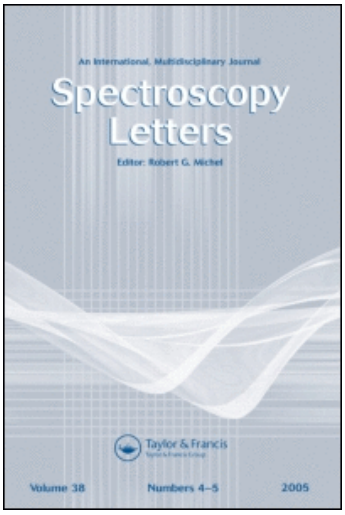

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Spectrophotometric Determination of Ammonia in Estuarine Waters by Hybrid Reagent-Injection Gas-Diffusion Flow Analysis

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Abstract: A flow-injection gas-diffusion technique is described for the online determination of ammonia in estuarine waters covering a salinity range of S = 0 to 36. The flow analysis system, which is a hybrid of reagent injection and conventional sample-injection flow systems, avoids the need for a rotary injection valve. Whereas gas-diffusion techniques have been widely applied in conventional sample-injection flow analysis, reagent-injection flow analysis involving gas diffusion has been little used because it is susceptible to interference from dissolved gaseous species such as carbon dioxide coexisting with ammonia in the sample. This source of interference has been overcome by online adjustment of sample to pH 8.4 prior to the injection of the base that initiates gas diffusion of ammonia. The pore sizes of hydrophobic membranes used in gas diffusion were characterized by a bubble-point test prior to use in the flow analysis system. These showed wide variation in pore size, and grading and careful selection was necessary in order to obtain reliable gas diffusion measurements of ammonia. The proposed flow-injection system can be operated in a continuous flow mode, at a sample throughput of 135 measurements hr^{-1} with a typical limit of detection (LOD) of 9 $\mu g\,N\,L^{-1}$, or in stopped-flow mode at 60 measurements hr^{-1} with a LOD of 3 $\mu g\,N\,L^{-1}$. The technique was validated using water samples containing a wide range of dissolved carbon dioxide concentrations,

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salinity, and pH. Excellent agreement ($r = 0.999$) was observed between results obtained using the reagent-injection system and an approved reference method.

Keywords: Ammonia, dissolved carbon dioxide, estuary, flow analysis, gas diffusion, reagent injection

INTRODUCTION

Ammonia occurs in natural and waste waters as dissolved NH_3 and as the cationic species, NH_4^+, with the latter form predominating at the pH of most natural waters. In aquatic systems, ammonia is produced by the decomposition of nitrogenous organic substances under anaerobic conditions, such as those that exist in aquatic sediments. Ammonia, as NH_3, is acutely toxic to some fish at concentrations of $\geq 200\ \mu g\ N\ L^{-1}$;[1] it is also more available to plants and phytoplankton than nitrate and at elevated concentrations may contribute to eutrophication. Consequently, the ammonia concentration is both an important indicator of water quality and an ecological stressor, and these are the drivers for development of improved methods of analysis.

Automated flow analysis techniques are increasingly being used to measure nutrient concentrations in online mode in the field.[2] Direct spectrophotometric flow injection approaches used for the analysis of ammonia in a wide range of water and wastewater samples include the use of Nessler's reagent[3] and the indophenol blue method.[4] Both of these methods employ toxic reagents, and where possible it is desirable from both environmental and occupational safety perspectives to replace these with more benign reagents. The indophenol blue method also suffers from interference by Mg^{2+} in marine waters, and an empirical correction based on Mg^{2+} concentration, pH, or salinity must be applied to compensate for this effect.[5] Furthermore, direct spectrophotometric flow injection measurements of marine and estuarine waters may suffer from pronounced salt effects on either the detection chemistry or due to the Schlieren (refractive index) effect.[6]

These interferences can be minimized by the use of gas-diffusion flow-injection techniques. In a normal (sample injection) flow-injection gas-diffusion manifold, sample containing ammonium ions is injected into an alkaline stream, and the ammonia that forms then diffuses through a hydrophobic gas-permeable membrane into an acceptor stream,[7] where it can be directly detected using potentiometry,[8] conductimetry,[9] or by spectrophotometry[10] or fluorimetry[11] if suitable chromogenic or fluorogenic reagents are present. Sample matrix effects are not encountered when gas-diffusion flow-injection is used because the membrane in the gas-diffusion cell provides a physical barrier, effectively excluding potential interferences such as nonvolatile ionic species (i.e., salt), and color from the measured detection zone.[10] A comparison of selected examples of various flow injection and segmented continuous flow methods for measurement of ammonia in various aquatic matrices is given in Table 1.

Table 1. Comparison of various detection chemistries employed in the determination of ammonia in waters by flow analysis techniques

Technique	Basis for detection	Sample type	Calibration range	LOD	Throughput injections hr^{-1}	Estimated reagent usage per measurement[a]	Estimated waste generated per measurement[b]	Notes	Ref.
FIA–spectrophotometry	Indophenol blue	River water	5 $\mu g\ L^{-1}$–1 $mg\ L^{-1}$	5 $\mu g\ L^{-1}$	90	1.6 mL	6.5 mL	Zone-trapping technique	[11]
FIA–spectrophotometry	Indophenol blue	Waste water	0.1–10 $mg\ L^{-1}$	0.03 $mg\ L^{-1}$	Not reported	Unable to estimate	Unable to estimate	On-line preconcentration	[12]
SIA–spectrophotometry	Indophenol blue with salicylate	River water	0.1–2 $mg\ L^{-1}$	25 $\mu g\ L^{-1}$	60	1.1 mL	5.6 mL	Multicommutation	[13]
FIA–spectrophotometry	Nessler's reagent	Rain water	50–200 $mg\ L^{-1}$	<200 $\mu g\ L^{-1}$	40	40 μL	6.8 mL	Ion exchange in sample loop	[3]
FIA–spectrophotometry	Nessler's reagent	Natural	50–500 $\mu g\ L^{-1}$	50 $\mu g\ L^{-1}$	45	50 μL	6.0 mL	Preconcentration with cation exchange resin	[14]
SFA–fluorimetry	Orthophthaldialdehyde, OPA	Sea & estuarine	1.6–9.9 $\mu g\ L^{-1}$ 41–206 $\mu g\ L^{-1}$	1.2 $ng\ L^{-1}$ 5.7 $ng\ L^{-1}$	20 20	8.2 mL 8.2 mL	8.3 mL 8.3 mL	Low range calibration High range calibration	[15]
FIA–spectrophotometry	Gas diffusion	Waste	0.5–20 $mg\ L^{-1}$	0.03 $mg\ L^{-1}$	Not reported	Unable to estimate	Unable to estimate	On-line preconcentration & stopped flow	[12]
FIA–spectrophotometry	Gas diffusion	River water	>2000 $\mu g\ L^{-1}$	17 $\mu g\ L^{-1}$	Not reported	Unable to estimate	Unable to estimate	Portable system	[7]
FIA–spectrophotometry	Gas diffusion	Rain & river water	0.25–25 $\mu g\ L^{-1}$	0.25 $\mu g\ L^{-1}$	40	2.0 mL	2.1 mL		[16]

(*continued*)

Table 1. Continued

Technique	Basis for detection	Sample type	Calibration range	LOD	Throughput injections hr^{-1}	Estimated reagent usage per measurement[a]	Estimated waste generated per measurement[b]	Notes	Ref.
FIA–spectrophotometry	Gas diffusion	Industrial effluent	1–100 mg L^{-1}	0.6 mg L^{-1}	13	2.9 mL	5.1 mL	Portable system	[9]
FIA–conductimetry	Gas diffusion	River water	30–500 mg L^{-1}	5 mg L^{-1}	60	1.6 mL	6.7 mL		[17]
FIA–fluorimetry	Gas diffusion, orthophthaldialdehyde (OPA)	Sea	>0.82 μg L^{-1}	5.8 ng L^{-1}	30	1.9 mL	5.6 mL	Shipboard analysis	[10]
Reagent injection FIA–spectrophotometry	Gas diffusion	Estuarine water	20–160 μg L^{-1}	9 μg L^{-1}	135	1.16 mL, i.e. (0.58 mL buffer + 0.58 mL indicator + 0.006 mL NAOH)	2.2 mL	Continuous flow	This work
			20–160 μg L^{-1}	3 μg L^{-1}	60	As above		Stopped flow	

[a]Reagents only.
[b]Reagents + Carrier + Sample.

Gas-diffusion methods using conventional flow-injection have been reported for measurements in a wide range of water sample types, including river waters,[7] rain waters,[17] lake waters,[18] effluent discharges,[10] and seawater.[19]

This work aims to exploit the gas-diffusion technique and its tolerance to sample matrices of varying salinity for the determination of ammonia in the development of a flow analysis method that can be used for rapid online monitoring on small sampling craft and larger marine vessels for extended periods.

We have previously reported a compact, portable flow analysis system for the rapid photometric determination of dissolved reactive phosphate in marine and estuarine waters.[20] This is a multiple reagent-injection, or multicommutation system, in which microliter volumes of reagents are injected into a continuously flowing stream of filtered sample. The flow system comprises a single-channel peristaltic pump for sample delivery, and injection of reagents is achieved by the use of microsolenoid valves that precisely deliver reagents in defined volumes and sequences from helium-pressurized reagent reservoirs. The advantages of this approach include simplicity of design, mechanical robustness, and rapid sample throughput, with minimal reagent consumption and waste production. The system has been applied to surface mapping of dissolved reactive phosphate in marine and estuarine systems.[21]

This approach has proved advantageous in the monitoring of nutrients in surface waters, and we wished to adapt the compact reagent-injection flow analysis system for the analysis of ammonia in marine and estuarine waters.

The fundamental difference between this work and previous flow-injection gas-diffusion determinations of ammonia (Table 1) is that we have used *reagent*-injection gas-diffusion flow injection. In this mode, base is injected into a continuously flowing stream of sample to liberate ammonia gas. This mode of flow-injection has been little used for gas diffusion because of the potential for interference from other dissolved gaseous species present in the sample, such as dissolved carbon dioxide. However, if this source of interference can be overcome, the use of reagent injection has several advantages. This is particularly the case if microsolenoid valves are used for injection because they enable rapid sequenced reagent addition and minimize the consumption of the alkaline reagent and the production of waste solutions (see Table 1). Use of this mode of injection also avoids the use of a rotary injection valve, which is preferable in a field instrument designed for long-term field application because of power consumption and reliability issues.

In the case of a gas-diffusion flow-injection system operated in the *normal* (sample injection) mode, the presence of $H_2CO_3^*$ will not interfere with ammonia detection. The injection of sample into a strongly alkaline donor stream ensures that any $H_2CO_3^*$ ($=[H_2CO_3] + [CO_{2(aq)}]$) present is completely converted to monohydrogencarbonate and/or carbonate before the sample reaches the gas diffusion membrane, and thus only ammonia gas will diffuse into the acceptor stream. However, in *reagent*-injection mode, the

sample stream flows continuously over the membrane and any dissolved gaseous carbon dioxide present at this pH in the sample donor stream (see Fig. 1) will also pass across the membrane into the acceptor stream. Diffused dissolved carbon dioxide gas will therefore tend to decrease the pH of the weakly buffered indicator stream, causing a shift in the background absorbance against which the ammonia signal is measured, giving rise to a sizeable error. However, by appropriate pH control of the sample, we have shown that this interference can be controlled.

MATERIALS AND METHODS

Reagents

All reagents were prepared from analytical grade materials and ultrapure water (Continental Water Systems Corp, Modulab Analytical, Seven Hills, NSW, Australia). Ammonia working standards in the range 20–3000 $\mu g\ N\ L^{-1}$ were prepared daily from a 1 $g\ N\ L^{-1}$ ammonium chloride stock solution (1.9104 g per 500 mL, previously dried at 105°C) that was stored at less than 4°C in the dark. Alkalinity working standards in the range 20–160 mg $CaCO_3\ L^{-1}$ were prepared daily from stock solution of 1.6769 g of $NaHCO_3$ per 100 mL.

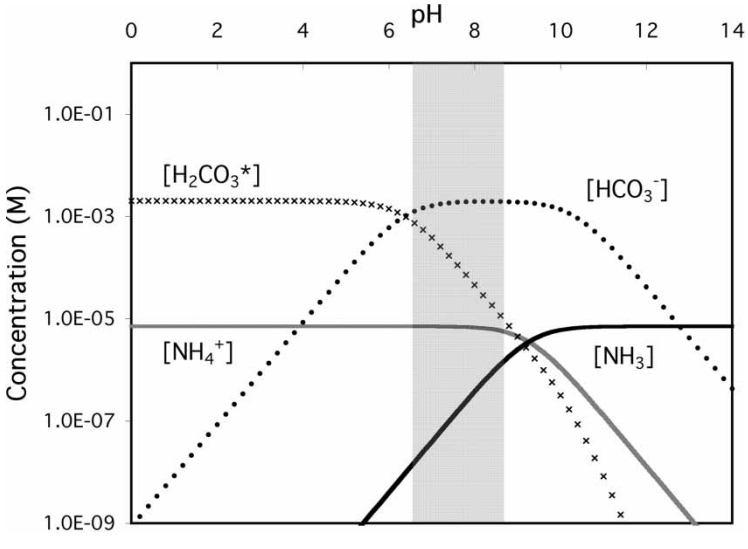

Figure 1. Equilibrium speciation diagram for $NH_{4\ (aq)}^{+}/NH_{3(g)}$ ($pK_a = 9.26$) and $H_2CO_{3\ (aq)}^{*}/HCO_{3\ (aq)}^{-}$ ($pK_a = 6.37$).[22] The enclosed region indicates the typical pH range of natural waters. Typical concentrations of ammonia (100 $\mu g\ NH_3\text{-}N\ L^{-1}$) and dissolved carbon dioxide (100 mg $CaCO_3\ L^{-1}$) species in natural waters are shown.

The indicator stream, an 8×10^{-5} M bromothymol blue solution[13] ($pK_{In} = 7.1$),[22] was prepared in 0.1 mM phosphate buffer at pH 6.1. A 0.1 M Tris buffer of pH 7.8 or 8.4 was used to adjust the pH of the sample stream, and 0.7 M NaOH solution was used for reagent injection. Sodium hydroxide and weakly buffered indicator reagents were stored in gas-tight containers with CO_2 traps to ensure the best day-to-day reproducibility in extended operation.

Two standard natural water reference materials (reference numbers 4023 and 2185) for ammonia with the consensual mean values of 2.100 ± 0.180 mg NH_3-N L^{-1} and 14.99 ± 1.03 mg NH_3-N L^{-1} (diluted to 2.998 ± 0.206 mg NH_3-N L^{-1} for this exercise) were supplied by Analytical Products Group, Inc. (Belpre, Ohio, USA).

Instrumentation

The flow-injection system employed for the development of this ammonia detection technique was constructed in-house, based on a design described by Lyddy-Meaney et al.[20] for measurement of filterable reactive phosphorus. Figure 2 shows the manifold configuration used for ammonia determination by gas diffusion. Indicator, sample, and buffer were pumped continuously through the manifold. Immediately prior to an injection sequence, valve V1 was switched to divert sample to waste, and 0.7 M NaOH was injected into the stationary sample stream for 150 ms (≈6 μL) using a miniature solenoid valve, V2 (Lee Company, Westbrook, CT, USA, LFVA series). The NaOH solution was held in a small machined reservoir (volume ca. 15 mL) that was pressurized with helium, as per the approach of Lyddy-Meaney et al.[20] This avoids the use of another peristaltic pump and ensures that the NaOH does not absorb atmospheric carbon dioxide throughout the period of deployment. Following reagent injection, valve V1 was

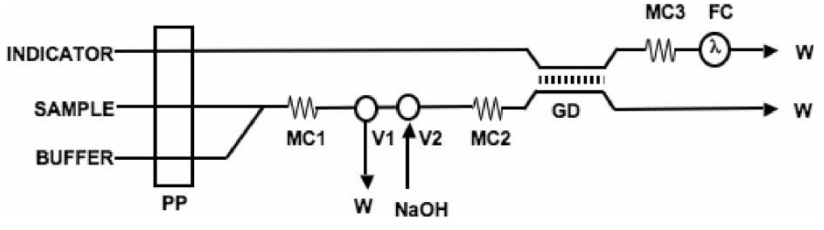

Figure 2. Manifold diagram for the determination of NH_3. PP, peristaltic pump (indicator [2.4 mL min^{-1}]; sample [1.3 mL min^{-1}]; buffer [1.3 mL min^{-1}]); V1, valve 1 (miniature solenoid valve) feeds mixed sample and buffer into the gas diffusion unit; V2, valve 2 NaOH reagent injection; MC1, mixing coil, 40-cm knitted; MC2 and MC3, 20-cm knotted; GD, gas diffusion unit; FC, flow cell and flow-through detector; W, waste.

switched to resume sample flow through the manifold. The sample-reagent zone containing ammonia gas generated by injection of NaOH was then passed over PTFE plumbing tape (0.1 mm thickness, 0.11 μm pore size)[23] that was supported on either side by polymethylmethacrylate blocks, each with a linear engraved channel, 75-mm long, 2-mm wide, and 0.3-mm deep. Ammonia that diffused through the membrane caused a change in the color of the bromothymol blue indicator acceptor stream, the absorbance of which was monitored using a low-dispersion, multireflection flow cell that had an effective optical pathlength of ca. 20 mm.[24] The light source used for absorbance measurements was a super bright red LED, ($\lambda_{max} = 654$ nm), and the transmitted signal was detected using a solid-state photodiode photometer with a ± 10 V output[20] or an Ocean Optics S2000 miniature CCD spectrometer. All valve and pump switching and data acquisition was performed using a program written in the programming language LabVIEW (National Instruments, Austin, TX, USA).

Membrane Characterization

PTFE plumbing tape was used as the hydrophobic membrane for gas diffusion in these experiments. Initial observations suggested that the gas-diffusion performance of these membranes was quite variable, probably due to variation in pore size caused by stretching of the tape during manufacture. In order to determine the variability in the pore size of different brands and batches of PTFE tapes, a "bubble-point" test was used to determine the maximum pore size.[23] The bubble-point test measures the minimum gas pressure required to force liquid out of a capillary tube, which is inversely related to the pore diameter. Thus by determining the bubble point, the membrane pore size can be calculated from:

$$d = \frac{4k \cos \theta \sigma}{P} = \frac{x}{P}$$

where d is pore diameter (μm), k is shape correction factor, θ is liquid–solid contact angle, σ is surface tension of water, and P is pressure (kPa). The experimental factor, x, was determined by finding the pressure required for the bubble point of a membrane of known pore size (Fluoropore PTFE Membrane Filter, Millipore, 0.45 μm, P/N FHLP04700). Each bubble point was measured in triplicate using nitrogen gas.

Sample Collection

Samples were collected from sites on the Yarra River estuary, Brushy Creek, and the mouth of the Werribee River, in Victoria, southeast Australia. These sites provided samples with a wide range of ammonia concentrations and salinities.

Samples were filtered on-site (0.2-μm filter, PALL Acrodisc Syringe Filters, or Gelman Vivaflow 50), and stored at 4°C in prewashed polypropylene bottles, and were analyzed within 24 hr of collection. Salinity and pH were measured at each site using a calibrated Horiba (Minami-ku, Kyoto, Japan) model U10 water quality checker.

RESULTS AND DISCUSSION

Manifold and Reagent Conditions

A single injection of 6 μL of NaOH did not produce sufficient ammonia to cause a measurable change of pH, and hence absorbance, in the bromothymol blue stream. However, when two injections of 6 μL NaOH were interspersed with 6 μL of sample, large, reproducible peak responses were obtained, which gave excellent sensitivity. When further reagent injections were added to the sequence, or larger injection volumes were used, this caused a pressure pulse that resulted in stretching and distortion of the membrane, which resulted in increased baseline noise and, in extreme cases, in membrane leakage. Consequently, a multiple reagent injection sequence of 6 μL NaOH, 6 μL sample, and 6 μL NaOH was used.

A number of flow rates were investigated for both the carrier and acceptor streams, and it was observed that peaks were more reproducible when the flow rates of both the carrier and acceptor streams were well matched. This is consistent with the findings of Cardoso de Faria and Pasquini,[18] who reported that in order to avoid distortion of the membrane (i.e., due to stretching), the hydrostatic pressures on either side of the diffusion cell must be similar. Hence, the acceptor stream was set at a flow rate of 2.4 mL min^{-1}, and the sample and buffer streams were 1.3 mL min^{-1} each.

A 40-cm knotted coil was used to mix the sample and buffer streams. Mixing coils were also added immediately after the injection valve V2 to pre-mix the injected reagent with the sample and also prior to the detector to ensure that the pressure was balanced on either side of the membrane and that there was adequate mixing between ammonia and indicator.

The manifold described is a hybrid of reagent injection and conventional sample injection flow systems. Although it would be preferable to use reagent injection for all reagents (multicommutation), the same detection limits or reproducibility could not be achieved as those that are reported here for the hybrid system.

Effect of Sample pH on Selectivity

The speciation of dissolved ammonia is highly dependant on pH, and over the pH range of natural waters (6.5–8.5),[1] the solvated ammonium cation, NH_4^+

($pK_a = 9.26$ at 25°C in freshwater) is the dominant species (Fig. 1).[22] However, at the pH of many natural waters, small, but detectable amounts of dissolved carbon dioxide may also be present as a result of the equilibrium between dissolved atmospheric CO_2 and the aqueous sample. This dissolved carbon dioxide is present as an equilibrium mixture between "true" carbonic acid, H_2CO_3, and dissolved carbon dioxide, $CO_{2(aq)}$, (such that $H_2CO_3^* = [H_2CO_3] + [CO_{2(aq)}]$),[25] and dissolved monohydrogen carbonate and carbonate (Fig. 1). The presence of dissolved gaseous ammonia and carbon dioxide in the sample stream prior to reagent injection can cause error in determination of ammonia using reagent-injection flow analysis. In this mode, the sample stream passes continuously over the gas-diffusion membrane. Under normal pH conditions, a measurable fraction of the analyte is present as dissolved ammonia gas, in addition to a small concentration of dissolved CO_2 (see Fig. 1). Continuous diffusion of ammonia through the membrane into the indicator stream causes an increase in the baseline from that which is set using a blank, while the effect of diffused acidic gaseous species such as CO_2 will cause a baseline decrease at this particular wavelength.

Injection of NaOH into the sample stream shifts the ammonia/ammonium equilibrium in favor of ammonia, which then also diffuses through the membrane, giving the analyte peak. However, depending on the relative concentrations of ammonia and dissolved CO_2, the shift of baseline varies from sample to sample and when added to or subtracted from the analytical signal can cause a significant error in the measured ammonia concentration.

Therefore, to minimize the problems of dissolved carbon dioxide interference, and baseline shifts due to continuous ammonia diffusion, the sample stream was initially buffered at a pH of 7.8 and then later at 8.4. At pH 7.8, the proportions of gaseous ammonia and carbon dioxide are small (approximately 3.5% ammonia and 3.7% carbon dioxide; Fig. 1). Hence buffering at this pH ensures that little ammonia or carbon dioxide passes through the membrane from the continuously flowing sample stream prior to base injection.

To determine the selectivity of the gas-diffusion method for ammonia, in the presence of dissolved carbon dioxide, random mixtures of known amounts of ammonia and carbon dioxide were used to obtain a calibration plot for ammonia. While it is the $H_2CO_3^*$ species present in the water samples that diffuses through the membrane and interferes with the ammonia signal, total alkalinity (TA) was used as a surrogate measure of dissolved carbon dioxide species. Although it is recognized that the TA titration does not include $H_2CO_3^*$ species, the justification for use of TA in this manner is that if there is a high TA, there will be a correspondingly high concentration of $H_2CO_3^*$ species in equilibrium with monohydrogencarbonate and carbonate at the pH of natural waters.

If interference from dissolved carbon dioxide occurs, the subtractive effect of this interferent should cause considerable scatter in the ammonia

Table 2. Analytical figures of merit for calibrations at pH 7.8 and 8.4. NH_3 concentrations expressed as μg NH_3-N L^{-1}

NH_3 range (μg NH_3-N L^{-1})	Tris buffer pH	Alkalinity (mg L^{-1}) as $CaCO_3$	Calibration equation (Correlation coefficient)	Limit of detection (μg NH_3-N L^{-1})	%RSD (n = 3) 100 μg NH_3-N L^{-1}
20–160	7.8[a]	20–160	$PkHt = 0.621[NH_3] + 42$ (r = 0.989)	21	4
	8.4[a]		$PkHt = 0.602[NH_3] + 34$ (r = 0.997)	9	3
	8.4[b]		$PkHt = 0.636[NH_3] + 133$ (r = 0.999)	3	2
100–3000	7.8[a]	5–35	$PkHt = 0.722[NH_3] + 31$ (r = 0.999)	52	3
	8.4[a]		$PkHt = 0.635[NH_3] + 31$ (r = 0.999)	12	5

[a]Continuous flow mode.
[b]Stopped-flow mode (30 s stop).

calibration graph. This is illustrated by the data in Table 2, which shows that a low concentration range (20–160 μg NH_3-N L^{-1}) ammonia calibration graph obtained using a pH 7.8 buffered sample had considerable scatter (r = 0.989), whereas the corresponding high concentration range (100–3000 μg NH_3-N L^{-1}) calibration, which should be less susceptible to interference from dissolved carbon dioxide, showed much less scatter (r = 0.999).

Although at pH 7.8 there is only an estimated 3.7% of carbonate species present (Fig. 1), under typical concentrations of ammonia (140 μg N L^{-1}) and dissolved carbon dioxide species (160 mg $CaCO_3$ L^{-1}), the proportion of $H_2CO_3^*$ would exceed that of the ammonia, and this can amount to a relatively large concentration with respect to ammonia, as shown in Fig. 1. Subsequently, we have buffered sample at pH 8.4, and predictably this has further suppressed interference from $H_2CO_3^*$, even at low ammonia concentrations where interference is most likely (Table 2; r = 0.997). At this pH, the detection limit[26] of 9 μg NH_3-N L^{-1} was somewhat better than that achieved at pH 7.8 (21 μg N L^{-1}; Table 2).

To quantify the extent of interference from dissolved carbon dioxide, a multiple linear regression analysis was performed for the ammonia response from randomly mixed standards of ammonia (20–160 μg N L^{-1}) and TA (20–160 mg $CaCO_3$ L^{-1}), at both pH 7.8 and 8.4. The following multiple linear regressions equations were obtained for the ammonia signal, and hence the selectivity (k) for ammonia with respect to TA can be calculated:

$$\mathrm{PkHt}_{\mathrm{pH7.8}} = 0.587[\mathrm{NH_3}] + 0.0817[\mathrm{TA}] + 37.9 \quad (r = 0.995) \qquad (1)$$
$$k[\mathrm{NH_3}]/[\mathrm{TA}] \approx 7.2$$

$$\mathrm{PkHt}_{\mathrm{pH8.4}} = 0.588[\mathrm{NH_3}] + 0.0365[\mathrm{TA}] + 32.6 \quad (r = 0.999) \qquad (2)$$
$$k[\mathrm{NH_3}]/[\mathrm{TA}] \approx 16$$

where k is gradient[NH_3]/gradient[TA], and [NH_3] is expressed as μg N L^{-1} and dissolved CO_2 species expressed in terms of the total alkalinity [TA] reported as mg $CaCO_3$ L^{-1}.

At pH 7.8, the effect of dissolved CO_2 species in the randomly mixed standards caused appreciable scatter in the regression plot, as shown by the correlation coefficient of 0.989. However at the higher buffer pH of 8.4, there was much less scatter of data about the regression line (r = 0.997) indicating the reduced affect of dissolved CO_2 species on the ammonia signal. The selectivity, k, of ammonia with respect to dissolved carbon dioxide species can be determined from regression equations (1) and (2), and it is evident that k has more than doubled as a result of changing pH from 7.8 to 8.4. Based on these results, a Tris buffer pH of 8.4 was employed for all further sample analyses.

Similar interference might also be expected in highly polluted samples containing higher concentrations of sulfide or cyanide, but arguably the pH control process described here would also suppress the effects of these ions on ammonia determination.

Membrane Characterization

Bubble-point tests were performed on eight different brands of PTFE plumbing tape. These varied in thickness from 0.05 to 0.15 mm and had pore sizes that ranged from 0.18 to <0.04 μm. Samples of the same membranes were also used to determine the gas-diffusion response for a 1000 μg NH_3-N L^{-1} standard. Predictably the thinner, larger pore sized membranes gave the largest signals but were also the least reproducible in their operation. Indeed some of the thinnest membranes gave signal responses that were off-scale, indicating that membrane leakage was occurring. Bubble-point testing was therefore used as a means of screening the suitability of membranes before use for gas-diffusion measurement of ammonia, and on this basis a batch of PTFE tape with a thickness of 0.10 mm and pore size of 0.11 μm was used for all subsequent analyses.

The Effect of Stopped-Flow Operation

The use of stopped flow, in which the injected reagent slug is held in the gas-diffusion unit for a defined period (5–60 s), allows a larger quantity of ammonia gas to diffuse through the membrane. A 30-s stopped-flow time was chosen as a compromise between enhanced sensitivity and decreased sample throughput (from 135 injections hr^{-1} to 60 injections hr^{-1} under stopped-flow conditions). Stopped-flow operation also resulted in even less scatter of data points for standards containing randomly mixed concentrations of dissolved CO_2 species ($r = 0.999$; Table 2), leading to an improved limit of detection (i.e., from 9 to 3 μg NH_3-N L^{-1}). Analytical figures of merit for the flow analysis system operated at both pHs and under continuous and stopped-flow conditions are shown in Table 2.

Validation

Samples spanning a wide range of ammonia concentrations in matrices varying from wastewaters to saline estuarine waters were collected from Brushy Creek (an urban stream that receives wastewater effluent), the Yarra River, and Werribee River (both estuarine waters of varying salinity) in southeast Australia. Each sample was analyzed using an appropriate calibration range of standards that also contained randomly mixed concentrations of dissolved CO_2 species and compared with results obtained by a NATA[1] accredited laboratory, using the indophenol blue method of analysis.[27] The alkalinity of each sample was determined by titration[27] and used as a surrogate measure of the

[1]NATA: The National Association of Testing Authorities, which is the body responsible for accreditation of Australian analytical laboratories.

Table 3. Comparison of results obtained using the proposed method and a reference method for a range of real samples and Standard Reference Materials

Sample	Salinity (S) Practical salinity scale 1978[27]	Total alkalinity (mg L^{-1}) as $CaCO_3$	Sample pH	This method NH_3 (μg NH_3-N L^{-1})	RSD% (n = 3)	Reference method (μg NH_3-N L^{-1})	RSD% (n = 3)
Yarra R. 1[a]	0.10	28.8	8.15	23	4	25	3
Yarra R. 2[a]	0.10	36.2	8.35	39	5	36	1
Yarra R. 3[a]	4.2	40.8	7.95	68	6	66	1
Yarra R. 4[a]	6.1	43.3	8.04	75	4	79	1
Yarra R. 5[a]	32	93.9	8.21	66	5	67	1
Yarra R. 6[a]	36	120	8.15	10	5	9	8
Brushy Ck 1[a]	0.000	14.7	7.69	112	4	111	5
Brushy Ck 2[a]	0.020	60.5	7.07	3320	1	3300	1
Brushy Ck 3[a]	0.020	104	7.45	13600[c]	4	13700	1
Brushy Ck 4[a]	0.020	92.8	7.17	12200[c]	1	12100	1
Brushy Ck 5[a]	0.020	93.0	7.18	11400[c]	1	11400	1
Werribee R. 1[b]	36	131	7.74	82	6	93	1
Werribee R. 1 Spiked[b]	36	131	7.74	171	4	172[d]	N/A
Werribee R. 1 Spiked[b]	36	131	7.74	254	1	252[d]	N/A
Werribee R. 2[b]	36	131	7.71	118	2	137	2
Werribee R. 2 Spiked[b]	36	131	7.71	247	3	255[d]	N/A
Werribee R. 2 Spiked[b]	36	131	7.71	377	1	374[d]	N/A
SRM[a]	0	0	6.26	2110[c]	1	2100[e]	9 (n = 46)
SRM[b]	0	0	6.24	2980[c]	2	2998[e]	7 (n = 76)

[a]Analysed using continuous flow.
[b]Analysed using 30 s stopped-flow, and with CCD detection.
[c]Samples diluted prior to analysis.
[d]Calculated concentration based on NH_3 spike addition.
[e]Consensual mean values.

likely $H_2CO_3^*$ interference. Standard reference materials (SRMs) were analyzed using both continuous and stopped-flow methods (Table 3).

The comparative data show that even in the presence of a wide range of alkalinity (28.8–131 mg $CaCO_3$ L^{-1}), and salinity (S = 0.02 to 36), ammonia can be detected successfully and quantified with minimal interference by dissolved carbon dioxide.

The very strong agreement between the gas-diffusion reagent-injection flow analysis method and the laboratory method is indicated by the weighted regression equation:

$$[NH_3]_{\text{Reagent Injection}} = 0.997[NH_3]_{\text{Lab}} + 0.235 \ (r = 0.999, n = 19) \quad (3)$$

A paired *t*-test was performed on the data, and the results indicated that there was no significant difference between this proposed method and the laboratory reference method (paired $t = 2.03$, $p = 0.999$, $DF = 36$).

CONCLUSIONS

The flow-injection system described in this paper is a hybrid insomuch that it embodies reagent injection into a flowing stream of sample and continuous delivery of buffer and indicator in a manner akin to conventional gas-diffusion flow analysis. This approach obviates the need for a mechanical rotary injection valve, which in a portable monitoring system confers advantages of compactness, lower power consumption, and greater reliability. Reference to Table 1 shows that this proposed hybrid flow method has the lowest reagent consumption and waste production per measurement of the gas-diffusion methods listed; this is an important practical consideration in the design of an instrument and associated method that are intended for automated monitoring applications.

The method developed using this hybrid flow analysis system for ammonia is sufficiently sensitive for estuarine and coastal marine monitoring (LODs of 3 and 9 μg NH_3-N L^{-1} for stopped-flow and continuous-flow techniques, respectively) and is applicable to waters with a widely varying salinity (S = 0 to 36). Interferences in this reagent-injection method from dissolved carbon dioxide are controlled by online adjustment of sample pH.

The method developed has applications for field analysis in both fixed-point temporal monitoring and in shipboard or underway surface mapping of ammonia.

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