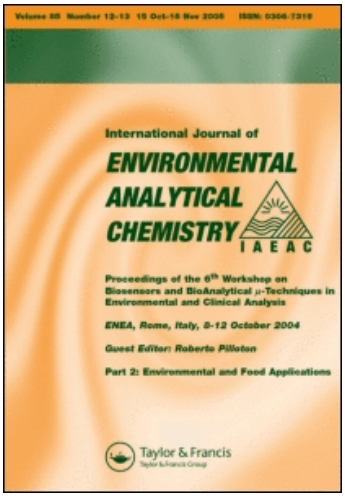

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Miniaturized flow-injection-analysis (μFIA) system with on-line chemiluminescence detection based on the luminol-hypochlorite reaction for the determination of ammonium in river water

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A miniaturized flow-injection-analysis system constructed from glass and polydimethylsiloxane was employed for the determination of ammonium in river water. The sample was filtered and delivered to the reactor chip electro-osmotically using a disposable fritted capillary, while reagents were delivered to the system by gravity. Ammonia was mixed with the hypochlorite, to form a monochloramine. Once the alkaline luminol (3-aminophthalhydrazide) was delivered to the system, it was oxidized by the unconsumed hypochlorite emitting a bright blue light ($\lambda_{max} \sim 440$ nm) that was detected using a miniaturized photomultiplier tube (PMT) located directly under the chip. The calibration model for ammonium standards was linear up to 0.1 μg mL^{-1} ($y = -8.96x + 1.02$; correlation coefficient, $r^2 = 0.9715$) over a working range of 0.0–0.5 μg mL^{-1}. A detection limit of 10 ± 6 μg mL^{-1} was achieved with a precision value of (RSD $\leq$ 6.4%), for $n = 5$. A direct and standard addition method were used to determine the concentration of ammonium in a river-water sample (from the Humber Estuary, UK) which was found to be 0.075 ± 0.005 μg mL^{-1}, with a precision value of (RSD $\leq$ 3.7%), for $n = 9$. The results obtained showed good agreement with the average concentration 0.065 μg mL^{-1} (provided by the local environmental agency), for the analysis of ammonia at different sample points on the estuary.

Keywords: μFIA system; Chemiluminescence; Luminol-hypochlorite reaction; Ammonium; Electro-osmotic pumping and filtration device; River water

1. Introduction

The significant role of ammonium in the nutrition chain of aquatic microorganisms explains its great importance in studies involving biology and ecology [1–4]. Ammonia can be found at various levels in wastewaters generated from industrial and agricultural activities (e.g. fertilizers, production of nitric acid, urea, and nitrogen compounds). Since ammonia can also be produced from the biodegradation of organic nitrogen compounds like urea and protein, it can also be found in domestic wastewater (e.g. in sewage or agricultural wastes) [5]. Aquatic life and fish also contribute to ammonia

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levels in streams. The toxicity of ammonia is attributed mainly to its principal form (NH_3), which has been reported to be toxic to freshwater organisms in concentrations in the threshold range 0.53–22.8 $\mu g\,mL^{-1}$. Here, toxicity is both pH- and temperature-dependent. Lower pH and temperature values enhance the toxic effect of ammonia. A seasonal chronic exposure limit of non-ionized ammonia has been established in the United States. Exposure limits of 0.05 $\mu g\,mL^{-1}$ for April through October, and 0.025 $\mu g\,mL^{-1}$ for November through March have been determined as the safest concentration limits for the protection of aquatic organisms [6]. Plants are more tolerant of ammonia than animals, while invertebrates are more tolerant than fish. Hatching and growth rates of fishes may be affected, and during development, changes in tissues of gills, liver, and kidneys may also occur. In humans, toxic levels of ammonia cause loss of equilibrium, convulsions, coma, and death [6]. Due to its adverse effect on the oxygen balance in the aquatic environment, ammonia has been classified as a list II substance under the European Community Dangerous Substances in Water Directive [7], and the discharge of wastewaters containing ammonia has been brought under legislative control [5].

Investigations carried out by the water industry on the environmental impact of ammonia have led to the requirement for new techniques capable of analysing large number of samples for ammonia. Among many techniques used, flow-injection analysis (FIA) has proved to be sufficient not only because of its well-known advantages in terms of rapid analysis, robustness, minimal consumption of reagents and analytes, and low operational costs, but also for its potential for on-line monitoring [5, 8–12]. Many FIA methods for the analysis of ammonia with a variety of detection process have already been reported [13–23].

Among many FIA procedures, those employing UV-Vis spectrophotometric detection are the most frequently used due to their high throughput capability [24], a feature that is required when many samples need to be processed. Andrew *et al.* [5] for instance developed a portable flow-injection monitor for the on-line analysis of ammonia in industrial effluents. The method exploited the diffusion of gaseous ammonia from an alkaline sample stream, through a microporous membrane, into a bromothymol blue indicator stream. The colour change was measured spectrophotometrically with a red LED lamp being used as the light source. A linear response in the range 1–100 $\mu g\,mL^{-1}$ NH_3–N was achieved. Icardo *et al.* [25] proposed a flow-spectrophotometric procedure for the determination of ammonium ion. In this method, the sample, in basic media, was forced through a solid-phase reactor containing immobilized AgCl on polymeric beads. The released Ag^+, complexed with the ammonia, gave a blue ternary complex with the aid of bromopyrogallol red and 1,10-phenanthroline, which was monitored spectrometrically at 636 nm. The limit of detection achieved was 0.35 $\mu g\,mL^{-1}$, and a relative standard deviation of 1.9% ($n = 7$) was obtained. Tsuboi *et al.* [26] developed a sensitive flow-injection spectrophotometric method for the determination of ammonia in samples taken from the exhaust gas of thermal power plant. The ammonia in the exhaust gas was absorbed into a boric acid solution prior to its analysis. The method was based on an indophenol blue reaction with salicylate and hypochlorite in the presence of manganese (II) as a reaction promotion catalyst, and the limit of detection achieved was 5 $\mu g\,mL^{-1}$. Fernandes *et al.* [4] reported a flow system for the simultaneous determination of ammonium and phosphate in river water at the $\mu g\,mL^{-1}$ level which employed a low-cost LED-based photometer. For the determination of ammonium, the method was based on the reaction of hypochlorite and

salicylic acid, and the measurement wavelength used was 660 nm. A high sample throughput of 112 samples per hour was achieved, with a relative standard deviation of 1.1% ($n = 6$). The method was highly sensitive for ammonium giving a detection limit of 7 $\mu g\,mL^{-1}$.

FIA systems with chemiluminescence (CL) detection have proved to be useful analytical methods not only for metal ions but also for important chemical species like ammonia. The major advantages that FIA–CL methods offer are a high sensitivity, wide determination range, inexpensive reagent and apparatus, easy and rapid measurements, robustness, portability, low contamination risk, and redox speciation capability [27, 28]. Li *et al*. [29] used a liquid core waveguide (LCW)-based CL detector for the determination of ammonium with electrogenerated hypochlorite based on the luminol-hypochlorite reaction. The fast CL reaction of luminol with hypochlorite was used to test the performance of the detection arrangement. A detection limit of 120–260 nM NH_4^+ was achieved with an inexpensive PMT detector and a constant current source.

The emergence of micro-machining techniques in combination with processes such as electro-osmotic pumping has led to a new generation of miniaturized flow injection analysis (μFIA) utilizing lab on a chip technology [30–38]. These systems allow greater portability, have lower energy requirements, and use very small volumes of reagents (μL), thus producing less waste. The aim of this work was to develop a prototype μFIA for the determination of ammonia in river water to show the feasibility of using this approach for water analysis. The system reported is constructed from glass and polydimethylsiloxane (PDMS) and utlilizes the luminol-hypochlorite reaction to give highly sensitive detection by chemiluminescence. A novel introduction system is described consisting of a disposable microporous silica frit, to which an electrical field is applied, and in this mode the system acts as both a filter and pumping device.

2. Experimental

2.1 *Reagents*

All the reagents and standards were of analytical grade unless otherwise stated, and all dilutions were made with ultra-high-purity (UHP) deionized water (18 $M\Omega\,cm^{-1}$ resistivity).

A 0.1 M Na_2CO_3 buffer solution was prepared by dissolving 5.3 g of sodium carbonate (Na_2CO_3 99%, supplied by Riedel-deHaën, Germany) in 500 mL of UHP water, the pH of the solution was adjusted to 10.50 by the addition of 0.1 M sodium hydrogen carbonate (prepared from dissolving 4.2 g of $NaHCO_3$, supplied by Fisher Scientific Co., Loughborough, UK, in 500 mL of UHP water), and the final dilution was made to 1 L. The solution was then stored at 4°C in a polyethylene bottle.

A 0.01 M solution of Luminol (3-aminophthalhydrazide supplied by Fluka Chemie GmbH, Buchs, Switzerland) was prepared by dissolving 0.177 g of luminol in 100 mL of carbonate buffer, pH 10.50, followed by sonicating for 30 min. The solution was kept refrigerated for 24 h prior to use in order to obtain a maximum and stable sensitivity for an optimum CL reaction. Luminol solutions are stable for at least 1 month [39].

A 1.0% (v/v) of sodium hypochlorite in carbonate buffer solution (pH 10.50) containing 0.5 M of KCl as an electrolyte solution was prepared by dissolving 3.728 g of KCl (supplied by Fisher Scientific Co., Loughborough, UK) in 8.33 mL of sodium hypochlorite solution 12% (w/v), (supplied by BDH, Poole, UK), and the total volume was made up to 100 mL by using a carbonate buffer at pH 10.50. The solution was stored at 4°C in a polyethylene bottle. This solution was prepared on a weekly basis.

A 1000 $\mu g\,mL^{-1}$ stock solution of ammonia was prepared by diluting 0.49 mL of ammonium standard solution 22% (w/v) (supplied by ROMIL, Cambridge, UK), to 100 mL with UHP water. Serial dilutions of the ammonium stock solution were then carried out in the range 0.025–0.50 $\mu g\,mL^{-1}$. These solutions were stored at 4°C in polyethylene bottles.

A real sample was collected from the Humber Estuary (Hull, UK) in polyethylene bottles. The sample was filtered through a Whatman filter article (No. 541) to remove course sediment and then stored in the refrigerator at 4°C.

2.2 *Preparation of electro-osmotic pumping device*

To construct the electro-osmotic pumping device, a microporous silica frit was fabricated following a procedure reported by Wilson *et al.* [40] with slight modification to the composition. These were designed to be disposable and easily replaced in the system. A 100 µL aliquot of formamide (98%, Avocado Research Chemicals Ltd, Heysham, Lancs, UK) was mixed thoroughly with 400 µL of distilled water and a 700 µL aliquot of potassium silicate (21% SiO_2, 9% K_2O, Prolabo, Manchester, UK). The mixture was driven into a 10-cm capillary tube of 1.5 mm i.d. by means of a peristaltic pump set at the minimum pump speed (0.25 $\mu L\,min^{-1}$). Thorough mixing was required prior to loading the capillary tubes in order to ensure a homogenous mixture. The tubes were closed at one end and allowed to stand vertically in an oven set 60°C for 1 h. After cooling, the tubes were cut into 2-cm-long pieces and washed sequentially with phosphate buffer solution (0.1 M, pH 5), UHP water, 10% (v/v) of hot HNO_3 solution (AR grade) and finally with UHP water. Washing the frit was necessary to remove any unreacted reagent that could block the frit after rehydration [41]. The washed frits were then returned to an oven at 80°C and left overnight. The fritted capillaries were then stored in a desiccator to maintain their dryness until required.

2.3 *Construction of the µFIA system*

The schematic diagram of the system can be seen in figure 1. The chip was fabricated according to a previously reported methodology [42] with the channel network etched into a glass base plate and the reservoirs located in a removable PDMS top plate. The glass base plate (Photonics, UK) was coated with a layer of chromium and photoresist. This was photolithographically patterned then etched in 1% hydrofluoric acid/ammonium fluoride for 10 min at 60°C. The glass chip consisted of two intersecting channels forming a T-pattern, the dimension of the channels being 20 mm long × 200 µm wide × 50 µm deep.

The positions of the reservoirs are labelled A–D. The top plate was made from PDMS (ISL, West Midlands, UK), which was prepared according to instructions, vacuum-degassed, and then moulded with the reservoirs in place. Reservoirs C and D

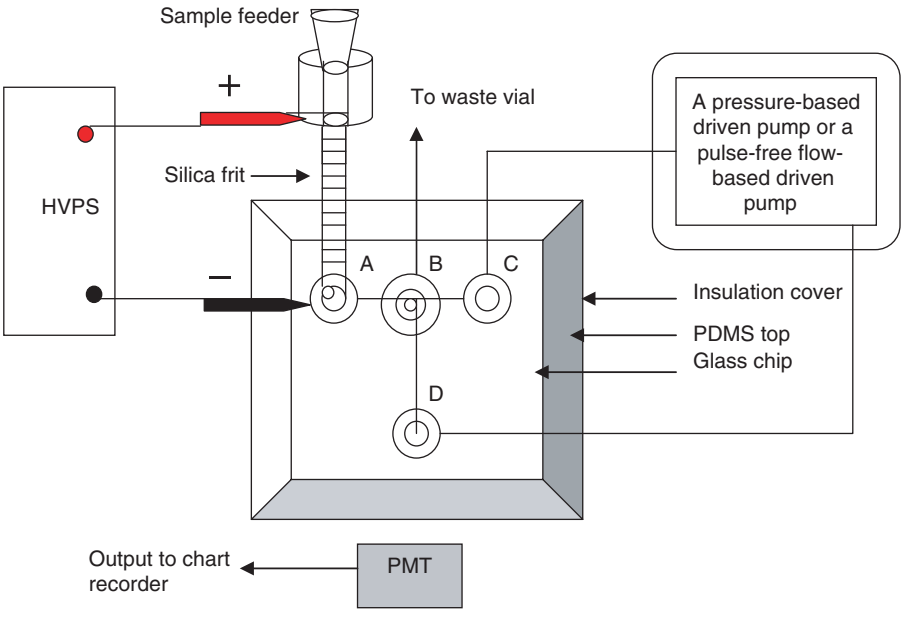

Figure 1. A schematic diagram of the μFIA system with on-line CL detection based on the luminol–hypochlorite reaction for the determination of ammonia in river water samples. The bottom plate of the chip was made from borosilicate glass with three equal channels forming a T shaped pattern. The channels had dimensions of 20 mm long $\times$ 200 µm wide and 50 µm deep and they were sealed by a PDMS top plate. The reservoirs were labelled A–D. Reservoirs C and D were used for the delivery of reagents to the reactor chip. Reservoir A held the frit unit firmly with the platinum electrodes facing directly the frit ends. Reservoir B resembled reservoir A in size, and functioned as a reaction well to drain the μFIA system of waste solution by directing it to the waste vial.

were used to introduce the reagents and had a diameter of 1.5 mm to permit PTFE tubing and a miniature stop-flow valve to be connected, thus allowing the reagent mixture to be delivered to the system by gravity. The sample was introduced through reservoir A, the top half of which had a diameter of 1.5 mm. This allowed the fritted capillary to be secured in the reservoir with a platinum electrode (cathode) positioned so that it directly faced the lower end of the fritted capillary. The lower part of reservoir A was 0.5 mm in diameter to reduce the dead volume after the frit. The anode (a platinum electrode) was located at the upper face of the fritted capillary. To obtain electro-osmotic pumping, the selected voltage was applied across the electrodes, and the current was monitored on an AVO digital autoscaling multimeter (Thurnby Thunder Instruments, Huntington, UK), which was connected in parallel with the device.

Reservoir B (0.5 mm) was where the CL reaction occurred, and so this was directly above the photomultipler tube. Waste sample and reagents were then removed from the top of reservoir B via a 0.5-mm-diameter draining channel moulded in the sidewall of the reservoir. The top of the reservoir was open to allow produced gases to escape.

Prior to using the electro-osmotic flow system, a syringe pump (Bioanalytical Systems Inc., model MD-1001, West Lafayette, IN) was used as a pulse-free flow delivery system for both reagents and analytes to optimize the CL detection system. PTFE tubing was used to link the syringes A, C, and D to their correspondence reservoirs. The two halves of the microreactor were held together by a simple perspex clamp with four corner bolts

to apply pressure. Thin rubber gaskets were cut from a suba-seal and inserted between the reservoirs inlets and the perspex clamp in order to prevent any possible seeping from reagents and/or analyte to the system top that could result from the PDMS expansion. The miniaturized system could easily be disassembled for cleaning whenever required.

A miniaturized photomultiplier tube (model H5784 Hamamatsu Photonics, Enfield, UK) contained in a custom built, light tight, insulation box and linked to a dual power supply (RS Components, Northants, UK) was located under reservoir B such that it was exposed to the light emitted from the chemiluminescence reaction. The edges of the chip were covered with black insulation tape to minimize light scattering, and all experiments were conducted in a dark room. The analogue output from the PMT detector was connected to a chart recorder (Chessel BD40-04, Kipp & Zonen, Netherlands).

3. Results and discussion

3.1 *Luminol–hypochlorite reaction for the chemiluminescence detection of ammonium in aqueous samples*

The CL reaction used to determine the ammonia was based on the luminol–hypochlorite reaction described by Fernandez [4]. In this reaction, the ammonium and hypochlorite react in alkaline media ($CaCO_3$ buffer pH 10.50) to form the monochloramine as shown in equation (1) below. This reaction is a first-order reaction in both ammonia and sodium hypochlorite:

$$NH_3 + OCl^- \rightarrow NH_2Cl + OH^-. \quad (1)$$

The measurement of ammonia is therefore indirect, as the CL signal decreases as the ammonia reacts with hypochlorite reagent present [17, 29, 43, 44]. The rate of monochloramine decomposition decreases as the pH increases. Below pH 10.50, monochloramine decomposition becomes extensive; above pH 11.50, the rate of monochloramine formation becomes very slow unless there is a large excess of hypochlorite relative to the ammonia concentration, according to Patton and Crouch [45]. For this reason, all the experiments were carried out at pH 10.50 [17]. It is important to avoid dilute solutions of hypochlorite because of their instability. The CL reaction of luminol with hypochlorite is shown in figure 2. In the first step, the luminol is converted by the basic carbonate buffer solution (pH 10.50) to a resonance-stabilized dianion (a deprotonated enol tautomere of luminol) denoted '1', which is then oxidized by the oxygen generated from the hydrolysis of sodium hypochlorite (prepared with carbonate, pH 10.50, buffered KCl), to form the dicarboxylate ion (unstable endoperoxide). The endoperoxide will split to form N_2 and an electronically excited aminophthalate denoted '2', which loses its energy by emitting a bright blue light, $\lambda_{max} \sim 440$ nm, as it returns to the ground state denoted '3' [17].

It is well known that halide ions enhance CL reactions [46], and from figure 2 we can infer that in the absence of ammonia, the Cl^- ions will enhance the CL efficiency. There are two pathways for the reaction, the major being non-chemiluminescent, this is why the luminol reaction has a low quantum yield. It is thought that the halide ions increase the CL efficiency by increasing the rate constant for the CL pathway. The key

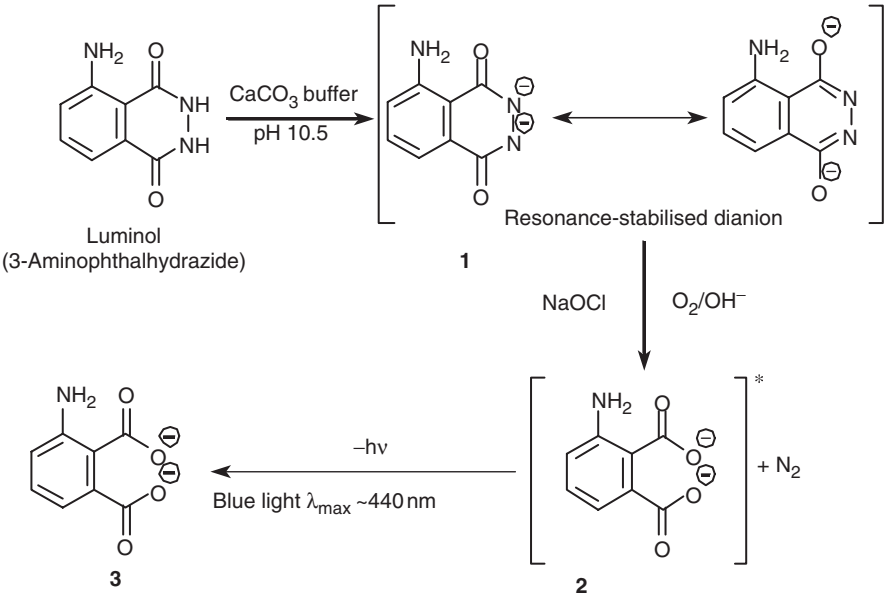

Figure 2. A proposed scheme for the luminol–hypochlorite reaction [17].

step for this mechanism is catalytic cleavage of an activated molecular complex, which produces more excited aminophthalate ions. According to Chang and Patterson [46], therefore, more excited aminophthalate ions are produced per unit time with halide ions, thus enhancing the CL signal. They proved their case by showing that the CL spectra was the same for the catalysed reaction in the presence and absence of halide ions, indicating that the same emitting species, aminophthalate ions, were being produced [47]. Once ammonia is introduced into the reaction mixture, the chloride ions (Cl^-) will be consumed (equation (1)), reducing the background signal generated from the luminol–hypochlorite reaction [29]. In order to maintain a stable and constant CL signal over many hours, avoiding the rapid decrease in hypochlorite concentration to zero level with time, it is therefore necessary to generate the hypochlorite from alkaline carbonate buffer media (pH 10.50) containing 0.5 M of KCl (electrolyte), thus allowing a gradual decrease in the hypochlorite concentration [29].

3.2 *A pulse-free flow based delivery system for reagents and analytes*

3.2.1 Optimization tests for the CL detection of ammonium in river water using a syringe pump as a pulse-free flow-delivery system. A syringe pump was used to deliver both the reagents (luminol and sodium hypochlorite) and the analyte (ammonium standard solutions) to the chip (see figure 1) to conduct preliminary optimization experiments. A concentration range of 0.00–0.50 $\mu g\,mL^{-1}$ ammonium standard solutions were prepared and loaded into syringe (A) while both sodium hypochlorite and luminol were loaded into syringe C and D with the pump flow rate set at 20 $\mu L\,min^{-1}$. To ensure that the ammonium solution consumes the hypochlorite prior to reaction with the luminol, the syringe pump was set up to allow the ammonium standard solutions and the

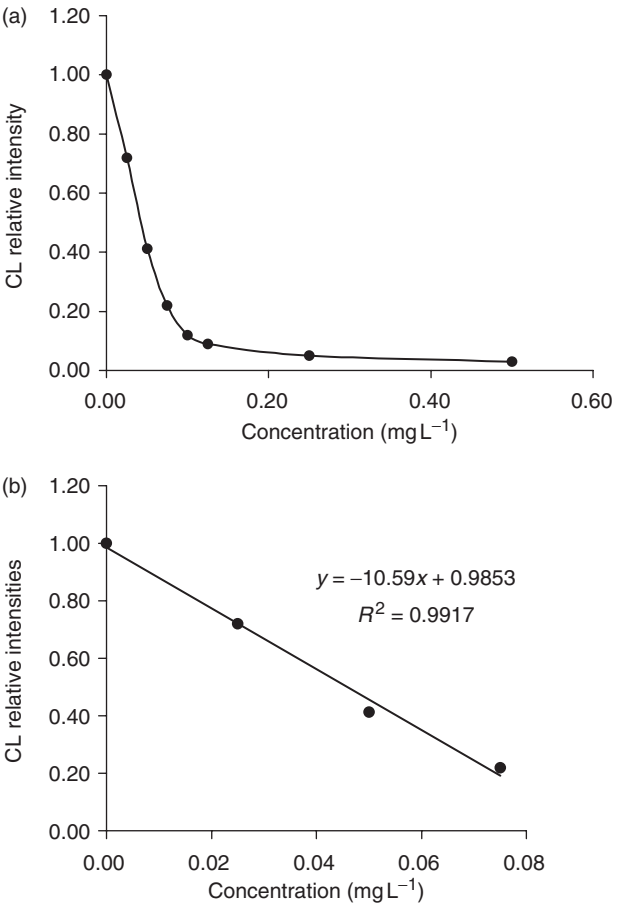

Figure 3. (a) The standard calibration plots for the CL detection of ammonium using μFIA system with a syringe pump as a pulse-free flow based driven system. The detection was measured under the channel. (b) The linear part of the standard calibration plots for the CL detection of ammonium using μFIA system with a syringe pump as a pulse-free flow based driven system. The detection was measured under the channel.

hypochlorite to be delivered to B first. Once the ammonium standard solutions and the hypochlorite were mixed inside the channel, the luminol was allowed to flow to the reactor chip via reservoir D. As the mixture of the reagents and standard solutions made their way to exit the system via reservoir B, a short-lived bright blue light was produced (0.8 s). The CL signal obtained was noisy, and the reason behind this was in part the formation of nitrogen bubbles that occurred during the reaction [36], but the enhancement effect of chloride ion (Cl^-) on the CL signal produced may also have contributed [39]. A washing step with UHP water and carbonate buffer, pH 10.50, was carried out between measurements to prevent carryover.

Figure 3(a) shows the calibration plot for the relative CL intensity values *versus* the ammonium concentrations in $\mu g\,mL^{-1}$ between 0 and $0.5\,\mu g\,mL^{-1}$. The plot was found to be linear up to $0.075\,\mu g\,mL^{-1}$, as shown in figure 3(b), with a correlation coefficient of 0.9917 for the equation $y = -10.59x + 0.9853$. The detection limit achieved

was 4.5 μg mL^{-1} with a% RSD of less than 7.4 for $n = 5$. From this preliminary work using the syringe pump, it has been demonstrated that that it is possible to use a μFIA system with CL detection to quantify ammonia.

3.2.2 Determination of ammonium in a river-water sample. A real sample was collected from the Humber Estuary in polyethylene bottles and treated as discussed in section 2.1. The results obtained using the calibration shown in figure 3b found that the concentration of ammonium in the estuarine water was 0.067 μg mL^{-1} with a precision (3.7%) RSD for $n = 10$. Although the sample had been filtered to remove course particulates the presence of colloidal particles in the river water sample could cause blocking of the μFIA system. The inclusion of the disposable capillary frit into the system in which EOF pumping (which would create negligible backpressure) was utilized for sample introduction would help overcome this problem.

3.3 *A pulse-free flow-based delivery system for reagents and analytes*

3.3.1 Use of electro-osmotic flow and gravity as a pulse-free flow delivery system for the μFIA CL detection of ammonium. The μFIA system was constructed as stated in section 2.3, and 3.2.1, but the aim of this work was to develop a low-cost system that would have potential for portability. To reach this aim, the syringe pumps were replaced by gravity feed systems for the reagents, and the sample was introduced using EOF via the novel fritted caplliary system. To achieve this, platinum electrodes were carefully inserted at the respective ends of the frit unit in reservoir A, an electrical field of 300 V was applied, and a typical flow rate of 5 μL min^{-1} was obtained.

Both sodium hypochlorite and luminol were delivered to the reactor chip by gravity via reservoirs C and D, respectively. The reagent reservoirs were set at a height of 50 cm to obtain a flow rate of 20 μL min^{-1} for the reagent. Both ammonium standard solutions and sodium hypochlorite in carbonate (pH 10.50) buffered KCl solution were allowed to flow to the system first, so the sodium hypochlorite could be consumed by ammonia as described previously. Ammonium standard solutions over an appropriate calibration range were prepared freshly (0.00–0.50 μg mL^{-1}) and loaded into the sample feeder consecutively, while the reagents (sodium hypochlorite and luminol) were loaded separately into their corresponding reservoirs. A washing step was included between each sample to prevent carryover. Figure 4(a) shows the calibration plot of relative CL intensity values *versus* ammonium concentrations in the range 0.0–0.5 μg mL^{-1}. The plot was found to be linear up to 0.1 μg mL^{-1}, as shown in figure 4(b), with a correlation coefficient of 0.9715 for the equation $y = -8.96x + 1.02$. The limit of detection was evaluated using the blank + $3S_{y/x}$ (the error calculated in the y direction) as defined by Miller and Miller [48] and was found to be 0.016 μg mL^{-1} with a precision of ($\leq$6.4%) RSD for $n = 5$ for the concentration range (0.0–0.5 μg mL^{-1}). Each sample run was implemented in 1.5 min, after washing and priming reactor chip with UHP water and carbonate buffer pH 10.50 consecutively.

This particular study was focused on the design and fabrication of μFIA system for the determination of ammonia in an estuarine water sample, and so the effects of any interfering species must be considered. The mechanism of the luminol–hypochlorite reaction (discussed in section 3.1) relies on the consumption of hypochlorite

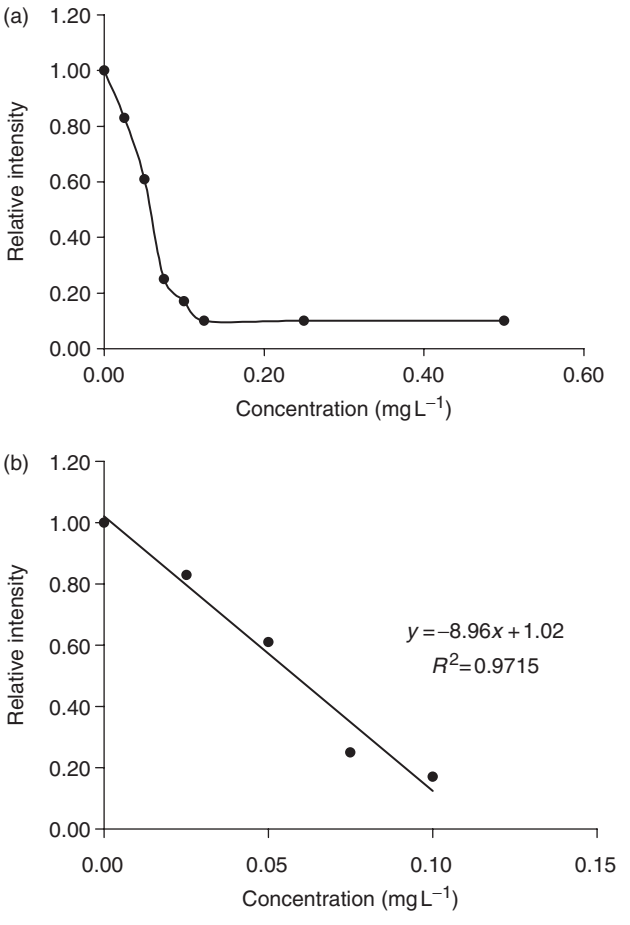

Figure 4. (a) The calibration graph for the CL detection of ammonium using μFIA system with electro osmotic flow and gravity as a pulse-free flow based driven systems. The detection was measured under the channel. (b) The linear part of the calibration graph for the CL detection of ammonium using μFIA system with electro osmotic flow and gravity as a pulse-free flow based driven systems. The detection was measured under the channel.

by ammonia. The presence of reducing substances such as Fe^{2+}, S^{2-}, and SO_3^{2-} in equal amounts is known to affect the results according to Qin *et al.* and would need to be removed [43]. Interferences due to the presence of transition metal cations, namely Ni^{2+}, Fe^{3+}, Fe^{2+}, Cu^{2+}, Mn^{2+}, and Co^{2+} that catalyse the luminol reaction in the presence of peroxide, are also well documented and are known to interfere at concentrations greater than 10 mM; these can be removed by a microcolumn containing chelating agents (Kraus and Crouch) [17]. Other compounds were also tested for their interference effect on the luminol–hypochlorite reaction; Na_2CO_3, for instance, suppressed the CL signal when presented in a tenfold excess over ammonia, while the CL signal was slightly increased in the presence of a 100-fold excess of $MgCl_2$. This was probably due to a pH effect as noted by Kraus and Crouch [17]. Potassium chloride and NaCl were found not to interfere when presented in a 100-fold excess over ammonia.

3.3.2 Determination of ammonium in estuarine water. To overcome concerns over the presence of interferences in the water samples, the standard addition method was used. A 0.5-mL aliquot of $0.075\,\mu g\,mL^{-1}$ of ammonium standard solution was used to spike 10 mL of the sample. The concentration of ammonium was found to be $0.078\,\mu g\,mL^{-1}$ with a precision ($\leq 0.5\%$) RSD for $n = 9$. This value was slightly lower than that obtained by direct analysis ($0.092\,\mu g\,mL^{-1}$) with a precision (3.18%) RSD for $n = 9$, suggesting that there were interference problems that could be overcome by standard additions. The precision values obtained using standard additions were high, thus showing that this was the preferred calibration method. This result showed a good agreement with the average ammonia concentration of $0.065\,\mu g\,mL^{-1}$ for the Humber Estuary (taken from different sampling points), reported by the Environment Agency, Leeds, UK.

4. Conclusions

In this study, the feasibility of using a μFIA system for the determination of ammonium in estuarine water has been demonstrated using CL detection. A disposable microporous silica frit along with the application of high electrical field has proved to be an efficient filtration and pumping device for the sample delivery to the reactor chip. Under optimized conditions, an estarine sample (from the Humber Estaury) was analysed, and the concentration of ammonium was found to be $0.078\,\mu g\,mL^{-1}$ with a precision ($\leq 0.5\%$) RSD for $n = 9$, showing good agreement with the average concentration, $0.065\,\mu g\,mL^{-1}$ (provided by the Environment Agency, Leeds, UK). A detection limit of $10 \pm 6\,\mu g\,mL^{-1}$ was achieved, with a precision value of RSD $\leq 6.4\%$ for $n = 5$. This means that a reliable and reproducible system for the determination of ammonium in river water has been constructed and without the need for any mechanical devices for the sample delivery to the reactor chip. This design can be exploited for the construction of a portable device that can be used for water quality control purposes.

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References

[1] H. Temmink, B. Petersen, S. Isaacs, M. Henze. *Water Sci. Technol.*, **34**, 1 (1996).
[2] J. Meinhold, H. Pedersen, E. Arnold, S. Isaac, M. Henze. *Water Sci. Technol.*, **38**, 97 (1998).
[3] B.W. Touchette, J.M. Burkholder. *J. Exp. Mar. Biol. Ecol.*, **250**, 133 (2000).

[4] R.N. Fernandes, B.F. Reis. *Talanta*, **58**, 729 (2002).
[5] K.N. Andrew, P.J. Worsfold, M. Comber. *Anal. Chim. Acta*, **314**, 33 (1995).
[6] Ammonia and water quality. Available online at: http://www.google.com/search/. Available online at: http://kywater.org/ww/ramp/rmnh4.htm (accessed 10 April 2004), River Monitoring Project Data. Available online at: illinois.sierraclub.org/RPG/watermonitorproj.htm (accessed 10 April 2004).
[7] J. Gardiner, G. Mance. UK Quality Standards Arising from EEC Directives, WRC Technical Report TR204, Medmenham, UK, 1984.
[8] J. Ruzicka. *Anal. Chim. Acta*, **190**, 155 (1986).
[9] M. Gisin, C. Thommen. *Anal. Chim. Acta*, **190**, 165 (1986).
[10] R.L. Beson, P.J. Worsfold, F.W. Sweeting. *Anal. Proc.*, **26**, 385 (1989).
[11] M.D. Luque de Castro. *Talanta*, **36**, 591 (1989).
[12] K.N. Andrew, N.J. Blundell, D. Price, P.J. Worsfold. *Anal. Chem.*, **66**, A916 (1994).
[13] M.E. Meyerhoff, Y.M. Fraticelli. *Anal. Lett.*, **14**, 415 (1981).
[14] S. Alegret, J. Alonso, J. Bartrolí, E. Martínez Fàbregas. *Analyst*, **114**, 1443 (1989).
[15] Z. Genfa, P.K. Dasgupta. *Anal. Chem.*, **61**, 408 (1989).
[16] J.J.R. Rohwedder, C. Pasquini. *Analyst*, **116**, 841 (1991).
[17] P.R. Kraus, S.R. Crouch. *Anal. Lett.*, **20**, 183 (1987).
[18] C.A. Georgiou, M.A. Koupparis. *J. Auto Chem.*, **13**, 199 (1991).
[19] A.C. Ariza, P. Linares, M.D. Luque de Castro, M. Valcárcel. *J. Auto Chem.*, **14**, 181 (1992).
[20] F. Cañete, A. Ríos, M.D. Luque de Castro, M. Valcárcel. *Analyst*, **113**, 739 (1988).
[21] J.S. Cosano, J.L. Pinillos, P. Linares, M.D. Luque de Castro. *Anal. Chim. Acta*, **221**, 173 (1989).
[22] M. van Son, R.C. Schothorst, G. den Boef. *Anal. Chim. Acta*, **153**, 271 (1983).
[23] J.R. Clinch, P.J. Worsfold, F.W. Sweeting. *Anal. Chim. Acta*, **214**, 401 (1988).
[24] J. Ruzicka, E.H. Hansen. *Flow Injection Analysis*, 2nd Edn, Wiley, New York (1988).
[25] M.C. Icardo, I.G. Torro, L.L. Zamora, J.M. Calatayud. *Anal. Chim. Acta*, **398**, 311 (1999).
[26] T. Tsuboi, Y. Hirano, Y. Shibata, S. Motomizu. *Anal. Sci.*, **18**, 1141 (2002).
[27] K. Tsukagoshi, M. Hashimoto, R. Nakajima, A. Arai. *Anal. Sci.*, **16**, 1111 (2000).
[28] E.P. Achterberg, T.W. Holland, A.R. Bowie. *Anal. Chim. Acta*, **442**, 1 (2001).
[29] J. Li, P.K. Dasgupta. *Anal. Chim. Acta*, **398**, 33 (1999).
[30] I. McKelvie, FIA Research Group, Monash University 'What is FIA?' (Recent Developments). Available online at: http://www.google.com/search/ Copyright © I.D. McKelvie, 1999. Available online at: http://www.wsc.monash.edu.au/fia/WhatisFIA/What_is_FIA_5.html (accessed 10th April 2004).
[31] A.G. Hadd, S.C. Jacobson, J.M. Ramsey. *Anal. Chem.*, **71**, 5206 (1999).
[32] Y.H. Chen, W.C. Wang, K.C. Young, T.T. Chang, S.H. Chen. *Clin. Chem.*, **45**, 1938 (1999).
[33] G.M. Greenway, S.J. Haswell, P.H. Petsul. *Anal. Chem. Acta*, **387**, 1 (1999).
[34] G.N. Doku, S.J. Haswell. *Anal. Chem. Acta*, **382**, 1 (1999).
[35] T. McCreedy, N.G. Wilson. *Analyst*, **126**, 21 (2001).
[36] L.J. Nelstrop, P.A. Greenwood, G.M. Greenway. *Lab On a Chip*, **1**, 138 (2001).
[37] X-. Zheng Wu, M. Suzuki, T. Sawada, T. Kitamori. *Anal. Sci.*, **16**, 321 (2000).
[38] K. Tsukagoshi, Y. Obata, R. Nakajima. *J. Chromatogr. A*, **971**, 255 (2002).
[39] A.R. Bowie, E.P. Achterberg, R.F.C. Mantoura, P.J. Worsfold. *Anal. Chim. Acta*, **361**, 189 (1998).
[40] N.G. Wilson, T. McCreedy, G.M. Greenway. *Analyst*, **125**, 237 (2000).
[41] P.D. Christensen, S.W.P. Johnson, T. McCreedy, V. Skelton, N.G. Wilson. *Anal. Commun.*, **35**, 341 (1998).
[42] T. McCreedy. *Anal. Chim. Acta*, **427**, 39 (2001).
[43] W. Qin, Z.J. Zhang, B. Li, Y. Peng. *Talanta*, **48**, 225 (1999).
[44] R.T. Balciunas. An automated reagent preparation system for fast reaction rate. PhD thesis, Michigan State University (1981).
[45] C.J. Patten, S.R. Crouch. *Anal. Chem.*, **49**, 464 (1977).
[46] C.A. Chang, H.H. Patterson. *J. Anal. Chem.*, **52**, 653 (1980).
[47] P.D. Wildes, E.H. White. *J. Am. Chem. Soc.*, **95**, 2610 (1973).
[48] J.N. Miller, J.C. Miller. *Statistics and Chemometrics for Analytical Chemistry*, 4th Edn, p. 120, Prentice Hall, Dorchester, UK (2000).