Direct electrochemical ammonia monitor

By Elizabeth A. Moschou, Nikolas A. Chaniotakis, Nikos Papandroulakis, and Pascal Divanach

AMMONIA IS ONE of the most toxic by-products in the nitrogen cycle. As protein and amino acids are metabolized, there is a direct excretion of ammonia from the living organisms to their surrounding environment (for example, fish produce 28 g of ammonia per kg of food eaten). Although fish excretion is the primary source of ammonia in aquaculture systems, other natural processes may contribute ammonia. The decrease in phytoplankton and the denitrification processes in sediments are two examples. In addition, decaying organisms that may come from runoff waters and bacterial decomposition of animal waste products also contribute considerably to the level of ammonia in a water body.

Once generated, ammonia can be absorbed by algae and aquatic vascular plants as their nitrogen source, it can be oxidized to nitrite and nitrate, or it can escape into the air. Because ammonia removal is dependent on natural microbial processes in most aquaculture facilities, changes in either bacterial metabolism and/or population can drastically increase the ammonia levels. Such increases can usually occur rapidly, since the process is related to the feeding rates, external water supply, and health of the overall aquaculture environment.

Changes in the levels of ammonia in aqueous environments can have drastic effects on the population. Ammonia has been reported to be toxic and inhibitory for mammalian cell cultures for many years. Undesired processes such as the reduction of growth rates and maximal cell densities in batch cultures, changes in metabolic rates, perturbation of protein processing, and virus replication take place in environments with elevated ammonia concentrations.[1] Additionally, high levels of ammonia lead to fish stress or mortality, and it has been speculated that ammonia may affect the biotic interactions and nutrient dynamics in these environments.[2] At the same time, the so-called nitrogenous biochemical oxygen demand (NBOD) (which refers to the bacterial conversion of ammonia to nitrite and nitrate) is a process that utilizes dissolved oxygen. NBOD and carbonaceous biochemical oxygen demand (CBOD) are the two most important types of oxygen-consuming wastes that must be regulated. Point source discharges (such as wastewater treatment plants) with high NBOD and CBOD also introduce large amounts of pollutants, and are highly dangerous to the receiving environment.

It is therefore evident that in any aquaculture, be it a pond, coastal area, or recirculating system, water quality is a major concern, and in order to sustain and expand life, a healthy environment is mandatory. Ammonia is the first indication of contamination, and, unless it is minimized, can set into motion processes that lead, in addition to direct toxicity, to high bacterial counts, oxygen depletion, fish disease, and mortality.[3] Management of an aquaculture system must thus begin with the continuous and precise analysis of the ammonia levels, since its concentration for normal fish growth must be kept below 2.5 ppm.[4]

Existing methods for the analysis of ammonia include colorimetry, ion chromatography, cathodic stripping voltammetry, and fluorimetry. The colorimetric indophenol blue method is based on the Berthelot reaction between ammonia, phenol, and hypochlorite leading to the formation of an indophenol dye.[5] One of the major drawbacks of this method is the required sample pretreatment, which includes a mandatory filtration step. This step is the main obstacle in using the colorimetric method for the continuous monitoring of ammonia in all samples of light-absorbing matter. In addition, this method is subjected to the relatively low range of detection, giving rise to large errors when measurements are obtained with samples of relatively high ammonia concentrations (above 5 ppm). In flow injection analysis (FIA)-ion chromatography, ammonia is transmembrane diffusing into an acidic media and determined as solvated ammonium.[6] The range of detection is 0–17 ppb and the analysis time is 15 min per sample. In FIA-cathodic stripping voltammetry, ammonia reacts with formaldehyde to form the determined methylenimine.[7] The range of detection is 0.17–51 ppb while the analysis time is in the order of 20–35 min, depending on the type of sample analyzed. Finally, in FIA-fluorimetry, ammonia is determined after derivatization with o-phthaldialdehyde and sulfite.[8] The range of detection is 4.25 ppb–0.34 ppm and the analysis time is 2.4 min per sample.

Although some of the above techniques can offer low detection limits of ammonia, none is capable of the direct and continuous measurement if NH_3 levels in water samples with high salinity and large amounts of suspended matter such as seawater in aquacultures and wastewaters.

This paper describes a portable flow analysis system for the direct and continuous monitoring of total ammonia levels in seawater samples from aquaculture. The analyzer incorporates the advantages of the NH_3 ion selective electrode (ISE) as the sensor, with the capabilities of the flow analysis (FA) manifold. The internal reference solution of the sensor has been optimized to eliminate potential drifts and to allow for a linear range of detection between 0.05 and 100 ppm of NH_3. The FA manifold was designed to eliminate any precipitate formation from calcium and magnesium oxides, enabling continuous NH_3 measurement over long periods of time with minimum sample and reagent consumption. The complete system offers high stability, accuracy, and precision, while requiring a short analysis time.

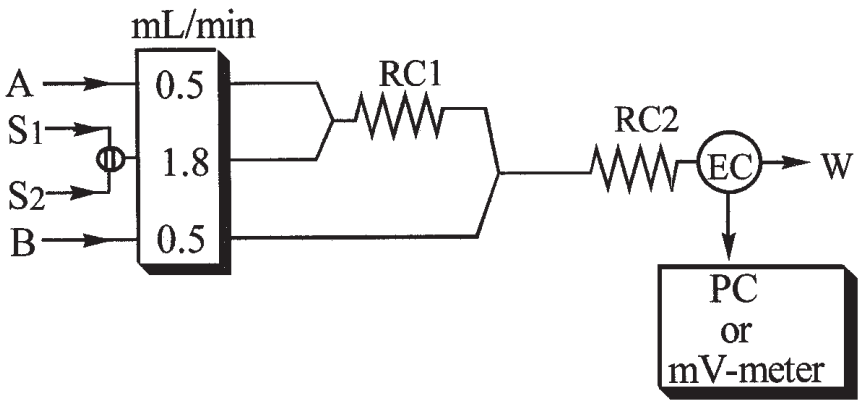

Figure 1 *Schematic diagram of the FA system used for the continuous analysis of NH_3. S1: sample, S2: calibrating solution, A: EDTA reagent, B: NaOH reagent, RC: reaction coil, EC: flow-through potentiometric detector, W: waste.*

Table 1

Electrode's characteristics for various filling solutions

Filling solution	Composition NH_4^+	Composition NaCl	Slope	R	% RSD of 1-ppm sample
A	0.1 *M*	—	47 ± 1 mV	0.9993	1.88
B	0.05 *M*	250 ppm	52 ± 2 mV	0.9989	2.00
C	0.05 *M*	0.1 *M*	51 ± 3 mV	0.9972	3.40
D	0.02 *M*	—	51 ± 1 mV	0.9997	2.70
E	0.02 *M*	250 ppm	53 ± 1 mV	0.9997	0.17

Table 2

Ion content in synthetic seawater

Cation	Concentration	Anion	Concentration
K^+	0.3672 g/L	F^-	1.356 mg/L
Na^+	10.53 g/L	Cl^-	19.06 g/L
Ca^{2+}	0.4008 g/L	Br^-	67.12 mg/L
Mg^{2+}	12.29 g/L	OH^-	4.222 mg/L
Sn^{2+}	7.976 mg/L	SO_4^{2-}	2.705 g/L
		SiO_3^{2-}	5.356 mg/L

Experimental

Apparatus

The flow analysis instrument was designed and built in-house, based on the system shown in *Figure 1*. It consists of an ALITEA U4-T four-channel peristaltic pump (**Alitea AB,** Stockholm, Sweden) for sample and reagent delivery operating between 9 and 16 V, delivery tubing (**Alitea AB,** R52125 i.d. 1.60 cc/M and Q76265 i.d. 0.030 in.), a V-100D diagonal flow selection valve (**Upchurch Scientific,** Oak Harbor,

Ms. Moschou and Mr. Chaniotakis are with the Department of Chemistry, University of Crete, Iraklion 71 409 Crete, Greece; tel.: +30 81 238 468; fax: +30 81 210 951; e-mail: nchan@talos.cc.uch.gr. Mr. Papandroulakis and Dr. Divanach are with the Aquaculture Department, Institute of Marine Biology of Crete, Iraklion, Crete, Greece.

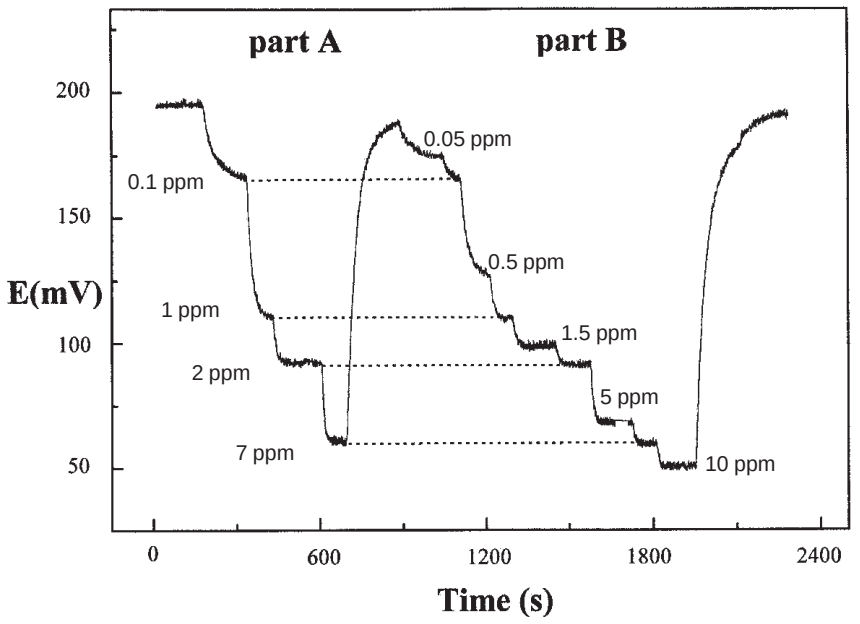

Figure 2 *Analysis of calibrating solutions (part A) and seawater (part B) samples.*

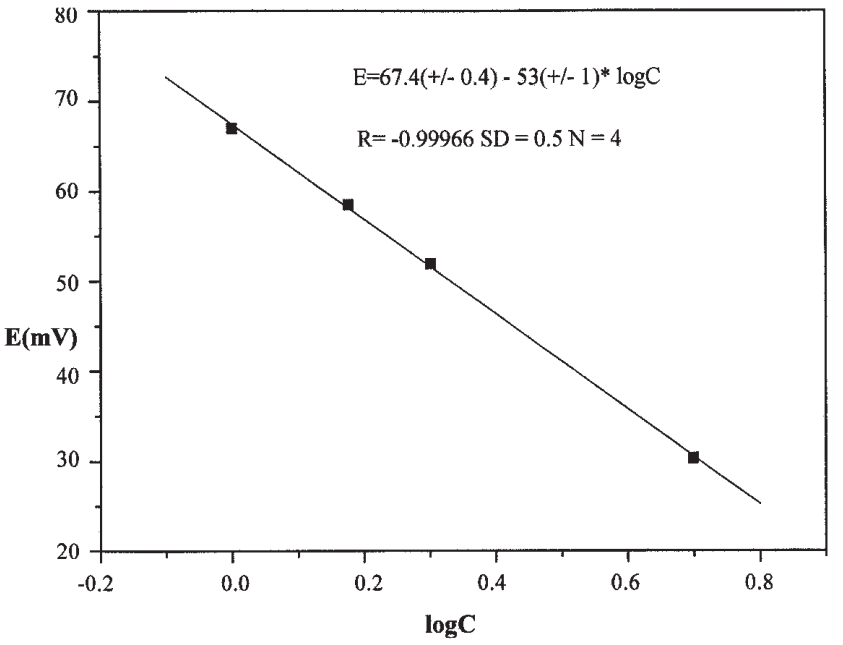

Figure 3 *Calibration curve obtained using synthetic seawater standard solutions.*

Table 3
Analysis time of samples with various amounts of ammonia

NH_3 (ppm)	t95% (min)
7.00	1.67
5.00	1.72
3.00	2.38
1.00	3.28
0.50	3.64

Table 4
Signal reproducibility of the FA system (the time between each measurement of each sample is 2 hr)

NH_3 (ppm)	E_1 (mV)	E_2 (mV)	E_3 (mV)	Average
1.00	67.0	67.0	66.8	66.9 ± 0.1
1.50	58.5	58.8	58.5	58.6 ± 0.2
2.00	51.9	51.9	51.7	51.8 ± 0.1
5.00	30.3	30.3	30.3	30.3 ± 0.0

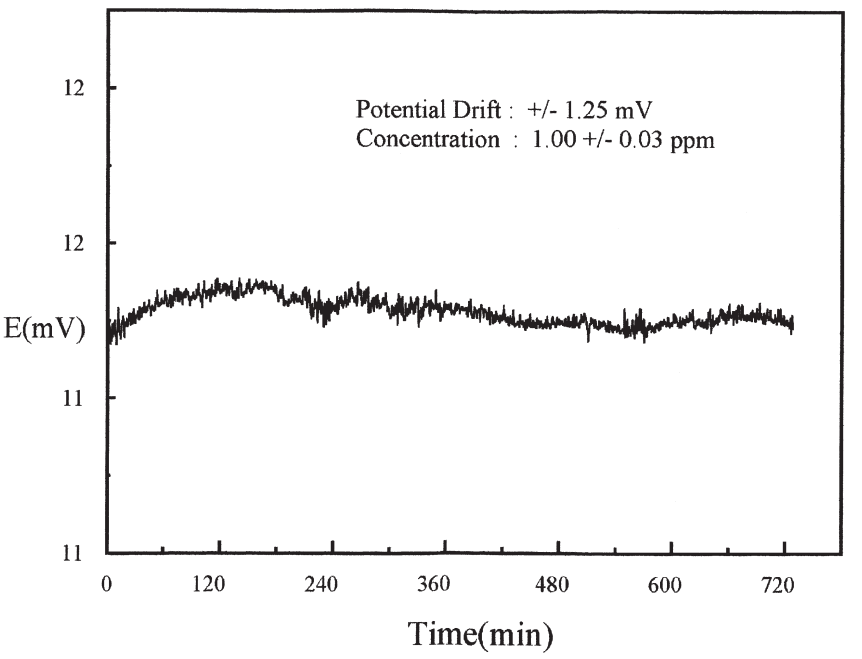

Figure 4 *Signal stability of the FA system. The signal was monitored with 1 ppm of NH_3 in seawater for 12 hr.*

WA) reaction coil 1 (0.030 in. i.d. × 50 cm length) and reaction coil 2 (0.30 in. i.d., 100 cm length). All other tubing, connectors, and tees were also of PEEK for minimum ammonia loss. The potentiometric flow-through cell was constructed of DERLIN® (**Goodfellow Cambridge Ltd.,** Cambridge, U.K.). The ammonia ISE used was the model 95-12 ammonia gas sensing electrode (**Orion Research Inc.,** Beverly, MA). The potential was monitored using a model 290-A pH/mV meter (**Orion**) with datalogging capabilities, operating at 9 V dc. All laboratory experiments were performed at 24 ±1 °C.

The rearing medium from the aquaculture seawater samples contained on average 200 larvae per liter, of 4–12 mm in length; 5–10 zooplankton per mL, and 0.5–1 million phytoplankton cells, 1–2 mm in length per mL.

Reagents

For all experiments, deionized water (NAN-O-Pure, **Barnstead,** Dubuque, IA) and chemicals of puriss p.a. grade were used. Ammonia 100-ppm stock solution was prepared from NH_4Cl (**Merck,** Darmstadt, Germany).

The synthetic seawater standards consisted of ammonia 100-ppm stock solution, NaCl (**Merck**), $MgCl_2(H_2O)_6$ (**Fluka,** Ronkonkoma, NY), and NaOH (**Merck**). All standard solutions were prepared fresh daily.

The ethylenediaminetetraacetic acid (EDTA) reagent was prepared from the disodium salt of EDTA (**Serva,** Hauppauge, NY) and the NaOH reagent from sodium hydroxide pellets (**Merck**).

Results and discussion

Optimization of sensor and FA parameters

In order to obtain the required range of detection and stability, as well as the sensitivity, the electrode's internal filling solution was initially optimized, since the commercially available filling solution supplied with the NH_3-ISE showed extensive drift and low slope when seawater samples were measured. The composition of some of the internal filling solutions were examined, and the performance of the sensors is shown in *Table 1*. The effect of the filling solution on the slope, the linear range of detection, as well as the relative standard deviations obtained for three measurements of 1 ppm NH_3 in seawater samples are listed in Table 1. It is evident from these data that the use of solutions A, B, C, and D gave irreproducible and unstable measurements, while the electrode with the filling solution E exhibited very good stability and reproducibility.

The next optimization step entailed obtaining calibrating solutions with the appropriate ionic strength and ionic composition. It was found that use of

Table 5
Determination of ammonia in filtered and nonfiltered seawater samples from rearing medium (comparison of the data obtained with FA system and colorimetric technique)

Spiked samples (ppm)	Photometry (ppm)	% Recovery	Filtered FA (ppm)	% Recovery	Nonfiltered FA (ppm)	% Recovery
0.300	0.344	115	0.310	103	0.280	93.3
0.800	0.830	104	0.884	110	0.892	112
0.900	0.881	97.9	0.983	109	0.912	101
1.00	0.969	96.9	1.05	105	1.00	100
1.40	1.28	91.4	1.49	107	1.46	104
1.50	1.57	104	1.54	102	1.50	100
1.90	2.35	124	1.92	101	1.90	100
2.50	2.49	99.7	2.53	101	2.50	99.9
3.00	—	—	3.09	103	3.05	102
5.00	—	—	5.17	103	5.00	100

synthetic seawater solution with the ions and concentrations shown in *Table 2* gave very reproducible background signal, and very small potential drift. Similar results were also obtained with calibrating solution containing 3.5×10^{-5} *M* NaOH, 0.4123 *M* NaCl, and 0.0911 *M* $MgCl_2(H_2O)_6$. Due to its simplicity, the second solution was finally used as the matrix of the required calibrating solutions with ammonia concentrations between 0.8 and 10 ppm.

Analysis procedure

The analyzer design is shown in Figure 1. The samples (S1) or standards (S2) are continuously pumped by the peristaltic pump via the selection valve to the first reaction coil (RC1). It was found that the sample must be thoroughly mixed first with the EDTA solution in the reaction coil RC1 before the NaOH reagent is introduced. The initial reaction of the sample solution with the EDTA reagent is essential, since the chelation process with the earth metal cations (mostly Ca^{2+} and Mg^{2+}) must be completed at low pH, eliminating the formation of any significant amounts of $Ca(OH)_2$ or $Mg(OH)_2$. As a result, the complexation of the generated NH_3 with the Ca^{2+} or Mg^{2+} is minimized, while at the same time the flow stream is not congested. The amount of the NaOH used is sufficient to cause a rise in the solution pH above 12, ensuring complete conversion of ammonium ions into gaseous ammonia. The length of the reaction coils are such that the reaction is completed before the solution reaches the flow cell where the measurement is obtained.

Analytical results

Figure 2 shows untreated recordings of the system in operation when samples with different ammonium concentrations are injected. Part A consists of standard solutions, while part B consists of spiked seawater samples with the indicated amounts of ammonia in ppm. The range of detection presented is between 0.05 and 10 ppm NH_3, but higher concentrations (more that 100 ppm) can also be accurately measured. The analysis time (shown in *Table 3*) is on the order of 1.7–3.6 min depending on the sample analyzed, while the sensitivity of the system is 53 mV/decade of NH_3 concentration (*Figure 3*). The reproducibility of the system is shown in *Table 4*, where the time between two consecutive measurements of the examined samples is 2 hr. Finally, the stability of the NH_3 monitor is presented in *Figure 4*. A seawater sample with an ammonia concentration of 1 ppm was monitored continuously for 12 hr. The drift of the measurement was only 1.25 or ± 0.03 ppm, an exceptionally low value.

Comparison between FA and colorimetric methods

In order to evaluate the performance of the FA system described, samples containing ammonia were measured and compared with results obtained using the well-established colorimetric indophenol blue method in synthetic seawater, aquaculture media, and effluent from the wastewater treatment plants. Initially, clean synthetic seawater was used to complete the recovery studies. The solutions were filtered through a 0.4-µm filter for the photometric method, while the same samples were also analyzed with the FA system. Additionally, samples that were not filtered were analyzed with the FA system. The results are shown in *Table 5*, which clearly shows that the results obtained with the FA system were very close to the theoretical values of ammonia concentrations, with lower deviations for both the filtered and unfiltered samples than those obtained with the photometric method. The 3- and 5-ppm samples could not be analyzed with the specific colorimetric method due to the limitations in its range of detection. The samples from aquaculture containers as well as those from the effluents from wastewater treatment plants were also measured for ammonia content from the chemistry laboratory of the Institute of Marine Biology. The results showed large deviations with poor reproducibility and accuracy. This was attributed to the cumbersome technique, which required the filtration of the highly contaminated with suspended matter samples through a 0.4- µm filter with subsequent color-generating reaction, which caused the accumulation of serious random errors.

Conclusions

This paper demonstrates the design and performance of a new, portable flow analysis system for the continuous, fast, and accurate measurement of total ammonia of difficult samples such as salty water rearing medium and effluents of wastewater treatment plants, with large amounts of earth metal ions and suspended matter, without the need for any pretreatment or filtration. The complete system has been optimized to operate within the ammonia concentration range above 0.05 ppm. The system offers good reproducibility (<5%) and stability (<0.02 ppm/hr) at constant temperature, while the analysis time is on the order of 1.5–4 min, depending on the sample analyzed. The results from the comparison of the FA system with the standard colorimetric method establish the suitability of the analyzer for precise and continuous measurements of untreated samples for both field and laboratory applications. In addition, its size and weight offer the advantage of portability, while its datalogging capabilities allow for independent monitoring.

References

1. Schneider M, Marison IW, von Stockar U. J Biotechnol 1996; 46(3):161–85.
2. Browder RG, Garman GC. Transactions of the American Fisheries Society 1994; 123:993.
3. Tarazona JV, Munoz MJ, Carbonell G, Carballa M, Ortiz JA, Castano A. Arch Environ Contam Toxicol 1991; 20:480.
4. Wajsbrot N, Gasith A, Diamant A, Popper DM. J Fish Biol 1993; 42:321.
5. Searle PL. Analyst 1984; 109:549.
6. Gibb SW, Wood JW, Fauzi R, Mantoura C. J Automat Chem 1995; 17:205.
7. Harbin A, Van Den Berg CMG. Anal Chem 1993; 65:3411.
8. Genfa Z, Dasgupta PK. Anal Chem 1989; 61:408.