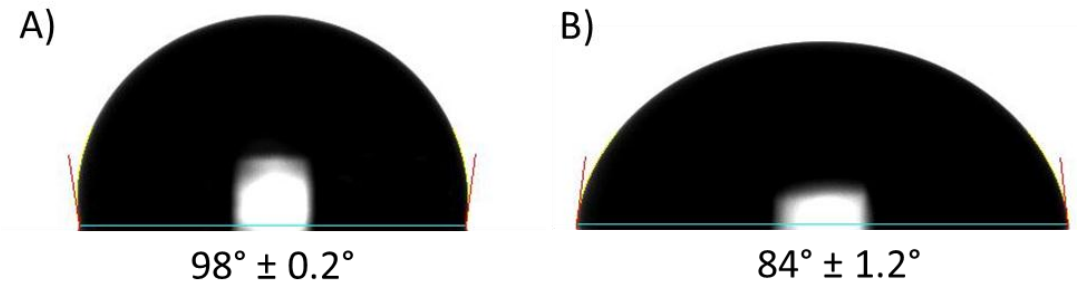

Figure SIA.2.
Contact angle measurments of water on COP surfaces before (A) and after (B) direct UV irradiation (n = 3).

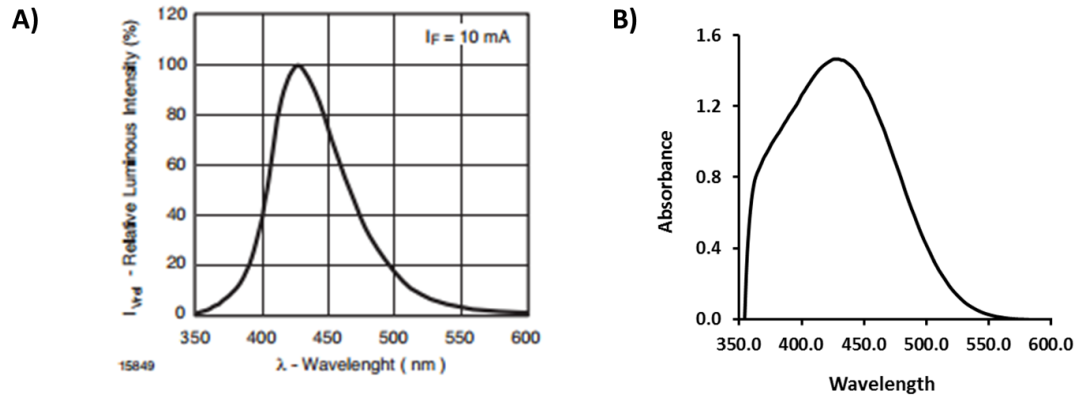

Figure SIA.3.
A) UV-LED light output (Vishay Semiconductors, 2004) and **B)** the UV spectrum of 80 mg/L NO_3^- - chromotropic reagent complex.

Figure SIA.4.
Peristaltic pump containing Tygon tubing showing degradation and discoloration, caused by decomposition of the polymer due to the highly acidic nature of the reagent (chromotropic acid and 98% sulphuric acid) and the high temperature caused by the exothermic reaction produced by the addition of the aqueous solution to the sulphuric acid. This unit had been in use for a period of three weeks.

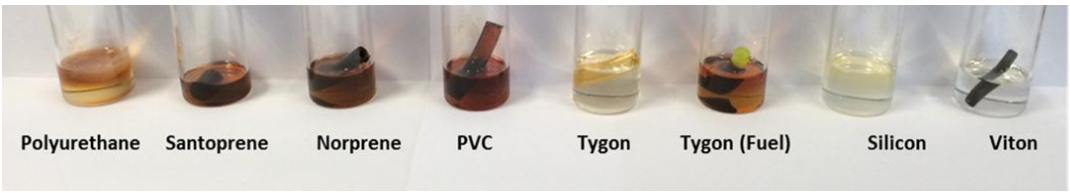

Figure SIA.5.a.
Range of elastomers exposed for a period of one week to 98% sulphuric acid. Polyurethane and silicon tubing have almost completely dissolved in the acid while Santoprene, Norprene, PVC and Tygon (fuel) show severe discolouration. Tygon shows slight discolouration while Viton shows no sign of discolouration or corrosion.

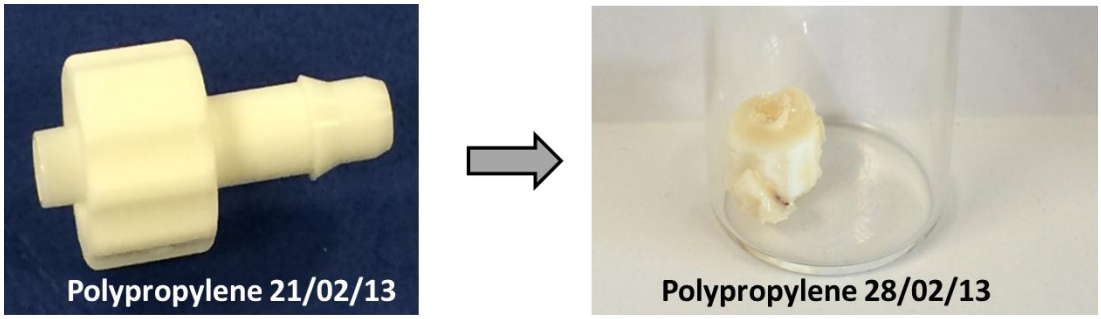

Figure S5.b.
New polypropylene luer (left), and severe damage after exposure for a period of one week to 98% sulphuric acid (right).

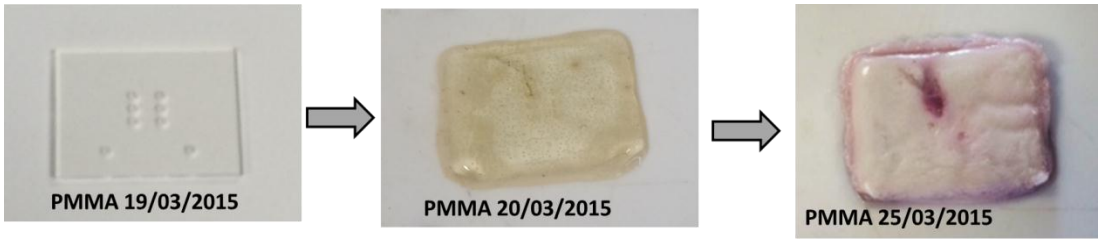

Figure SIA.5.c.
PMMA (Poly(methyl methacrylate)), a material commonly used for the production of microfluidics chips, showing severe effect of exposure for one week to 98% sulphuric acid.

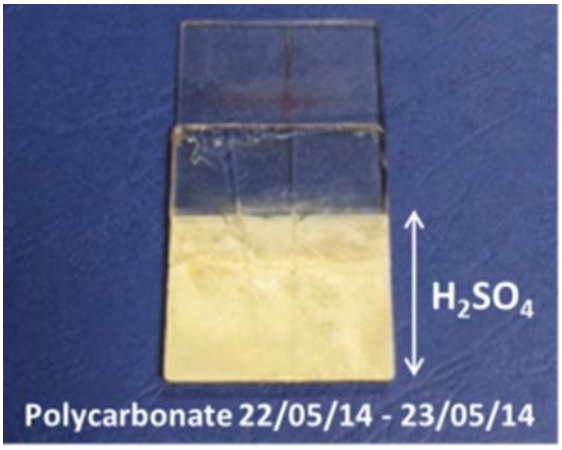

Figure.SIA.5.d.
Polycarbonate (PC), a commonly used material for the production of microfluidics chips, showing severe effect of exposure for one week to 98% sulphuric acid.

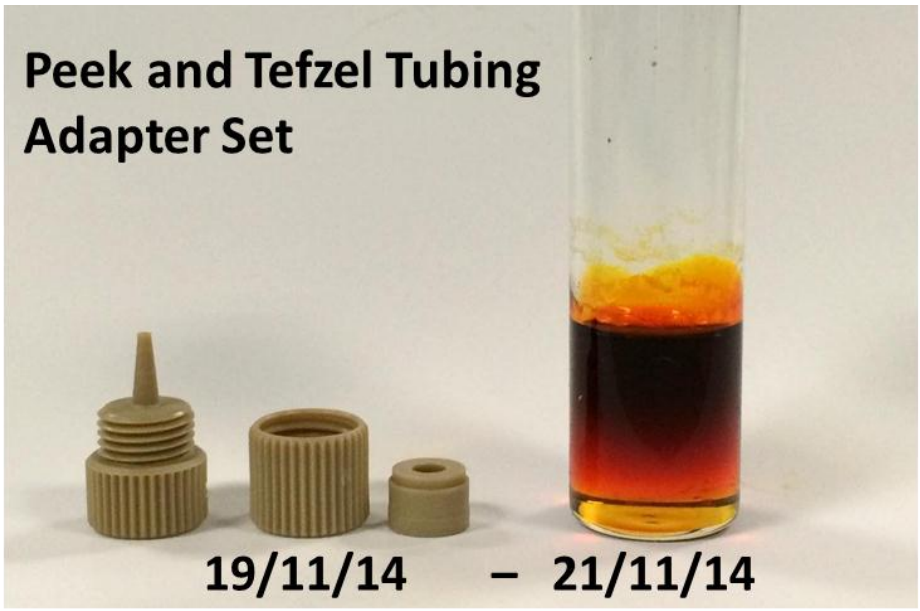

Figure SIA.5.e.
Polyether ether ketone (PEEK) and Tefzel tubing connectors for peristaltic pumps commonly used in flow analysis systems: new (left) and completely dissolved after exposure for three days to 98% sulphuric acid (right).

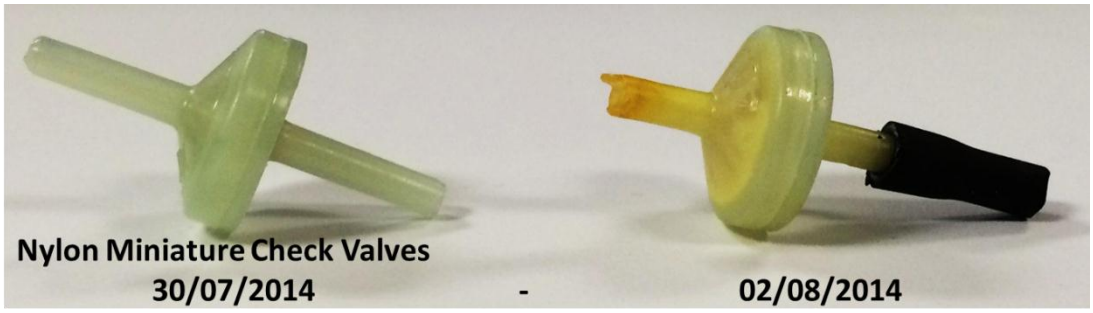

Figure SIA.5.f.
New nylon miniature check valve with fluorosilicone diaphragm (left), and a similar valve after being used in nitrate-chromotropic acid flow analysis system for a period of one week.

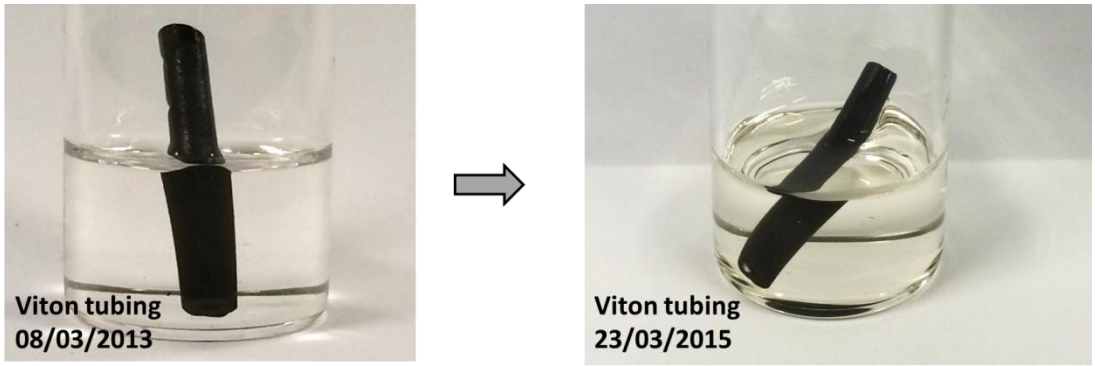

Figure SIA.5.g.
Viton tubing exposed for 2 years to 98% sulphuric acid showing no signs of discolouration of degradation.

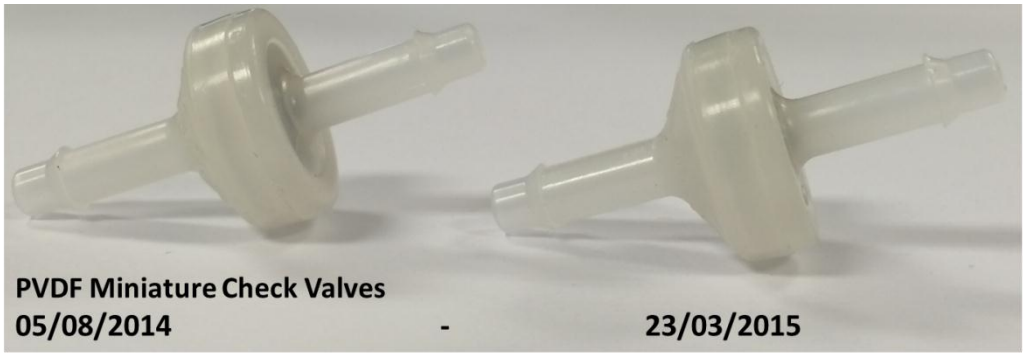

Figure SIA.5.h.
PVDF check valves with Viton diaphragm used in nitrate-chromotropic acid flow analysis system after a period of 9 months to 98% sulphuric acid showing no signs of discolouration of degradation.

Video SIA.1.
Real-time response (3 min) of PEEK (commonly used in flow analysis platforms as tubing and tubing connectors) to 98% sulphuric acid demonstrating the immediate discolouration and degradation of PEEK on contact with the acid.

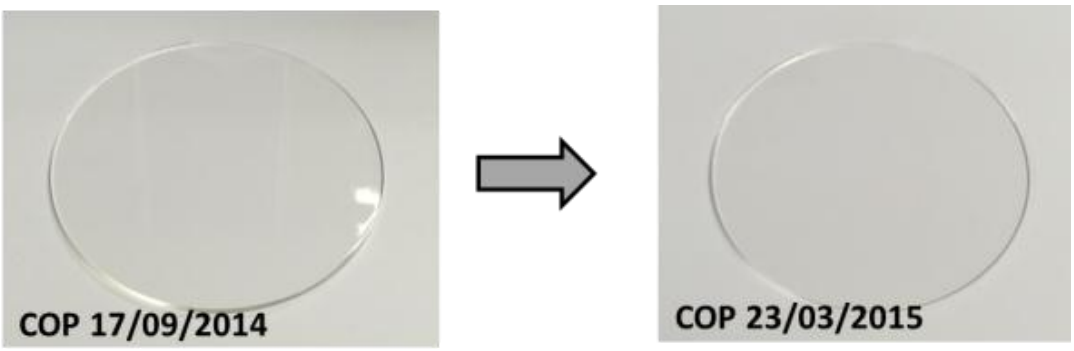

Figure SIA.6.
COP (Zeonex mcs- COP- 04) discs before (left) and after (right) immersion in 98% sulphuric acid (8 months) showing excellent chemical resistance to the highly concentrated acid.

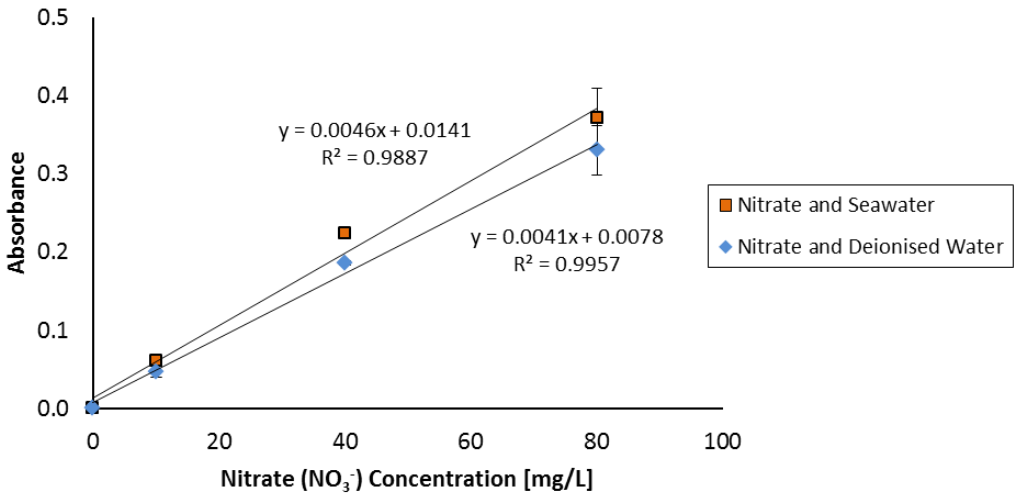

Figure SIA.7.
Calibration curves using microfluidic nitrate analyser, using nitrate standards ranging from 0 – 80 mg/L nitrate with the chromotropic acid reagent using both deionised water and seawater matrices. The error bars represent the standard deviations for $n = 3$ replicates.

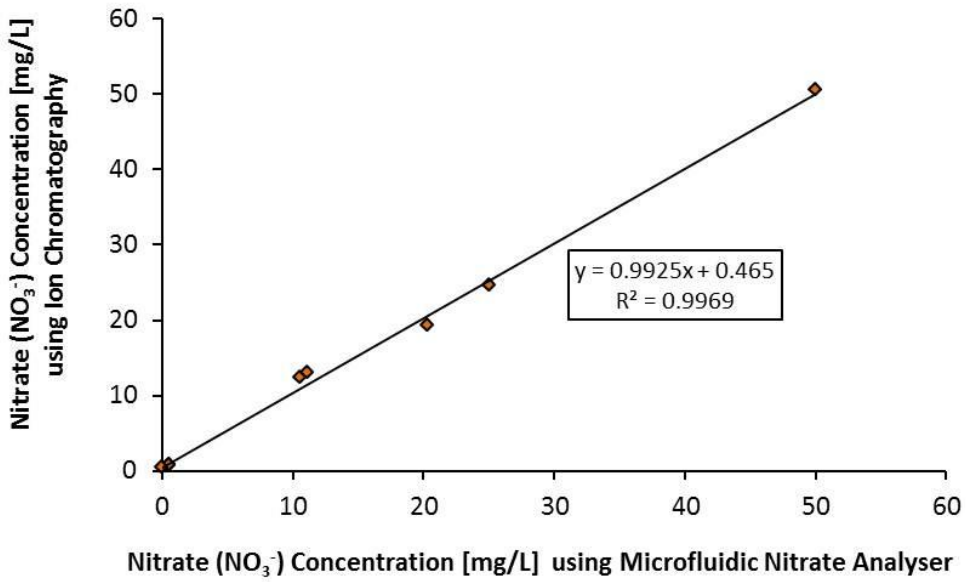

Figure SIA.8.
Correlation plot of sample concentrations obtained using the microfluidic nitrate analyser and ion chromatography.

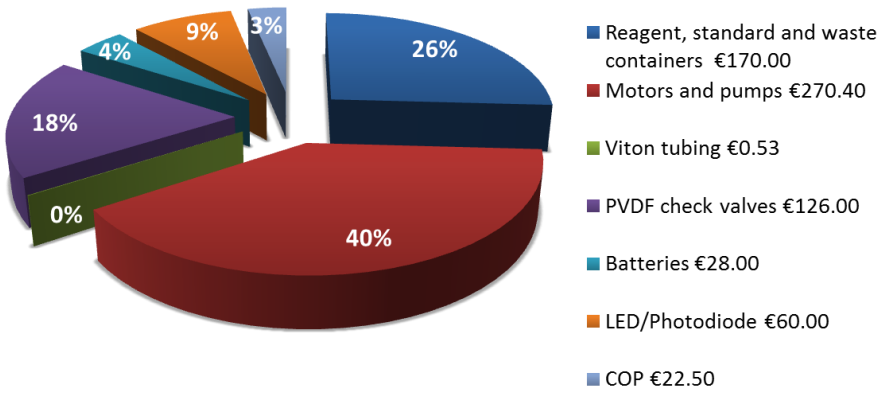

Figure SIA.9.
Cost breakdown of nitrate sensor components.