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Automatic colorimetric analyzer prototype for high frequency measurement of nutrients in seawater

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Abstract

This paper describes the characteristics of a prototype of a modular multiparametric analyzer (MicroMAC FAST MP3) for automatic monitoring of seawater and analytical methods for nutrients.

The MicroMAC FAST reactor is an evolution of the basic LFA (Loop Flow Analysis) reactor. It has been conceived to assay ammonium, nitrate–nitrite and orthophosphate at low concentration in seawater samples. A sample analysis is 3–4 times faster than that obtainable with a standard LFA reactor. With respect to the previous analyzer a temperature control (30–52 °C) on the measurement cell has been added (only for modules NH_4 and PO_4), while the colorimeter and the related links for transporting the sample have been moved beyond the Loop and form a hydraulic-optical set almost completely independent from the main LFA. All the steps of a wet-chemical colorimetric analysis method are carried out in an analysis cycle sequentially. The hermetic closed Loop provides full protection against background interference, which is a basic requirement for stable trace analysis. At the start of a cycle the loop is washed and filled with sample. The sample color is measured for compensation. Small amounts of concentrated reagents are added and mixed with high intensity. This new technique allows the preparation of two products of reaction which can be introduced at intervals of 150 s in the measurement cell. The intensity of the color of the reaction product is measured on the colorimeter using a monochromatic light beam of specific wavelength. The statistical test shows that the results of automated and manual analyses agree for all the examined parameters. Precision of all three analyses is ≤4% RSD.

Multiparametric online analyzer: it is possible to connect the analytical modules to a data logger with analogue and digital signals, in order to have online simultaneous analysis of the sample. A typical application is used during research at sea which vessel does not require an operator.

Keywords: Seawater; Ammonia; Nitrates; Orthophosphate; Colorimetric techniques

1. Introduction

In the course of the last 30 years an ever increasing number of automatic tools for Colorimetric analysis, principally using "Continuous Flow Analysis" (Barwell-Clarke and Whitney, 1996; Alvarez-Salgado et al., 1992) and "Flow Injection Analysis" (Hiray et al., 1980; Ranger, 1981; Johnson and Petty, 1983) have been devised. The analyzers of this kind, employed for the determination of phosphorus and nitrogen salts, generally have detection limitations, insufficient to satisfy the analytical demands concerning seawater, generally characterized by poor nutrients.

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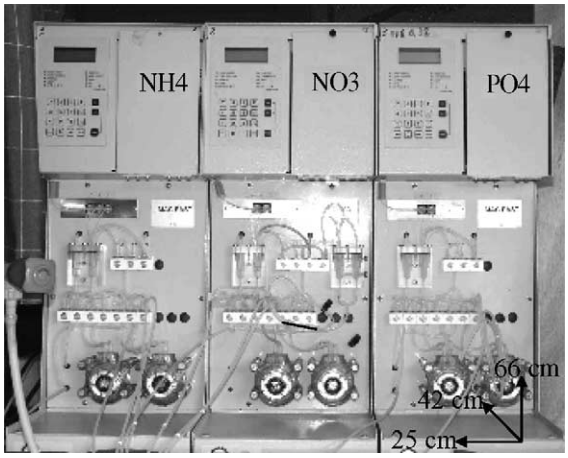

Fig. 1. MicroMac Fast MP3; module ammonia, nitrate and orthophosphate.

The need to have automatic monitoring operations during real-time determination of some chemical parameters (such as nutrients) in environments at risk, has made the realization of an automatic chemical analyzer suitable for onboard installation.

The MicroMac Fast MP3 (MultiParametric) is an automatic chemical analyzer for rapid measurement of seawater (Fig. 1), based on analytical technology developed by Systea (an Italian company) and named LFA (Loop Flow Analysis). LFA has permitted levels never reached before in analytical automation and in analyzer miniaturization. All the steps of a wet-chemical colorimetric analytic method are carried out in an analysis cycle sequentially. The LFR reactor components are sequentially connected to form a Loop, that can be opened (sampling position) or closed (analysis position) by means of 2×3 ways valve. The hermetic closed Loop provides full protection against background interference, a basic requirement for stable trace analysis. At the beginning of a cycle the loop is washed and filled with a sample. The sample color is measured for compensation. Small amounts of concentrated reagents are added and vigorously mixed.

2. Materials and methods

2.1. MicroMac Fast description

The MicroMac Fast is an evolution of the basic reactor LFA and has been conceived for the fast determination of nutrients in seawater. In fact, the frequency of the measurements is 4 times faster than that achieved with previous versions of the instrument μMAC 1000 (Azzaro et al., 1994).

With respect to the LFA standard reactor, the colorimeter and the circuit hydraulic connections to bring the sample and the wash of the flowcell, has been shifted

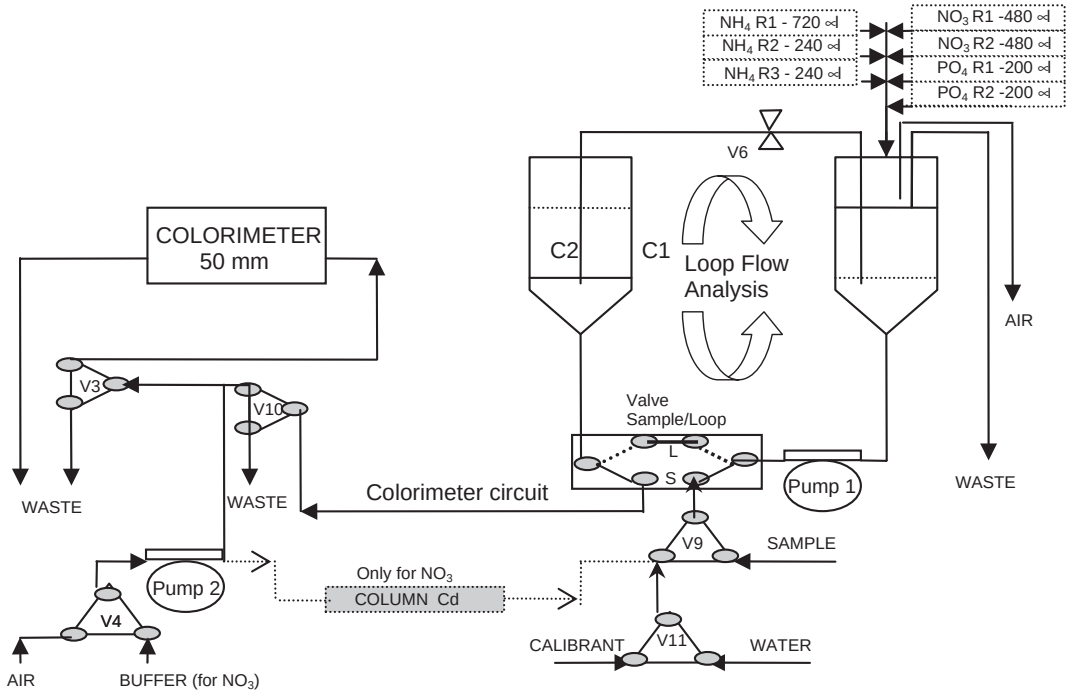

Fig. 2. Hydraulic schematic diagram for determination of ammonia, nitrate and orthophosphate.

outside the loop and make an hydraulic-optical assembly independent from the normal dosing loop (Fig. 2). In the Fast system, the LFA standard reactor can make normal and maximum speed, sampling and reagent dosing, similar to the normal LFA reactor. To speed up the reaction, a heating bath (30–52 °C) has been inserted in the circuit, which is made of a teflon tube coiled around an aluminium heater (only for modules NH_4 and PO_4). As in the normal LFA reactor, a SAMPLE/LOOP valve positioned in sample S and the peristaltic pump P1 is activated in direct to draw the sample. The sample is drawn up through the V9, starts to flow inside the Loop, washing out the liquid present here. At the end of the sampling, the S/L valve is placed in the Loop and the reagents are injected and mixed as in the normal LFA reactor. At the end of the mixing, the S/L valve is positioned in Sample, the valves V10 and V3 are activated realising a link between LFA base and the colorimeter circuit. The pump P1 is activated in direct for a convenient time to transfer the product of reaction inside the colorimeter. The V10 and V3 are deactivated, disconnecting the colorimeter circuit from the LFA base, which is connected to the waste line through of V10 valve. An extra prolonged activation of the P1 pump, with V9 connected to the sample, allows for complete disposal of the product. A part of this has been transferred to the colorimeter to fill the LFA base circuit with the next sample. The steps required for sampling are the dosing of reagents and their mixing with the sample just aspirated, the product of reaction of the previous sample which is still left inside the colorimeter. This allows sufficient time for complete color development that reaches maximum efficiency during the mixing and heating phases in the LFA part of the reactor.

Before transferring the product of the reaction into the colorimeter, the raw optical density of the sample is read and stored. The following wash cycle of the flowcell occurs: activation of V3 valve, activation of V4, activation of P2 (to allow the aspiration of a small portion of air to increase the cleaning efficiency of the flowcell), deactivation of V4 and activation of P2 (to better clean the flowcell), deactivation of P2 and V3 and measuring of the sample blank. As previously said, the new product of reaction can be transferred to the flowcell by the modality described above. This new technique allows the preparation of two products of reaction which can be introduced at 150 s intervals in the measurement cell. The analytical time from introducing a sample to having a result is 300 s. The intensity of the color of the reaction product is measured on the colorimeter using a monochromatic light beam of specific wavelength.

The Fast MP3 is a modular analyzer and each analytical module is independent. Numeric results are stored in a system memory together with analytical graphics for further evaluation. The results are given in concentration units; all measured values are stored with date, time and sample optical density.

On line monitor: each module can be used as a stand alone on line monitor or portable analyzer with IP65 protection, the built-in software includes all the functions needed for on line analysis (16-key keyboard): results on display (2 × 16 row LCD), internal memory up to 100 results, remote printout of stored results, analog output for recorder connection, RS232 serial port. The PC software, expressly developed under Win 95/98 OS, allows a simple and complete management of all the analyzer functions, from autocalibration to results presentation.

2.2. Reaction and OD reading

The reaction takes place in all points of the reactor and therefore in the colorimeter flow cell, thus allowing the monitoring of the reaction from time 0 (reagents injection) to the end point.

If methods require heating, to speed up the colour development (i.e. for NH_4, PO_4) only the colorimeter flow cell will be heated at the requested temperature.

At the reaction end point, measured OD is stored.

2.3. Calculations

Reagent blank OD: stored and used to calculate the calibration factor.

Calibrant OD: stored and used to calculate the calibration factor.

Sample OD: stored together Sample Blank OD and used to calculate Sample concentration.

Sample *concentration*
= (*Sample* OD − Sample blank OD
− Reagent blank OD) × Calibration factor.

The wet chemistries used in Fast MP3 are those recommended by international standards (Strickland and Parsons, 1972).

2.4. Determination of ammonia in seawater

In this automated method, the ammonia ions present in the sample react with phenol and hypochlorite in an alkaline medium according to Bethelot's reaction; trisodium citrate and EDTA are added to the sample to

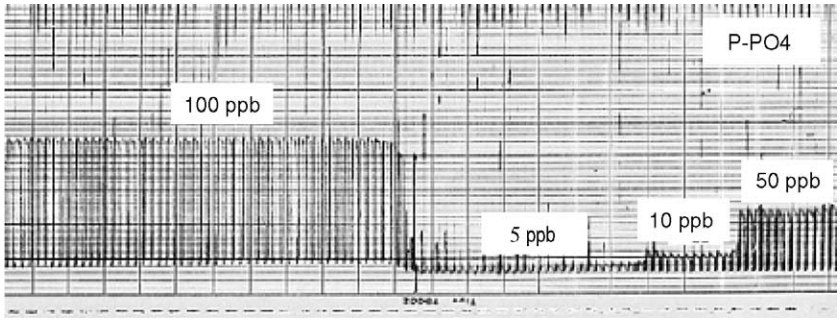

Fig. 3. Recorder traces-orthophosphate product of reaction in 300 s, frequency samples each 150 s.

avoid the precipitation of alkaline hydroxides, while nitroprusside acts as a catalyst. The indophenol blue is measured at 630 nm.

2.5. Ammonia reagents

The complexing agent for seawater was trisodium citrate, dihydrate—25 g, EDTA, disodium salt—2 g, sodium nitroprusside—0.4 g, deionized water (DIW) q.s.—100 ml. The phenol reagent was phenol, solid and colorless—6.4 g, sodium hydroxide—2.75 g, DIW q.s.—100 ml. The Chlorine reagent was dichloroisocianuric acid (D.I.C.), sodium salt—1 g, sodium hydroxide—4 g, DIW q.s.—100 ml.

2.5.1. Ammonia standard solutions

The standard stock 1000 mg/l NH3 as N (solution A) was ammonium sulphate anhydrous—4.714 g, chloroform—10 drops, DIW q.s.—1000 ml. One milliliter of solution A when diluted to 100 ml of water gives a working standard of 10 mg/l ammonia (solution B). 5 ml of solution B when diluted to 500 ml of natural low nutrient seawater (LNSW) gives a working standard of 100 ppb NH3 as N. That is actually introduced into the analyzer for calibration.

2.6. Determination of nitrate–nitrite in seawater

In this automated method, the nitrate present in the sample is reduced to nitrite in a coppered cadmium column, in a buffered medium. The nitrites formed and the ones already present in the sample, react with sulphanilamide and *N*-(1-naphtyl) ethylendiamine in acid medium to give a colored diazonium salt, which is measured at 550 nm.

2.6.1. Nitrate reagents

The washing water was deionized water. The sulphanilamide (SAA) reagent was sulphanilamide 2.5 g, concentrated hydrochloric acid 25 ml, DIW q.s. 250 ml. The naphtylethylendiamine (NED) reagent was *N*-(1-naphtyl) ethylendiamine $\times 2$ HCl—0.375 g, DIW q.s.—250 ml. Buffer solution was imidazole—4 g, concentrated hydrochloric acid—1 ml, DIW q.s. —1000 ml.

2.6.2. Nitrate standard solutions

The standard stock 1000 mg/l NO_3 as N (solution A) was sodium nitrate anhydrous—6.068 g, chloroform—10 drops, DIW q.s.—1000 ml. One milliliter of solution A when diluted to 100 ml of water gives a working standard of 10 mg/l nitrate (solution B). 5 ml of solution B when diluted to 500 ml of LNSW gives a working standard of 100 ppb NO_3 as N. That is actually introduced into the analyzer for calibration.

2.7. Determination of phosphate in seawater

In this automated method, the orthophosphate present in the sample reacts with molybdate in an acid medium to form phosphomolybdate, and then with ascorbic acid to form molybdenum blue, whose intensity is measured at 880 nm. The antymonium catalyzes the reaction.

2.7.1. Phosphate reagents

The molybdate reagent was antimony potassium tartrate—120 mg, ammonium heptamolybdate tetrahydrate—4.3 g, concentrated sulphuric acid—27 ml, DIW q.s.—250 ml. The ascorbic acid reagent was ascorbic acid—10 g, DIW q.s.—100 ml.

2.7.2. Phosphate standard solutions

The standard stock 1000 mg/l PO_4 as P (solution A) was potassium hydrogen phosphate anhydrous—4.394 g, chloroform—10 drops, DIW q.s.—1000 ml. One milliliter of solution A when diluted to 100 ml of water gives a working standard of standard 10 mg/

Table 1
Analytical protocol for the ammonia, nitrate+nitrite and phosphate determinations

Test	1	2	3
Name	Ammonia	Nitrate+nitrite	Phosphate
Sample introduction	Aspiration	Aspiration	Aspiration
First reagent	Citr. hypoch.	SAA	Ammon. molyb.
Second reagent	Phen. alk+DIC	NED	Ascorbic acid
Mixing conditions	Turbulent		
Colorimeter	630 nm	550 nm	880 nm
Light path	50 mm	50 mm	50 mm
Range	0.001÷1.3 absorbtion units		
Kind of measurem.	End point	End point	End point
Uses blank	Yes	Yes	Yes
Sample blank	Automatic zeroing at the end of sampling		
Calibration	Automatic preparation of the working calibrant		
Units	ppb	ppb	ppb
Decimal digits	4	4	4
Calib. ratio mode	Factor	Factor	Factor

l phosphate (solution B). 5 ml of solution B when diluted to 500 ml LNSW gives a working standard of 100 ppb PO_4 as P. That is actually introduced into the analyzer for calibration.

3. Results and analyses

The colorimetric methods examined have different reaction rates reaching the maximum value of optical density for ammonia after 60 min, nitrate after 8 min and phosphate after 25 min (Cardellicchio and Luzzana, 1993). The difference between the value of optical density read at time T2 and that recorded at time T1 is proportional to the nutrient concentration under examination. The Fast MP3 is a sensitive instrument, also able to detect variations in optical density in the order of a tenth of mAU; therefore, it is not necessary for the colorimetric dose to wait for the complete development of the color (Fig. 3). The value of the time T2 usually results from the compromise between the need for the maximum development of color and the number of analysed samples per unit of time.

An important aspect of the system concerns the possibility sample blank measurement and colorimeter zeroing, measuring the optical density immediately after the addition of reagents and the aspiration in the cell, making the approximation that at T1 the colorimetric reaction has not yet occurred. With this stratagem it is possible to correct the value of the optical density, compensating for variations linked to the instability of the lamp, salinity or turbidity of the matrix (Trodelich and Pilson, 1978), without resorting to dual ray systems. In Table 1 the analytical protocols for the three considered parameters in seawater are summarised.

The calibration of the analyzers may be done either with a reading of a standard (100 ppb) located in the reaction chamber or the factor may be set from a series of standard readings at various concentrations, aspirated as samples.

3.1. Accuracy and precision

The precision of the analysis was evaluated by analyzing a series of standard solutions (15, 10, 7, 5 and 2.5 ppb) 15 times for all the methods and calculating the relative standard deviation % (RSD) of the measurements (Table 2).

The analysis precision proves satisfactory with a % RSD average of 2.5 for the ammonia, 4.0 for the nitrates and 2.7 for the phosphates.

3.2. Comparison of manual and automated chemical analyses

Intercalibration of manual and automated procedures is necessary to detect systematic bias in either method, and to ensure that data from automated analyses are compatible with data from manual analyses. Stock Standards (15, 10, 7, 5, 2.5, 2 ppb prepared in low-nutrient seawater) were used to test the agreement between the Fast MP3 (lightpath 50 mm) and the spectrophotometer determinations Varian Mod. Cary 50 (lightpath 50 mm).

3.3. Correlation coefficient

Results indicate good agreement of the two methods with a correlation coefficient $R^2=0.94$ for NH_4, 0.98 for NO_3 and 0.96 for PO_4, calculated on $n=86$ (Fig. 4). The determinable minimal concentration for methods nitrate

Table 2
Accuracy and precision for each method

Nutrient species		Known concentration (ppb)				
		15	10	7.5	5	2.5
Ammonia	Mean concentrat.	15.01	9.64	7.30	4.68	2.10
	Stand. deviation	0.13	0.16	0.10	0.20	0.10
	% RSD	0.84	1.68	1.37	4.13	4.55
Nitrate	Mean concentrat.	15.07	10.01	7.46	5.01	2.47
	Stand. deviation	0.37	0.32	0.18	0.29	0.15
	% RSD	2.36	3.18	2.45	5.85	6.23
Phosphate	Mean concentrat.	15.00	10.00	7.52	4.97	2.46
	Stand. deviation	0.11	0.23	0.15	0.18	0.13
	% RSD	0.74	2.35	2.01	3.54	5.11

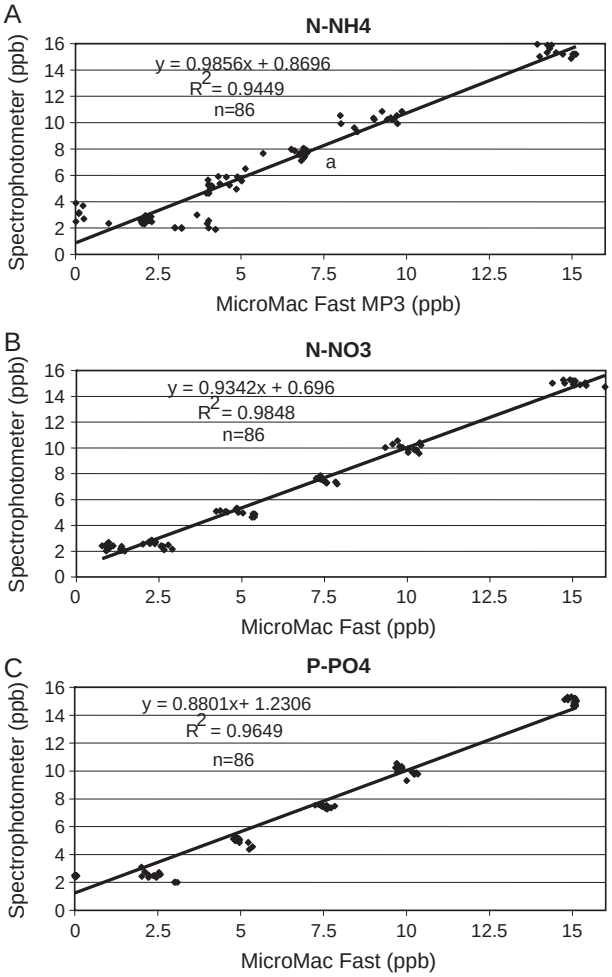

Fig. 4. Linear regression analysis relationship between automated and manual determinations of ammonia (A), nitrate (B) and orthophosphate (C).

and orthophosphate is 2.5 ppb while for ammonium it is 5 ppb, considering that the readings below that value were not perfectly repeatable as they were for other concentrations. If the values of 2 ppb were removed then the correlation coefficient would be $R^2 = 0.99$ for NH_4, 0.99 for NO_3 and 0.99 for PO_4, calculated on $n = 71$.

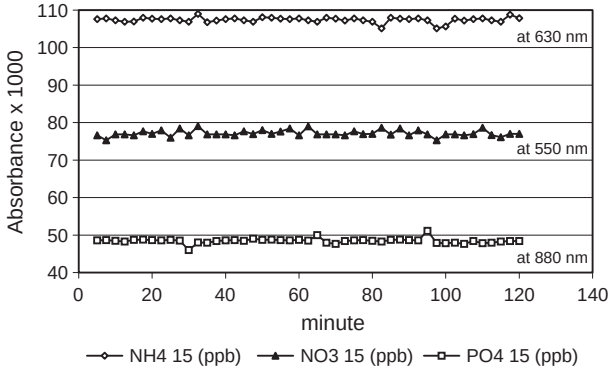

Fig. 5. Instrument drift-a standard solution (15 ppb) for ammonia, nitrate and orthophosphate in seawater was analyzed, every 150 s.

Table 3
Main characteristics for each method

Parameter	Detect. limit (ppb)	Time analys. (s)	Analysis rate samples/h	Vol. samp (ml)	Vol. reag (μl)
Ammonia	5	300	24	10	1200
Nitrate	2.5	240	30	11	960
Phosphate	2.5	240	30	10	400

3.4. Instrumental drift

A standard solution (15 ppb) for each method in seawater was analyzed, every 150 s, per 2 h. The solutions were prepared in a 5 l container and stirred with a magnetic stirred. The results, shown in Fig. 5 do not reveal any instrumental drift.

3.5. Carryover studies

The effects of carry-over which may be present when solutions at highly varied concentrations are sequentially analyzed have been evaluated. The Broughton carryover percentage (K) was calculated according to the equation: carryover (%)$=[(L_1-L_3)/(H_3-L_3)]\times 100$ (Broughton et al., 1974). Three consecutive samples with high (H) concentrations (ex. 500 ppb of NH_4) were measured, followed by three samples with low (L) concentrations (ex. 10 ppb of NH_4), and this sequence was repeated five times. All the measurements made as well as for the three analyzed parameters, a carry-over coefficient superior than 0.3% has not been highlighted.

The three chemical parameters examined have different analysis times. The main characteristics of the devised automatic methods are summarised in Table 3.

Anomalous values were recorded occasionally during the various experiments, probably due to air bubbles in the circuit that created errors in reading the sample; these determinations were not included in the elaborations.

4. Conclusions

The results obtained suggest that the MicroMac Fast MP3 autoanalyzer is reliable for the automatic performing of chemical analyses of ammonium, nitrate–nitrite and phosphates in seawater. The correlation with traditional methods is optimal, also considering the other positive experiments such as the repeatability of data and the absence of drift. In fact, the cleaning of the loops and the colorimeter is a very important feature for the seawater analysis. Indeed, in flow instrumentation the drift is a limiting factor especially for the orthophosphate analysis.

In the future, with regard to the analysis rate, it would be better that all the modules had the same analysis time in such a way to achieve coastal monitoring with the same analysis rate (every 150 s).

In conclusion, the μMAC-FAST MP3 meets the required characteristics for repeatability, accuracy and reading speed, with universally recognised analytical methods. Analysis of nutrients on an equipped boat is the typical application of MicroMAC Fast MP3 that can run a fully automated analysis of nutrients in seawater. Near real-time results, the possibility of connection with the boat positioning system and georeferencing, are the main features of the application.

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