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

Deirdre Cogan[a], Cormac Fay[a], Aoife Rickard[a], John Cleary[a,*], Thomas Phelan[a], Kamil Jankowski[a], Mark Bowkett[b] and Dermot Diamond[a]

[a]Insight, Centre for Data Analytics, National Centre for Sensor Research, Dublin City University, Dublin, Ireland [b]T.E. Laboratories, Tullow, Carlow, Ireland

*Corresponding author at: Insight, Centre for Data Analytics, National Centre for Sensor Research, Dublin City University, Glasnevin, Dublin 9, Ireland. Tel.: +353 1 700 6009. *E-mail address:* john.cleary@dcu.ie

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

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 $\leq$ 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 1. In the presence of hypochlorite, ammonia forms a monochloramine (Eq. 1), which is converted to 5-aminosalicylate by the salicylate (Eq. 2). This is oxidised and complexes with salicylate to produce the highly conjugated blue indophenol complex (Eq. 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.

$$NH_3 + OCl^- \longrightarrow NH_2Cl + OH^- \qquad \text{Eq. 1}$$
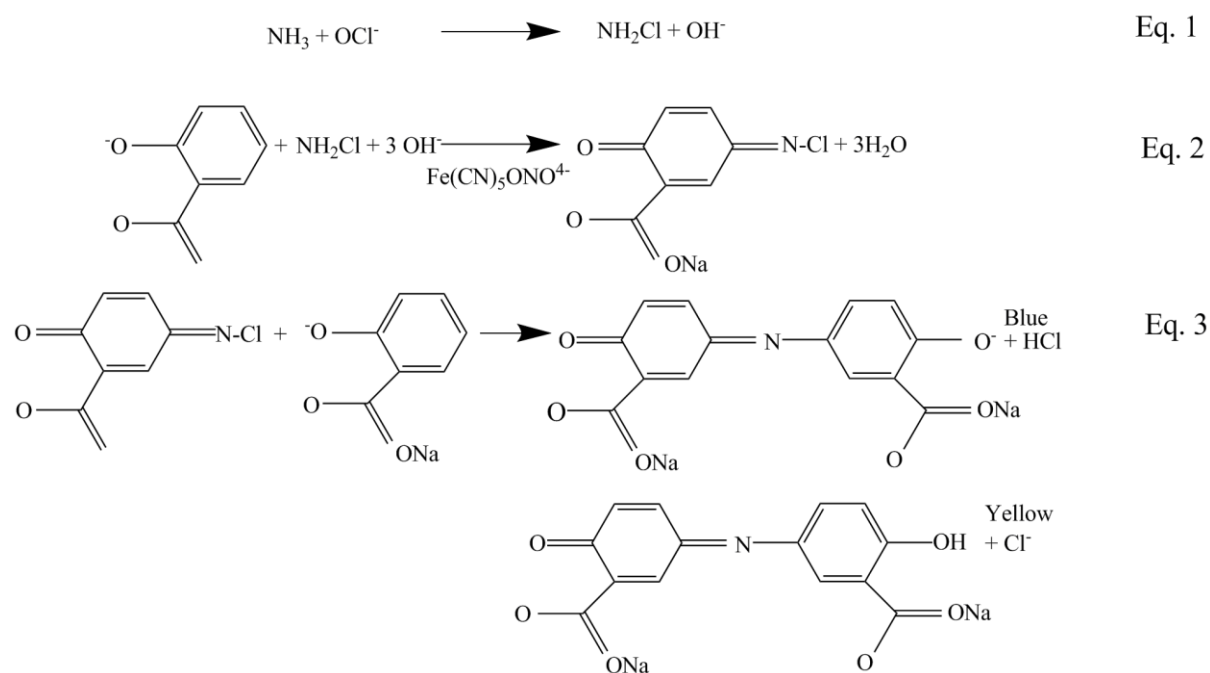

Eq. 2

Eq. 3

Figure 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

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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.
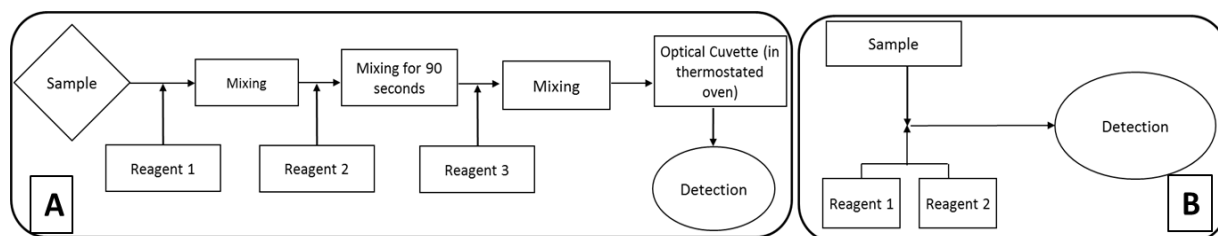

Figure 2. (A) Traditional Berthelot method incorporating a multistage process for generating the coloured complex; (B) Modified Berthelot method incorporating a reagent mixing stage prior to sample introduction at a 1:1 v/v reagent to sample ratio.

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 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.

2. Experimental and fabrication of automated ammonia analyser and optical detection system
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.

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.

2.3 Instrumentation design and measurement procedure

The ammonia analyser shown in figure 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.
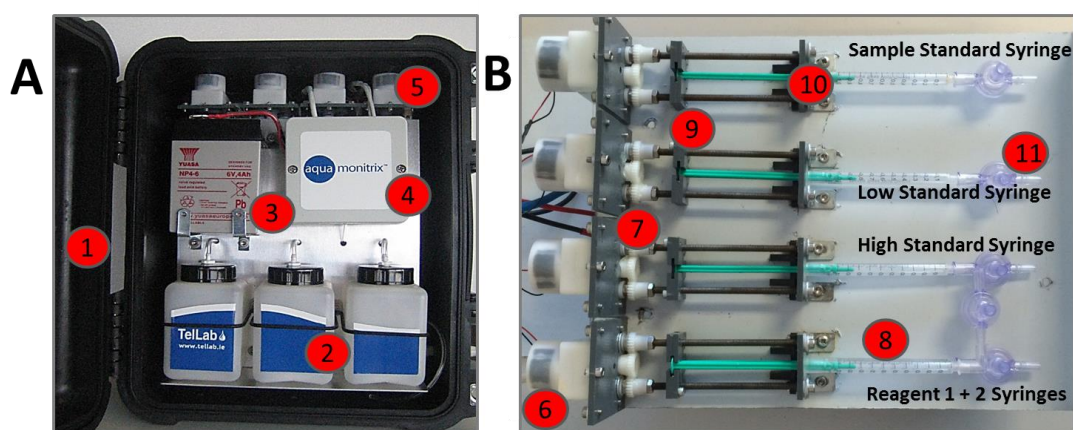

Figure 3. (A) Integrated ammonia analyser; [1] Waterproof housing [2] reagent and standard containers [3] 6V battery [4] optical detector [5] syringe pump array. (B) The syringe pump array for fluid control; [6] motors for syringe pumps [7] gears [8] syringe [9] syringe holder [10] proximity sensor [11]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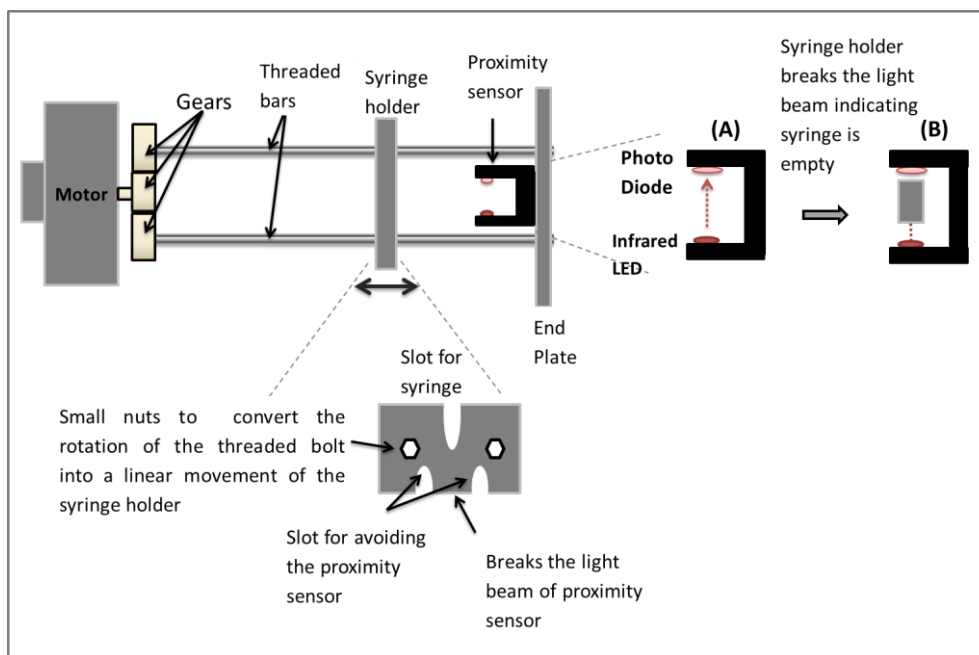

Figure 4. Schematic of syringe pump.

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 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).
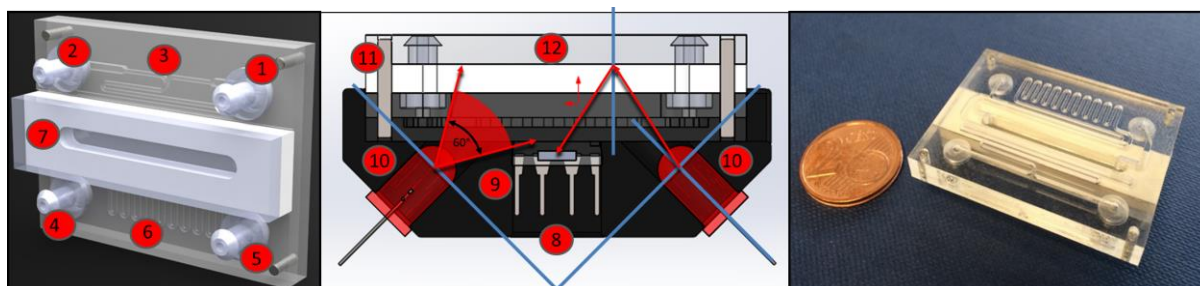

Figure 5. Microfluidic chip (40 x 30 mm) with reagent and sample mixing channels and detector channel with mirror (28 x 3 mm). [1] Reagent 1 inlet [2] Reagent 2 inlet [3] Reagent mixing channel [4] Sample inlet [5] Waste Outlet [6] Sample and reagent mixing channel [7] Detection channel with mirror [8] 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 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 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.

3. Results and Discussion

3.1 Optimisation of 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.
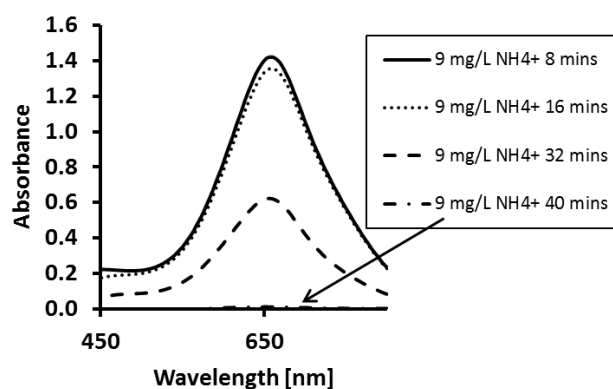

Figure 6. Mixed reagent stability over time.

As shown in Figure 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 (R.S.D) (n = 3) of 0.5%. The method gave a linear response to ammonia concentrations up to 12 mg L^{-1} 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.

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:

$$T = a\,(1 - e^{-kt}) + b \hspace{5cm} \textit{(Equation 1)}$$

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 NH4+ average RSD= 7.19%, 6 mg/L NH4+ average RSD= 3.92% and 9mg/L NH4+ average RSD= 3.29%) are shown in figure 7 with the corresponding rate constants shown in table 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.
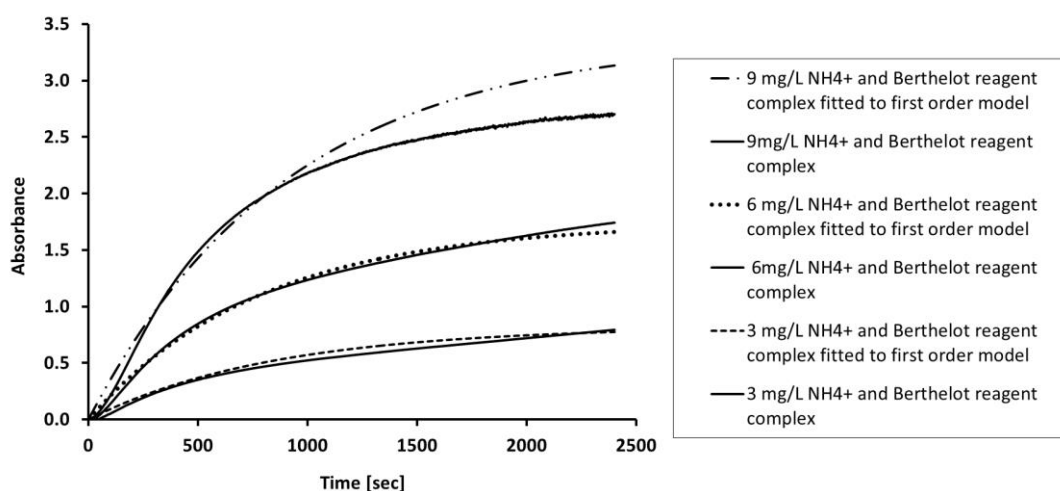

Figure 7. Kinetic study of the Berthelot- ammonia complex formation using standards ranging from 3 – 9 mg/L NH_4^+ with corresponding first order model fits (23.0 °C).

Table 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

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 2:

$$y_{dl} = y_{blank} + 3s \qquad\qquad (Equation\ 2)$$

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 3:

$$Detection\ limit = 3s/m \qquad\qquad (Equation\ 3)$$

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 8 illustrates the signal obtained from the reagent blank and the minimum detectable signal and also signals from sample concentrations and reagent.
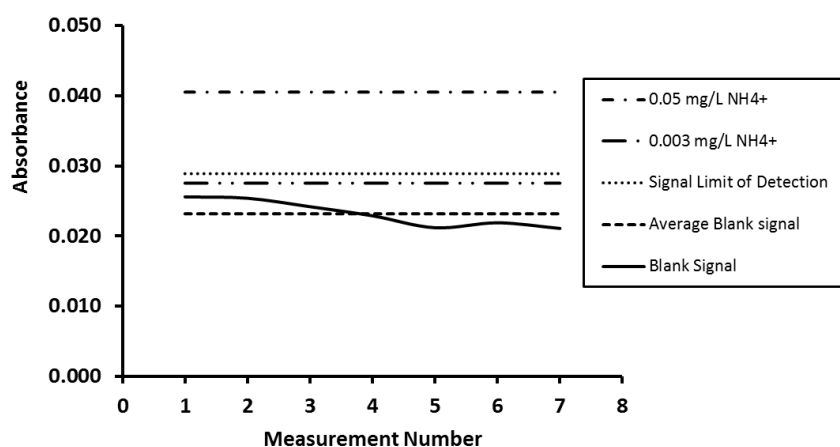
Figure 8. Replicate absorbance measurements on blank.

3.4 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 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 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 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 form 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 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)	0.0340	0.0017	0
Open cap	0.0293	0.0018	13.8235
Container with ammonia source (open cap)	0.0362	0.0020	6.3725

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.

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.

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. 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 x 10^{-04}	1.00
Std. 0.5 mg/L	0.48	1.20 x 10^{-03}	0.46
Effluent	0.31	5.66 x 10^{-04}	0.33
Effluent	0.02	7.07 x 10^{-05}	0.02
River water	0.01	0.00	0.01
Effluent	0.09	1.06 x10^{-03}	0.09
River water	0.17	2.83 x10^{-03}	0.13

3.6 Integration of chemistry into autonomous sensing platform

The modified Berthelot reagents and a range of standards were prepared as described in section 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 3 with a correlation coefficient value of 0.9983 and an average relative standard deviation (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. 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.

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6. References

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