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An Autonomous Nutrient Analyzer for Oceanic Long-Term in Situ Biogeochemical Monitoring

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An autonomous nutrient analyzer in situ (ANAIS) has been developed to monitor nitrate, silicate, and phosphate concentrations while deployed at sea at pressure (down to 1000 m). Detection is made by spectrophotometry. The instrument uses solenoid-driven diaphragm pumps to propel the sample, the standards, and the reagents through a microconduit, flow injection-style thermostated manifold. The analyzers are placed in an equipressure container filled with oil. The analyzers operate until a pressure of 100 bar and show a linear response up to 40 μM nitrate, 150 μM silicate, and 5 μM phosphate with a detection limit less than 0.1, 0.5, and 0.1 μM and an accuracy of 1, 1, and 3% for nitrate, silicate, and phosphate, respectively. The measurement protocol includes three steps over 13 min: rinsing with the sample stream, reagents introduction, and absorbance detection. Field tests comprise ANAIS nitrate, silicate, and phosphate testing alone in the surface ocean. Phosphate results are not yet fully satisfactory. The instrument implemented on top of a YOYO vertical eulerian profiler was then deployed successfully in the northwestern Mediterranean Sea acquiring 30 nitrate profiles between 200 and 1100 m over a 15-day period. This chemical analyzer can be a valuable observing asset adapted on any type of oceanographic platform.

The ocean is a vital component in the metabolism of Earth and an important player in global change. Its vast storage of heat and gases has decisive impacts on the climate; it harbors the most extensive and least known biosphere and contains key living and mineral resources. The ocean usually reacts more slowly in comparison to the land or atmosphere, and it is considered as that part of the Earth system which buffers, modulates, or amplifies physical and biogeochemical signals. Understanding the climatic evolution of the Earth requires long-term monitoring of its main components, the atmosphere, the continental biosphere, and the ocean. Our ability to fully follow and characterize many aspects of the biogeochemical cycles in the marine system requires an improvement of the quantity and quality of measurements of key variables in order to distinguish natural versus human-induced changes. The requisite data need to be collected simultaneously and span time and space scales spanning up to 10 orders of magnitude to observe relevant oceanic processes. Ships and satellites will always be valuable observing assets in international programs such as GOOS, GODAE, CLIVAR, SOLAS, or OCEANS. However, there is an increasing international awareness of the importance and need for development and deployment of novel analyzer and sensor systems in marine studies.[1−3]

Chemical sensors and analyzers[1,2] will play a key role in the study of ocean nutrient dynamics in relation to global carbon fixation and sequestration through the carbon pump with climate implications. They may be carried on various vectors/platforms to operate in situ: buoy systems, eulerian profiling vehicles, lagrangian floats, autonomously underwater vehicles, or remotely operated vehicles. Autonomous multidisciplinary in situ observatories constitute one of the essential players of climatic monitoring of the ocean in conjunction with satellite observations. Building such oceanic observatories, however, remains a significant technological and scientific challenge of the 21st century, as such in situ instrumentation must be autonomous, reliable, precise, of miniature size, not compromised by biofouling, and able to operate on the long term (>3 months of deployment) and at pressure (down to 5000 m) with low energy consumption.

Submersible nutrient analyzers such as the SCANNER,[4] the ALCHEMIST,[5,6] NAS-2E (W.S Ocean Systems),[2] or DPA (Systea) use either peristaltic pumps or piston pumps to propel reagents, calibration standards, and sample, which require significant quantities of power and reagents. The tubing also requires periodic replacement. Such analyzers are thus best suited for short-term, high-resolution spatial mapping of dissolved chemicals. An osmotically pumped continuous-flow analyzer was developed specifically

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for monitoring dissolved species in marine environments for longer times.[7] This analyzer has a 90% response time of less than 30 min and consequently was not ideally suited for implementation on a profiling vehicle.

Our team is particularly interested in the possibility of measuring dissolved macronutrients, key elements in the oceanic carbon cycle. This led us to develop the autonomous nutrient analyzer in situ (ANAIS[8,9]) whose objective is to measure simultaneously dissolved nitrate, silicate, and phosphate. This autonomous chemical analyzer is adapted onto a vertical YOYO profiler[10,11] developed by Laboratoire d'Océanographie Dynamique et de Climatologie[9] (LODYC), within the European MAST III project "YOYO 2001: The Ocean Odyssey". This vehicle is able to profile up and down from 1000 m to the sea surface with a 400 cycles autonomy and is equipped with many chemical, biological, physical, and biooptical sensors and analyzers. As such, this instrument can be considered as a first step to in situ multidisciplinary autonomous oceanic observatories.

ANAIS fulfills the specific requirements to be adapted onto such observatories: long-term autonomy, low energy consumption, miniature size, and ability to sustain 100-bar pressure by equipressure maintenance between the sample and the analyzer. The technique used is the reverse-flow injection analysis technique (rFIA),[12] derived from the FIA.[13] Chemical reagents are injected into the sample stream in rFIA; the reverse occurs in FIA. FIA and rFIA techniques involve microflow rates, minimizing the volumes of required reagents and calibration standards, a prerequisite for long-term in situ deployment. Our system uses solenoid-driven diaphragm pumps to propel the sample, the standards and the reagents through a microconduit, flow injection-style manifold. Nitrate is determined by reduction to nitrite in the presence of a Cd surface, followed by reaction of nitrite to form an azo dye that is detected colorimetrically. Silicate determination is based on the formation of the yellow β-silicomolybdic acid reduced in the silicomolybdenum blue complex. To eliminate interferences with phosphate and arsenate, oxalic acid is added. Phosphate determination is based on the formation of the yellow complex phosphomolybdic reduced with stannous(II) chloride.

We will first describe the ANAIS instrument and then laboratory and initial field results.

EXPERIMENTAL SECTION

The ANAIS instrument is composed of three analyzers determining concentrations of nitrate, silicate, and phosphate, an equipressure container where all analyzers are assembled, a system for in situ seawater sampling, a set of calibration standards and reagent bags for the three nutrients, and a set of four electronic cards.

Apparatus. Each analyzer consists of the following three parts: the solenoid minipumps, the flow manifold, and the colorimetric detector.

(A) Solenoid Pumps. We use solenoid pumps from the Lee Co., of small size, light, maintenance free, self-priming, and individually controlled.[14] The solenoid minipumps function by a solenoid-driven piston pressing or releasing a diaphragm that is integrated with a dual check valve, resulting in a pulsating flow. The standard wetted surfaces within the pump are made of PEEK and Viton. In ANAIS, the sample circulation and the introduction of reagents in the engraved circuit are allowed by Lee Co. solenoid pumps, model LPLA1220050L, originally specified to provide a 50-μL pulse of fluid per stroke and to stand a maximum pressure of 0.3 bar. We modified them to work in oil at equipressure and to offer an adjustable pulse. For equipressure work, four vents were drilled within the PEEK body to allow penetration of the oil in the inner part of the pump. To yield an adjustable pulse, the armature holding was transformed with a M6 thread and we added a mounting fixation in pure iron. A solenoid valve from Lee Co., model LFYA1216032H, which we also modified to work at equipressure by adding four vents within the PEEK body, is also used. In each ANAIS analyzer, the pumps P1, P2, and P3 deliver sample and two calibration standards, which need to be extremely stable over time. Our modification yielding adjustable pulses allowed us to assign different flow rates and check the chemical reaction performance.

(B) Flow Manifold. The manifold is the hydraulic circuit from the sample inlet to the waste outlet. Figure 1 shows the diagrams of the nitrate, silicate, and phosphate flow manifolds from the inlet to the flow cells. The nitrate analyzer is composed of eight pumps (sample, standard solution 1, standard solution 2, reagent 1, reagent 2, reagent 3, $CuSO_4$ for cadmium reconditioning, and deionized water for rinsing) and one solenoid valve. Seven pumps are set up in the silicate manifold (sample, standard solution 1, standard solution 2, reagent 1, reagent 2, reagent 3, and deionized water for rinsing) and only six for the phosphate analyzer (sample, standard solution 1, standard solution 2, reagent 1, reagent 2, and deionized water for rinsing). All nominal flow rates of pumps for the three nutrients, the fluid volume, and the engraving areas of the different sections of the three nutrient manifolds to the flow cell are provided in Figure 1. The different lengths and diameters of the engraved circuit are determined to provide the best shape and intensity of the absorbance peaks. The microconduits are engraved in two poly(methyl methacrylate) (PMMA) plates for nitrate and phosphate analyzers and within two polyetheretherketone (PEEK) plates for silicate. For nitrate analysis, a sinusoidal microconduit has been engraved in a solid cadmium sheet (Goodfellow, Cambridge, U.K.) (50 × 28 × 5) (Figure 2). A 1 mm by 10 mm detector flow cell is bored directly into the manifold perpendicular to the plane of the graved flow path. It is in PMMA for nitrate and phosphate and in PEEK for silicate. The ensemble is sandwiched between two aluminum (Au 4G) plates onto which the pumps and valve are fixed. Threaded screws with nuts hold the ensemble and ensure a proper clamping for perfect adhesion of PMMA or PEEK plates and fluid tightness (Figure 3). Dead volumes within the manifold were minimized to reduce a potential carryover, in particular on reagent introduction within the circuit.

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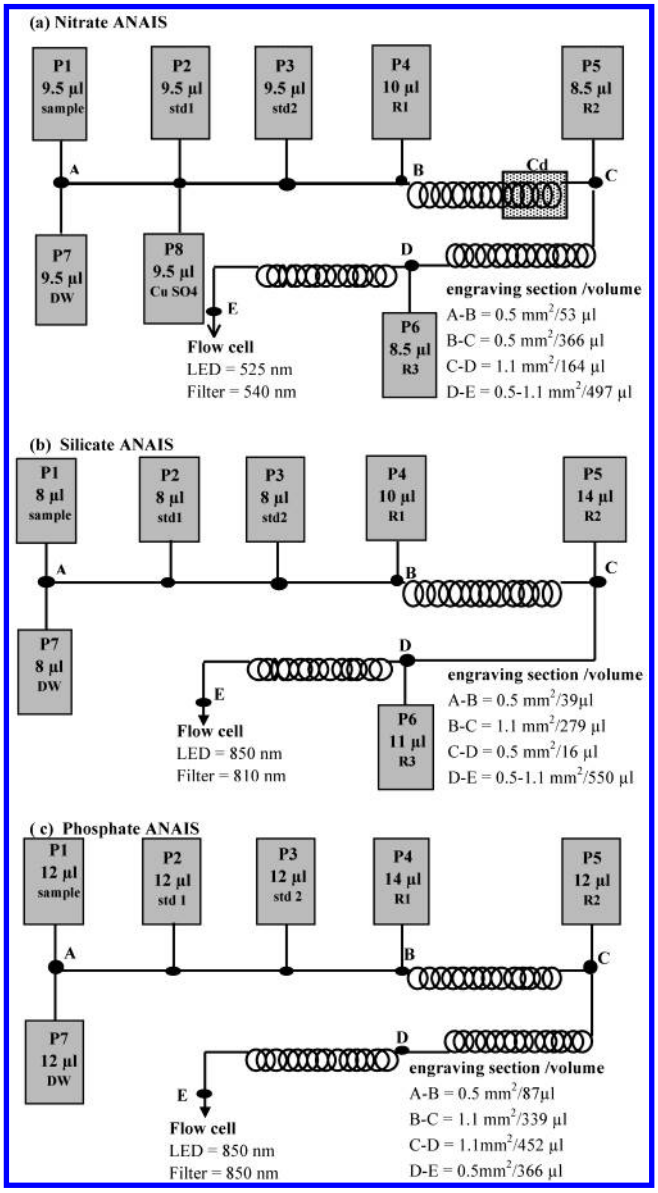

Figure 1. Scheme of the nutrient flow manifold. P1, P2, and P3 deliver sample and two standard solutions, respectively. (a) Nitrate manifold: P4, P5, and P6 deliver the three reagents; P7 deionized water for rinsing; P8 $CuSO_4$ for cadmium reconditioning. (b) Silicate manifold: P4, P5, and P6 deliver the three reagents; P7 deionized water for rinsing. (c) Phosphate manifold: P4 and P5 deliver the two reagents; P7 deionized water for rinsing.

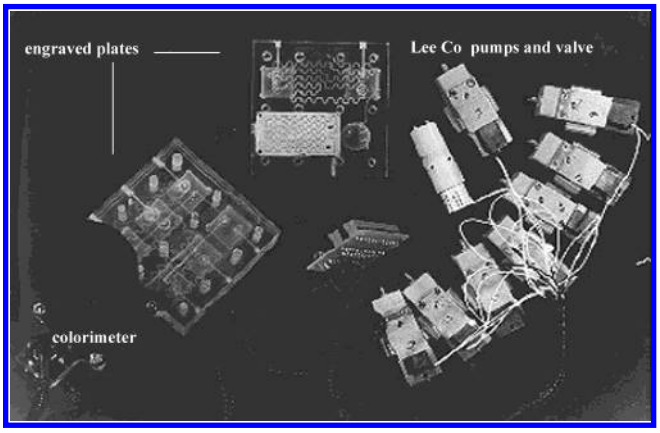

Figure 2. Photograph of the different components of the nitrate manifold.

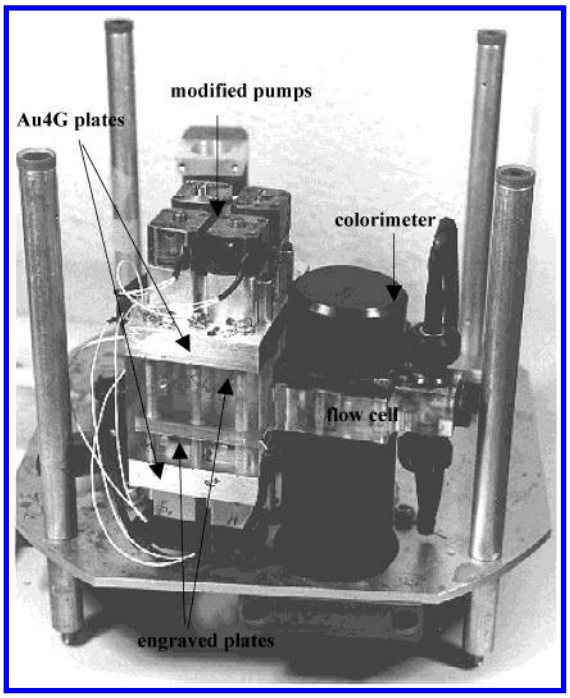

Figure 3. Photograph of the phosphate ANAIS on the container support.

Kinetic reactions are highly temperature dependent; therefore, we choose to thermostat our manifolds within the 20−25 °C temperature range. A thin Minco heating plate is inserted between both PMMA or PEEK plates covering their whole surface for phosphate and silicate (HK 22299-78 * 45 * 0.06) and between PMMA plates for nitrate (HK 5294−39 × 20 × 0.06) uniquely under the cadmium sheet. A temperature sensor (PT 1000) inserted within the manifold allows accurate control and regulation of the temperature within the chemical reaction zones.

(C) Colorimetric Detector. Colorimetric detection is based on Beer−Lambert's law, which links the solution absorbance A of the light by the solution to the analyte concentration c by the relationship $A = \epsilon lc$, where ϵ is the molar absorptivity and l is the length of the flow cell. The colorimetric detectors use a simple LED and photodiode detector to determine the concentration of the analyte product. For nitrate, we use an ultrabright green LED (525 nm, angle of diffusion 15° and dissipated power 120 mW, Radiospares), and for silicate and phosphate, we use an infrared LED (850 nm, angle of diffusion 15° and dissipated power 150 mW, Radiospares). An interference filter (540, 810, and 850 nm for nitrate, silicate, and phosphate, respectively, Melles Griot) is set up close to the LED to obtain a monochromatic beam. Two photodiodes detectors (Hamamatsu S2386-5K), one for the reference signal and one for the measurement signal, are mounted on either side of the flow cell. Convex lenses (Melles Griot) are fixed between the LED and the flow cell to better focus the light signal after the LED. The flow cell is isolated from the spectrophotometer by two quartz glasses (10-mm diameter and 10-mm thickness, Melles Griot) mounted by tightening on either side of the flow cell ensuring fluid tightness between the manifold and the colorimeter down to 100 bar. The ANAIS colorimeter works at atmospheric pressure, and the cylindric body is thus pressure

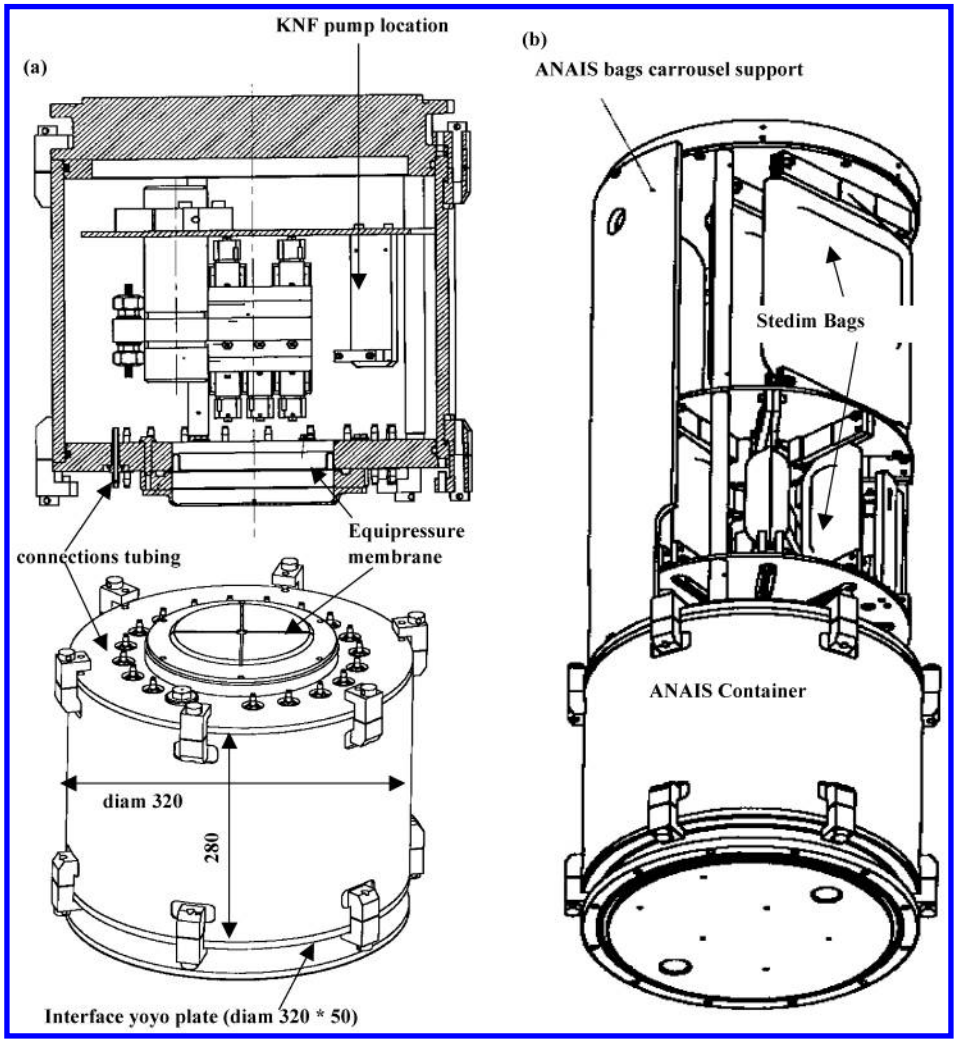

Figure 4. (a) Nitrate ANAIS within the cylindrical container, filled with Isopar M oil; (b) ANAIS assembled for integration onto the YOYO vehicle.

resistant. Two pressure-resistant connectors (SVT 6 pins) allow signal transmission. The output signals of the two photodiodes are converted to voltages and amplified using an electrometer circuit. These currents to voltage circuits are located directly adjacent to the photodiodes to reduce noise. We measure simultaneously the light intensity of the reference and measurement photodiodes. Raw data correspond to the ratio of the sample signal to the reference signal. The amplified signal is digitized with a 18-bit A/D converter with serial interface and stored on a FLASH mass memory card on one of the electronic cards set.

(D) Equipressure Container. The three analyzers (except the electronic package) are placed in a 5086 aluminum alloy cylindrical container, filled with Isopar M oil (Figure 4). Isopar M oil is a very low viscosity oil (density at 15 °C is 0.790 kg/dm^3) and has a dielectric constant of 2.51 at 25 °C.

Success of measurements at great depth highly depends on equipressure maintenance of the container−analyzers ensemble. Consequently, no air bubble can be present in this ensemble. After the container is filled with Isopar M, the container is vacuum degassed for over 48 h. A Viton membrane (Bellofram DC 130 H 55 LJF) allows us to compensate for variation of oil volume due to temperature changes in the container environment.

Reagents and calibration standards are stored in sampling flexible Flex Boy (Stedim) bags of different volumes (150 mL and 2 L). Introduction of the reagents within the manifold in the container is ensured through Teflon inert tubing. The bags are fixed on a carousel support on top of the container and are protected by a careening to guarantee the hydrodynamics of the profiler vehicle (Figure 4).

(E) Seawater Sampling. Seawater sampling is ensured through a KNF pump (Ref NF 11 DC, 0.1 L/min) which allows us to sample swiftly at the desired depth through a sample reservoir made of flexible Teflon tubing of 15 mL of volume. A KNF check valve is fixed near the outlet. The KNF pump is sampling during 40 s in order to allow proper rinsing of the reservoir. The KNF pump is installed within the oil-filled pressure-compensated container. The sample reservoir is attached to the reagent and calibration standard bag carousel.

(F) Measurement Protocol. The measurement protocol for all nutrients comprises three steps: (1) rinsing with the sample stream, (2) reagent introduction, and (3) absorbance detection. For each analyzer, we introduce 2 mL of sample to be analyzed (pumped with the KNF pump) to rinse the circuit. For ANAIS nitrate, we introduce a mixture of sample and buffer (R1) (9 + 1 (v/v)). After the rinsing step, we introduce 26 μL of R2 and 26 μL of R3 for nitrate, 30 μL of R1, 56 μL of R2, and 66 μL of R3 for silicate, and 56 μL of R1 and 48 μL of R2 for phosphate. The colored reaction is transported to the flow cell by introducing the

sample during 5 min for nitrate and 6 min for silicate and phosphate with a flow rate of 0.15 mL/min. The measurement duration is 13 min for each nutrient. The three analyzers work simultaneously. The two standard solutions (pumps P2 and P3) are introduced before and after each series of samples to be analyzed.

(G) Control and Data Logging Electronics. Each analyzer has its own electronic card, which comprises a microcontroller, a FLASH memory card, the optical components, and the supply for all pumps and valve. The Motorola 68HC11 microcontroller drives individually each solenoid pump with a chronogram (software where all dosage parameters are defined). Data recording and storage are performed by a 20-Mbyte FLASH mass memory card (Intel), which also contains the chronogram. Five different chronograms L (rinsing), C (standard 1), D (standard 2), R (cadmium reconditionning), and E (sample) are planned and stored at different addresses on the FLASH card. Chronograms are stored from 0 to C00 addresses. After C00, all data are recorded. Each data heading includes the nutrient type, date, hour, pressure, temperature, data, and an end code for signaling data transmission. A master card ensures the dialogue between the YOYO main brain and the ANAIS cards. A power of 21 mAh is required for one nutrient−one measurement when the manifold is thermostated at 20 °C. In the YOYO configuration, the electronic package is placed within the YOYO body with lithium pack batteries. When ANAIS is working alone, the package is placed in a watertight cylindrical container attached below the ANAIS container.

Reagents and Calibration Standards. (A) Reagents. All solutions were prepared in Milli Q water (Millipore Milli-Q water system) with reagent grade salts (Aldrich and Merck). Prior to preparing the solutions, Milli Q water was autoclaved for 30 min at 120 °C in high-density polyethylene bottles. The prepared solutions were stored within the Flex Boy bags and degassed under vacuum to eliminate potential bubble formation.[7]

The three reagents used for nitrate determinations are an imidazole buffer (6.81 g of imidazole and 1.9 mL of concentrated HCl in 1000 mL of autoclaved deionized water), an acidified sulfanilamide reagent (1 g of sulfanilamide and 10 mL of concentrated HCl in 100 mL of autoclaved deionized water), and a (*N*-(1-naphthyl)ethylenediamine dihydrochloride (NED) solution (0.1 g of NED in 100 mL of autoclaved deionized water). The surface cadmium was initialized by successive passages through the manifold of the following solutions: 10% HCl, imidazole buffer, a mixture of a 2% $CuSO_4 \cdot 5H_2O$ solution and imidazole buffer (1 + 1(v/v)), and imidazole buffer[7].

The three reagents used for silicate determinations are an acidified ammonium heptamolybdate solution (15 g of ammonium heptamolybdate and 6.4 mL of concentrated H_2SO_4 in 500 mL of autoclaved deionized water), an oxalic acid solution (17.5 g of oxalic acid dihydrate and 5 mL H_2SO_4 in 500 mL of autoclaved deionized water), and an ascorbic acid solution (3.25 g of L-ascorbic acid and 0.7 mL of H_2SO_4 in 500 mL of autoclaved deionized water).[15]

The two reagents used for phosphate determinations are an acidified ammonium heptamolybdate solution and a stannous chloride solution (90 mg of stannous chloride, 2.6 g of hydrazine sulfate, and 28 mL of concentrated H_2SO_4 in 1000 mL of autoclaved deionized water[16]). To prepare the final ammonium heptamolybdate solution, three solutions with the following concentrations need to be prepared beforehand: 250 mL of concentrated H_2SO_4 per 1000 mL, 9.5 g of ammonium heptamolybdate per 100 mL, and 3.25 g of potassium antimony oxytartrate per 100 mL. For the final solution, we combine 400 mL of the previous H_2SO_4, 88 mL of ammonium heptamolybdate, and 9.6 mL of potassium antimony oxytartrate solutions.[17] Reagents stability is known to be about one month.[18] A strict preparation protocol allows us to increase their stability for several months (>3 months, Table 1).

Table 1. Stability of ANAIS Nitrate Reagents (R) between March and August 1997[a]

nitrate sample dates	absorbance signal with R in bottles	absorbance signal with R in bags	(R bag − R bottle)/ R bottle (%)
3/5/1997	0.1867	0.1814	−2.8
	0.1702	0.1729	1.6
4/3/1997	0.1895	0.1915	1.1
	0.1815	0.1830	0.8
7/11/1997	0.4820	0.4830	0.2
	0.4800	0.4800	0.0
7/15/1997	0.4780	0.4690	−1.9
	0.4685	0.4590	−2.0
7/22/1997	0.4890	0.4865	−0.5
	0.4720	0.4650	−1.5
8/5/1997	0.4970	0.5020	1.0
8/5/1997	0.4840	0.4995	3.2

[a] R in bottles indicate reagents prepared on the day of the intercomparison, and R in bags are reagents prepared on 3/1/97 with autoclaved LNSW, stored in bags, and degassed

(B) Calibration Standards. Low-nutrient seawater (LNSW) was obtained from northwestern Mediterranean surface nutrient-depleted waters and underwent the same preparation protocol as the reagents (autoclave, storage in Flex Boy bags, and degassing). All working nitrate, silicate, and phosphate calibration standards are prepared as described in the WOCE operations and methods manual with LNSW.[18] For field deployments in the Mediterranean Sea, low and high concentrations of the calibration standards were 4 and 10, 4 and 20, 1 and 4 μM for nitrate, silicate, and phosphate, respectively. Standard solution stability is effective for one year.[19]

RESULTS AND DISCUSSION

Laboratory Results. (A) Figures of Merit. All laboratory tests are performed with filtered (0.45-μM Durapore filter) LNSW. Accuracy[18] tests performed on 10 measurements of the same seawater sample located in the calibration range for nitrate (40 μM), for silicate (80μM), and for phosphate (4 μM) show that the achieved accuracy is approximately 1, 1, and 3%, respectively. Calibration curves obtained with six different calibration standards exhibit perfect linearity with average linear regression coefficient (r^2) of 0.9994 for the 0−25 μM nitrate range, 0.9996 for the 0−30

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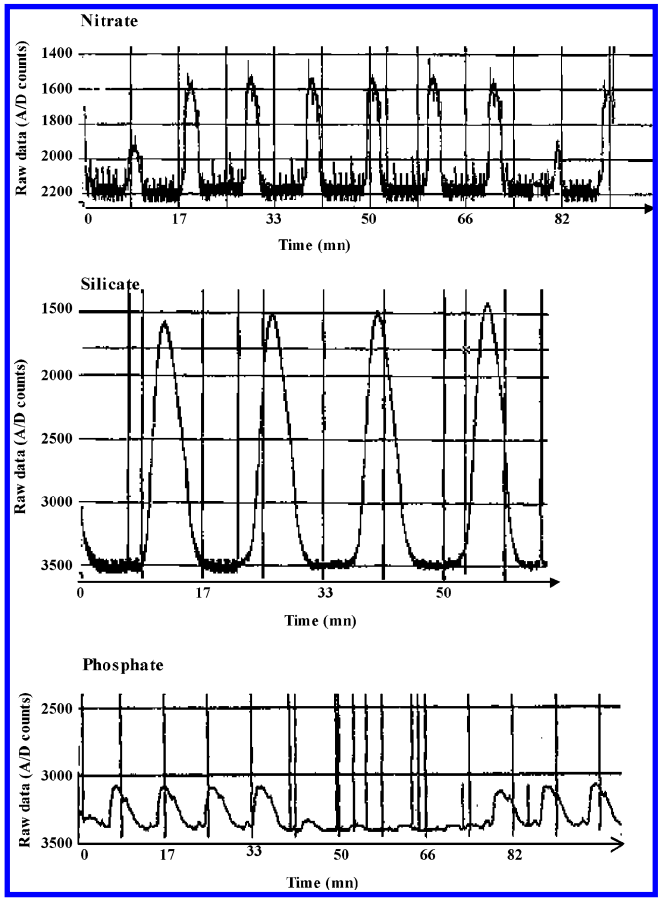

Figure 5. Typical examples of raw data (A/D counts) for nitrate, silicate, and phosphate samples (concentration of 6, 150, and 4 μM, respectively), from top to bottom.

μM silicate range, and 0.9997 for the 0−5 μM phosphate range. Typical detector responses for analysis of various nitrate, silicate, and phosphate standards are shown in Figure 5. Detection limits are less than 0.1 μM nitrate, 0.5 μM silicate, and 0.1 μM phosphate as calculated from 6σ of 10 or 20 replicates of 0.25 μM nitrate, 0.8 μM silicate, and 0.5 μM phosphate standards. To validate our analyzer, systematic comparison was performed in the laboratory between ANAIS and a Technicon Auto-Analyzer II: unknown sample concentrations were determined simultaneously with both apparatus with the same working standards. Tests were carried out with a large range of concentrations for the unknown sample. Average difference between both apparatus is 0.6% for nitrate, 0.3% for silicate, and ~5% for phosphate at the present time.

As for any analyzer, the absorbance had to be corrected for a "carryover" error.[18] This error is highly dependent upon the presence of poorly flushed "dead volumes" in the flow stream and upon the sheer length and complexity of the flow stream. It depends on the differences in concentrations of successive samples entering the flow stream. Thus, an error is present in any given absorbance reading. For monitoring ANAIS performance and ensuring data quality control, the carryover coefficient[18] *k* was recorded over time for each analyzer. These tests confirmed the reliability of ANAIS.

(B) Cadmium Reduction Efficiency. Initial cadmium conditioning yields a cadmium efficiency of 90%. It decreases within a month to 80%. We thus decided to carry out an autonomous partial reconditionning on a monthly basis to bring it back to 90% with a passage of a mixture of a 2% $CuSO_4 \cdot 5H_2O$ and imidazole buffer (1 + 1 (v/v)).

Table 2. Flow Rate (μL/Stroke) of the Pumps during a Three-Month Period (a) for Nitrate, (b) for Phosphate, and (c) Variation of Absorbance (50 μM Nitrate Sample) as a Function of Sample Pump Volume

(a) Nitrate ANAIS[a]

date	P1	P2	P3	P4	P5	P6	P7	P8
3/25/2001	10.1	9.3	9.1	11.5	6.6	8	9.5	7.8
3/26/2001	11.1	10.5	9.9	12.3	8	9.1		
3/28/2001	10.7	10.6	10.4	12.4	8.5	9.2	9.4	9.4
4/25/2001	9.8	10.8	10.4	12	8.3	8.4	10.0	10.0
5/17/2001	9.8	10.2	10.7	11.7	7.4	8.1	10.0	10.1
5/17/2001	9.8	10.2	9.8	11.7	7.4	8.1	10.0	10.1
5/29/2001	9.9	10.2	9.7	12	7.6	7.9	10.0	10.0
5/29/2001	9.9	10.4	9.1	11.7	7.7	7.7		
6/1/2001	9.7	10.2	8.5	11.6	8	8.7	9.6	9.8
6/5/2001	9.6	10.5	9.3	12.2	7.6	8.7	9.8	10.0
average	10.0	10.3	9.7	11.9	7.7	8.4	9.8	9.7
Std/average (±%)	5	4	7	3	7	6	3	8

(b)Phosphate ANAIS[b]

date	P1	P2	P3	P4	P5	P6
8/3/2001	12.4	11.4	11.1	13.4	11.9	11.8
8/20/2001	12.0	11.7	10.5	13.7	12.0	11.5
8/22/2001	12.7	11.4	10.6	14.4	12.0	
8/28/2001	12.3	12.1	12.0	13.3	10.4	11.6
8/31/2001	12.7	12.7	12.2			11.6
9/4/2001	13.7	12.2	13.7	14.8	13.1	
9/6/2001	13.8	12.0	12.9			
9/10/2001	13.6	12.4	13.4	14.4	13.0	
9/18/2001	13.3	13.9	12.7	11.9	11.9	
9/19/2001	13.4	12.9	12.9	10.8	12.1	
9/20/2001	13.3	13.1	12.9			
9/28/2001	13.6	13.9	13.6			
11/21/2001	13.0	13.5	13.2			
average	13.1	12.5	12.4	13.7	12.0	11.6
Std/average (±%)	5	7	9	7	7	1

(c) Variation of Absorbance (50 μM Nitrate Sample)

pump	(μL/stroke)	absorbance
P1(1)	9.5	0.3305
P1(2)	10.1	0.3290
difference (%)	7	0.5
P1(1)	9.5	0.3370
P1(2)	8.8	0.3343
difference (%)	7	0.8

[a] Flow rate of the pumps during three months (March to June 2001) after degassing, container closed. [b]Flow rate of the pumps during three months (August to November 2001) after degassing, container closed.

(C) Pump Stability. Solenoid pumps used in our apparatus were calibrated every month over one-year period in order to check the stability of the volume delivered. This issue is crucial for ensuring measurement reproducibility. A maximum average change of 7% in the injection volume was observed over a period of three months for the main pumps P1, P2, and P3 (sample and calibration standards) (Table 2). This variation does not affect our measurements and proves the reliability of the microsolenoïd pumps. Indeed, an upper limit difference of 7% on the pump volume per stroke can be tolerated for these three pumps, the colorimetric absorbance remaining stable (resulting variation <0.5%) for the precision requirements defined in the WOCE

Table 3. Effect of Temperature on Signal Absorbance (*A*)

	nitrite ANAIS (03/98)		nitrate ANAIS (03/98)		silicate ANAIS (03/00)	
T (°C)	*A*	*A* loss/ 20 °C (%)	*A*	*A* loss/ 20 °C (%)	*A*	*A* loss/ 20 °C (%)
20	0.3075		0.3015		0.4104	
10	0.2915	5	0.2160	28	0.3306	19
5	0.2835	8	0.1898	37	0.1924	53

Table 4. Influence of Temperature: Origins of the Absorbance Loss for a Nitrate Sample

	temperature (°C)		
sample	manifold	sample	absorbance
nitrate 40 μM	5	5	0.1900
	5	10	0.1896
	5	5−10	0.2%
nitrate 40 μM	10	10	0.2180
	5−10	10	13.0%
nitrate 10 μM	10	10	0.0513
	10	20	0.0515
	10	10−20	0.3%
nitrate 10 μM	20	20	0.0630
	10−20	20	18.3%

operations and methods manual[18] (Table 2c as a representative example). Note that change in reagents and $CuSO_4$ pumps volume (P4 and P8) is slightly higher (up to 8%).

(D) Temperature Tests. Immersion and temperature tests were carried out in an aquarium where the water temperature can be varied between 4 and 30 °C. For a given sample, we obtain a significant variation of the absorbance as a function of temperature. Between 20 and 5 °C, a decrease of absorbance of 37% on a 40 μM nitrate sample and of 53% on a 80 μM silicate sample is observed (Table 3). Several experiments were carried out to investigate the causes of the absorbance loss. Indeed, it could be due either to the sample temperature decrease or to the manifold temperature fluctuation. The temperature manifold was held constant, and the temperature of the sample was varied. Deviations of absorbance were ∼0.3% (Table 4, example of nitrate). The temperature sample was then held constant, and the temperature manifold was allowed to vary. Deviations of absorbance ranged between 13 and 18% (Table 4). This led us to the conclusion that if we maintain the manifold to a rather constant temperature with an inserted heating plate, we will avoid any major effect of temperature on the sample or standard absorbance.

Nitrate determination involves reduction to nitrite on a cadmium surface. Between 20 and 5 °C, a decrease of absorbance of 8% on 40 μM nitrite is measured, much lower than that obtained with a 40 μM nitrate sample (Table 3). This indicates that the chemical reaction occurring with the cadmium surface is the most sensitive to temperature. To conclude, heating of the manifolds is performed with an integrated resistance plate all over the surface for both silicate and phosphate ANAIS, whereas for nitrate ANAIS, the heating plate is located only under the cadmium sheet.

(E) Pressure Tests. Pressure tests were made to verify mechanical resistance. During these tests, ANAIS functioning did not undergo any alteration. Each ANAIS individual part and the whole ANAIS system were tested successfully at pressure (up to 120 bar) in a pressure tank facility. All ANAIS parts were working and behaving perfectly well at pressure. As an example, over a 24-h period, the nitrate colorimeter gave a signal difference between 1 and 100 bar of only 0.1%. Reproducibility of the measurement of the same standard between 1 and 100 bar was 0.2%.

Table 5. Summary of Results Collected for Nitrate, Silicate, and Phosphate Determinations with ANAIS Alone in Situ

ANAIS testing alone		surface sample (2−8 m)	deep sample (80−100 m)
nitrate (μM) Nov−Dec 1998	in situ	0.58 ± 0.04	3.24 ± 0.13
	Niskin	0.51 ± 0.16	3.63 ± 0.20
silicate (μM) July 2000	in situ	0.97 ± 0.07	2.78 ± 0.02
phosphate (μM) Oct 2001	in Situ		0.28
	Niskin		0.17 ± 0.01

Field Results. All field tests are carried out without filtration of seawater samples.

(A) ANAIS Testing Alone. A series of tests under real conditions, as part of the YOYO 2001 EC project milestones, was performed with ANAIS alone (either nitrate, silicate, or phosphate) offshore Blanes (Spain) and offshore Banyuls sur Mer (France). ANAIS was tested fixed on a cable from a ship through a shackle attached to an inox rectangular frame. Measurements were made close to the surface and at greater depth. Seawater samples collected with Niskin bottles at the same site were stored at 4 °C and analyzed in the laboratory in Blanes or Banyuls sur Mer for an intercomparison exercise. Table 5 provides a summary of all results collected for nitrate, silicate, and phosphate determination.

In the case of nitrate, the water samples were taken at the very surface and ANAIS samples were taken at ∼8-m depth.[8] For the deep test, all samples analyzed with a TRAACS 2000 in the Blanes laboratory were taken from the same Niskin bottle while the five ANAIS samples might not be exactly from the same location due to the ship drift during the experiment. It is then delicate to call this exercise a rigorous intercomparison exercise. However, one can note that the standard deviation on ANAIS results is much lower than that achieved on the Niskin samples. In the case of silicate, we cannot provide a result for Niskin samples. Samples were taken and measured with a TRAACS 2000 in the Blanes laboratory. Silicate values were abnormally lower as compared with those determined by ANAIS. The reason this happened was attributed to the fact that deionized water with which baseline silicate values were computed contained an excess of dissolved silica due to a strong salinization problem in the water supply in the Blanes laboratory.[20] No surface testing was performed with ANAIS phosphate due to the extremely low level of phosphate offshore Banyuls sur Mer. As can be noticed, the comparison for phosphate determination between samples and ANAIS measurements is not yet satisfactory in the field. We need to duplicate this field trial with a longer sampling period.

(B) ANAIS onto the YOYO Vehicle. A cycle sequence of ANAIS onto the YOYO vehicle comprises three phases: a rest

(20) Provost, C.; et al. YOYO Consortium, YOYO 2001: OCEAN ODYSSEY, Contract MAS3-CT97-0130, Third Annual Report, 2001.

phase (at 1000 m depth), an ascending phase where acquisition of data occurs, and a lowering phase. Two in situ calibrations are performed: one at rest before the ascent and another one upon arrival at the shallowest depth.

The ANAIS container is fixed on the upper hemisphere of the YOYO vehicle with an intermediate plate (Figure 4). In this profiling configuration, the ANAIS electronic package is placed within the YOYO body with lithium pack batteries. A communication software was developed to dialogue with the YOYO central brain. The YOYO central brain sends the order of *on* and *off* and the pressure information to ANAIS. Several dialogue tests were carried out prior to the in situ mooring deployment.

Dialogue tests took place initially in the laboratory and then from the Casablanca oil rig on September 26−27, 1999, in the Mediterranean Sea (40°34′04″ N, 1°21′34″ E) to check that the communication between both instruments was performing adequately.[20] Five vertical profiles were performed successfully over the depth interval 0−140 m during 24 h. Calibration took place at 36 and 138 m, and three samples were determined for nitrate concentrations at 36, 87, and 138 m.[20] In addition to the FLASH card, a second data storage is ensured in the buffer of the YOYO.

Another field test was carried out in the Blanes canyon on the northwestern Mediterranean shelf off the Catalan coast. The YOYO was installed on a subsurface mooring, the top of which was at 100 m below the surface to avoid any interference with local fishermen. The mooring was deployed from the *R/V Garcia del Cid* on October 14, 1999, at 41°31′402″ N, 2°50′740″ E. Only nitrate ANAIS was implemented onto the subsurface YOYO vehicle. The programmed profiling sequency was two cycles a day, starting the ascent at 6 a.m. and 6 p.m. After its last measurement in the surface ocean, the YOYO was programmed to begin its descent. A time out was set up at 8 h on the ascent. The originally planned eight sampling depths for ANAIS were 1000, 800, 600, 400, 200, 150, 100, and 50 m. The mooring line was deployed slightly too deep: the lower stop on the cable was at 1090 m and the top one at 140 m. Therefore, the YOYO was blocked at 140 m and could not go higher. It remained at 140 m blocked by the stop until the time out of 8 h on the ascent was reached. The YOYO central brain sends the *on* order to nitrate ANAIS based on a 200-m pressure decrement until the YOYO reaches 200 m. Above 200 m, the decrement is 50 m. This explains the five resulting sampling depths at which nitrate ANAIS operated: 1090, 890, 690, 490, and 290 m (Figure 6).

The KNF pump did not work during the cycles due to a connections problem, thereby seawater sampling was performed with the solenoid pump which normally pumps the sample water from the sample reservoir. Over the time needed to draw the seawater sample, the vehicle has traveled upward a distance of 20 or 45 m, based on the vehicle speeds, which varied throughout the cycles between 5 and 10 cm/s, respectively. Consequently, nitrate concentrations determined at a depth level Zl reflect the nitrate content of the water column between Zl and Zl-20 (45) m. This implies that nitrate concentrations at depth Zl may be slightly underestimated as compared to their true values. In case of a proper functioning of the KNF pump, the water column sampled, assuming the previous vehicle speed range, would be much smaller, between 1 and 2 m.

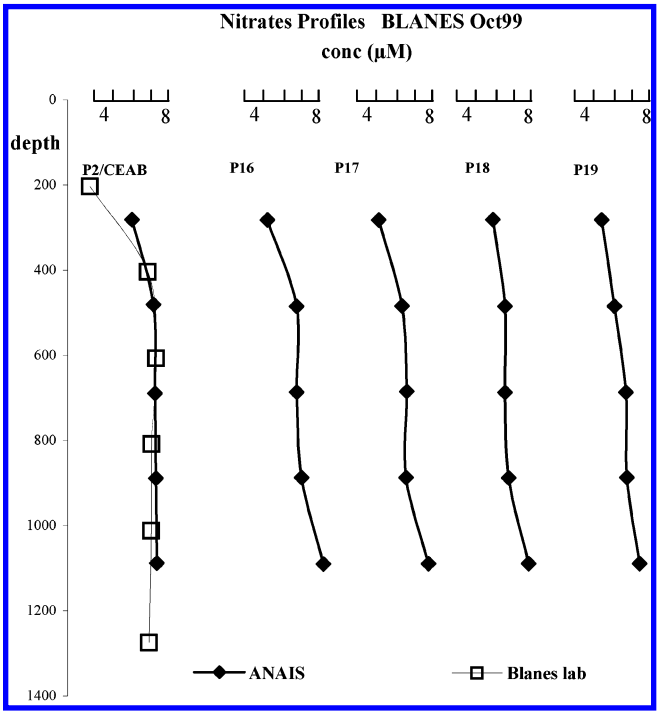

Figure 6. Nitrate vertical profiles obtained in the Blanes canyon. Diamonds indicate ANAIS in situ nitrate concentrations, and open squares indicate nitrate concentrations measured on frozen samples with a TRAACS 2000 collected with Niskin bottles.

The YOYO/nitrate ANAIS profiled twice a day between 1090 and 140 m between October 14 and 29, 1999, so 30 vertical nitrate profiles were obtained. A series of meteorological (short and strong storm) and hydrographical (passage of an anticyclonic feature at the YOYO site) events decomposed the 15-day sampling period in three distinct phases. Five profiles collected between October 21 and 23, 1999, during the third phase are shown in Figure 6. The water column is homogeneous with respect to nitrate concentrations around 5−8 μM down to 1100 m. The nitracline lies above with less than 1 μM at 100 m. An intercomparison exercise, performed at the onset of the mooring deployment on October 15, between NO_3 concentrations measured on samples collected by conventional means (General Oceanics rosette) and ANAIS in situ NO_3 concentrations, is also presented in Figure 6. ANAIS nitrate profile on October 15 nicely overlays that obtained by the classical method. Due to the homogeneity of the profiles, the KNF issue did not turn out to be a severe one. However, the sampling depths are not identical due to the deeper lower stop on the cable by 90 m as initially planned.

During the 15-day deployment, biofouling was not a problem, the ensemble YOYO/nitrateANAIS remained at great depth most of the time, and the system seems immune to internal biofouling for at least the time frame ANAIS has been deployed so far.

CONCLUSION

The analyzers operate until a pressure of 100 bar, show a linear response up to 40 μM nitrate, 150 μM silicate, and 5 μM phosphate with a detection limit less than 0.1, 0.5, and 0.1 μM and an accuracy of 1, 1, and 3% for nitrate, silicate, and phosphate, respectively.

Reliability of ANAIS for the nitrate determinations is fully established for a midterm oceanic deployment. To confirm it over

a longer term deployment, further in situ testing onto the YOYO will take place early 2003 offshore Argentina in the subtropical convergence zone of the Southwest Atlantic ocean. The subsurface YOYO/ANAIS nitrate, profiling once a day, will allow us to monitor water mass characteristics variability over six months in the upper 1000 m. Silicate determinations are fully satisfactory whereas the in situ phosphate results indicate that more field trials need to be conducted. An avenue for exploiting ANAIS is to now measure simultaneously nitrate, silicate, and phosphate. The equipressure container is built for this objective. As such, ANAIS will be ideally suited for in situ oceanic biogeochemical observatories.

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