Determination of ammonium in marine waters using a gas diffusion multicommuted flow injection system with in-line prevention of metal hydroxides precipitation

Sara Melo Oliveira, Teresa Isabel Marques da Silva Lopes, Ildikó Vargáné Tóth and António Osmaro Santos Silva Rangel*

Received 23rd July 2008, Accepted 6th October 2008
First published as an Advance Article on the web 20th October 2008
DOI: 10.1039/b812624k

A multi-commuted flow system coupled to a gas diffusion device was developed for the spectrophotometric determination of ammonium nitrogen in sea and estuarine waters. The efficiency of complexing agents to prevent precipitation of metallic hydroxides, due to the high pH value of the carrier solution, was studied. Under the optimised conditions, no interference was observed from different expected interfering ions as well as volatile amines. The proposed method provided the determination of NH_4^+ in concentrations ranging from 50 to 1000 μg L^{-1}, with detection and quantification limits of 18 and 35 μg L^{-1}, respectively. A determination rate of 20 h^{-1} was achieved, with good repeatability for 10 consecutive injections of sea and estuarine samples (relative standard deviations lower than 2.0%). Accuracy of the methodology was assessed through recovery assays in 10 samples and also by analysis of certified reference material.

1. Introduction

Ammonium ion represents one the most commonly used nutrients by marine phytoplankton and its ocurrence in coastal waters is directly related with the presence of ammonia in the natural atmosphere as well as in the air-sea interface.[1,2] Ammonia concentrations higher than 200 μg N L^{-1} can lead to direct toxicity, increased microbiological growth and oxygen depletion, resulting in disease and mortality of the marine population.[3] The rise in ammonia is one of the first indicators of toxicity in aquatic systems[4] and its impact can be significant on the eutrophication process. Therefore, ammonium ion is one of the most important parameters to control in fish farming plants.[5]

To cope with this increasing demand for ammonium determination in a large number of coastal water samples, several automatic methods based on flow procedures like segmented flow analysis (SFA) and flow injection analysis (FIA) were developed. The methods without a separation device employ spectrophotometric,[2,5–8] fluorimetric[9,10] or potentiometric[4] detection. To overcome the problems inherent to the nature of saline samples, these methods required matrix matching of the standard solutions with NaCl, artificial seawater or low nutrient seawater.[2,4–11] The level of interferences and matrix effects of saline waters can be significantly reduced by the introduction of a gas diffusion device in the flow system.[3,11–14] Some of these works also involved ion chromatography separation to quantify ammonia and methylamines.[13,14] Nevertheless, the inclusion of a chromatographic separation step compromises drastically the sample throughput, and contributes to a higher analysis cost.

In this work, an alternative method for the ammonium determination in marine waters exploiting the multicommutation concept,[15] and using low toxicity reagents, is presented. The multicommuted approach offers a high degree of automation, and low reagent consumption and waste generation, since solutions are introduced in the flow network only when they are required for the determination, returning to the respective reservoirs during the rest of the time of the analytical cycle. The developed method was based on the spectrophotometric monitoring of the colour change caused by a pH variation of a bromothymol blue solution, after diffusion of ammonia to the acceptor channel of the gas diffusion cell. In order to apply the system to samples with a wide salinity range, a systematic study with different solutions to eliminate interferences from coastal water samples was carried out. To accomplish this objective, the use of several complexing agents was evaluated, and an extensive study of possible interfering species was performed.

2. Experimental

2.1. Reagents and solutions

All the reagents were of analytical grade and all solutions were prepared in deionized water with a conductivity lower than 0.1 μS cm^{-1}. The carrier and acceptor solutions were prepared with previously boiled water.

The carrier solution was prepared weakly by dissolving 70 g of potassium sodium tartrate tetrahydrate and 20 g of sodium hydroxide in 1000 mL of water.

To prepare the bromothymol blue stock solution, 0.2002 g of the indicator was dissolved in 100.0 mL of ethanol. The 250.0 mL acceptor solution was prepared daily by appropriate dilution of the stock solution with water, resulting in a solution containing 0.06 mmol L^{-1} of bromothymol blue. The pH of this solution was adjusted to 6.8 with NaOH 0.25 mol L^{-1}.

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal. E-mail: aorangel@esb.ucp.pt; Fax: +351 225090351; Tel: +351 225580064

A 1000 mg L^{-1} stock standard solution of ammonium was prepared by dissolving in water 0.2967 g of NH_4Cl, previously dried for 2 h at 105 °C. This solution was adjusted to pH 2 with H_2SO_4, in order to avoid loss of analyte by its conversion to NH_3 and the final volume was adjusted to 100.0 mL. Working standard solutions were daily prepared from the above solution, by dilution in water, resulting in ammonium concentrations of 50.0, 100, 400, 700 and 1000 μg L^{-1}.

For the study of the complexing agents, solutions were prepared from dissolution in water of the required amounts of sodium citrate dihydrate, ethylenediaminotetracetic acid disodium salt dihydrate (EDTA), potassium sodium tartrate tetrahydrate, and boric acid.

A certified reference material QC RW1 (VKI Reference Materials) was prepared by dilution of 1.00 mL of the concentrated reference solution in 100.0 mL of the sample, according with the manufacturer's specifications.

All samples were collected in previously washed and dried polyethylene bottles and filtered through a 0.45 μm cellulose acetate membrane filter (Whatman). Samples were introduced in the flow system without the need to carry out any further treatment.

2.2. Instrumentation

All solutions were propelled by a Gilson (Villiers-le-Bel, France) Minipuls 3 multi-channel peristaltic pump equipped with PVC Gilson and Cole-Parmer (Illinois, USA) pumping tubes. All connections were made of PTFE tubing with 0.8 mm i.d. (W025953, Omnifit, Cambridge, United Kingdom) attached to Gilson end-fittings and connectors. A Perspex x-shaped joint (W018483, Omnifit) was used as confluence.

To control the direction of the solutions, five three-way solenoid valves (NResearch, 161 T031, New Jersey, USA) were used. The solenoid valves were operated by means of a power drive (CoolDriveTM, NResearch). A 386 personal computer (SD700, Samsung, Seoul, South Korea) equipped with an interface card (PCL-818L, Advantech) running a lab-made software written in QuickBasic 4.5 (Microsoft, USA) controlled the switching of the solenoid valves.

The gas diffusion device consisted of two separate Perspex blocks, pressed against each other by 6 screws.[16] A hydrophobic membrane (HVHP09050, Millipore Durapore®, Madrid, Spain) with a porosity of 0.45 μm was placed between the two blocks, being replaced weekly.

Absorbance measurements were carried out by a UV/Vis spectrophotometer (Unicam 8625, Cambridge, United Kingdom) set at 620 nm, equipped with a flow-through cell with 18 μL of internal volume and 1-cm flow path (Hellma 178.712-QS, Mullheim/Baden, Germany). A chart recorder (Kipp & Zonen BD111, Delft, Holland) connected to the spectrophotometer was used to register the analytical signals.

2.3. Manifold and flow procedure

The system components were arranged as shown schematically in Fig. 1. The protocol and time sequence used for the spectrophotometric determination of ammonium in sea and estuarine waters is given in Table 1.

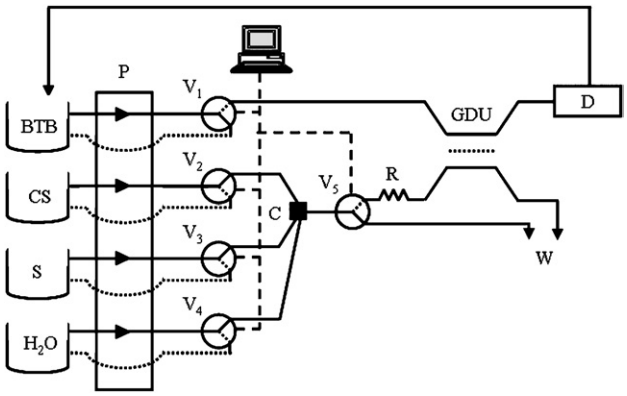

Fig. 1 Schematic configuration of the multicommuted flow system for the spectrophotometric determination of ammonium in marine waters. BTB: bromothymol blue 0.06 mmol L^{-1}, 1.7 mL min^{-1}; CS: potassium and sodium tartrate 70 g L^{-1} + NaOH 0.5 mol L^{-1}, 0.84 mL min^{-1}; S: sample or standard, 0.85 mL min^{-1}; H_2O, 0.82 mL min^{-1}; P: peristaltic pump; V_i: solenoid valve; C: confluence; R: reaction coil (100 cm); W: waste; GDU: gas diffusion unit; D: detector (620 nm). In the valves, the position "on" is represented by a continuous line and the position "off" is represented by a dotted line.

Table 1 Analytical protocol for the spectrophotometric determination of ammonium in sea and estuarine waters[a]

		Position of the commutation valves					
Step	Description	1	2	3	4	5	Time/s
1	Wash connection between valves V_3 and V_5	N	F	N	F	N	15
2	Wash connection between confluence and valve V_5	N	F	F	N	N	15
3	Wash acceptor and donor channels	F	N	F	F	F	20
4	Sample introduction	F	N	N	F	F	18
5	Sample introduction and stop BTB flow	N	N	N	F	F	12
6	Stop BTB flow	N	N	F	F	F	48
7	Propel BTB toward the detector. Signal registration	F	N	F	F	F	90

[a] The letters N and F correspond to positions "on" and "off" of the commutation valves, respectively.

The analytical cycle started with the manifold washing steps. First, the connection between valves V_3 and V_5 was washed and filled with the new sample (step 1). Then, the sample remaining in the tubing between the confluence and valve V_5 was removed with the carrier solution (step 2). Finally, donor and acceptor channels were washed with the respective solutions (step 3). The above-mentioned steps were only necessary when a new sample was introduced in the flow system. Afterwards, the sample was introduced in the system with the carrier solution, over 30 s (steps 4 and 5). To increase the efficiency of the diffusion process, the acceptor stream was stopped during 60 s, as soon as the donor solution reached the gas diffusion area (steps 5 and 6). In the last step, the acceptor solution with the diffused analyte was sent towards the detector, and then recirculated to the acceptor solution flask. This recycling was considered as this solution did

not suffer any significant alteration during the analytical cycle, as previously shown in a systematic study.[16] To ensure baseline stability the volume of the acceptor solution was relatively large (250 mL) and was maintained under constant stirring.

3. Results and discussion

3.1. Flow system and evaluation of complexing agents

The flow system configuration and physical parameters were studied before.[16] However, chemical parameters were studied and modified to enable application of the flow system to saline water samples. The final conditions are presented on Fig. 1.

Applying the previous flow system to saline samples, recovery percentages close to 120% were obtained (Table 2), and after several injections, a poor precision of the results was noticed. Moreover, the formation of a white precipitate inside the tubing was observed, leading to the deterioration of the flow system performance. This fact is probably due to the precipitation of some metal ions in alkaline medium, present in high levels in saline waters, as metal hydroxides, such as $Ca(OH)_2$ and $Mg(OH)_2$. Despite this precipitation occurs in the donor channel and so the colour detection is not affected, the decrease in repeatability affects the overall method performance.

Table 2 Results obtained (μg L^{-1} of NH_4^+) in recovery studies with sea and estuarine waters, using the multicommuted flow system developed for fresh water samples[16]

	$[NH_4^+]$ (μg L^{-1})		
Sample	Added	Found[a]	Recovery[a] (%)
Estuarine water	0	175 ± 6	—
	50.0	219 ± 3	88.0 ± 5.0
	200	415 ± 8	120 ± 4
	500	753 ± 17	116 ± 3
	800	1175 ± 5	125 ± 1
Seawater	0	< LOQ (25.6 ± 2.1)	—
	50.0	58.9 ± 3	118 ± 6
	200	230 ± 0	115 ± 0
	500	598 ± 8	120 ± 2
	800	989 ± 9	124 ± 1

[a] n = 3.

To overcome these problems, preliminary off-line studies were carried out to evaluate the efficiency of different complexing agents. However, addition of this complexing agents to the NaOH solution caused a significant decrease on pH of the carrier solution. In order to maintain pH values above 12 and ensure total conversion of ammonium to ammonia, NaOH concentration was increased to 0.5 mol L^{-1}. Evaluation of complexing agents was carried out by adding 2.00 mL of saline samples to 2.00 mL of a solution containing NaOH 0.5 mol L^{-1} plus the complexing agent, followed by the measurement of the absorbance of the formed mixture at 420 nm. In the first set of experiments, several concentrations of sodium citrate, EDTA and potassium sodium tartrate were tested separately.

The results, presented in Fig. 2 revealed that the use of EDTA or potassium sodium tartrate in concentrations of 70 g L^{-1} prevented occurrence of precipitation. However, citrate did not prevent precipitation, even when a concentration of 180 g L^{-1} was employed. Citrate is typically applied in ammonia determination by the indophenol blue method at a pH value of 10.5. However, in this method, pH values higher than 12 are required to promote the maximum conversion of ammonium to ammonia, and consequently increase the diffusion efficiency. According to others,[13] the ability of citrate to chelate Mg^{2+} and Ca^{2+} and thereby inhibit the precipitation of these metals as hydroxides,

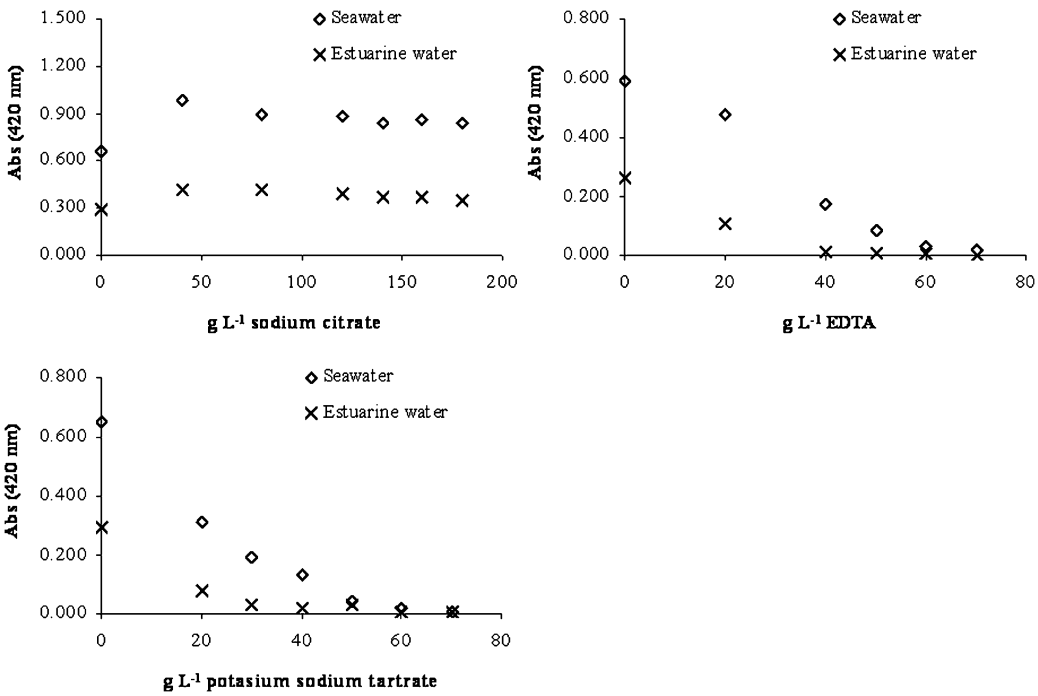

Fig. 2 Evaluation of different complexing agents on precipitation prevention in sea and estuarine waters, in alkaline medium.

was only effective at pH values below 11. This was confirmed by measuring the pH of the solutions containing sample, hydroxide and citrate. For solutions containing all citrate concentrations tested, pH values > 12.7 were obtained.

In a second stage, studies to evaluate the efficiency of the combination of EDTA with citrate were also carried out, using fixed EDTA concentrations of 30 and 40 g L^{-1}, while the citrate concentration was varied up to 180 g L^{-1}. Results revealed (Fig. 3) that the use of citrate combined with EDTA does not prevent precipitation completely, although a higher efficiency of citrate on precipitation impediment was observed when this complexing agent was combined with a concentration of 40 g L^{-1} of EDTA.

Considering an International Standard,[17] the efficiency of a solution composed by EDTA plus boric acid was studied. In a first approach, fixing EDTA concentration to 30 g L^{-1}, the concentration of boric acid was studied in a range of 0–20 g L^{-1}. Afterwards, using a concentration of 16 g L^{-1} of boric acid, the EDTA concentration was evaluated from 0 to 40 g L^{-1}. Results, depicted in Fig. 4, revealed that the addition of boric acid allows the reduction of EDTA concentration. Taking in consideration the overall results, three efficient complexing agents were found to be successful to prevent precipitation of metal hydroxides in saline waters: EDTA, tartrate and EDTA in combination with boric acid. However, tartrate is a more environmental friendly option and was referred by the U.S. Environmental Protection Agency[18] as an advantageous alternative to EDTA. Therefore, tartrate was selected as the complexing agent and its concentration was studied in the flow system. This evaluation was carried out by adding different concentrations of tartrate to the carrier solution containing NaOH 0.5 mol L^{-1}. Tartrate concentrations ranging from 70 to 120 g L^{-1} were studied. Baseline instability was observed with the tartrate concentration increase. Thus, a tartrate concentration of 70 g L^{-1} was chosen for further work.

3.2. Interference study

The selection of the potential interferents to be involved in this study was based on the content usually found in this type of waters. In order to assess the effect of each species, a known concentration of the possible interfering compound was added to a standard solution containing a concentration of 100 μg L^{-1} of ammonium. A species was considered to interfere if a relative deviation higher than 5%[19] of the peak signal for an ammonium standard of 100 μg L^{-1} was obtained. The relative deviations, indicated in Table 3 demonstrate that none of the tested species interfere in the methodology, even when present in concentrations higher than those expected in seawaters.

3.3. Figures of merit

The linear range for the spectrophotometric determination of ammonium in saline waters by the presented method was from 50.0 to 1000 μg NH_4^+ L^{-1} (recorder output presented in Fig. 5). Expressing ammonium concentration in mg L^{-1}, the typical calibration curve was as follows: A = 0.524 (±0.016) [NH_4^+] + 0.176 (±0.034), R = 0.9993 (±0.0003). Reproducibility was

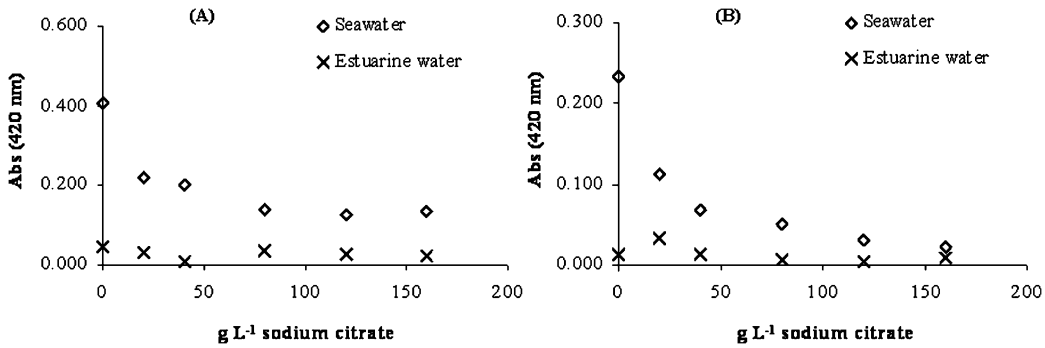

Fig. 3 Study of the efficiency of different concentrations of citrate on precipitation prevention of coastal waters in alkaline medium, using EDTA concentrations of 30 (A) and 40 g L^{-1} (B).

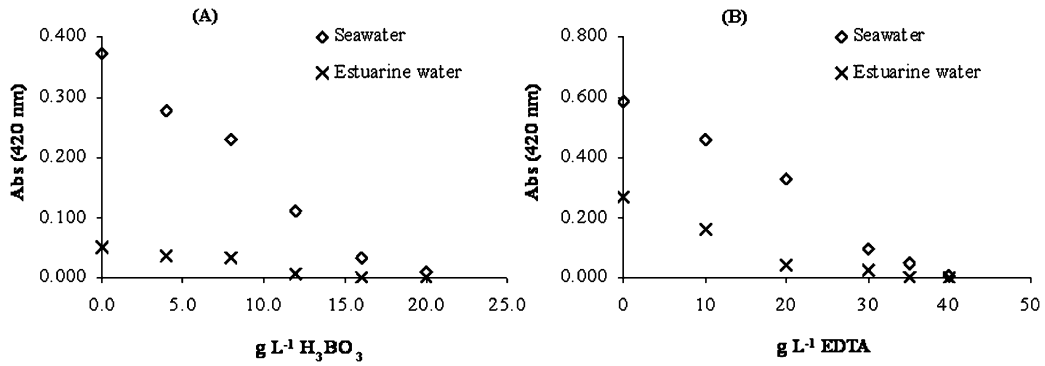

Fig. 4 Study of the efficiency of different concentrations of boric acid on precipitation prevention of coastal waters in alkaline medium, using 30 g L^{-1} of EDTA (A) and different concentrations of EDTA, using 16 g L^{-1} of H_3BO_3 (B).

Table 3 Study of interferences, respective concentration tested and relative deviation obtained, using a standard solution containing 100 μg NH_4^+ L^{-1}[a]

Species studied	Concentration tested	Relative deviation (%)	Expected concentration in seawater
Methylamine (nmol L^{-1})	800	3.4	587[20]
Dimethylamine (nmol L^{-1})	2000	2.7	360[12]
Trimethylamine (nmol L^{-1})	1000	2.4	514[20]
Ethylamine (nmol L^{-1})	1500	3.0	1400[20]
Diethylamine (nmol L^{-1})	3000	2.1	71[20]
Triethylamine (nmol L^{-1})	3000	4.3	N/A
Triethanolamine (nmol L^{-1})	8000	−3.1	N/A
Urea (μmol L^{-1})	10	−0.5	3[20]
HCO_3^- (mg L^{-1})	200	4.1	140[21]
CO_3^{2-} (mg L^{-1})	100	4.4	N/A
Cu^{2+} (mg L^{-1})	1	−2.2	0.001[22]
Al^{3+} (mg L^{-1})	1	0.6	0.001[22]
Fe^{3+} (mg L^{-1})	1	1.1	0.004[22]
Hg^{2+} (mg L^{-1})	1	2.2	0.0002[22]
S^{2-} (mg L^{-1})	10	1.6	0.096[23]
Ca^{2+} (mg L^{-1})	500	0.6	412[21]
Mg^{2+} (mg L^{-1})	1500	1.9	1284[21]
K^+ (mg L^{-1})	500	4.0	399[21]
Sr^{2+} (mg L^{-1})	20	3.7	7.94[21]
SO_4^{2-} (mg L^{-1})	3000	4.5	2712[21]
Br^- (mg L^{-1})	100	0.5	67[21]
H_3BO_3 (mg L^{-1})	50	2.0	25[21]
F^- (mg L^{-1})	10	−0.5	1.3[21]

[a] N/A - not available.

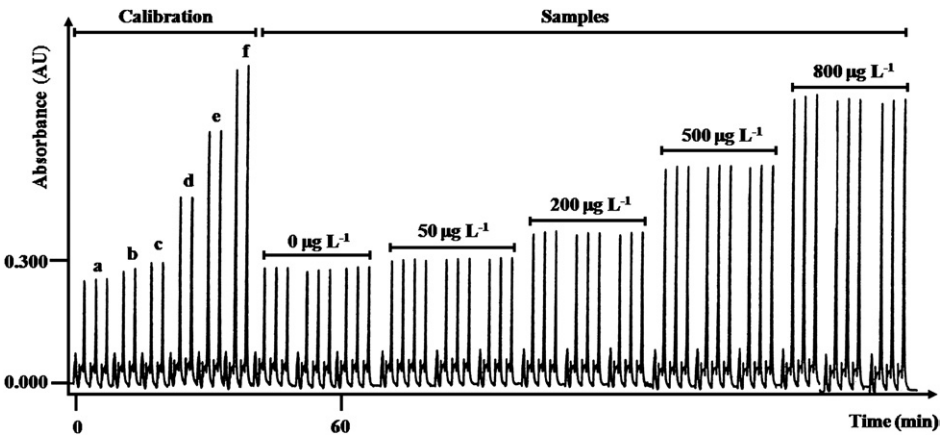

Fig. 5 Recorder output obtained in the spectrophotometric determination of ammonium, corresponding to the injection of a set of standard solutions (a = 0, b = 50, c = 100, d = 400, e = 700, and f = 1000 μg L^{-1}), and recovery assays with addition of 50, 200, 500 and 800 μg NH_4^+ L^{-1}, using a seawater sample.

assessed from the standard deviation of the parameters of 12 calibration curves carried out over a period of one month. Each calibration curve incorporated 6 ammonium standards, injected in duplicate.

The repeatability of the procedure was assessed from the relative standard deviation (RSD) calculated from ten consecutive injections of the certified reference material prepared in four different samples, providing RSD values less than 2.0%.

The detection and quantification limits were calculated as recommended by the IUPAC.[24] Detection and quantification limits of 18 and 35 μg NH_4^+ L^{-1} were obtained, respectively.

The determination throughput was calculated considering the time spent in all steps of the analytical cycle. A time of 168 s was needed for each determination, and a 50 s washing time was required between different samples, resulting in a determination frequency of 20 h^{-1}.

3.4. Application of the flow system to real samples

After establishing the working conditions, the system was applied to analysis of estuarine and seawater samples.

Table 4 Results obtained (μg L^{-1} of NH_4^+) by the proposed system in recovery studies using estuarine (1–3) and sea (4–10) waters

Sample	Sample characteristics	$[NH_4^+]$ (μg L^{-1}) Added	Found[a]	Recovery[a] (%)
1	pH = 7.71 S = 11.2[b]	0	61.4 ± 1.9	—
		50	111 ± 3	98.7 ± 5.5
		200	250 ± 5	94.5 ± 2.3
		500	560 ± 2	99.8 ± 0.5
		800	858 ± 4	99.6 ± 0.5
2	pH = 7.77 S = 9.6[b]	0	49.7 ± 5.6	—
		50	98.0 ± 2.9	96.5 ± 5.8
		200	247 ± 9	98.8 ± 4.4
		500	562 ± 7	102 ± 1
		800	853 ± 11	100 ± 1
3	pH = 7.93 S = 10.0[b]	0	75.6 ± 1.5	—
		50	125 ± 1	99.0 ± 2.2
		200	267 ± 2	95.7 ± 1.1
		500	570 ± 3	98.9 ± 0.6
		800	872 ± 5	99.5 ± 0.6
4	pH = 7.69 S = 34.2[b]	0	81.5 ± 2.2	—
		50	132 ± 1	101 ± 2
		200	283 ± 9	101 ± 4
		500	579 ± 9	99.5 ± 1.7
		800	884 ± 6	100 ± 1
5	pH = 7.82 S = 32.4[b]	0	70.0 ± 2.4	—
		50	120 ± 2	101 ± 5
		200	274 ± 3	102 ± 2
		500	587 ± 8	103 ± 2
		800	889 ± 8	102 ± 1
6	pH = 8.00 S = 34.8[b]	0	38.5 ± 1.3	—
		50	88.1 ± 1.4	99.2 ± 2.8
		200	228 ± 4	94.9 ± 1.8
		500	520 ± 2	96.4 ± 0.5
		800	830 ± 5	98.9 ± 0.6
7	pH = 8.00 S = 33.0[b]	0	119 ± 3	—
		50	169 ± 2	99.8 ± 3.4
		200	313 ± 4	96.9 ± 2.0
		500	602 ± 5	96.7 ± 1.0
		800	909 ± 6	98.9 ± 0.8
8	pH = 7.96 S = 33.6[b]	0	88.7 ± 2.2	—
		50	141 ± 2	105 ± 5
		200	281 ± 5	96.3 ± 2.5
		500	604 ± 10	103 ± 2
		800	891 ± 8	100 ± 1
9	pH = 7.97 S = 31.9[b]	0	< LOQ (21.9 ± 2.5)	—
		50	51.5 ± 2.1	103 ± 4
		200	196 ± 6	98.1 ± 3.0
		500	481 ± 9	96.2 ± 1.9
		800	776 ± 12	96.9 ± 1.5
10	pH = 8.00 S = 32.4[b]	0	61.1 ± 4.3	—
		50	112 ± 2	102 ± 3
		200	256 ± 5	97.6 ± 2.5
		500	564 ± 7	100 ± 1
		800	866 ± 6	100 ± 1

[a] n = 9. [b] Salinity.

Ammonium concentration of the samples was calculated by interpolation in the previously established calibration curve.

Conductivity and pH measurements were carried out for each sample. Salinity values of the samples were calculated based on the practical salinity scale,[25] using the conductivity values and the measurement temperature (21 °C). These values are presented in Table 4.

The accuracy of the proposed method was assessed by recovery tests and analysis of certified reference material.

Recovery studies consisted in the addition of 1.00 mL of standard solutions of ammonium to volumetric flasks of 25.00 mL, adjusting the volume with the respective sample. Ammonium concentrations of 50.0, 200, 500 and 800 g L^{-1} were added to all samples. Each concentration level was prepared in triplicate, and all assays were analysed also in triplicate, resulting in 9 peaks for each concentration level.

Recoveries between 94.5 and 105% were obtained (Table 4). Statistical test (t-test) was used to evaluate if the mean recovery value did not significantly differ from 100%.[19] The results demonstrated that the recovery values were not statistical different from 100% at a 95% confidence level, since the calculated t-value (1.81) was lower than the correspondent t-critical value (2.02) (n = 40), thus indicating the absence of systematic errors.

Concerning the certified reference material QC RW1, the certified value is 100.9 ± 1.3 μg $N–NH_4^+$ L^{-1} and the acceptance limit is 100.2–101.5 μg $N\text{-}NH_4^+$ L^{-1}. Each sample was injected in the flow system ten times. Analysis of the certified sample material prepared using samples 2, 3, 5 and 6 provided concentrations of 100.7 ± 1.3, 101.2 ± 2.0, 101.1 ± 1.6, and 100.5 ± 1.6 μg L^{-1} of $N–NH_4^+$, respectively. For all samples tested, the values obtained were within the acceptance limit specified by the reference material.

4. Conclusions

Compared to the previously described automatic flow procedures, the present method offers an environmentally friendly alternative, since avoids the use of toxic reagents, such as OPA and those used by the indophenol blue method. Additionally, since the BTB solution is recirculated, only 3.2 mL of effluent is produced per determination. The inexpensive instrumentation, easy manipulation and high versatility of the flow system represent valuable qualities concerning its implementation in routine analysis laboratories. Moreover, the small dimensions and consequent portability of the system make it suitable for in-situ determination.

The majority of the flow methods for ammonium determination in sea or estuarine waters require the preparation of the working standard solutions in NaCl, artificial seawater or LNSW. In the herein described method, the standards are prepared in deionized water. Despite the absence of salinity in the standard solutions used for calibration, accurate results were attained when samples with salinities ranging from 9.6 to 34.8 were analysed.

Acknowledgements

The authors thank Fundação para a Ciência e a Tecnologia (FCT) for financial support through Project PTDC/AMB/64441/2006. Sara Oliveira and Ildikó Tóth acknowledge financial support from FCT and FSE (III Quadro Comunitário) through the grants SFRH/BD/23782/2005 and SFRH/BPD/5631/2001, respectively.

References

1 J. N. Galloway, *Environ. Pollut.*, 1998, **102**(S1), 15–24.
2 Q. P. Li, J. Z. Zhang, F. J. Millero and D. A. Hansell, *Mar. Chem.*, 2005, **96**(1–2), 73–85.

3 S. M. Gray, P. S. Ellis, M. R. Grace and I. D. McKelvie, *Spectrosc. Lett.*, 2006, **39**(6), 737–753.
4 E. A. Moschou, U. A. Lasarte, M. Fouskaki, N. A. Chaniotakis, N. Papandroulakis and P. Divanach, *Aquacult. Eng.*, 2000, **22**(4), 255–268.
5 H. Muraki, K. Higuchi, M. Sasaki, T. Korenaga and K. Toei, *Anal. Chim. Acta*, 1992, **261**(1–2), 345–349.
6 M. Jodo, K. Kawamoto, M. Tochimoto and S. C. Coverly, *J. Autom. Chem.*, 1992, **14**(5), 163–167.
7 A. Tovar, C. Moreno, M. P. Mánuel-Vez and M. García-Vargas, *Anal. Chim. Acta*, 2002, **469**(2), 235–242.
8 F. Azzaro and M. Galletta, *Mar. Chem.*, 2006, **99**(1–4), 191–198.
9 R. Kérouel and A. Aminot, *Mar. Chem.*, 1997, **57**(3–4), 265–275.
10 A. Aminot, R. Kérouel and D. Birot, *Water Res.*, 2001, **35**(7), 1777–1785.
11 R. J. Watson, E. C. V. Butler, L. A. Clementson and K. M. Berry, *J. Environ. Monit.*, 2005, **7**(1), 37–42.
12 P. O. Hall and R. C. Aller, *Limnol. Oceanogr.*, 1992, **37**(5), 1113–1119.
13 S. W. Gibb, R. F. C. Mantoura and P. S. Liss, *Anal. Chim. Acta*, 1995, **316**(3), 291–304.
14 S. W. Gibb, J. W. Wood, R. Fauzi and C. Mantoura, *J. Autom. Chem.*, 1995, **17**(6), 205–212.
15 B. F. Reis, M. F. Giné, E. A. G. Zagatto, J. L. F. C. Lima and R. A. Lapa, *Anal. Chim. Acta*, 1994, **293**(1–2), 129–138.
16 S. M. Oliveira, T. I. M. S. Lopes, I. V. Tóth and A. O. S. S. Rangel, *Anal. Chim. Acta*, 2007, **600**(1–2), 29–34.
17 International Organization for Standardization, *Water quality - Determination of ammonium nitrogen - Method by flow analysis (CFA and FIA) and spectrophotometric detection*, 2005, ISO 11732, Geneva, Switzerland.
18 http://www.uga.edu/~sisbl/epa-nh4.html.
19 J. C. Miller, J. N. Miller, *Statistics for Analytical Chemistry*, 3rd ed., Ellis Horwood, New York, USA, 1993, pp. 53–54.
20 X. H. Yang, C. Lee and M. I. Scranton, *Anal. Chem.*, 1993, **65**(5), 572–576.
21 http://www.ocean.washington.edu/courses/oc400/Lecture_Notes/CHPT4.pdf.
22 http://www.seafriends.org.nz/oceano/seawater.htm.
23 R. Al-Farawati and C. M. G. vandenBerg, *Mar. Chem.*, 1997, **57**(3–4), 277–286.
24 L. A. Currie, *Pure Appl. Chem.*, 1995, **67**(10), 1699–1723.
25 L. S. Clesceri, A. E. Greenberg, A. D. Eaton, (Eds.), *Standard methods for the examination of water and wastewater*, 20th ed., American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC, USA, 1998, Method 2520.

