Automated of flow injection gas diffusion–ion chromatography for the nanomolar determination of methylamines and ammonia in seawater and atmospheric samples

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The automation and improved design and performance of Flow Injection Gas Diffusion–Ion Chromatography (FIGD–IC), a novel technique for the simultaneous analysis of trace ammonia (NH₃) and methylamines (MAs) in aqueous media, is presented. Automated Flow Injection Gas Diffusion (FIGD) promotes the selective transmembrane diffusion of MAs and NH₃ from aqueous sample under strongly alkaline (pH > 12, NaOH), chelated (EDTA) conditions into a recycled acidic acceptor stream. The acceptor is then injected onto an ion chromatograph where NH₃ and the MAs are fully resolved as their cations and detected conductimetrically. A versatile PC interfaced control unit and data capture unit (DCU) are employed in series to direct the solenoid valve switching sequence, IC operation and collection of data. Automation, together with other modifications improved both linearity (R² > 0.99 MAs 0–100 nM, NH₃ 0–1000 nM) and precision (<8%) of FIGD–IC at nanomolar concentrations, compared with the manual procedure. The system was successfully applied to the determination of MAs and NH₃ in seawater and in trapped particulate and gaseous atmospheric samples during an oceanographic research cruise.

Introduction

Nitrogen, a bio-essential element in the marine environment [1, 2], is found in a variety of inorganic and organic forms in ocean seawater, ranging from the thermodynamically most stable species, nitrate (NO₃⁻), to the reduced compounds such as ammonia (NH₃) and nitrite (NO₂⁻), to the reduced nitrogen into the troposphere [3, 4, 11–13]. Here, through processes such as their dissolution into cloud or rainwater, reaction with H₂SO₄ aerosol particles and photochemical oxidation to NOₓ, NH₃ and the MAs may influence the redistribution of both nitrogen and sulphur, the acid-base chemistry of the atmosphere and, ultimately, climatic parameters such as the number density and chemical composition of cloud condensation nuclei [3, 4, 11–13].

Methylamines are of additional importance due to the conversion of secondary amines (for example DMA) to their N-chloro-derivatives in chlorine disinfected wastewaters [14] and the implication of secondary and tertiary amines in the synthesis of carcinogenic nitrosamines in aqueous media, air, soils and foodstuffs [15].

Aspects of the marine biogeochemistry of NH₃ and MAs have been studied over many years (for example [3, 5, 8]). However, understanding their distribution and transformations has been restricted by the absence of an analytical technique capable of their selective quantification at the nanomolar levels typical of the marine environment.

Ammonia is often determined in natural waters potentiometrically by ion-selective electrodes [16, 17], or colorimetrically, typically by a version of the indophenol blue method [18]. However, away from bacterially active, anoxic or anthropogenically influenced regions, concentrations of NH₃ often fall below the sensitivity of these techniques (~0.1 μM). Only recently have cathodic stripping voltammetry [19] and o-phthalaldehyde (OPA) [20] fluorescence techniques with limits of detection <10 nM been reliably applied to the analysis of ammonia in pristine and open oceanic waters.

Methylamines, meanwhile, are normally studied by Gas or High Performance Liquid Chromatography (GC or HPLC). In practice, many GC techniques suffer from peak asymmetry [21–23], ghosting phenomena [24–26] and detector response quenching [21, 23, 27], while HPLC techniques require derivatization of MAs before detection. While derivatization is advantageous in overcoming the often problematic polar, volatile nature of the MAs, there is no single derivatizing agent available for primary, second and tertiary amines. Only in ion chromatography (IC) are these problems overcome and it is possible to simultaneously analyse ammonia and MAs (as solvated ammonium (NH₄⁺) and methylammonium cations) [21, 28, 29].

The authors recently described a novel technique, Flow
Injection Gas Diffusion coupled to Ion Chromatography (FIGD-IC), which exploits the advantages of IC and permits the simultaneous analysis of NH$_3$ and the MAs at nanomolar levels by a single analytical technique in natural waters [30]. This paper reports on the improvement, automation and computer interfacing of the technique which has increased its precision and reliability. The applicability of the technique is demonstrated through the analysis of MAs and ammonia in seawater and atmospheric samples during a research cruise in the northwestern Indian Ocean.

System design

Reagents

Ammonia and MA stock solutions (0–10 M) were prepared from their hydrochloride salts (Fluka). Single and mixed standards were prepared daily from these stocks. Internal standards (ISs) cyclo-propylamine (c-PA) and secbutylamine (s-BA) were prepared from serial dilution of laboratory grade reagents (Sigma, UK). Cyclo-PA was chosen as an IS since its occurrence in the natural environment has not been reported.

The alkaline-chelating reagent, 1 M ethylene diamine \(\text{N}_2\text{N}_2\text{'N}'\text{'N}^+\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2\) (EDTA)/0.11 M NaOH was prepared from the tetra-sodium salt of EDTA and ACS grade sodium hydroxide pellets (Sigma, UK). Eluent (40 mM MSA) was routinely prepared via a 1 M stock from ‘Aristar’ grade concentrate (BDH, UK).

Water taken freshly from a Milli-Q Water Purification System (Millipore, UK) with a specific resistivity of >18 M\(\Omega\) and further passed through a sealed ion-exchange column packed with Amberlite IR-120+ (proton form) was used as the diluent or solvent in the preparation of standards and reagents. Further ion-exchange was used to reduce the interference of alkali metals (Na$^+$ and K$^+$) and the contribution of blanks (NH$_3$ and MAs).

Ion chromatograph and suppression system

Chromatography was performed on a Dionex DX-100 IC equipped with conductimetric detection (Dionex, UK). Resolution of MAs was effected under isocratic elution (40 mM Methane Sulphonic Acid, MSA, 1 ml/min) by two IonPac CG-10 cation-exchange columns (surface sulphonated, cross-linked styrene–divinyl benzene; 4 x 50 mm; Dionex, UK). A Dionex Cation Self Regenerating Suppressor (CRS) operated with Self-Regenerating Suppressor (SRS) current controller (Dionex, UK) was used to chemically suppress the background conductivity. Injections were carried out by a pneumatically actuated injection valve fitted with a 200 µl injection loop.

Flow injection gas diffusion system

The diffusion and stripper cells were custom designed and fabricated from milled perspex [30]. Each composite module consists of a pair of mirror image blocks into which a zig-zag shallow rectangular cross-section channel was cut (length 1004 mm, width 2.0 mm, depth ~0·1 mm). The two blocks were secured using stainless-steel screws and reproducibly and uniformly tightened using a calibrated torque-limiting screwdriver.

Microporous PTFE was used as a the gas-exchange membrane (Goreten MF/002/PM–pore size 1 µm, thickness 0·076 mm, porosity 78% ; W. L. Gore, UK). Supplied in sheet form, this material was cut to a template while sandwiched between sheets of paper to facilitate easy handling.

A full analytical description and evaluation of the original FIGD-IC technique has previously been published [30].

A series of normally closed solenoid operated valves were used to control and direct liquid flow in the FIGD system (Biochem Valve Corp., USA).

(1) One four-inlet/one-outlet isolation mixing valve (080T4 12-62-4 pps, 0·062 in inlet) used to switch between sample, standard and wash solutions as required (see figure 1, V1).

(2) Two two-inlet/one-outlet isolation mixing valves (08T2 12-54-4 pps, 0·054 in inlet) coupled together to effect switching of the acceptor stream from enrichment to transfer (see figure 1, V2/V3).

(3) One three-way normally closed isolation valve (075T3MP 12-32-3 PEEK, 0·032 in inlet (see figure 1, V4).

Two single-speed, dual-channel peristaltic pumps (Ismatec type CA-2E 840, Ismatec, Switzerland) fitted with compatible pump tubing were used to generate liquid flow. All other flow lines were of Teflon (0.3, 0.5 and 0.7 mm i.d.). connected with Teflon faced grippers and 1/26 screw Tefzel end fittings and couplers (Anachem, UK). Diffusion times employed in the analysis of standards and samples were 20–60 min.

Software, PC and automation

The analogue output signal (10 µS range; 10% offset/1 V output) from the IC was converted to digital (2 Hz) using a Philips PU6031/10 data collection unit (DCU) and then processed using ATI-Unicam 4880 chromatographic software run on a Compaq 4/25 portable PC within a Windows environment. The 4880 software was also used to predetermine the timed events of valve and IC switching.

Control of solenoid valves: The DCU has seven external reed relays which are individually addressable from within the 4880 software, each with a set of uncommitted normally open contacts and available for external operation. The contacts are light duty and unsuitable for direct operation of the solenoid valves. It is thus necessary either to provide additional heavier duty relays, or more efficiently, to use an electronic switching circuit. The latter approach is advantageous since the circuit can be designed to be more flexible and made able to handle open collector (transistor switched) or TTL levels, as well as contact closure inputs. The solenoid valves are not then limited to use with the DCU, but could alternatively be operated by digital control systems.

The electronic interface is quite simple and is shown in...
The control input is connected to a Schmitt NAND gate (four of which are available in one CMOS 4093 series package). The output of the gate is connected to a high current VMOS field effect transistor which acts as the switch for turning the solenoid on and off. When the input reed relay is open, the two inputs of the NAND gate are at logical 1 due to the two resistors which act as ‘pull-ups’ to the 5 V line. The output of the NAND gate will therefore be at logical 0, the transistor will be turned off, and the solenoid will not be energized. The diode in parallel with the solenoid prevents the build-up of back e.m.f. when the solenoid is turned off.

When the reed relay contacts close, after a software command, one input of the NAND gate will go to logical 0 thus forcing the output to go to logical 1. This turns on the transistor and the solenoid will be energized. The action is the same if the ‘contact closure’ is performed by an open collector transistor, or the control line is forced to logical 0 by a TTL logic level. The interface is therefore quite versatile. The use of a Schmitt NAND gate ensures positive on-off switching, even if the control input changes slowly. The built-in hysteresis of this gate prevents solenoid ‘chatter’ during switching, which would otherwise generate considerable electrical and mechanical noise.

The development of the circuit for use with the flow injection gas diffusion-ion chromatography system (FIGD-IC) is shown in figure 3 (note that this is very similar to figure 2).
Outline of the technique

In the automated FIGD-IC system (see figure 1), seawater is pumped continuously and treated with the mixed EDTA (1.0 M)/NaOH (0.11 M) reagent to chelate alkaline earth cations (Ca$^{2+}$, Mg$^{2+}$, Be$^{2+}$, and Ba$^{2+}$) and, at the same time, to raise the pH of the mixture to >12. Under these conditions, >95% of the total dissolved NH$_4^+$ and MAs are deprotonated to their volatile gaseous forms and capable of diffusion from the sample stream, across the hydrophobic diffusion cell membrane, into an acidic acceptor stream (40 mM MSA) in which they are re-protonated. Recycling the acceptor, via coupled valves V2 and V3, promotes selective accumulation of the analytes in the enrichment loop. Following an enrichment period of between 20 and 60 minutes, the amine enriched accept is transferred to the IC via valve V4 and 200 μl is injected. Analytes and internal standards are chromato-
Table 1. Regression data for automated FIGD-IC (spiked seawater) and comparison of response linearity and FSDs for manual and automated FIGD-IC systems.

<table>
<thead>
<tr>
<th>Species</th>
<th>Parameter</th>
<th>NH₃</th>
<th>MMA</th>
<th>DMA</th>
<th>c-PA</th>
<th>TMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression data</td>
<td>Regression range (nM)</td>
<td>0–1000*</td>
<td>0–100</td>
<td>0–100</td>
<td>0–100</td>
<td>0–100</td>
</tr>
<tr>
<td></td>
<td>No. of points, N</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Normalized response†</td>
<td>3:12</td>
<td>2:87</td>
<td>2:48</td>
<td>1:00</td>
<td>2:09</td>
</tr>
<tr>
<td></td>
<td>Normalized intercept‡</td>
<td>61:8</td>
<td>2:32</td>
<td>1:75</td>
<td>1:00</td>
<td>2:33</td>
</tr>
<tr>
<td>Linearity (R²)</td>
<td>Manual system (0–1000 nM)**</td>
<td>0:988</td>
<td>0:992</td>
<td>0:992</td>
<td>0:996</td>
<td>0:990</td>
</tr>
<tr>
<td></td>
<td>Automated system</td>
<td>0:992</td>
<td>0:997</td>
<td>0:992</td>
<td>0:998</td>
<td>0:992</td>
</tr>
<tr>
<td>Relative Standard Deviation (% at 100 nM spike)</td>
<td>Manual system**</td>
<td>10:5</td>
<td>6:09</td>
<td>4:06</td>
<td>n &lt; a</td>
<td>5:09</td>
</tr>
<tr>
<td></td>
<td>Automated system</td>
<td>7:99</td>
<td>6:86</td>
<td>2:77</td>
<td>3:83</td>
<td>2:14</td>
</tr>
<tr>
<td>Limit of detection (nM)</td>
<td>Manual system (20 min diffusion)</td>
<td>20–40</td>
<td>~ 5</td>
<td>3–4</td>
<td>n/a</td>
<td>3–4</td>
</tr>
<tr>
<td>Automated system (40 min diffusion)</td>
<td>30</td>
<td>4</td>
<td>1–2</td>
<td>n/a</td>
<td>1–2</td>
<td></td>
</tr>
</tbody>
</table>

All data Gulf of Oman except * Ammonia regression data, which were English Channel seawater [21]; ** Spiked Mediterranean deep water (= 3); † normalized wrt c-PA (IS); n/a not applicable.

graphically resolved using isocratic elution (40 mM MSA). The monovalent cations are conductimetrically detected with chemical suppression of the background signal. The analogue signal (which is proportional to the analyte concentration) is digitized by the DCU and collected and processed by the 4880 software system.

Since FIGD-IC response has previously been shown to relate linearly to diffusion time ($R^2 > 0.99$, 1–60 min [21]), diffusion times can be selected in accordance with analytes concentration. As a consequence, with the appropriate switching sequence, utilization of diffusion or enrichment times in excess of the 15-min chromatographic run time, permits enrichment of one sample and chromatography of the preceding sample to proceed in parallel [5]. This significantly increases sample throughput and operational efficiency.

Chemical suppression systems, such as the CSRS employed here, greatly enhance signal to noise ratio by effectively decreasing background eluent conductivity, increasing relative analyte detector response and eliminating system peaks by removing counter ions. The result is a significant improvement in analyte detection limits. CSRS is a high capacity, automatic suppression system in which the use of detector cell effluent as a water source eliminates the need for chemical regenerant solutions or ion-exchange cartridges. With a dead volume of only 30 μl analyte dispersion effects are minimal.

Since all reagent and sample bottle headspace is fed with acid-scrubbed air fed through PTFE lines, atmospheric contamination is minimized and reagent life is prolonged. Using a second diffusion or ‘stripper’ cell (SC in figure 1) minimizes the NH₃ and MAs’ content of the EDTA/NaOH reagent before its addition to the sample. Eluent and FIGD acceptor solution (both 40 mM MSA) are prepared concurrently, ensuring the two are matched and therefore minimizing IC base-line perturbation upon acceptor injection.

The two internal standards incorporated into the analytical scheme were used to monitor FIGD-IC performance (figure 1). Sec-BA, added to the acceptor (typically 10 μM), to monitor IC stability and reproducibility, while c-PA spiked into the sample was generally used to quantify determinant concentrations through predetermined relative response factors. It was also possible to determine a range of other low molecular weight alkylamines from aqueous samples [21].

**Calibration, precision and sensitivity**

The automated FIGD-IC system gave a highly linear response to NH₃ and MAs in both standards and spiked seawater with relatively good precision in the concentration range of interest (see table 1). Both $R^2$ and RSD coefficients show, in general, an improvement over those achieved with the manual system. This is particularly important in the trace determination of MAs.

On-column detection limits for IC alone were 0:20 ng NH₃, 0:37 ng MMA, 0:54 ng DMA, 0:95 ng TMA and 0:72 ng c-PA for a 200 μl injection volume [21]. Limits of detection for the coupled FIGD-IC system are dependent upon the diffusion time employed, base-line stability, the relative abundance of analytes and the influence of the system blank. Since NH₃, in particular, gives a significantly non-zero blank, this makes trace analysis of NH₃ more difficult than for the MAs. In practice, improvements in MA detection limits (but not NH₃) are achieved using the automated system with a 40 min diffusion time (table 1). Detection limits are improved most noticeably for DMA and TMA, since these occur in regions of greater base-line stability.

**Applications**

The automated FIGD-IC system was deployed for the analysis of NH₃ and MAs during Cruises 210 and 212 of
Figure 5. Automated FIGD-IC chromatogram and corresponding valve switching sequence for duplicate analysis of a seawater sample spiked 1 μM in NH₃, MAs and c-PA (IS) based on a 20 min diffusion.

RRS Discovery in support of the UK’s ARABESQUE programme as part of the Joint Global Oceanic Flux Study (JGOFS) community research programme in the Arabian Sea (August–December 1994).

Seawater samples

Seawater samples were collected in gas tight bottles, either directly from a CTD rosette (equipped with 12 bottle sampler, chlorophyll fluorimeter, oxygen electrode, transmissometer and underwater light meter) or from an on-line subservible pump (at 2 m depth). Samples were spiked with c-PA (typically 20 nM) and analysed unfiltered using 20–60 min diffusion times.

Atmospheric samples

Particulate and gaseous phase atmospheric samples were collected in tandem using a cyclonic filter air-sampling technique [31] adapted for use with automated FIGD-IC. Aerosols were collected on PTFE pre-filters (47 mm diameter, 1 μM pore size; Costar, UK), while gaseous species were collected downstream on pairs of oxalic acid-soaked paper filters (47 mm diameter, Whatman 40, Whatman Scientific, UK). Samples were collected in triplicate over periods of 10–100 h (at ~50 dm³/min) from a height of ~8 m above sea level. The exact volume of air sampled was determined by in-line gas meters (B.S.S., UK). Filters were extracted [31] and diluted in MQ water (to 50 ml) and analysed by FIGD-IC using a 20 min diffusion time. Both particulate and gaseous concentrations were background corrected using ‘blank’ filters treated in the same manner as the sampled filters but without air pumping (see figure 6).

Results

The automated FIGD-IC system was successfully applied to the determination of NH₃ and MAs at nanomolar concentrations in a seawater (see figure 7) and in trapped atmospheric samples (table 2). However, in atmospheric samples, the relative abundance of NH₃ with respect to MMA made quantification of MMA difficult since it appeared only as a shoulder to the NH₃ peak (figure 6). Furthermore it was not possible to quantify DMA in the gaseous phase due to an interfering peak arising from processing the acid soaked paper filter. In general, NH₃ was the dominant species occurring at 5–1000 times greater concentrations than those of any MA (whose levels occasionally fall below the detection limits of the system). Monomethylamine was generally observed to be the dominant MA in Indian Ocean seawater, while TMA was normally detected at concentrations of <5 nM. Highest concentrations in oceanic vertical profiles were
Figure 7. Depth profile of MA concentrations at ‘ARABESQUE’ sampling station A1-42 (RRS Discovery Cruise 210).

Table 2. Selected particulate and gaseous phase atmospheric concentrations for the northeastern Indian Ocean (August–December 1994) determined by automated FIGD-IC.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Concentration range (pmol/m³)</th>
<th>(No. of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH₃</td>
<td>MMA</td>
</tr>
<tr>
<td>Particulate (1 μM Teflon filter)</td>
<td>940–4880</td>
<td>49–190</td>
</tr>
<tr>
<td>Gaseous (Acid-soaked paper filter)</td>
<td>350–3190</td>
<td>36–169</td>
</tr>
</tbody>
</table>

Techniques for the analysis of nanomolar concentrations of MMA, DMA and TMA generally require prolonged preconcentration times (up to 36 h) [24, 32, 33], also they are labour intensive [21, 32, 33] or they are unsuitable for use on ship [32]. FIGD-IC, on the other hand, allows determination of up to four samples per hour and is also less susceptible to contamination than the other techniques.

The prerequisite of a chromatographic step for simultaneous determination of NH₃ and MAs, results in a considerably lower sample throughput than may be achieved for a single determinant. Recently it has been possible to determine NH₃ by OPA-fluorimetry using flow injection principles and a gas diffusion cell [20]. This technique gives a sensitive (~2 nM) and precise (2%) analysis, and, with a throughput of up to 60 samples/hour, is ideally suited to high resolution, ‘real’ time analysis of NH₃. The only major drawback of this technique is that primary amines (for example MMA) are positive interferents. The ability to chromatographically resolve these analytes is an advantage of FIGD-IC. (Note that previous studies have shown manual FIGD-IC to be in good agreement with OPA fluorimetry [20] for the determination of NH₃ [R² = 0.995, n = 24; slope = 0.904, intercept = 8 nM].)

Comparison with other analytical techniques

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Conclusion

The automated FIGD-IC system proposed in this paper permits the near real-time determination of MAs and NH₃ in seawater and aqueous extracts of atmospheric gaseous and particulate samples. Simultaneous analysis of nanomolar concentrations are possible without the need for derivatization schemes or lengthy preconcentration procedures. While the manual FIGD-IC system required constant supervision (for switching valves, injecting samples etc.), automation significantly reduced this need. Automation improved the analytical reproducibility and sample throughput of the system by increasing precision and accuracy valve switching and by reducing the scope for human error.

The flexible nature of the automation control system described allows considerable scope for broader applications of the FIGD-IC and also for other automated analytical applications, for example other natural waters such as rainwater and fresh waters.
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