

Automated nutrients analysis for buoys in sea-water and intercalibration

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Abstract: The Nutrient Probe Analyser-4 (NPA-4) is an automatic chemical analyser for “in-situ” and continuous measurements, based on a new improvement of the exclusive analytical technology developed by Systea and named LFA (Loop Flow Analysis); LFA has permitted levels never reached before in analytical automation and in analyser miniaturisation. With NPA-4 probes, it is possible to analyse directly in the water up to four parameters with the same instrument, using a particular configuration of LFA technology, which allows the sequential analysis of more than one analytical method in a single analytical reactor (30 minutes for a complete 4 parameters cycle). It has been conceived to execute ammonium, nitrate-nitrite, nitrite and orthophosphate analysis at low concentration in sea water samples. The hermetic closed Loop provides full protection against background interference, a basic requirement for stable trace analysis. At the start of a cycle the loop is washed and filled with sample. The sample colour is measured for compensation. Small amounts of concentrated reagents are added and mixed with high intensity. The intensity of the colour of the reaction product is measured one or two colorimetric dual beam with silicon detector, depending on requested methods. The standard wet chemistries for nutrients analysis used in NPA-4 are the ones recommended by international standard. The statistical test show that the results of automated and manual analyses agree for all the examined parameters. Precision of all four analyses is $\leq 3\%$ RSD. The Limit of detection of 5, 5, 2.5 and 2.5 $\mu\text{g/L}$ were reported for N-NH_4 , N-NO_3 , N-NO_2 and P-PO_4 , respectively. Multiparametric on line analyzer: it is possible to connect to a data logger with analogue and digital signals, in order to have on line simultaneous analysis of the sample. A typical application is on line analysis of seawater on an oceanographic research buoy (Max -5m). It does not require any operator.

Keywords: Seawater, Ammonia, Nitrates, Nitrites, Orthophosphate, Colorimetric Techniques

1. Introduction

Continuous monitoring of a highly dynamic system such as coastal waters requires frequent sampling at a precise place in order to catch short-term events which might have a strong impact on the coastal ecosystem, such as exceptional phytoplankton blooms or change caused by storms.

The lack of monitoring systems that provide continuous observations of the marine environment, especially concerning chemical species like nutrients in the coastal areas and shelf seas of Europe, is a serious hindrance. Instruments for in-situ dissolved nutrient analyses are needed by oceanographers. In-situ measurement techniques have many advantages, like avoiding the use of storage bottles to be shipped to land-based laboratories, reducing the risk of sample contamination. In any case, additional and collateral sampling can be performed when special phenomena are

identified.

Automated procedures for the analysis of water sample for nutrients are preferable manual methods when large number of samples and mainly on an oceanographic research buoy. In the course of the last thirty-five years an ever increasing number of automatic tools for Colorimetric analysis, principally using “Continuous Flow Analysis” [1,2] and “Flow Injection Analysis” [3-7] have been devised. The analysers of this kind, employed for the determination of phosphorus and nitrogen salts, generally have detection limitations, insufficient to satisfy the analytical demands concerning sea-water, generally characterized by poor nutrients.

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

Measured parameters:	NH ₃ , PO ₄ , NO ₃ +NO ₂ , NO ₂
Detectors:	two colorimetric dual beam with silicon detector, depending on requested methods (660 and 525 nm)
Type of analysis:	sequential batch
Analysis interval:	programmable
Measuring time:	30 minutes for a complete 4 parameters cycle
Number of parameters:	up to 4
Maximum in-situ depth:	5m (2m suggested)
Body material:	Aluminium
Operating temperature:	8°-27 °C
Hydraulic connections:	std. 3.2x1.6 mm
Waste:	directly discharged in water; optional waste bag available on request
Reagent expiring:	3 to 10 weeks, depending on the method and water temperature
Reagent compartment cooling:	Refrigerated by surrounding water; Peltier refrigeration available on request
Autonomy:	up to one month, depending on measurement interval time

Hardware:	industrial PC-104 CPU, externally programmable by serial communication port
Data output:	RS 232 serial port; RS 422 or RS 485 available on request
NPA control functions:	a) PC: Control Software Windows based, connection on serial port b) External keyboard: IP65 with 16 keys keyboard and 2x16 LCD display, internal connection, do not use on field.
External connections:	6-pole submergible cable for serial data communication and power supply; parallel connector for the control panel
Power supply:	12 or 24 Vdc, provided through the main connecting cable; look to the s/n label inside the NPA before connecting power
Power absorption:	4 W in stand by, 10 W during analysis (mean), 20 W peak absorption
Weight:	25 Kg without reagents (in air)
Dimensions:	250 mm diameter x 700 mm height

2.3. Calibration Cycle

Calibration with working standard:

The mixed working standard is normally placed inside the NPA reagents container; it will be used as it is, instead of sample, during calibration cycles. For each method, the calibration cycle analyse a standard to calculate the calibration factor

2.4. Analytical Cycle Description

2.4.1. LFR Reactor – Main Components

A simple LFA monoparametric analytical reactor includes several devices (mechanical, hydraulics, optics) sequentially interconnected to form a ring defined LOOP FLOW REACTOR (LFR). A standard LFR hydraulic schematic diagram is shown in the previous drawing (Fig. 2); components shown are enough to run up to four chemistries, with the possibility to select two different wavelength.

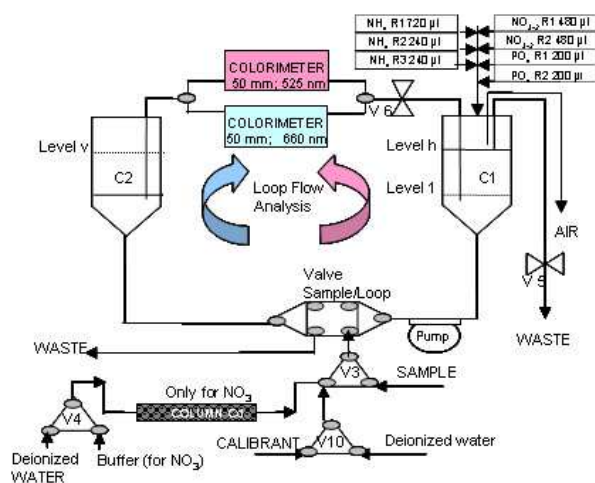


Figure 2. Hydraulic schematic diagram for determination of ammonia, nitrate+nitrite, nitrite and orthophosphate. V (valves); C (chambers).

Valves S/L allows to open the LFR to pump sample, standard or blank solution in (SAMPLE position) or to keep the LFR closed in LOOP, to allows mixing of reaction product. The cylinder C1 allows the injection of the reagents and the calibrant (only in case of calibration using a stock calibrant see 2.3 Calibration cycle) in the LFR, and contributes to create a strong turbulence inside the circuit, in order to speed up the mixing phase.

The valve V6, when activated, interrupts the LFR hydraulic connection and contributes to the vacuum production inside C1. The pump P is a single tube peristaltic pump that can be activated in direct or reverse mode. The valve V4 are activated only when a sample reduction is needed before analysis (i.e. for NO₃-NO₂ reduction). The valves V10 and V3 are used to select Sample, Diluent and Calibrant.

The cylinder C2 is normally filled with air and contributes to the vacuum production in C1, containing temporarily the volume of liquid pumped out from C1 by the pump P.

2.5. Sampling

With valves S/L and V3 on, the pump P is activated in direct mode; the sample is pumped in the cylinder C1, and once reached the level Lh it goes up on the tube, passes through V6, through the colorimeters flow cells, and then reaches C2 and flows out to the waste through the opposite section of the valves S/L. After an appropriate sampling time, the cylinder C1 is filled up to the level Lh by closing V6 and opening V5.

All the LFR components, including the flow cell, are now filled with sample; the system can read and store the Sample Blank value that will be subtracted from the final OD of the reaction product.

2.6. Analysis

Following operation are valid for Sample analysis, Reagent Blank and Calibration

To start the analysis valves S/L are switched off to close LFR in LOOP.

2.6.1. Reagent Injection

To inject reagents into the LFR the valve V6 is closed and the pump P is started in reverse mode until the liquid in C1 will reach the level Ll.

The volume of liquid between Lh and Ll has been moved in C2 where has reached the level Lv; the strong vacuum produced with this operation in C1 will allows to suck the reagents, with a thin and fast flow-out, activating for a precise time (proportional to the volume), the microvalves V1-7 connected to the reagents containers.

2.6.2. Pressure Compensation

Opening the valve V6 will restore the original levels in C1 and C2.

To compensate the overpressure, after reagents injection, valves S/L are switched on for few seconds allowing discharge of excess of liquid contained in the circuit.

2.6.3. Mixing

Valves S/L are positioned again in LOOP, the pump P is activated in direct mode, to allow a fast mixing of sample and the reagents.

The special position of the IN and OUT tubes inside C1 will contribute to produce a strong turbulence thus allowing a fast mixing and the start of the chemical reaction.

2.6.4. 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.

2.6.5. 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) x Calibration factor.

2.6.6. Wash

Valves S/L are switched on, all other valves are off, to allows pumping of diluent (usually deionized water). Pump P is activated in direct mode, water fill up LFR washing hydraulics.

After a proper washing time (about 1 min) LFR, remains in stand by filled with distilled water ready for a new run.

2.6.7. Analytical Procedure

The NPA-4 nutrients probe was configured to measure the nutrient parameters ($\text{NH}_3\text{-N}$, $\text{PO}_4\text{-P}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$) in sea water using the analytical sequence and the standard wet chemical methods reported in [9] and those recommended

by international standards [12,13]. The general analytical sequence and the procedures are summarized in Table 2.

2.7. 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 avoid the precipitation of alkaline hydroxides, while nitroprusside acts as a catalyst. The indophenol blue is measured at 660 nm.

2.8. Determination of Nitrate+Nitrite and Nitrite

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) ethylenediamine in acid medium to give a coloured diazonium salt, which is measured at 525 nm.

2.9. 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 660 nm. The antymonium catalyzes the reaction.

3. Results and Analyses

The colorimetric methods examined have different reaction rates reaching the maximum value of optical density for ammonia after 60 minutes, nitrate-nitrite after 8 minutes and phosphate after 25 minutes [14]. The difference between the value of optical density read at time T1 and that recorded at time T0 is proportional to the nutrient concentration under examination. The NPA-4 is a sensitive instrument, also able to detect variations in optical density in the order of a tenth of milli-adsorption units (mAU); it is not therefore necessary for the colorimetric dose to wait for the complete development of the color (Fig. 3). The value of the time T1 usually results from the compromise between the need for the maximum development of color and the number of analysed samples per unit of time.

Table 2. Analytical protocol for the ammonia, nitrate+nitrite, nitrate and phosphate determinations.

Name	Phosphate	Ammonia	Nitrate+Nitrite	Nitrite
Filtration	Yes			
Sampling	90 sec			
Sample blank reading	Yes (Deionized water)			
First reagent	Acid molybdate	Citrate alk, Hypochlorite alk	Sulfanilamide	
Second reagent	Ascorbic acid	Phen.alk + Dichloroisocyanuric	Naphthyl-ethylenediamine	
Mixing conditions	yes			
Colorimeter	660 nm		525 nm	
Temperature condit.	25°C	45 °C	----	----
Light path	50 mm			

Name	Phosphate	Ammonia	Nitrate+Nitrite	Nitrite
Range LOD-LOL	2,5-500 µg/L	5-200 µg/L	5-500 µg/L	2,5-200 µg/L
Kind of measurem	End point			
Sample blank	Automatic zeroing at the end of sampling			
Calibration	Automatic preparation of the working calibrant			
Calib. ratio mode	factor			
Time analys	5 minutes	9 minutes	7 minutes	5 minutes
Vol. reag. (µl)	960	1200	400	400
Wash cycle	50 sec			

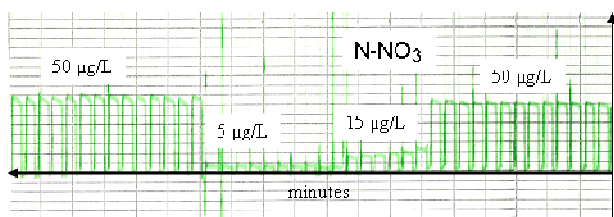


Figure 3. Standard recorder traces-nitrates product of reaction in 7 minutes

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 T0 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

[15], without resorting to dual ray systems.

The general analytical sequence and the procedures for the four considered parameters in sea water are summarized in Table 2.

The calibration, for each method, may be done either with a reading of a standard 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 analysing a series of standard solutions (15, 10, 7, 5 and 2.5 µg/L) 15 times for all the methods and calculating the relative standard deviation % (RSD) of the measurements (Table 3).

The analysis precision proves satisfactory with a % RSD average of 2,96 for the ammonia, 2,99 for the nitrates, 2,24 for the nitrites, and 2,49 for the phosphates.

Table 3. Accuracy and precision (RSD- relative standard deviation) for each method.

Nutrient Species		Known Concentration (µg/L)				
		15	10	7.5	5	2.5
Ammonia	Mean Concentrat.	14.98	9.78	7.45	4.89	2.10
	Stand. Deviation	0.15	0.17	0.12	0.16	0.13
	% RSD	1.00	1.73	1.61	4.29	6.19
Nitrate	Mean Concentrat.	15.06	9.89	7.56	5.06	2.46
	Stand. Deviation	0.32	0.21	0.19	0.19	0.11
	% RSD	2.12	2.12	2.51	3.75	4.47
Nitrite	Mean Concentrat	15.00	9.99	7.51	4.98	2.49
	Stand. Deviation	0.1	0.14	0.16	0.15	0.10
	% RSD	0.66	1.4	2.13	3.01	4.01
Phosphate	Mean Concentrat	14.99	10.02	7.49	5.06	2.44
	Stand. Deviation	0.10	0.18	0.16	0.19	0.19
	% RSD	0.67	1.80	2.14	3.75	4.09

3.2. Limit Range

Limit of detection (LOD) and limit of linearity (LOL), were calculated using approved validation methods and statistically based estimation and the results shown in Table 2.

3.3. Carryover Studies

The effects of carry-over or washout error which may be

present when solutions at highly varied concentrations are sequentially analysed have been evaluated.

The Broughton [16] carryover percentage (K) was calculated according to the equation: carryover (%) = [(L1 - L3)/(H3 - L3)] x 100. Three consecutive samples with high (H) concentrations (ex 200 µg/L of NH₄) were measured, followed by three with low (L) concentrations (ex 10 µg/L of NH₄), and this sequence was repeated five times.

All the measures you make and for the four analysed parameters a carry-over coefficient superior than 0,4 % has not been highlighted.

3.4. 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 (Fig. 4).

Stock Standards (15, 10, 7, 5, 2.5 $\mu\text{g/L}$ prepared in low-nutrient seawater) were used for test the agreement between the Nutrient Probe Analyser (lightpath 50 mm) and the spectrophotometer determinations Varian Mod. Cary 50, (lightpath 50 mm).

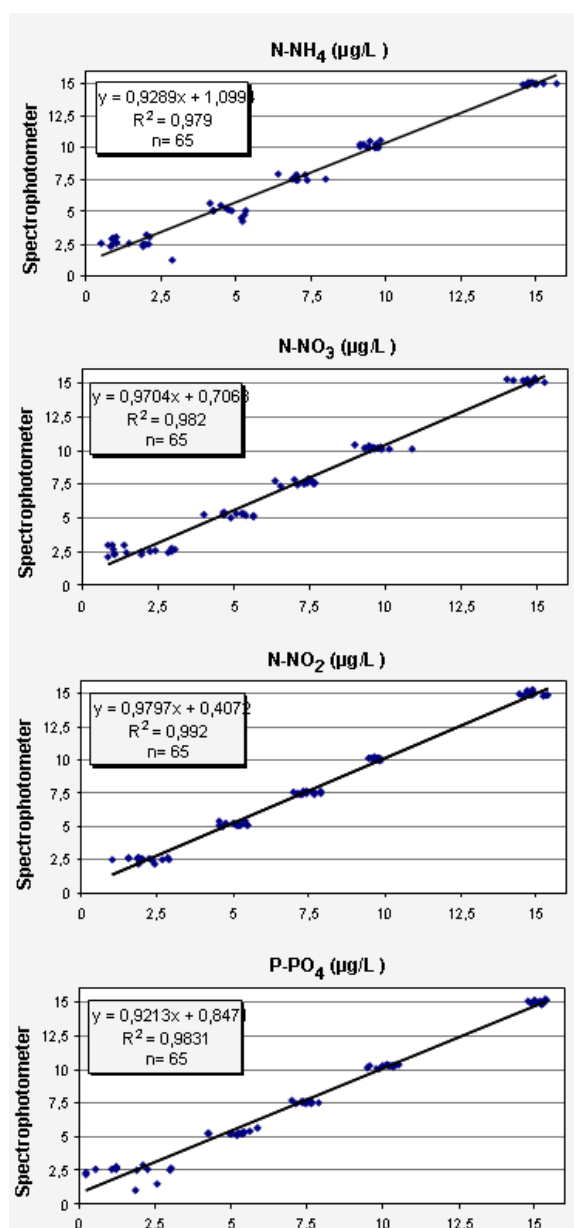


Figure 4. Linear regression analysis relationship between Nutrient Probe Analyser-4 and manual determinations (Spectrophotometer) of ammonia, nitrate+nitrite, nitrite and orthophosphate

3.5. Correlation Coefficient

Results indicate good agreement of the two methods with a correlation coefficient $R^2 = 0,979$ for NH_4 , $0,982$ for NO_3 , $0,992$ for NO_2 and $0,983$ for PO_4 , calculated on $n = 65$ (Fig. 4). The Limit of detection (LODs) for methods nitrite and orthophosphate is of $2.5 \mu\text{g/L}$, while for ammonium and nitrate it is $5 \mu\text{g/L}$, considering that the readings below that value were not perfectly repeatable as they were for other concentrations.

If the values of $2.5 \mu\text{g/L}$ were removed then the correlation coefficient would be $R^2 = 0,98$ for NH_4 and $0,985$ for NO_3 , calculated on $n = 54$.

3.6. Instrumental Drift

The evaluation of repeatability was tested through measurement two standards solution in seawater containing $15 \mu\text{g/L}$ (orthophosphate and nitrites), $30 \mu\text{g/L}$ (ammonia and nitrates) was analyzed for 15 days.

The solutions were prepared in a 5 liters container and shaken with a magnetic shaker. The results, shown in Figure 5 do not reveal any instrumental drift.

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.

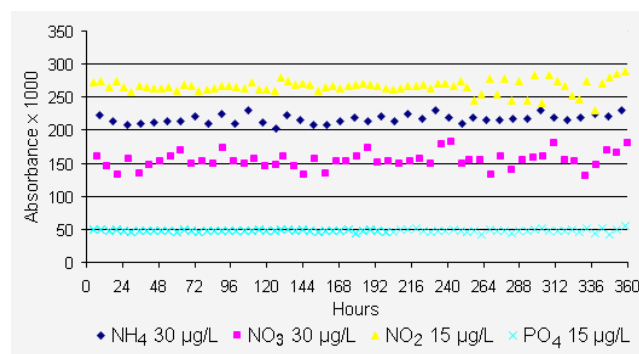


Figure 5. Linear regression analysis relationship between Nutrient Probe Analyser-4 and manual determinations (Spectrophotometer) of ammonia, nitrate+nitrite, nitrite and orthophosphate

3.7. NPA-4 on Automatic Coastal Platform

The study was performed over 11 months (from February to December) in the context of Cluster 10 – SAM Programme (Advanced Monitoring Systems) funded by the Italian national Ministry for Scientific Research. A test of implementation has been done at IAMC-CNR Messina with Nutrients Probe Analyzer and it was later installed in the Gulf of Milazzo (Sicily) in the coastal platform station starting from August 13th, (Fig. 6). This station was measuring nutrients, salinity, seawater and air temperature, fluorescence, turbidity, dissolved oxygen, PAR, and wind speed [17, 18]. The NPA-4 nutrients in-situ probe was configured to measure automatically and unattended the nutrient parameters ($\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$) in sea water using the analytical sequence and the standard wet chemical methods

reported in this paper. The sampling frequency chosen was adapted to the amount of reagents and standards that were filling the flexible bags (500 ml). A about 3-month field test was planned with a 6 hours sampling frequency to run cycles of four nutrients measurements. Online working standards were respectively of 100 $\mu\text{g/L}$ for ammonia, 50 $\mu\text{g/L}$ for phosphates, 20 $\mu\text{g/L}$ for nitrites and 100 $\mu\text{g/L}$ for nitrates. Regular in situ calibration checks were made using on-board concentrated standard automatically diluted by the probe before the measurement.

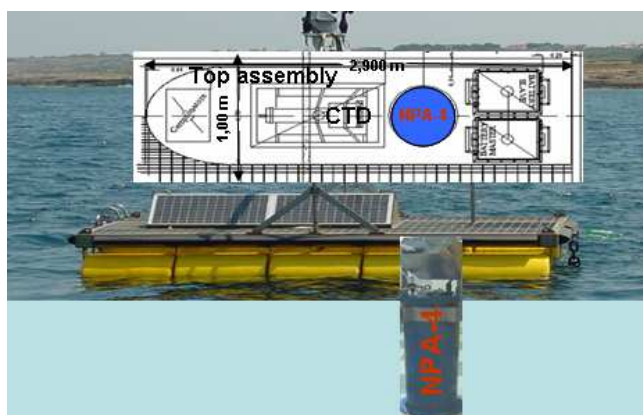


Figure 6. Nutrient Probe Analyser-4 installed on oceanographic platform (buoy).

During the sample intake, the sample is filtered through a 10 μm metal filter screen and during cleaning cycles the filtering unit is flushed several times with compressed air to prevent any back clogging of the filter.

Change of reagents and calibration standards were done every 10 days. Two analyzers were used making a rotation every 20 days during maintenance operations, in order to ensure a complete clean up and recalibration.

The results, presented in Fig. 7 shows the concentration nutrient trend provided by the probe from August 13th to November 11th. Very low values were obtained, typical from this period of the season and of the oligotrophic water on area Gulf of Milazzo (Sicily). The good stability of the PO_4 (range 2.4-18.5 $\mu\text{g/L}$, median 9.2 $\mu\text{g/L}$) and NO_2 (range 0.2-7.8 $\mu\text{g/L}$, median 2.04 $\mu\text{g/L}$) concentrations in the Gulf is highlighted.

Due to wind-driven upwelling (three periods) between September 16th and October 16th an increase in salinity, NH_3 and NO_3 concentration was observed (up to about 30 $\mu\text{g/L}$ for ammonia, with a median of 6.21 $\mu\text{g/L}$ and up to about 23 $\mu\text{g/L}$ for nitrates, with a median of 11,96 $\mu\text{g/L}$).

4. Conclusions

The results obtained suggest that the Nutrient Probe Analyser is reliable for the automatic performing of chemical analyses of ammonium, nitrate, nitrite and phosphates in sea water. The correlation with traditional methods is optimal, considering also 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 sea water analysis.

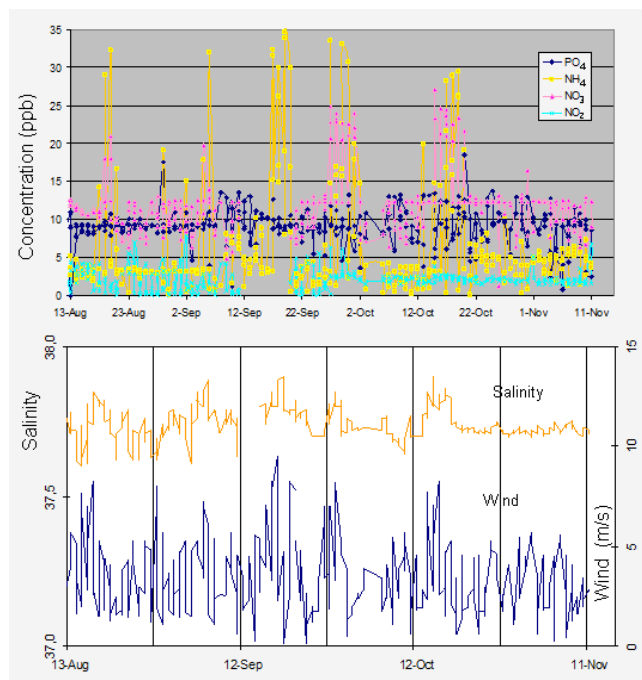


Figure 7. Platform data: automatic hourly series of meteoceanographic parameters (windspeed, salinity) and N-, P-nutrients at the surface, every 6 hours.

Indeed, in flow instrumentation the drift is a limiting factor especially for the orthophosphate and ammonium analysis.

The compactness and the robustness, together with the low reagents consumption, allow the system to be easy used directly for environmental monitoring and research activities. Another important result was the deployment of the Nutrient Probe Analyser on in situ conditions in the coastal platform station. It has been successfully performed with the demonstration of the capability of such system to work on an automated mode with a good accuracy. The probe deployed was loaded with reagents for 2 weeks autonomy and it carried sufficient quantity of standards and DI water to analyze four nutrient parameters continuously at 6 h intervals during the reported period.

In conclusion, the Nutrient Probe Analyser meet the required characteristics, both for repeatability, accuracy and precision, with universally recognised analytical methods. Analysis of nutrients on an equipped coastal buoy is the typical application of Nutrient Probe Analyser that can run a fully automated analysis of nutrients in sea-water.

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