Talanta 118 (2014) 186–194

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A highly reproducible solenoid micropump system for the analysis of total inorganic carbon and ammonium using gas-diffusion with conductimetric detection

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ARTICLE INFO

Article history:
Received 30 May 2013
Received in revised form
30 September 2013
Accepted 3 October 2013
Available online 9 October 2013

Keywords:
Multipumping flow system
Solenoid micropumps
Gas difussion
Total inorganic carbon
Ammonium
Conductimetric detection

ABSTRACT

In this work, a simple, economic, and miniaturized flow-based analyzer based on solenoid micropumps is presented. It was applied to determine two parameters of high environmental interest: ammonium and total inorganic carbon (TIC) in natural waters. The method is based on gas diffusion (GD) of CO_2 and NH_3 through a hydrophobic gas permeable membrane from an acidic or alkaline donor stream, respectively. The analytes are trapped in an acceptor solution, being slightly alkaline for CO_2 and slightly acidic for NH_3. The analytes are quantified using a homemade stainless steel conductimetric cell. The proposed system required five solenoid micro-pumps, one for each reagent and sample. Two especially made air bubble traps were placed down-stream of the solendoid pumps, which provided the acceptor solutions, by this increasing the method's reproducibility. Values of RSD lower than 1% were obtained. Achieved limits of detection were 0.27 μmol L^{-1} for NH_4^+ and 50 μmol L^{-1} for TIC. Add-recovery tests were used to prove the trueness of the method and recoveries of 99.5 $\pm$ 7.5% were obtained for both analytes. The proposed system proved to be adequate for monitoring purpose of TIC and NH_4^+ due to its high sample throughput and repeatability.

1. Introduction

Ammonium and carbon dioxide are analytes of special interest in environmental monitoring since they are involved in nearly all biological processes, are transferable between all environmental compartments, such as atmosphere, hydrosphere, biosphere, and pedosphere. Their concentration levels in all compartments are influenced further by human activity. In natural waters, ammonium is an important nitrogen pool. It is the product of bacterial nitrogen fixation, decomposition of organically bound nitrogen, direct nitrogen elimination, and can easily be taken up by phytoplankton [1].

Anthropogenic emissions, especially originated from agriculture, industry, and urban waste contribute significantly to the global budget of atmospheric ammonia with estimation over 50% or 41 Tg of N per year [2]. This problematic has created the interest in effective NH_3 monitoring and analysis in different aqueous compartments including groundwater, surface water, and seawater.

Carbon dioxide is a key component of the carbon cycle [3] and is involved in the metabolism of all organisms [4]. It plays an important role in the occurrence of the photosynthesis and pH of natural waters [5]. Increasing atmospheric CO_2 through human activity with about 24% over the last 50 years especially decrease the surface seawater pH (ocean acidification) and by this increases the solubility of carbonates, which affects the calcification process of mollusk's larvae [4]. For this reason, the development of methods for free CO_2 and total inorganic carbon (TIC) monitoring in natural waters and seawater is of pressing interest.

Gas diffusion (GD) is the separation technique "per excellence" for the determination of volatile species in complex matrix [6], while flow techniques (FT) such as flow injection analysis (FIA) [7], sequential injection analysis (SIA) [8], and multi-syringe flow injection analysis (MSFIA) [9] are the ideal tools to automate GD. Only by this combination, the construction of simple but yet reliable analyzer systems based on GD has become feasible achieving high selectivity, promising sensitivity, excellent precision, and rapidness [10–13].

Solenoid micro-pumps (SMP) are an economic alternative to the syringe and peristaltic pumps typically used for FIA, SIA, and MSFIA. A FT based on SMP has firstly been proposed and characterized Lapa et al. [14], denoted since then as multi-pumping flow systems (MPFS), although prior and successful use in FT has been reported [15].

SMP provide a semi-continuous flow with a highly pronounced pulsation causing intermediate turbulent conditions in the manifold. This improves the mixing efficiency compared to the former

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http://dx.doi.org/10.1016/j.talanta.2013.10.005

FT operating mainly in laminar flow conditions [16]. Moreover, SMP enable a high versatility and portability of the flow system due to their small size, independent operation, and their low energy consumption [12,17].

For the determination of NH_4^+ using GD, the sample is injected into, or merged with, an alkaline solution and the formed NH_3 diffuses across a gas-permeable membrane and is retained into an acidic acceptor solution. For the determination of TIC, the same principle is valid but the sample is mixed with acid and the formed CO_2 is trapped into an alkaline acceptor solution. The trapped analytes are mostly determined by spectrophotometry [10,11,13,18], by potentiometry [19], or by conductometry [3,12,20–23]. Here, conductometry has some important advantages over the other detection techniques without loss of sensitivity. First, the required equipment, i.e. conductimeter and conductometric flow cell is simple, economic and can easily be miniaturized. Second, reagents and waste are diluted mineral acids and sub-milliliter amounts are required per analysis, so that the final method can be considered as environmentally friendly.

Combining the advantages of the MPFS as flow technique, GD as separation method, and conductometry as detection method, a further denoted C-GD-MPFS system has been developed and applied to the determination of both $NH_4^{\pm}$ and TIC in different water samples.

This work was based on a previous one developed for the determination of ammonium with C-GD-MSFIA. Here, we intended to improve the former method in respect of rapidness, reproducibility, sensitivity, portability, and able to measure both ammonium and TIC in coastal waters. For these reasons, the MPFS technique was used instead of MSFIA.

2. Materials and methods

2.1. Reagents and solutions

All reagents and solutions were prepared with ultrapure Milli-Q water (Millipore SAS, Molsheim France). All reagents were purchased from Scharlau S.A. (Barcelona, Spain). A Teflon membrane provide by LACHAT (Loveland, USA) was used for GD [24].

For ammonium determination, the donor reagent was a mixture of sodium hydroxide ($25\ \text{mmol}\ L^{-1}$) and sodium citrate dihydrate ($200\ \text{mmol}\ L^{-1}$). A stock solution of $0.01\ \text{mol}\ L^{-1}$ HCl was prepared using hydrochloric acid 37% v/v. It was used to prepare the acceptor solution of $25\ \mu\text{mol}\ L^{-1}$ HCl. A $0.02\ \text{mol}\ L^{-1}$ NH_4Cl stock solution was prepared by dissolving 0.1070 g NH_4Cl in 100 mL of ultrapure water.

For TIC determination, concentrate sulfuric acid (reagent grade) was used to prepare a $5\ \text{mmol}\ L^{-1}$ of H_2SO_4 solution as donor reagent. A stock solution of $1\ \text{mol}\ L^{-1}$ of NaOH was prepared by dissolving an appropriate amount of NaOH (reagent grade). It was used to prepare the acceptor solution, $20.0\ \text{mmol}\ L^{-1}$. A $0.2996\ \text{mol}\ L^{-1}$ of $NaHCO_3$ stock solution was prepared by dissolving 2.516 g of dried $NaHCO_3$ in 100 mL of Milli-Q water.

All standard solutions were prepared daily by appropriated dilution of the respective stock solution with Milli-Q water. A commercial standard solution of $0.1\ \text{mol}\ L^{-1}$ HCl from Scharlau SA. was used for total alkalinity determination by potentiometric titration of 100 mL of sample at room temperature [25].

2.2. Samples

Drinking water, coastal seawater, well water, and tap water samples were used to evaluate the proposed methods. They were measured directly and as soon as possible, without any further pretreatment such as filtration to avoid loss of analyte.

Four samples of coastal surface seawater (coastal water 1–4) from Palma Beach (Mallorca, Balearic Islands, Spain) were collected in dark glass bottles. They were hermetically sealed avoiding trapping of any air bubbles, and immediately refrigerated, transported to the lab, and measured.

Two different well water samples were collected in the same way than the seawater samples and likewise measured immediately. Two different commercial drinking waters with medium (Drinking water 1) and low (Drinking water 2) mineralization and a tap water sample were used further. The freshwater samples were only used to evaluate the determination method for TIC.

Samples were simultaneously measured with the proposed method for TIC determination and the potentiometric titration for total alkalinity quantification [25].

2.3. Instrumentation and manifold

The analyzer manifold is given in Fig. 1. All parts in liquid contact were of polymers resistant to the used chemicals, namely

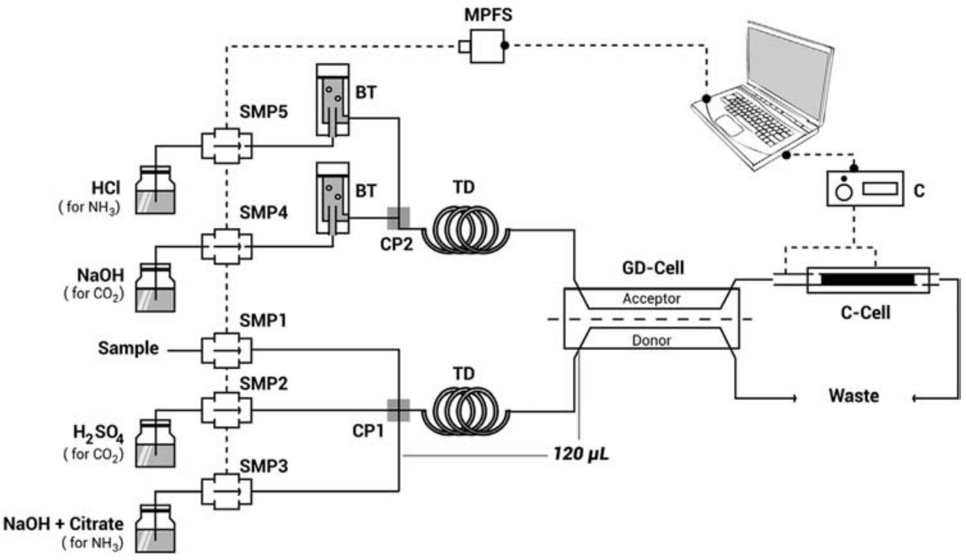

Fig. 1. Manifold of used analyzer system for TIC and NH_4^+ determination. Elements: SMP, Micropumping; MPFS, multipumping module; C, Conductimeter; C-Cell, Conductimetric Cell (Tubino's electrodes); GD-Cell, Gas-Diffusion Cell; BT, bubble trap device; CP1, 4-way confluent point; CP2, 3-way confluent point; M, Membrane (LACHAT Instrument); TD, Tygon pulsation dumpers; W: Waste.

PMMA (connectors and GD-Cell), PEEK (pump manifold), Tygon® (pulsation dumpers), and PTFE (connecting tubes). All PTFE tubes were of an inner diameter of 0.8 mm.

One SMP of 25 μL (Takasago, P/N090SP-12-25, SMP 1) was used for the sample while all other four were of a nominal volume of 20 μL (Takasago, P/N090SP-12-20, SMP 2–5) and used to propel the reagents. All SMP were from BIO-CHEM FLUIDICS (Boonton, NJ, USA). Valves calibration was done by weighting the dispense volume of 100 pulses and gave pulse volumes of 22.1 μL for SMP 1, 21.3 μL for SMP 2, 18.9 μL for SMP 3, 20.9 μL for SMP 4, and 19.9 μL for SMP 5. The SMPs were controlled by a MultiPumping module from Sciware System SL (Bunyola, Spain).

SMP 1 (sample), SMP 2 (acid solution: 5 mmol $L^{-1}H_2SO_4$), and SMP 3 (alkaline solution: 25 mmol L^{-1} of NaOH and 200 mmol L^{-1} of sodium citrate) were connected by a 4-way confluence point (CP1, Sciware Systems SL) and further connected to the donor channel of the GD-cell via a mixing coil made of a 40 cm long Tygon® tube (orange/orange 0.89 mm id) acting further as pulsation dumper (TD). SMP 4 (alkaline solution: 20 mmol L^{-1} NaOH) and SMP 5 (slightly acidic solution: 25 μmol L^{-1} HCl) were connected by a 3-way confluence point (CP2) and further connected to the acceptor channel via another pulsation damper. Then, the acceptor channel outlet of the GD-cell was connected to the C-cell (Sciware Systems SL) and further to waste. Two homemade bubble traps were placed between SMP 4 and SMP 5 and C2 with a scheme of the bubble trap (BT) given in Fig. 2(I).

The bubble trap consisted of a PMMA piece (A) with a cylindrical cavity (B) into which the flow enters from above and flows out via a lateral opening. By placing the outlet of the ingoing tube (D) higher than the outlet (E), air bubbles cannot pass the trap but float at the top. A screw cap (C) allowed the release of the trapped air volume, generally required once per day. The trapped air inside further acted as a pulsation damper.

Both the GD-cell and the C-cell were described in details elsewhere [20]. Schemes of both cells are depicted in Fig. 2(II) and (III). The GD-cell was made of two identical rectangular PMMA blocks, each one showing a U-shape flow channel of 180 μL dead volume. They were placed onto each other, holding in between a hydrophobic GD membrane from LACHAT Instrument (Loveland USA), separating the donor flow, i.e. the mixture of sample and the donor reagent, from the acceptor flow. Six stainless steel screws were used for fixation of the GD-cell. It was then connected to the manifold in a way that a countercurrent flow between donor and acceptor was achieved. It was observed that the membrane was usable for 2 weeks or more. It proves to be very rigid and did not need to be replaced using standards. However, when samples with high amount of organic matter or suspended particles were used the membrane was change every day in order to avoid the clog of the membrane pores.

The cell acceptor channel was connected downstream to the homemade conductometric cell, via a 12 cm long PTFE tube. Basically, it consists of two stainless steel tubes, one thinner and longer than the other one. They were placed concentrically one inside the other. The acceptor solution enters through the thinner tube but in the middle section the tube is blocked and forces the solution to flow into the cavity between both tubes through a small hole and back into the inner tube through a second hole at the outlet. The cavity between both the tubes acts as the conductometric cell and each tube as one of the electrodes. The relatively large electrode surfaces and the very short distance between them (0.2 mm) lead to a low cell constant and thus a high sensitivity. The cell constant was 0.06 cm^{-1}. It was determined using a KCl 0.01 mol L^{-1} conductivity standard solution from Scharlau SA of 1413 μS cm^{-1} at 25 °C.

The C-cell design was introduce in 2008 by Elsholz et al. [26] and the C-cell used was build by the authors as described in Ref. [20]. The C-cell, was connected to a commercial Crison 525 conductimeter (Crison Instruments S.A) with alligator clips. This instrument had six working ranges from 20.00 μS cm^{-1} to 2000 mS cm^{-1}, the range up to 20.00 mS with a resolution of 0.01 mS cm^{-1} was selected for TIC measurement, and the range up to 200.0 μS cm^{-1}, with a resolution of 0.1 μS cm^{-1} for NH_4^+. These ranges were

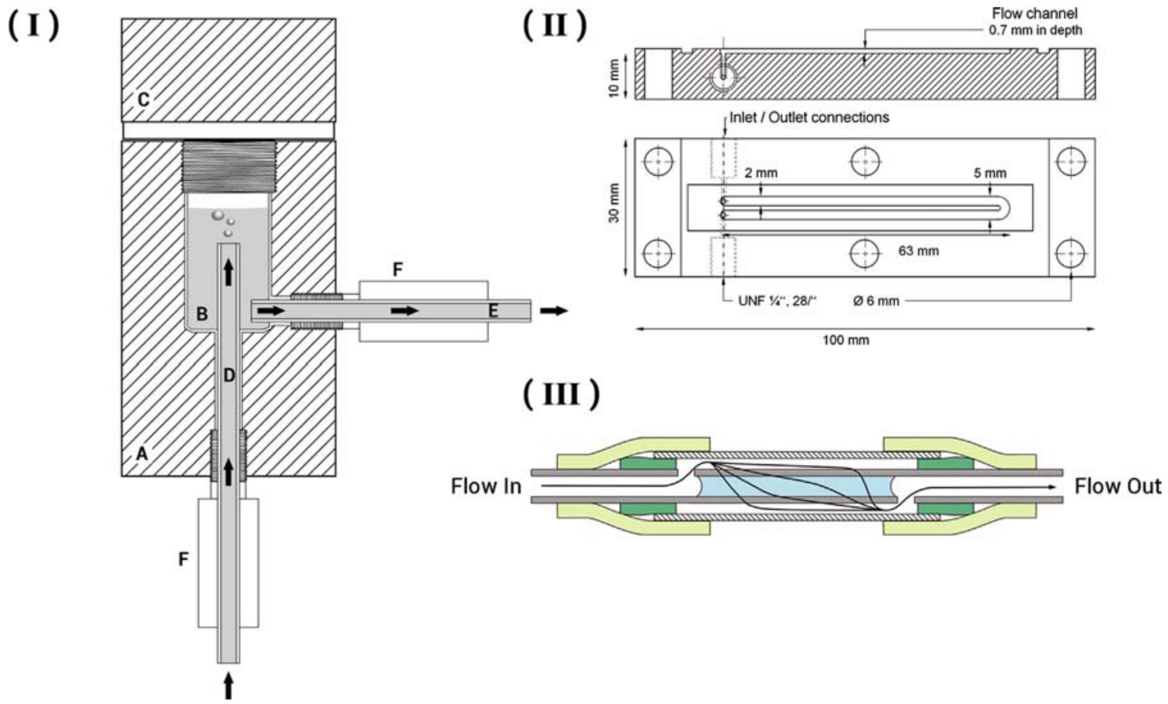

Fig. 2. Detail drawings of manifold components. (I) Scheme of air bubble removing device (Sciware Systems SL), made of PMMA, includes indications of the liquids directions: A, PMMA piece; B, cylindrical cavity; C, screw cap; D inlet tube; E, outlet tube; and F, sealed screws. (II) Technical drawing of one of the two identical parts of the used gas diffusion cell (Sciware Systems SL), made of PMMA. (III) Scheme of conductometric flow cell (Sciware Systems SL) following design. Both (II) and (III) are described elsewhere [19].

selected and baselines were adjusted to almost the maximum values of these scales in order to obtain the highest sensitivity and peak height. It was proven that the laboratory temperature was sufficiently stable, and samples were placed at laboratory temperature until thermal equilibrium before the measurement, so that in-system temperature correction was not required. As the analytical signal, the conductivity multiplied by factor (-1) was used to facilitate data processing.

A 45 positions autosampler from Crison Instruments S.A. was used for sample analysis and was connected to the inlet of SMP 1.

A pH-meter Crison micro pH 2002, (Crison Instruments S.A.) and a glass electrode were used for sample characterization and for potentiometric titration of alkalinity [25]. This titration together with the sample pH and temperature were used for DIC estimation according with [27], The estimated DIC was used as a comparative method for TIC.

2.4. Software

The software package AutoAnalysis 5.0 from Sciware Systems SL was used for instrumental control as well as for data acquisition and treatment. The basic software protocol is adaptable to each instrument by incorporating dynamic link libraries, which are able to communicate and control the individually assembled instruments (in this case the MultiPumping module, the autosampler, and the conductimeter) through a RS232 interface. User-friendly software tools for method development include loops, procedures, variables, user inquiries, waiting steps, and definition of conditional inquiries, among others.

2.5. Analytical protocol

The analytical protocol for TIC measurement is summarized in Table 1 and explained in details below. The analytical protocol for $NH_4^{\pm}$ determination is basically the same but changing SMP2 and SMP4 for SMP3 and SMP5, respectively. Further details or modifications are given in Table 1.

At sample change, the sample tube and the donor channel of the GD-cell were cleaned by propelling 1.2 mL of sample via SMP 1. Simultaneously, the acceptor solution inside the system was renewed by pumping 0.12 mL with SMP 4 (Step 1).

Before every injection (steps from 3 to 13), 0.8 mL of sample were propelled to clean the tubes and the donor channel (Step 3). Then, 0.12 mL of sample (SMP1) was mixed with 0.12 mL of the donor reagent provided by SMP 2 at 2 mL min^{-1} at C1 and in the mixing coil (Step 4). In this step, the donor mixture just reached the beginning of the donor channel of the GD-cell. Simultaneously, the acceptor channel was filled with 0.18 mL of acceptor solution by SMP 4. Then, 0.99 mL of sample and donor reagent for TIC measurement were propelled through the donor channel of the GD-cell at reduced flow rate of 0.33 mL min^{-1} (steps 5–8) for GD. Afterwards, 0.12 mL of sample were dispensed to flush the donor mixture from the tube D in the Fig. 1 completely, and by this, avoid peak tailing and obtain a rapid baseline recovery (Step 9). Subsequently, the conductivity measurement was initiated (Step 10) while 1.25 mL of acceptor solution were propelled through the C-cell at 0.25 mL min^{-1} (SMP 4) simultaneously with another 1.0 mL of sample (SMP1) to clean completely the manifold part for the donor and to recover the baseline in the manifold part for the acceptor (Step 11).

For NH_4^+ determination 0.66 mL of sample volume and donor solution were dispensed at 0.26 mL min^{-1} during the steps from 5 to 8. The volume dispensed in steps from 5 to 8 in Table 1 were calculated according to the SMP 1 calibration (0.022 mL per pulse) and by this, were 0.66 mL (0.022 mL per 30 pulse) at 0.264 mL min^{-1} (0.66 mL divided per 2.5 min) for NH_4^+ and 0.99 (0.022 mL per 45 pulses) at 0.33 mL min^{-1} (0.99 mL divided per 3 min) for TIC.

It should be pointed out that the donor mixture (sample plus donor reagent) flow through the GD-cell at almost 2-time the sample flow rate, 0.61 mL min^{-1} for TIC and 0.51 mL min^{-1}, for NH_4^+ according with the SMPs calibration. However, we will refer to just the flow rate of the sample SMP in order to facilitate the understanding and the comparison with other methods.

3. Result and discussion

3.1. Bubble traps and pulsation dampers

The SMP operate in suction on activation, which leads to the near-to-instantaneous aspiration of their nominal volume. Deactivation leads to solution and thus pressure release into the flow manifold with the flow rate given by the actual flow resistance. A detailed description of the operation of the used SMP can be found elsewhere [16].

Due to the rapidness of pulse motion and consequent pressure drop, spontaneous degasification of the reagent solutions and air bubble formation is likely. Continuous degassing is impractical due to costs and additionally required instrumentation. On the other side, air bubbles can greatly affect the flow pattern and mixing of sample and reagent in the manifold and thus decrease the method reproducibility. Using conductivity detection as done in this work,

Table 1
Analytical procedure for TIC (and for NH_4^+).

Step	Function	Operations
1	Cleans sample tube	SMP 1 [1.2 mL 4.0 mL/min]
2	Loop: A	
3	Dispense sample	SMP 1 [0.800 mL, 3.0 mL/min]
4	Fill DC with DS and AS	SMP 1 [0.120 mL, 2.000 mL/min]; SMP 2[a] [0.120 mL, 2.000 mL/min]; SMP 4[a] [0.180 mL, 2.500 mL/min]
5	Loop: B[b]	
6	Dispense DS	SMP 1[0.020 mL, 0.250 mL/min]; SMP 2 [0.020 mL, 0.250 mL/min]
7	Diffusion time	wait 3s for CO_2 (4s for NH_4^+)
8	End Loop B repeat 45 for CO_2 (30 for NH_4^+)	
9	Dispense sample	SMP 1 [0.120 mL, 2.000 mL/min]
10	Start measurement	Start conductimeter frec 5 Hz
11	Dispense AS to the detector	SMP 1 [1.000 mL, 2.000 mL/min]; SMP 4 [1.250 mL, 2.500 mL/min]
12	End measurement	Stop measurement
13	End Loop A repeat 3	

[a] The SMP 2 and SMP 4 were used for TIC determination; it should be substituted by SMP3 and SMP 5, respectively, to obtain the procedure for NH_4^+ determination.

[b] During the repetition of steps from 5 to 8 (Loop B) occurs the gas diffusion involving 0.9 mL of sample volume propelled at 0.6 mL min^{-1} for TIC and 0.6 mL at 0.48 mL min^{-1} for NH_4^+.

air bubbles in the acceptor solutions can also cause baseline disruption and false signals.

Therefore, passive bubble traps were placed at the pressure side of the SMP of the acceptor reagents (see Figs. 1 and 2(I)). The device prevented effectively the passage of the bubbles formed during the SMP operation. Moreover, it also fulfilled the function of a pulsation dumper, since the formed gas cushion in the bubble trap reduced the pulsation of the flow from the SMP.

With the objective of reducing flow pulsation and by this, signal noise, the acceptor and donor mixtures passed through flexible pumping tubes of Tygon® before they entered the GD-cell. The use of the passive bubble traps and the flexible tubes increased significantly the reproducibility reducing the relative standard deviation from, before, 10% to $<1\%$.

3.2. Theoretical considerations

In this work, two analytes were measured by the same technique, GD. These were total inorganic carbon and ammonium.

TIC, also called dissolved inorganic carbon (DIC), is defined as sum of all carbonate species in the water including carbonate, bicarbonate, carbonic acid and carbon dioxide, according with formula (1) [4].

$$\mathrm{TIC}=[\mathrm{CO_2}]+[\mathrm{H_2CO_3}]+[\mathrm{HCO_3}^-]+[\mathrm{CO_3}^{2-}] \tag{1}$$

The relation of all these species is regulated by pH equilibrium at given pressure and temperature according to formula (2) and (3).

$$\mathrm{CO_2}+\mathrm{H_2O}\rightleftharpoons\mathrm{H_2CO_3}\rightleftharpoons\mathrm{H}^+ +\mathrm{HCO_3}^- \rightleftharpoons 2\mathrm{H}^+ +\mathrm{CO_3}^{2-} \tag{2}$$

With $\mathrm{p}K_{a1}=6.35$ and $\mathrm{p}K_{a2}=10.32$ at 25 °C [28].

A pH equilibrium also regulate the ammonium/ammonia relation in natural waters systems.

$$\mathrm{NH_3}+\mathrm{H_2O}\rightleftharpoons\mathrm{NH_4}^+ +\mathrm{OH}^- \text{with } \mathrm{p}K_b=4.75 \text{ at } 25\ \text{¨C}\ [28] \tag{3}$$

At the usual natural water pH (7–8) ammonia is present mainly as $\mathrm{NH_4}^+$ and TIC is generally present as $\mathrm{HCO_3}^-$. In the proposed method, the analytes were forced into their volatile form by a strong pH change ($\mathrm{pH}>11$ for $\mathrm{NH_4}^+$, and $\mathrm{pH}<3$ for $\mathrm{HCO_3}^-$). The gaseous analytes ($\mathrm{NH_3}$ and $\mathrm{CO_2}$) were diffused through the membrane and trapped in an aqueous stream, in which the analyte equilibrium was inclined towards their ionic forms by an opposite pH change. The formation of the ionic species leads to a measurable change of the conductivity value of the acceptor solution. This variation on the solution conductivity is based in the different conductivities of the involved ions. The hydronium and the hydroxyl ions are the first and the second more mobile ion in the nature, with specific equivalent conductivities at 25 °C of $\lambda_{\mathrm{H_3O^+}}=349.6\ \mathrm{S\ cm^2\ mol^{-1}}$ and $\lambda_{\mathrm{OH^-}}=199.1\ \mathrm{S\ cm^2\ mol^{-1}}$, respectively. Thus, for both analytes, the analytical signal is a decrease of the total conductivity, because these ions have lower specific conductivity than H^+ and OH^-, respectively· specifically $\lambda_{\mathrm{NH_4}^+}=73.4\ \mathrm{S\ cm^2\ mol^{-1}}$ and $\lambda_{1/2\mathrm{CO_3}^{2-}}=69.3\ \mathrm{S\ cm^2\ mol_{eq}}^{-1}$.

For TIC, the conductivity of the acceptor solution can be calculated as formula (4). When GD takes place, the conductivity of the acceptor solution (only valid for lack of dispersion) is calculated by formula (5).

$$K_{acceptor}|_{25\,^\circ C}=[OH^-]\cdot\lambda_{OH^-}+[Na^+]\cdot\lambda_{Na^+} \tag{4}$$

$$K_{acceptor}|_{25\,^\circ C}=(1-X)\cdot[OH^-]\cdot\lambda_{OH^-}+X/2\cdot[OH^-]\cdot 2\cdot\lambda_{1/2CO_3{}^{2-}}+[Na^+]\cdot\lambda_{Na^+} \tag{5}$$

where X is the molar fraction of OH^- which reacts with the $\mathrm{CO_2}$ diffused, and then $X/2\cdot[\mathrm{OH}^-]$ is equal to the concentration of $\mathrm{CO_3}^{2-}$ formed. It is consider that all the $\mathrm{CO_2}$ diffused through the membrane is transformed in $\mathrm{CO_3}^{2-}$ when the acceptor solution has a $\mathrm{pH}>11$, like in this case.

For the ammonium system, the calculation of the conductivity is accordingly given by formula (6) and (7), And, then, $X\cdot[\mathrm{H_3O}^-]$ is equal to the concentration of $\mathrm{NH_4}^+$ formed.

$$K_{acceptor}|_{25\,^\circ C}=[H_3O^+]\cdot\lambda_{H_3O^+}+[Cl^-]\cdot\lambda_{Cl^-} \tag{6}$$

$$K_{acceptor}|_{25\,^\circ C}=(1-X)\cdot[H_3O^+]\cdot\lambda_{H_3O^+}+X\cdot[H_3O^+]\cdot\lambda_{NH_4{}^+}+[Cl^-]\cdot\lambda_{Cl^-} \tag{7}$$

The efficiency of the diffusion step depends on different factors. First, the strength of the donor reagent should be sufficiently concentrated to ensure the total conversion of the ionic analyte form into the neutral, volatile form. Second, the acceptor should be able to re-convert the gas diffused into the ionic form and to allow the detection of small variations in conductivity. Third, a slow flow rate of the donor mixture during the GD step favors the transfer but could also increase of the time of analysis. Considering these remarks, the three factors donor reagent concentration, acceptor reagent concentration, and flow rate were optimized.

The problem of optimization of GD consists in the possibility that a higher sensitivity can easily be achieved just by using a larger sample volume or a lower flow rate for the gas diffusion step. So, an optimum cannot be obtained as long as there are no other objectives pre-set such as an acceptable sample volume, time of analysis, working range, or limit of detection.

3.3. Optimization of acceptor and donor reagent for ammonium determination

The starting conditions for ammonium determination were based on a previous work [20]. The alkaline donor solution was a mixture of 25 mmol $\mathrm{L^{-1}}$ NaOH and 200 mmol $\mathrm{L^{-1}}$ sodium citrate. Citrate was used to avoid the precipitation of earth alkaline hydroxides and tube obstruction. The NaOH concentration was in great excess in respect to the typical ammonium concentration in coastal seawater samples, generally lower than 6 μmol $\mathrm{L^{-1}}$ in coastal seawater from Mallorca [20].

Low concentrated hydrochloric acid was selected as acceptor solution. Its concentration was studied from 10 to 150 μmol $\mathrm{L^{-1}}$. The peak height of 0.66 mL of $\mathrm{NH_4Cl}$ standard 5 μmol $\mathrm{L^{-1}}$, propelled at 0.264 mL $\mathrm{min^{-1}}$, was used as analytical response. Maximal peak height was obtained using 25 μmol $\mathrm{L^{-1}}$ of HCl as acceptor solution. A higher concentration of HCl increases the baseline and requires the use of a higher range of conductimeter scale (200.0 μS $\mathrm{cm^{-1}}$) which reduces the resolution and furthers the sensitivity, minimal unit 0.1 μS $\mathrm{cm^{-1}}$. At lower concentration than 20 μmol $\mathrm{L^{-1}}$ of HCl occurs the acid saturation with it consequent lack of linearity.

3.4. Selection of donor and acceptor solution for TIC determination

A diluted solution of sulfuric acid was selected as donor reagent for TIC determination. As $\mathrm{H_2SO_4}$ is not volatile, it cannot pass the GD membrane and its influence on the blank signal or baseline was therefore found as negligible. The effect of $\mathrm{H_2SO_4}$ concentration on the diffusion efficiency was studied from 10^{-4} to 10^{-1} mol $\mathrm{L^{-1}}$. At concentrations equal or higher than 5 mmol $\mathrm{L^{-1}}$, the peak height of a solution of 2.5 mmol $\mathrm{L^{-1}}$ $\mathrm{HCO_3}^-$, being a typical concentration in seawater, remains constant, with a variation around 2%. At lower concentrations, the peak height decreases drastically because the acid concentration is not sufficient to convert all bicarbonate and carbonate into gaseous $\mathrm{CO_2}$. Therefore, 5 mmol $\mathrm{L^{-1}}$ was used for the donor reagent for further work, using 0.99 mL of standard propelled at 0.33 mL $\mathrm{min^{-1}}$.

A solution of NaOH was selected as acceptor. As a first approximation, a wide range of the NaOH concentration was studied, due to the lack of knowledge about the membrane efficiency for CO_2. The maximum peak height for a 10 mmol L^{-1} HCO_3^- standard was obtained with NaOH concentration between 10 and 1 mmol L^{-1} propelling 0.99 mL of standard mixed with the same volume of H_2SO_4 5 mol L^{-1} at final flow rate of 0.61 mL min^{-1}. A more detailed study was done within a concentration range of 1–35 mmol L^{-1} NaOH. The ratio between the peak heights of a 2.5 and a 5.0 mmol L^{-1} bicarbonate standard was used as dependent variable during the optimization, in order to maximize the sensitivity in the HCO_3^- in a concentration range of greater interest. This ratio is also an indicative of the saturation of the acceptor solution. In the first part of this range, from 1 to 12.5 mmol L^{-1}, the ratio increased drastically. At higher NaOH concentration, from 12.5 to 35 mmol L^{-1}, the ratio did not vary significantly (2.19 ± 0.02). Finally, 20 mmol L^{-1} of NaOH was selected as working acceptor solution to avoid saturation during the analysis of samples with HCO_3^- concentration higher than 5 mmol L^{-1} such as very hard water.

3.5. *Flow rate optimization*

SMP flow is done by repeated pulses. So the effective flow rate was varied by changing the waiting time between two pulses and the volumes by changing the total number of pulses carried out with one SMP.

The flow rate and time, in which gas diffusion can take place, is crucial for the gas diffusion efficiency and sensitivity. Different modes and strategies for passing the donor and acceptor solution through the gas diffusion cell have been studied elsewhere [10,11,20,29], The most efficient option considering both sensitivity and the required time of analysis was that in which the acceptor solution stops meanwhile the donor is flowing.

In this work, the flow rate and the sample volume were optimized using a full factorial experimental design (2^2) including three central points. For both analytes, the experimental domains were: flow rate, 0.088–0.33 mL min^{-1} and sample volume, 0.264–1.06 mL.

For TIC, the dependent variables were the ratio of the peak heights obtained with a 5 mmol L^{-1} and a 2.5 mmol L^{-1} HCO_3^- standard and their absolute difference using 5 mol L^{-1} of H_2SO_4 as donor solution and 20 mmol L^{-1} of NaOH as acceptor solution. The peak height difference is then related to the sensitivity while the ratio is related to the linearity of the method. Ideal linearity would yield a ratio of 2 while taking a blank value into account, close but lower than 2.

For $NH_4^{\pm}$, the ratio and absolute difference between the peak height of 5 μmol L^{-1} of NH_4^+ standard solution and the blank (Milli-Q water) were used as dependent variables for flow rate and sample volume optimization. This optimization were carried out using 25 mmol L^{-1} of NaOH plus 200 mmol L^{-1} of sodium citrate as donor solution and 25 mmol L^{-1} of HCl as acceptor solution.

For TIC, the effect of the sample volume on the signal ratio was insignificant while the sensitivity increased nearly linearly. The flow rate was found to be the most significant factor and with a positive effect, increasing the signal ratio near linearly with higher flow rate while the effect on the absolute peak height difference was insignificant. This was because at low flow rates and by this higher diffusion efficiency, the acceptor could become saturated, especially close to the membrane surface and this effect would be more pronounced for higher standard. Second, there is a small share of CO_2, which will be converted into bicarbonate even at the acceptor pH of 12.3. However, the more concentrate the acceptor solution becomes, also the relative amount of formed bicarbonate increases leading to a loss of linearity. On the other side, a higher concentration of the acceptor was undesirable due to higher viscosity, reagent consumption or possible damage of the detection cell. Also, gas diffusion is fastest within the first seconds, i.e. when the high concentration gradient between acceptor and donor is highest.

The interaction coefficient of sample volume and flow rate was very small and positive indicating that either a small volume at low flow rate or a large volume at high flow rate is acceptable. A shorter time for gas diffusion, i.e. a higher flow rate, but eventually higher sample volume, is more favorable to achieve linear response and higher sensitivity, and faster analysis is possible as explained above. Also, because the pulsation of the donor flow and consequent membrane vibration decrease the solution's boundary layer on both sides of the membrane, a faster flow could improve the gas diffusion process. Therefore, and because the highest reproducibility was obtained at largest sample volumes, 0.99 mL and 0.33 ml min^{-1} were chosen as optimum sample volume and flow rate, respectively, for TIC determination.

For the ammonium determination, the interaction between flow rate and sample volume was the most significant factor with a positive effect followed by the flow rate and then the sample volume with a very low significance. Lower flow rates and higher sample volumes favored the method sensitivity while in respect of the signal ratio. Flow rate variations had a significantly higher effect on the sensitivity than sample volume variation. Nevertheless, a rather high flow rate of 0.264 mL min^{-1} was chosen since signal reproducibility improved with flow rate and a slightly better standard to blank ratio would yield better limit of detection. The selected sample volume was 0.66 mL establishing a compromise between the sample throughput and the increase of the sensitivity with the sample volume.

3.6. *Figures of merits*

Once the analytical parameters were optimized and the influences of the main operational variables were examined, the proposed methodology was evaluated for each analyte in terms of performance.

The analytical parameters obtained for TIC determination are summarized in Table 2. A typical conductometric response of TIC calibration obtained with the presented analyzer is depicted in Fig. 3.

For TIC determination, the peak height repeatability is the most important factor because a precision better than 1% is required to detect very small variation of this parameter. In this work, the average RSD was 0.7%. This is a very low value even for FT automation. This high reproducibility can most probably be related to the used bubble trap on the acceptor side, a very well mix of sample and donor reagent, and the turbulent flow conditions in the GD-cell. The injection throughput was also high 15 h^{-1}, and the LOD was 50 μmol L^{-1}, allowing the TIC quantification in seawater and low mineral water.

The analytical parameters for ammonium determination using the proposed system as well as in our previous work using C-GD-MSFIA for comparison are also summarized in Table 2. As it was mentioned before, the turbulent flow favors the GD step. This statement was confirmed by the comparison between the present method, C-GD-MPFS, and a previous one, C-GD-MSFIA [20], which used a multi-syringe pump as liquid driver.

The sample volume used for ammonium determination in C-GD-MPFS was 0.66 mL, 3.3-times higher than in the previous work (0.2 mL with C-DG-MSFIA) while the flow rates were similar (0.264 mL min^{-1} and 0.30 mL min^{-1}, respectively). However the MPFS do not require time for syringe refilling like MSFIA, then, the injection throughput with the MPFS configuration was less than 2-times lower (17 h^{-1} for C-GD-PMFS and 32 h^{-1} for C-GD-MSFIA).

Table 2
Analytical Parameters and optimal conditions: for NH_4^+ and TIC measurement with the C-GD-MPFS. Comparison with a C-GD-MSFIA system [20] for ammonium determination.

Analytical parameters	TIC C-GD-MPFS	NH_4^+ C-GD-MPFS	NH_4^+ C-GD-MSFIA [20]
Donor solution	H_2SO_4 5 mmol L^{-1}	NaOH 25 mmol L^{-1} NaCitrate 200 mmol L^{-1}	NaOH 25 mmol L^{-1} NaCitrate 200 mmol L^{-1}
Acceptor solution	NaOH 20 mmol L^{-1}	HCl 25 μmol L^{-1}	HCl 25 μmol L^{-1}
Sample volume (mL)	0.99	0.66	0.2
Flow rate (mL min^{-1})	0.33	0.264	0.30
Calibration curve			
Slope (μS L cm^{-1} $μmol^{-1}$)	0.314 ± 0.005 (mS L cm^{-1} $mmol^{-1}$)	3.75 ± 0.02	0.125 ± 0.002
Intercept (μS cm^{-1})	0.099 ± 0.028 (mS cm^{-1})	2.0 ± 0.1	6.2 ± 0.38
Linear working range	0.08–9.0 mmol L^{-1}	0.5–25 mol L^{-1}	4.2–20000 μmol L^{-1}
LOD (3σ/slope, $n=10$)[a]	50 μmol L^{-1}	0.27 μmol L^{-1}	2.5 μmol L^{-1}
LOQ (10σ/slope, $n=10$)	80 μmol L^{-1}	0.89 μmol L^{-1}	8.3 μmol L^{-1}
^{2}RSD (%)[b]	0.7%	0.9%	2.4%
Injection throughput	15 h^{-1}	17 h^{-1}	32 h^{-1}

[a] The LOD was calculated as three times the standard deviation of the peak height of the blank (distilled water).

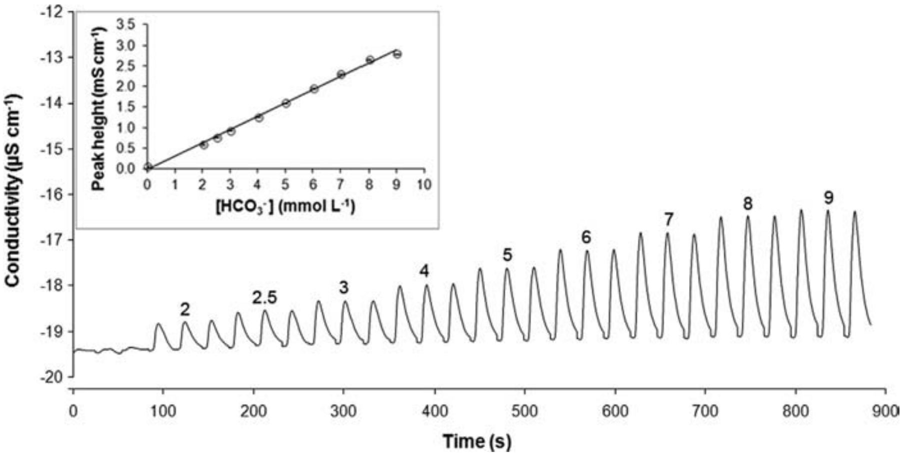

Fig. 3. Typical conductometric response for TIC calibration obtained under optimal conditions, using standards from 2 to 9 mmol L^{-1}.

The calibration curve slope was highly increased compared to the previous work with 3.75 ± 0.02 μS L cm^{-1} $μmol^{-1}$ for C-GD-MPFS and 0.125 ± 0.002 μS L cm^{-1} $μmol^{-1}$ for C-GD-MSFIA. In other words, the effective sensitivity was about 30-times higher than with the C-DG-MSFIA system. Fig. 4 shows the typical conductometric response for NH_4^+ calibration obtained with the C-GD-MPFS analyzer.

The repeatability of the MPFS was significantly higher than the one found in the previous work. The relative standard deviation (RSD) of 10 consecutive measurements of the same standard was almost 3-times lower than using C-GD-MSFIA (0.9% and 2.4%, respectively, both for 2.5 μmol L^{-1} of NH_4^+), so that, the limit of detection (LOD) was 10-times lower than in the previous work (0.27 μmol L^{-1} versus 2.5 μmol L^{-1}). The LOD was calculated as 3-times the standard deviation of 10 consecutively blank peak height divided by slope of the calibration curve.

It is necessary to point out that one of the objectives of C-GD-MSFIA work was the development of an analyzer system applicable to samples with a very wide ammonium concentration range. Meanwhile, the objective of the presented system was to develop a system with a higher sensitivity, able to quantify ammonium in coastal seawater and with higher reproducibility, able to detect small variation of TIC in seawater.

One way to estimate the NH_3 and CO_2 diffusion efficiency is the measurement of the absolute amount of the analyte in both, the acceptor and donor volume, after the diffusion step [20,30]. In this work, the GD efficiency ε was estimated from the signal value obtained, the specific conductivities λ_{ion} and its concentration [ion] of each participant ion. The decrease of the acceptor flow conductivity ($\kappa_{acceptor}$) can be described by formula (5) for TIC and 7 for ammonium and assuming that all diffused CO_2 is converted in CO_3^{2-} in the acceptor solution and that the dispersion of the acceptor solution zone is near to zero.

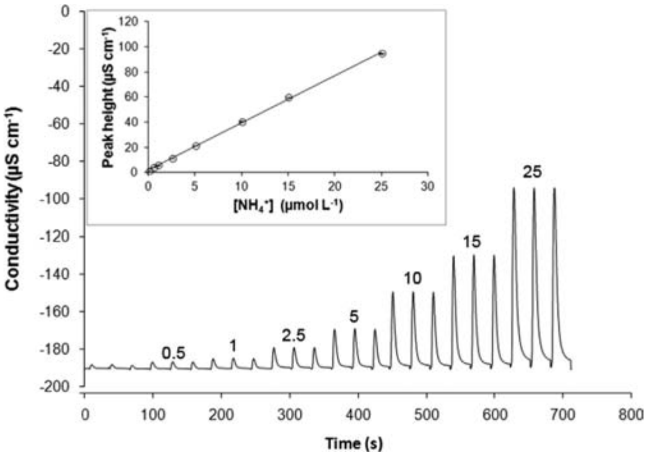

Fig. 4. Typical conductometric response for NH_4^+ calibration obtained under optimal conditions, using standards from 0.5 to 25 μmol L^{-1}.

X is the molar fraction of the initial hydroxyl or hydronium ion concentration ($[OH^-]$ or $[H_3O^+]$), which is neutralized by the GD-transferred carbon dioxide or ammonia, respectively. X can be calculated by isolation of X from formula (5) and (7), respectively, leading to formula (8) and (9).

$$X_{CO_2}=\frac{\kappa_{\text{Acceptor}}-[OH^-]\lambda_{OH^-}-[Na^+]\lambda_{Na^+}}{[OH^-]\,(\lambda_{CO_3{}^{2-}}-\lambda_{OH^-})} \tag{8}$$

$$X_{NH_3}=\frac{\kappa_{\text{Acceptor}}-[H_3O^+]\lambda_{H_3O^+}-[Cl^-]\lambda_{Cl^-}}{[H_3O^+](\lambda_{NH_4{}^+}-\lambda_{H_3O^+})} \tag{9}$$

Formula (8) and (9) allow the calculation of the concentration of the respective analyte in the acceptor flow from the measured peak heights, i.e. the final conductivity of the acceptor after the GD process, since the initial concentration of sodium hydroxide and the specific conductivities at given temperature are known.

Considering n_{Acceptor} the absolute amount of analyte molecules, which have passed the membrane and n_{Sample} the absolute amount of analyte molecules, which were available in the original sample, the GD-efficiency ε can be defined as the fraction of both numbers. With the known initial analyte concentration and the applied volumes of sample and acceptor, i.e. the acceptor channel volume, the GD-efficiency for carbon dioxide and ammonia can be calculated from formula (10) and (11), respectively.

$$\begin{aligned}\varepsilon&=\frac{n_{CO_3{}^{2-}\ \text{Acceptor}}}{n_{HCO_3{}^-\ \text{Sample}}}=\frac{V_{\text{Acceptor}}}{V_{\text{Sample}}}\cdot\frac{[CO_3{}^{2-}]_{\text{Acceptor}}}{[HCO_3{}^-]_{\text{Sample}}}\\&=\frac{X_{CO_2}}{2}\cdot\frac{V_{\text{Acceptor}}}{V_{\text{Sample}}}\cdot\frac{[OH^-]_{\text{Acceptor}}}{[HCO_3{}^-]_{\text{Sample}}}\end{aligned} \tag{10}$$

$$\begin{aligned}\varepsilon&=\frac{n_{NH_4{}^+\ \text{Acceptor}}}{n_{NH_4{}^+\ \text{Sample}}}=\frac{V_{\text{Acceptor}}}{V_{\text{Sample}}}\cdot\frac{[NH_4{}^+]_{\text{Acceptor}}}{[NH_4{}^+]_{\text{Sample}}}\\&=X_{NH_3}\cdot\frac{V_{\text{Acceptor}}}{V_{\text{Sample}}}\cdot\frac{[H_3O^+]_{\text{Acceptor}}}{[NH_4{}^+]_{\text{Sample}}}\end{aligned} \tag{11}$$

Insertion of the terms for X, given in formula (8) and (9), into formula (10) and (11), the efficiency can be estimated by formula (12) and (13), respectively.

$$\varepsilon=\frac{\kappa_{\text{Acceptor}}-[OH^-]\lambda_{OH^-}-[Na^+]\lambda_{Na^+}}{2[HCO_3{}^-]_{\text{Sample}}(\lambda_{CO_3{}^{2-}}-\lambda_{OH^-})}\cdot\frac{V_{\text{Acceptor}}}{V_{\text{Sample}}} \tag{12}$$

$$\varepsilon=\frac{\kappa_{\text{Acceptor}}-[H_3O^+]\lambda_{H_3O^+}-[Cl^-]\lambda_{Cl^-}}{[NH_4{}^+]_{\text{Sample}}(\lambda_{NH_4{}^+}-\lambda_{H_3O^+})}\cdot\frac{V_{\text{Acceptor}}}{V_{\text{Sample}}} \tag{13}$$

Inserting all known values, i.e. concentration and volumetric data, the estimated efficiencies were 10.6% for CO_2 and 21.4% for NH_3 using 0.99 mL and 0.66 mL of sample, respectively under optimal conditions. These values are in good agreement with the values reported by Van der Linden [30] of 20.2–24.6% for commercial GD modules and about 10% for a homemade GD module. However the efficiency is strongly affected by the membrane characteristics [12].

The calculated transfer efficiency for CO_2 (10.6%) also confirms that all the transferred CO_2 is converted in $CO_3{}^{2-}$ in the acceptor channel. Considering that the highest analyte concentration applied was lower than 5 mmol L^{-1}, a maximum of 0.5 mmol L^{-1} diffused through the membrane. Taking into account that the sample volume is about 5 times larger than the acceptor volume, there is still an eight-fold surplus of sodium hydroxide. The add-recovery test also confirms the linearity and by this, the independence of the transfer and neutralization of the CO_2 on the acceptor side of the membrane in the concentration range of interest for natural waters.

3.7. *Applications to samples*

To evaluate the trueness of the proposed method for both analytes, spiking/recovery tests were done following the IUPAC recommendations [31]. The results and the pH value of the samples are given in Tables 3 and 4 for TIC and ammonium respectively.

TIC determination was done in two coastal waters and one low mineralized drinking water. Two spiking levels of 0.5 and 2 mmol^{-1} $HCO_3{}^-$ were done for each sample obtaining recoveries between 92% and 106%.

Total alkalinity, pH, and temperature were also measured and used to estimate the DIC according with the calculations proposed by Dickson and Goyet [27] using an average of salinity (38 g L^{-1}) for Mediterranean seawaters. As can be observed in Table 3, the comparison between the DIC measured with the C-GD-MPFS and the DIC estimated did not presented significant differences.

The values obtained for drinking waters with the proposed method do not differ significantly from the certified values being 2.25 mmol L^{-1} of bicarbonate for low mineralized drinking water.

For ammonium determination (Table 4), two spiking levels of 5 and 10 μmol L^{-1} were done obtaining samples recoveries values between 92% and 107%. In coastal seawater samples from 1 to 3, values around 5 and 10-times higher than LOD were obtained. In coastal seawater 4, the $NH_4{}^+$ concentration was below but very close to the LOD of the method. This recovery values have been appropriated for ammonium analysis in coastal water samples.

Interference could arise from the presence of other volatile substances, which can pass through the membrane and change the conductivity of the acceptor solution. For the ammonium

Table 3
Result of TIC contents in different type of waters. Spike-Recovery test result and comparison with DIC estimated for seawater and certified value for drinking water.

Samples	MPFIA (mmol/L)			Estimated DIC for comparison[a]			
	$HCO_3{}^-$ added	TIC Found	Recovery (%)	TA (mmol/L)	pH	DIC[a] (mmol/L)	Recovery (%)
Coastal water 1	0	2.35 ± 0.01		2.60 ± 0.03	7.97	2.37	99
	0.5	2.81 ± 0.02	92				
	2	4.26 ± 0.03	96				
Coastal water 2	0	2.48 ± 0.02		2.71 ± 0.03	7.84	2.53	98
	0.5	2.94 ± 0.03	93				
	2	4.35 ± 0.04	94				
Drinking water	0	2.27 ± 0.01		2.30 ± 0.02	7.94	2.25[b]	101
	0.5	2.78 ± 0.007	101				
	2	4.38 ± 0.006	106				

[a] DIC estimated according with Dickson and Goyet [25] using the total alkalinity (TA) and the pH measured at 25 °C, and considering the salinity 38 g L^{-1}, the average of Mediterranean sea.

[b] Certified values reported for low mineralized drinking water.

Table 4
Ammonium determination with the MPFIA in coastal water samples. Result of spikes-recovery test.

Samples	Added (μmol L^{-1})	Measured (μmol L^{-1})	Recovery (%)	pH
Coastal Water 1	0	2.46 ± 0.01		7.97
	5	7.14 ± 0.05	94	
	10	12.47 ± 0.09	100	
Coastal Water 2	0	< **LOD**[a]		7.84
	5	5.57 ± 0.04	106	
	10	9.88 ± 0.01	96	
Coastal Water 3	0	1.51 ± 0.03		7.94
	5	6.13 ± 0.06	93	
	10	10.73 ± 0.015	92	
Coastal Water 4	0	3.80 ± 0.01		7.99
	5	8.51 ± 0.04	94	
	10	13.23 ± 0.09	94	

[a] The value is less but very close to the LOD, then 0.27 μmol L^{-1} was used to calculate the recovery.

method, volatile amines could give a similar analytical signal as the analyte. However, they are generally present in natural waters in much lower concentration than NH_4^+ and they also have smaller diffusion coefficients due to a larger molecular size [11]. Therefore, their effects as interference can most likely be neglected. For TIC determination, the volatile organic acids and oxides (e.g. formic and acetic acid, H_2S, NO_2,or SO_2) are able to permeate the membrane. Likewise, their low concentration levels in the studied types of sample make a significant interference of these substances unlikely.

4. Conclusions

The use of MPFS for the automation of gas diffusion allowed the very precise conductometric determination of ammonium and TIC in a single manifold. The insertion of a bubble trap also reduced the SMP pulsations and increased significantly the measurement's reproducibility, achieving a RSD of 0.7% for TIC and 0.9% for NH_4^+. In fact, to the best of our knowledge, it was the first method, which uses this kind of device to improve the reproducibility of MPFS and to obtain such low RSD. Moreover, the obtained limits of detection and working ranges allowed the quantification of TIC and NH_4^+. The results of add-recovery test for both parameters together with the comparison between the TIC measured and the DIC estimated according to Dickson and Goyet [27] were indicative for the adequate accuracy of the proposed method. Thus, the developed C-GD-MPFS is an automated analyzer with a high simplicity, a reduced size and economic instrumentation, enhanced reproducibility and sensitivity, and able for the robust and reliable determination of these two parameters in environmental samples.

Acknowledgments

This work was supported by the Spanish "Ministerio de Ciencia e Innovación", through the Project CTQ2010–15541, and the Conselleria d'Economia, Hisenda, i Innovació of the Government of the Balearic Islands through the allowance to competitive groups (43/2011) also provided by FEDER funds. C. Henríquez is very grateful with the Conselleriad´EducacióCultura I Universitat and the Fondo Social Europeo for funding her Ph.D. Grant. B. Horstkotte was further supported by a postdoctoral fellowship of the project CZ.1.07/2.3.00/30.0022 supported by the Education for Competitiveness Operational Program (ECOP) and co-financed by the European Social Fund and the state budget of the Czech Republic.

References

[1] D.G. Capone, D.A. Bronk, M.R. Mulholland, E.J. Carpenter, Nitrogen in the Marine Environmet, 2nd ed., Elsevier, Burlingtoon, USA. Amsterdam, The Netherlands.California, USA. London, USA, 2008.
[2] W.H. Schlesinger, A.E. Hartley, Biogeochemistry 15 (1992) 191–211.
[3] F.V. Almeida, J.R. Guimaraes, W.F. Jardim, Journal of Environmental Monitoring 3 (2001) 317–321.
[4] H.G. Schulz, J. Barcelos e Ramos, R.E. Zeebe, U. Riebesell, Biogeosciences 6 (2009) 2145–2153.
[5] F. Wenzhöfer, M. Adler, O. Kohls, C. Hensen, B. Strotmann, S. Boehme, H.D. Schulz, Geochim. Cosmochim. Acta 65 (2001) 2677–2690.
[6] L.N. Moskvin, T.G. Nikitina, J. Anal. Chem. 59 (2004) 6–22.
[7] J. Ruzicka, E.H. Hansen, Anal. Chim. Acta 78 (1975) 145–157.
[8] J. Ruzicka, G.D. Christian, Anal. Chim. Acta 234 (1990) 31–40.
[9] V. Cerda, J.M. Estela, R. Forteza, A. Cladera, E. Becerra, P. Altimira, P. Sitjar, Talanta 50 (1999) 695–705.
[10] J. Klimundova, R. Forteza, V. Cerda, Int. J. Environ. Anal. Chem. 83 (2003) 233–246.
[11] M.T. Oms, A. Cerda, A. Cladera, V. Cerda, R. Forteza, Anal. Chim. Acta 318 (1996) 251–260.
[12] J.N. Plant, K.S. Johnson, J.A. Needoba, L.J. Coletti, Limnol. Oceanogr. Methods 7 (2009) 144–156.
[13] I.C. Santos, R.B.R. Mesquita, A. Machado, A.A. Bordalo, A.O.S.S. Rangel, Anal. Chim. Acta 778 (2013) 38–47.
[14] R.A.S. Lapa, J.L.F.C. Lima, B.F. Reis, J.L.M. Santos, E.A.G. Zagatto, Anal. Chim. Acta 466 (2002) 125–132.
[15] D.A. Weeks, K.S. Johnson, Anal. Chem. 68 (1996) 2717–2719.
[16] B. Horstkotte, E. Ledesma, C.M. Duarte, V. Cerda, Anal. Chem. 82 (2010) 6983–6990.
[17] B. Horstkotte, C.M. Duarte, V. Cerdá, Int. J. Environ. Anal. Chem. 92 (2012) 344–354.
[18] S.M. Oliveira, T.I.M.S. Lopes, I.V. Tóth, A.O.S.S. Rangel, Anal. Chim. Acta 600 (2007) 29–34.
[19] L. Monser, N. Adhoum, S. Sadok, Talanta 62 (2004) 389–394.
[20] C. Henríquez, B. Horstkotte, V. Cerdá, Int. J. Environ. Anal. Chem. (2012) 1–17.
[21] M.T. Oms, A. Cerda, V. Cerda, Electroanalysis 8 (1996) 387–390.
[22] Z. Hoherčáková, F. Opekar, Anal. Chim. Acta 551 (2005) 132–136.
[23] H.L. Braz, D.T. Ito, J.A.F. da Silva, C.L. do Lago, J.J. Pedrotti, Electroanalysis 23 (2011) 2594–2600.
[24] LACHAT Instrument, Loveland, U.S.A., 〈http://www.lachatinstruments.com/index.asp〉, 2011.
[25] M.A.H. Franson, L.S. Clesceri, A.E. Greenberg, R. Rhodes Trussell, Métodos normalizados para el análisis de aguas potables y residuales. in: D.d.S. S.A. (Ed.), APHA, AWWA, WPCF, 1992.
[26] O. Elsholz, T. Rodrigues, V. Cerdà, M. Tubino, GIT Labor-Fachz. 2 (2008) 110–113.
[27] A.G. Dickson, C. Goyet, Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Sea Water, Department of Energy ORNL/CDIAC-74, Tennessee, USA, 1994.
[28] C. Weast Robert, Handbook of Chemistry and Physics 51st ed. The Chemical Rubber Co., Ohio, 1970–1971.
[29] M.I.G.S. Almeida, J.M. Estela, M.A. Segundo, V. Cerdà, Talanta 84 (2011) 1244–1252.
[30] W.E. Van Der Linden, Anal. Chim. Acta 151 (1983) 359–369.
[31] M. Thompson, S.L.R. Ellison, R. Wood, Pure Appl. Chem. 74 (2002) 835–855.