

# Research on automation of a reductive dissolved organic nitrogen analyser

Dissolved Organic Nitrogen  
Analysis  
Automation  
Reduction  
Total Nitrogen

Azote Organique Dissous  
Analyse  
Automatisation  
Réduction  
Azote Total

Christophe LE POUPON <sup>a</sup>, Ron Gen LIN <sup>b</sup>, Jean Y. BENAÏM <sup>a</sup> and  
Adri G. A. MERKS <sup>c</sup>

<sup>a</sup> Laboratoire RCMO, Université de Toulon et du Var, BP 132, 83957 La Garde,  
France.

<sup>b</sup> Institute of Oceanology, AS, 7 Nanhai Road, Qingdao, P. R. China.

<sup>c</sup> CEMO-NIOO, Vierstraat 28, 4401 EA Yerseke, Netherlands.

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## ABSTRACT

The accurate classical batch method of Duursma (1961), based on the conversion of Dissolved Organic Nitrogen (DON) into  $\text{NH}_3$ , is used as a basis for a new automatic DON analyser. After testing different possibilities through mechanical automation of the original batch technique, in which the conversion is preceded by the degassing of  $\text{NH}_3$  and drying of the sample, the procedure of autoanalyses has been selected. This procedure was simplified to the extent that a compact unit could be constructed, which would easily be applicable on board ship. The automatic method has a conversion phase in which in principle only DON is dissociated to  $\text{NH}_4^+$ , whereas both  $\text{NO}_2^-$  and  $\text{NO}_3^-$  are not reduced. This requires selective conditions and particularly high silver purity for the reactor. Consequently, a total reduction, including  $\text{NO}_2^-$  and  $\text{NO}_3^-$  is also possible, applying an efficient metal catalyst. Using distilled water, the accuracy of the method is at the level of  $3 \mu\text{M}$  (0.05 mg N/l), whereas the percent conversion of DON into  $\text{NH}_3$  is estimated between 80 and 100%, depending on the applied reference compounds. The method is applicable to both fresh and sea water.

## RÉSUMÉ

Recherche sur l'automatisation d'un procédé d'analyse de l'azote organique dissous en conditions réductrices.

La méthode classique et précise de Duursma (1961), basée sur la réduction de l'azote organique dissous, est utilisée comme base de travail pour l'élaboration d'un nouvel analyseur automatique d'azote organique dissous (NOD). Après avoir testé différentes possibilités au travers de l'automatisation du procédé, dans lequel la réduction est précédée par le dégazage de  $\text{NH}_3$ , une procédure d'auto-analyse a été choisie. Le principe a été modifié de façon à pouvoir construire une unité compacte qui doit être facile à mettre en application à bord d'un navire. La méthode automatique comprend une phase de réduction dans laquelle, théoriquement, seul l'azote organique est réduit en  $\text{NH}_4^+$  sans que les formes  $\text{NO}_2^-$  et  $\text{NO}_3^-$  soient réduites. Ceci demande des conditions particulières dans le réacteur qui doit être conçu en argent de très haute pureté. Il est possible également de procéder à la réduction totale de l'azote, y compris les nitrites et nitrates, à l'aide de catalyseurs métalliques spécifiques. La précision de la méthode est de l'ordre de  $3 \mu\text{M-N}$  (0,05 mg N/l), tandis que le pour-

centage de réduction varie de 80 à 100 %. Les résultats dépendent directement des composés de référence analysés. La méthode est applicable à la fois en eau douce et en eau de mer.

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## INTRODUCTION

A number of dissolved organic compounds, containing nitrogen, exist in the marine environment, where these compounds have an atomic carbon-nitrogen ratio between 18 and 2. The majority of the organic compounds are of living origin, and include urea, amino acids, nucleotides and proteins. They have surprisingly low C/N ratios between 2 and 6 in the deep sea (Duursma, 1965), whereas in the oceans DON (dissolved organic nitrogen) concentrations range from 0.05 to 0.42 mg N/l (Sharp, 1983; Hopkinson and Cifuentes, 1992; Koike and Tupas, 1993).

Most classical and modern methods for the determination of DON involve a transformation either to  $\text{NH}_4^+$  or/and to  $\text{NO}_3^-$ . The concentration of DON is subsequently found by subtracting the response obtained with the separately determined inorganic nitrogen. In this context it is supposed that all dissolved nitrogen not detected as inorganic nitrogen is organic nitrogen. As noted by Walsh (1989), certain compounds, such as ammonium, can be adsorbed by small particles and colloids, and thus escape inorganic nitrogen analysis. Most of the colloid material will pass through the filter, which separates DON from PON, causing an excessively high DON. Filtration is therefore necessary prior to DON analysis.

One of the first classical methods is the so-called Kjeldahl digestion of DON by sulphuric acid into ammonium. A major problem for the Kjeldahl detection is the low precision of the method, due to the ammonia contents of the reagents (D'Elias *et al.*, 1977). The method is therefore impractical for marine samples.

Other methods, such as the method of Koroleff (1970) and the modified one by D'Elias *et al.* (1977) use an oxidative digestion with persulphate in alkaline conditions under pressure. The oxides formed as nitrate are detectable by spectrophotometry after reduction to nitrite (Henriksen, 1970). In general, these techniques are satisfactorily applicable to fresh and waste waters with high DON concentrations, but less so in the case of sea water with much lower concentrations.

An improved system combines oxidation by UV irradiation and persulphate oxidation in oxygen gas, thereby increasing the completeness of the oxidation and sensitivity of the method. Ultraviolet photo-oxidation has, however, certain drawbacks with respect to the completeness of the reaction, due to the decreasing power of the UV irradiation source with time.

The first method using combustion at high temperature (Suzuki *et al.*, 1985) gave values higher than those determined earlier with other methods, and this caused considerable controversy in the literature. The most recently used method

involves combustion at high temperature, as in the case of DOC (dissolved organic carbon) analysis (Sugimura and Suzuki, 1988; Suzuki, 1993). The system uses a pyrolysis reactor where, in a flow of pure oxygen, organic and ammonia nitrogen are converted into nitrogen-oxide, which reacts with ozone produced by an ozone generator to an activated form of  $\text{NO}_2^-$ , which is detected by chemiluminescence. This method does not require the execution of blanks.

All these methods give the concentration of DON in an indirect manner. The different handlings and separate analyses of the inorganic nitrogen compounds are sources of errors which directly affect the accuracy and precision of the DON analysis. This is even more valid for deep-sea water samples, where the DON concentrations are of an order of magnitude lower than those of inorganic nitrogen. For example, in the North Pacific, DON ranges between 2-3  $\mu\text{M}$  (Walsh, 1989) and 6-8  $\mu\text{M}$  DON (Koike and Tupas, 1993), while nitrate ranges between 1 and 40  $\mu\text{M-N}$  (Sagi, 1970; Sharp, 1983; Maita and Yanada, 1990).

Although the modern techniques for  $\text{NO}_3^-$  and  $\text{NO}_2^-$  analysis are very sophisticated, the classical batch method of Krogh and Keys (1934), which was improved by Duursma (1961), avoids part of the problem of subtraction. Only ammonia has to be subtracted, which usually occurs at a much lower concentration than DON and nitrate. The precision of the method was, for 17 replicates of Atlantic surface water with 34.85 salinity, 0.037 mg DON/l for a DON concentration of 0.145 mg DON/l ( $10.4 \mu\text{M} \pm 2.6 \mu\text{M}$  DON; Duursma, 1961).

Despite the fact that the method is not very laborious, it has unfortunately not often been applied, due to a lack of automation. Nevertheless, the method has major advantages over the oxidative methods, and automation without loss of precision and accuracy would be of service to research on dissolved organic matter in the oceans.

## POSSIBILITIES FOR AUTOMATION

The basic parts of the batch DON analyser of Duursma (1961) were a digestion reactor consisting of a silver tube and a receptor for ammonia. The procedure was: a sample of 5 ml sea water is introduced into the reactor containing NaOH and being flushed with hydrogen. The reactor is slowly heated to 100 °C in order to evaporate the water, which is distilled into diluted HCl, together with the released initial ammonia. Subsequently the reactor is heated for 20 minutes at 500 °C, where DON is converted to  $\text{NH}_3$ . The ammonia obtained from both sources, as well as, separately, inorganic ammonia, is determined by the same method.

A first trial was carried out to conserve the above-mentioned batch procedure by automating the system of sample addi-

tions, the pre-heating procedure and the digestion phase. In order to speed up the treatment of the number of samples per day, a semi-automatic system was tested with batches of five or ten reactors, linked to the same number of absorption vials. These vials were subsequently placed in an autoanalyser for  $\text{NH}_4^+$  detection. However, this method proved as laborious as the original batch method and introduced risks of errors due to differences between the different reactors.

The second option involved a system containing computer-steered valves, which would regulate the absorption of the released ammonia during the two steps of evaporation and digestion. The major problem, however, was the regulation of the two-step heating procedure and the subsequent cooling phase, prior to the addition of a new sample. A single analysis might take even longer than the original batch analysis.

The final option envisaged the suppression of the evaporation phase. The sample is directly injected into the hot reactor, which is kept at  $500^\circ\text{C}$ . Hence, the water sample will evaporate abruptly where water vapour will temporarily expel the flushing hydrogen or helium gas. The immediate question was, whether the transformation of DON would still be complete and whether or not other nuisances, such as the reduction of nitrate, would occur.

By introducing a continuous flow of blank water, interrupted at regular intervals with samples, possibilities of errors were reduced to a minimum. The technical advantage was that a simple automatic analyser could be built with a reasonable performance, on which we will report in the following paragraphs.

## METHOD AND MATERIALS OF DON AUTOANALYSER

### DON reductive silver reactors

Two kinds of silver reactors, in which the transformation takes place, were tested (Fig. 1).

An original vertical tube reactor of Duursma (1961) and a new spiral one were used. Both were constructed from the highest quality pure silver. This is essential, since metal impurities may have a catalytic effect favouring nitrate reduction, while their dissolution causes porosity and sweating of the tubes after a certain period of time. The vertical reactor is heated in an ordinary oven, while the spiral one is wound around a heating element, the whole being in a tightly-sealed box. Both reactors contain temperature detectors and a sensitive thermostat which maintains the temperature within one or two degrees.

The spiral reactor was constructed in order to increase the surface contact of the evaporating and digesting sample with the reagent NaOH, which in the vertical reactor was only present at the bottom of the tube.

### Reagents

#### Reduction phase

Two reagents are required for the reduction: 100 mg/ml pure NaOH dissolved as stock solution, and hydrogen gas

of the highest quality. Should the hydrogen contain oxygen and ammonia impurities, it can be purified by heating over a platinum catalyser and a bubbler containing concentrated  $\text{H}_2\text{SO}_4$ .

Hydrogen serves as the reductive environment in the reactor and as carrier gas to the autoanalyser (for  $\text{NH}_3$ ). The hydrogen flux is set between 10 and 15 ml/min, depending on the size of the equipment.

#### Detection phase

All reagents were of highest available purity and all demineralized water used for dilutions and washings was from a Millipore Ro/Q Plus water system.

For HPLC detection, an eluent ( $10^{-2}$  N  $\text{HNO}_3$ ) was prepared in a 30% methanol solution and stored in a brown glass bottle to prevent light-assisted reduction to ammonium ion.

The analysis of solutions by the indophenol blue method was carried out as follows. The sodium citrate reagent was prepared in 1 litre of ammonia-free water, with 112.0 g of tri-sodium citrate, 2  $\text{H}_2\text{O}$  (AR), 6.18 g sodium hydroxide and 8.15 ml sodium hypochlorite (1 N in 0.1 N NaOH). The phenol solution contained 9.375 g phenol (AR) and 0.125 g sodium nitroprusside in 1 litre of ammonia-free water. The reagents were stable for at least 14 days in darkened reagent containers, stored at  $4^\circ\text{C}$ .

The analysis of ammonia by fluorescence used the  $\text{NH}_4^+$ -o-phthalaldehyde ( $\text{NH}_4^+$ -OPA) complex. The OPA solution containing borate buffer was made up daily in 1 litre of Milli-Q water and contained 0.25 M Borate (15.46 g of  $\text{H}_3\text{BO}_3$  in 1 litre of Milli-Q water, adjusted to pH 9.50 with 10 N NaOH), 100 mg of o-phthalaldehyde (Sigma Chem. Co.) in 2 ml of methanol and 500  $\mu\text{l}$  of 2-mercaptoethanol (Sigma). This solution was deoxygenated by bubbling 200 ml of  $\text{O}_2$ -free  $\text{N}_2$   $\text{min}^{-1}$  through the solution for 20 minutes. The NaOH-citrate solution contained 200 g of sodium citrate, 18.0 g of NaOH, and 950 ml of Milli-Q water. Carrier solution was prepared with 8 ml of

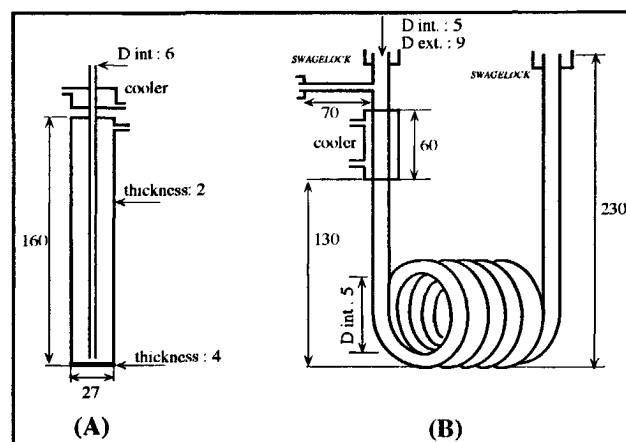


Figure 1

Scheme of the (A) vertical and (B) five-turn spiral silver reactors. (Numbers are in millimetres).

concentrated  $\text{H}_2\text{SO}_4$  and 4 litres of Milli-Q water. The reagents were stable for at least 72 h and are stored capped.

### Preparation of standards

A stock ammonium solution of 100 g/l  $\text{NH}_3\text{-N}$  was prepared by dissolving 76.429 g ammonium chloride (AR) in 200 ml of high purity water. A working solution of 100 mg/l  $\text{NH}_3\text{-N}$  was prepared from the stock solution by serial dilution. Standard solutions were prepared daily from the 100 g/l  $\text{NH}_3\text{-N}$ . All glassware was soaked in 10% hydrochloric acid and rinsed copiously with high purity water prior to use.

### $\text{NH}_3$ detection equipment

#### Analysis of $\text{NH}_3$ by HPLC

Because of its accurate and easy applicability, an HPLC equipment was first tested with the aim of detecting possible breakdown products produced in the reactor and transferred to the analyser. The equipment used a column containing a cation exchanger (type "VYDAC", charged with poly (styrene divinylbenzene) sulphonate, dimensions 4.6 x 50 mm), placed behind a pump (LC-9A, double piston, programmable, Shimadzu). Sample injection ranged from 20 to 100  $\mu\text{l}$ . The detection occurred by conductometry with a CDD-6A (Shimadzu) detector. The obtained signal was treated by an integrator, which permitted the determination of ammonia by the retention time and the peak area. The detection limit is about 3.6  $\mu\text{M-NH}_3$ .

#### Analysis of $\text{NH}_3$ by spectrophotometry

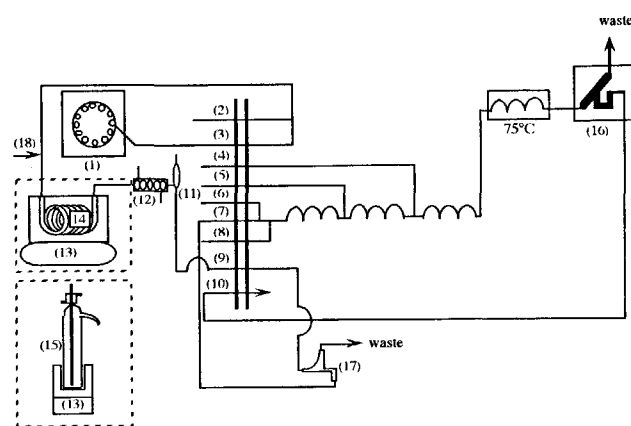
The second detection equipment tested was the SKALAR autoanalyser technique, using a spectrophotometer, applying a micro cell of 30 mm. Colouring occurred with the indophenol method according to Helder and de Vries (1979). The peristaltic pump and sampler were of Technicon. The signals were collected by an integrator as applied above. The detection limit is about 0.014  $\mu\text{M-NH}_3$ .

#### Analysis of $\text{NH}_3$ by fluorometry

The third detection method utilized as detector a SKALAR fluorescence detector, where  $\text{NH}_3$  could be detected at lower concentrations compared with the two other methods. The principles of this method are based on those of Genfa and Dasgupta (1989) and Jones (1991).  $\text{NH}_3$  is diffused across a microporous hydrophobic Teflon membrane into a flowing stream of o-phthaldialdehyde reagent to produce a fluorescent adduct. The detection limit is about 0.0015  $\mu\text{M-NH}_3$ .

#### Standard solutions

For calibration of the technique, the standard solutions used were  $\text{NH}_4\text{Cl}$ , Urea,  $\beta$ -alanine, caffeine, glycine and  $\text{NaNO}_3$ , in Milli-Q water and sea water.



- |                                   |   |
|-----------------------------------|---|
| (1) Sampler changer               | (10) Surplus exit (0.32 ml/min)           |
| (2) NaOH solution ( 0.1 ml/min)   | (11) First de-bubbler                     |
| (3) Sample (0.32 ml/min)          | (12) Cooler                               |
| (4) & (5) Na-citrate (0.1 ml/min) | (13) Temperature regulator and thermostat |
| (6) Air (0.32 ml/min)             | (14) Spiral silver reactor                |
| (7) Sample (0.32 ml/min)          | (15) Vertical silver reactor              |
| (8) Phenol (0.1 ml/min)           | (16) Flow cell 30 mm, filter 630 nm       |
| (9) Reactor exit (0.42 ml/min)    | (17) Second de-bubbler                    |
|                                   | (18) Hydrogen gas (15 ml/min)             |

Figure 2

Flow scheme of reductive DON autoanalyser, with spectrophotometric detection.

### Flow scheme of reductive DON analysis

Small units of some millilitres of sample are placed in a sample changer, interchanged with samples of fresh Milli-Q water and standard solutions. Subsamples are taken at regular intervals, ensuring a fixed amount of solution. This subsample is transferred among a continuous current of Milli-Q water flowing into the reactor, together with the carrier gas hydrogen. In Figure 2, the complete flow scheme of the DON autoanalyser is given for the detection system with spectrophotometry.

#### Discontinuous or continuous methods

##### Discontinuous method :

Injection of samples into the reactor is effected with a syringe. This technique requires the cleaning (by injecting Milli-Q water) of the reactor after each sample injection, in order to remove possible residues in the upper part of the reactor.

##### Continuous method :

By means of a peristaltic pump, a continuous flow of Milli-Q water (and NaOH solution) enters the reactor, thus ensuring stable conditions. This flow is interchanged with samples and standard solutions taken from the sample changer. Except for complete cleaning of the reactor after a defined number of samples (every five days) to remove NaOH and NaCl residues, the

Table 1

Comparison of continuous and discontinuous methods.

Method	Advantages	Disadvantages
Continuous	- Automatic - Rapid analysis	- Some effect of sea water matrix - Regular change of catalyst if applied
Discontinuous	- Less sea water matrix problems - Hydrogen more effective	- Semi-automatic only - High blanks - High limit of detection - Few analyses per hour

method requires a very simple flow scheme and attributes (Fig. 2).

Table 1 presents the advantages and disadvantages of both methods.

## RESULTS AND DISCUSSION

### On the different reagents and conditions

#### NaOH

Sodium hydroxide is essential for a complete transformation of DON to  $\text{NH}_3$ . At the reaction temperature of 500 °C, NaOH is in liquid form. Excessive amounts of NaOH in the reactor will cause a release of NaOH with the effluent, interfering with the detection system. After serial systematic tests, the most suitable form of addition was found to be a co-injection of 0.1 N NaOH with either the samples and/or the blank solutions.

#### Hydrogen

At the moment of sample introduction and water vapour formation, hydrogen is only present at 3%. However, conversion is still occurring during the experimental procedure, thereby demonstrating that conversion of DON to  $\text{NH}_3$  does not really depend on the presence of hydrogen. It is essential to avoid any oxidative conditions that would oxidize DON and  $\text{NH}_3$ . The hydrogen flow should be restricted to 15 ml/min in order to avoid NaOH aerosol release from the reactor at higher rates.

Table 2

Test results of different catalysts on the reduction efficiency of DON and  $\text{NO}_3^-$ .

Catalysts	Compound	DON			N- $\text{NO}_3$			Conditions		
		added (mg N/l)	found (mg N/l)	%	added (mg N/l)	found (mg N/l)	%	T (°C)	H <sub>2</sub> (ml/min)	NaOH (N)
Ni/Cu and Ag thread	Urea	0.140	0.119	85	0.700	0.630	90	500	0.16	0.1
	Glycine	0.140	0.119	85						
	$\beta$ -alanine	0.140	0.127	91						
Stainless steel dipping	Urea	0.280	0.224	80	0.700	0.630	90	500	6	0.1
	$\beta$ -alanine	0.350	0.333	95						
Stainless half-dipping	Urea	0.140	0.122	87	0.560	0.728	130	500	30	1.0
	Glycine	0.140	0.119	85						
	$\beta$ -alanine	0.140	0.148	106						

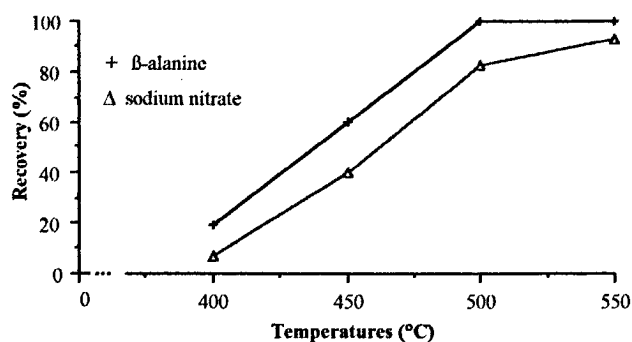


Figure 3

Response of different reactor temperatures on DON and sodium nitrate reduction.

#### Temperature

A high temperature is indispensable for the DON conversion, as can be seen from Figure 3 with, for example, the reduction of  $\beta$ -alanine. The optimal temperature is taken at 500 °C, for the direct outer side of the reactor, which is in a leak-free box to maintain a stable temperature throughout the oven. The sensor has to be attached to the reactor and not to the heating element.

#### Reactor

As explained above (Fig. 1), two types of reactors have been tested. Both the vertical and spiral reactor can give optimal results for the proper conditions involved. It is essential that the connections with the silver tubes be preferentially of silver, in order to avoid unnecessary catalytic effects by other metals which produce active hydrogen in NaOH medium. The highest purity silver tubes available on the market have been used in the design of the spiral reactor.

#### Catalysts

The presence of different catalysts has been tested. In principle, an injection tube of stainless steel will already cause a stimulation of the transformation of DON, where the peak response of the detector will improve. The point

is, however, whether or not  $\text{NO}_2^-$  and  $\text{NO}_3^-$  will be reduced as well. A number of catalysts have been tested, including Raney alloy (Ni,Al), NiO or zinc powder, copper on cadmium grains, vanadium, copper-zinc or nickel-copper alloys. Sometimes, results gave some difficulties to be reproduced. Table 2 presents a survey of the more representative tests with nickel-copper alloy and stainless steel.

From these results, two possibilities are offered by the continuous flow reductive method. First, as previously stated, a very high purity silver reactor can restrict the process to a specific DON conversion to ammonia, without any nitrate reduction. Second, it appears that when metal catalysts are used, nitrate is converted, and thus total dissolved nitrogen can be determined in the samples.

#### Detectors

**HPLC** : according to Noller and Currey (1990), the HPLC technique with ionic chromatography should be able to detect concentrations of  $\text{N-NH}_4^+$  between 0.05 to 500 mg/l  $\text{N-NH}_4^+$ , at least for fresh water. However, the adaptation of the HPLC to samples of sea water posed certain problems. The solutions arriving from the reactor contain sodium, which caused difficulties in detection. A peak signal of  $\text{Na}^+$  was observed to interfere with the  $\text{NH}_4^+$  signal, resulting in a bad resolution of the latter. All tests to eliminate  $\text{Na}^+$  evolution from the reactor failed with respect to this problem.

**Fluorometry** : the fluorometric method of Genfa and Dasgupta (1989) and Jones (1991) has been tested, using a fluorometer adjusted to the autoanalyser. The test series with  $\text{NH}_4\text{Cl}$  could not produce the same sensitivity of the measurements as claimed by the above authors. This remained even below that of the spectrophotometric detector, which impelled us for the moment to continue the research with the last detector only.

**Spectrocolorimetry** : the major part of the research has been carried out with the spectrocolorimetric detector. No interference by either sodium from NaOH or sea water of the reactor has been found. The detection limit was equivalent to 0.04 mg/l  $\text{NH}_4^+$ -N, for a setting with an upper limit of 2 mg/l  $\text{NH}_4^+$ -N. This detector was selected for the final version of the DON analyses.

#### Reproducibility of DON analyser

##### Conversion of DON to $\text{NH}_3$ in the absence of catalysts

A number of tests have been carried out with both types of silver reactors in order to determine the reproducibility of the conversion of DON to  $\text{NH}_3$ . It is essential that the reactors have not previously been used for tests with catalysts, since these are very difficult to remove by rinsing or special cleaning. As is shown in Table 3, the recoveries of DON range from 26% to 126%, demonstrating dependence on the kind of organic matter involved.

These results show that no reduction of nitrate has been observed when its concentrations is less than 0.70 mg N/l.

Table 3

*Examples of conversion in absence of catalysts with organic nitrogen compounds. (Reaction temperature: 550 °C, Hydrogen flux: 6 ml/min, Na OH (0.1N) flux: 0.1 ml/min, sample flux : 0.32 ml/min), \*solutions in sea water.*

Samples	mg N/l added	mg N/l found	Recoveries (%)
Urea	0.140	0.158	113
Urea	0.280	0.221	79
Caffeine	0.140	0.115	82
Caffeine	0.280	0.245	88
$\beta$ -alanine	0.070	0.075	108
$\beta$ -alanine	0.140	0.137	98
$\beta$ -alanine	0.210	0.176	84
$\beta$ -alanine	0.280	0.238	85
Glycine*	0.140	0.176	126
Glycine*	0.210	0.232	111
EDTA*	0.140	0.045	32
Caffeine*	0.140	0.036	26
Urea*	0.140	0.068	49
Sodium nitrate	0.140	0	0
Sodium nitrate	0.700	0	0

The sea water dissolved organic nitrogen standards give lower values than those dissolved in Milli-Q water. De Baar *et al.* (1993) observe the same result in their studying of organic carbon in fresh and sea water. This is probably due to a salt effect.

#### Possibilities of complete conversion/reduction of DON to $\text{NH}_3$ with catalyst(s)

Because of the difficulties in designing a high purity silver reactor, a number of tests have been carried out with the addition of a metal catalyst (stainless dipping steel for instance, Table 2). In these cases, the DON values are obtained by subtracting the inorganic N ( $\text{NH}_4^+$ ,  $\text{NH}_3$  and  $\text{NH}_2^-$ ) from total N.

Replicate series of Mediterranean sea water solutions (with 0.143 mg N/l) were used to estimate the accuracy of the method. The results show a variation coefficient of 8.8% and a standard deviation of 0.013 mg N/l. Blank standard deviation and standard error (D'Elia, 1983) with Milli-Q water ( $n = 55$ ) were 0.007 mg N/l and 0.010 mg N/l respectively (Tab. 4).

SD : standard deviation calculated by the formula  $\sqrt{\frac{\sum d^2}{2N}}$

2.ES : two standard errors :  $\frac{2SD}{\sqrt{2}}$

Table 4

*Blank determinations.*

Replicate analysis (n)	Mean concentration (mg N/l)	SD (mg N/l)	2SE (mg N/l)
7	0.066	0.007	0.009
10	0.059	0.008	0.011
10	0.076	0.007	0.009
14	0.081	0.004	0.006
14	0.059	0.010	0.014

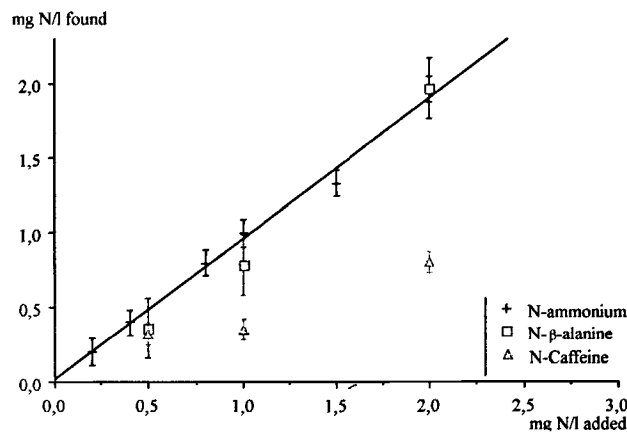


Figure 4

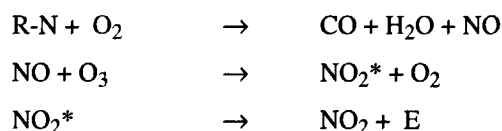
Control analyses of dissolved organic nitrogen determination.

Results show 100% recovery of nitrogen with  $\beta$ -alanine. Caffeine with C = N double bonds probably less reactive gives poor yields (Fig. 4). Le Poupon (1994) discuss catalytic effects with possibilities of complete nitrogen conversion.

#### Intercalibration step

An intercalibration exercise has been set up comparing our reductive DON analyser with the oxidative ammonia analyser ANTEK-7000, available at the Oceanographic Institute at Villefranche-sur-Mer of Dr. Copin-Montégut (Laboratoire de Physique et Chimie Marines, University of Paris-VI). This technique should analyse DON in sea water, ranging between 0.07-0.63 mg N/l.

The ANTEK analytical procedure involves the injection of 5 ml sea water into a quartz crucible containing quartz wool. The crucible is automatically placed in a furnace which is heated at 1100 °C. The carrier gas is pure oxygen and generated ozone:



After separation of water vapour, the  $\text{NO}_2^*$  produced in a high electronic state is detected by chemiluminescence. A

Table 5

Recovery of added DON to distilled water with the ANTEK-7000 technique and reductive method.

Solutions	n	Concentrations (mg/l)					
		ANTEK-DON			Reductive DON		
		Added	Found	Efficiency	Added	Found	Efficiency
urea	2	0.7	0.59	84%	0.7	0.69	99%
EDTA	2	0.7	0.67	96%	0.14	0.12	86%
caffeine	2	0.7	0.64	91%	0.28	0.25	89%
Mean				90 ± 6			91 ± 7

problem encountered is the interference of chlorine, which makes the analysis of low DON concentrations in sea water difficult. The calibration curve of  $\text{NO}_3^-$  given in Figure 5, shows a proportional response between 0.4 and 7 mg N/l.

Calibration with urea, EDTA and caffeine dissolved in distilled water showed, however, a lower response with respect to the added amounts (Tab. 5). The percentage recovery ranged between 84 and 96% for an added concentration of 0.7 mg DON/l. With the same compounds and lower concentrations, our reductive method gave as much as 86 to 99% of recovery. The means of each DON determination show similar results.

Continuous combined UV plus chemical oxidation analyser has been used as another comparative method. This apparatus has been set up in the RCMO laboratory (University of Toulon et du Var - France) with two parts of Technicon systems. One of this is a UV plus chemical oxidation compartement of the DOC AutoAnalyser and the other part is the classical nitrite and nitrate AutoAnalyser. Combination results in a continuous flow apparatus. DON is determined by difference, the accuracy is 0.02 mg N/l and the coefficient of variation is inferior to 5%.

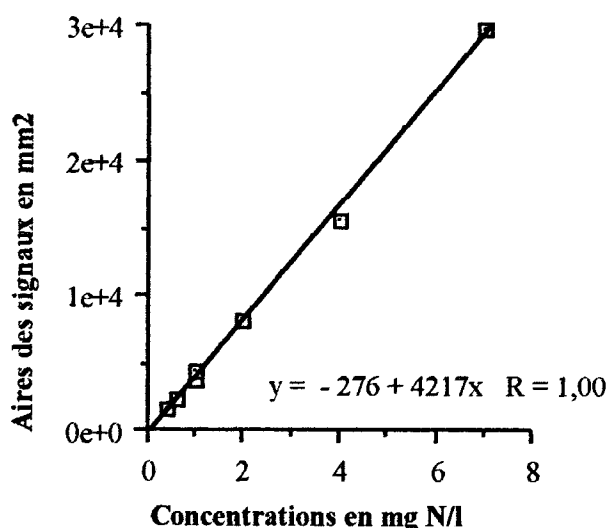


Figure 5

Calibration curve of  $\text{NO}_3^-$  using Antek analyser.

Table 6

Nitrogen concentrations in mg N/l by different techniques (\*, station at Antwerp). (1) : CEMO Yerseke (NL), (2) Lab.; RCMO Toulon (F), (3) Institut Villefranche-sur-Mer (F). WS = Western Scheldt, OS = Eastern Scheldt.

Stations	Ammonium (1)	Nitrate plus Nitrite (1)	Total Org. + Inorg.N by oxidation (2)	Total Org. + Inorg.N by oxidation (3)	DON by oxidation (2)	Total Org. + Inorg.N by reduction (2)	DON by reduction (2)
WS1	0.09	3.08	5.0	2.60	1.83	3.87	0.70
WS2	0.12	3.62	4.4	3.48	0.66	4.26	0.52
WS3	0.22	4.01	5.6	3.87	1.37	5.56	1.33
WS4	1.05	4.04	6.4	5.29	1.31	6.47	1.38
WS5	2.95	3.92	6.9	5.56	0.03	7.55	0.68
WS6*	4.25	3.12	7.0	5.66	–	8.16	0.79
WS7	4.62	2.78	5.8	5.69	–	7.64	0.24
OS1	0.34	0.37	0.81	0.50	0.10	0.61	–
OS2	0.13	0.37	0.51	0.35	0.01	0.47	–

The intercomparison, carried out with different sea water samples, between oxidative and reductive techniques showed some additional problems with the DON calculation (Tab. 6).

The samples were taken from the eutrophic river estuary system of the river Scheldt (Western Scheldt) and the much less eutrophic Eastern Scheldt (The Netherlands), the latter having no (or very little) fresh-water inflow (Fig. 6).

One-litre water samples were passed over a filter, prior to analysis. Conservation with, *e.g.*, HgCl<sub>2</sub> was avoided, since this might provoke interferences with the nitrate determination (Kremling and Wenck, 1986). Samples were rapidly frozen and analysed the following day.

### Comments on the results

Total nitrogen obtained with the different methods is shown (Fig. 7).

Relatively high concentrations of inorganic nitrogen in the Western Scheldt are due to urban waste releases from Antwerp. Small concentrations in Eastern Scheldt are mainly due to its open connections with the North Sea (Fig. 8).

In the two figures, total nitrogen values show relatively poor correlation between high temperature oxidative determination (ANTEK) and other methods. The difference may be related to a poor quick-freezing preservation of samples for Antek analysis, two months after sampling,

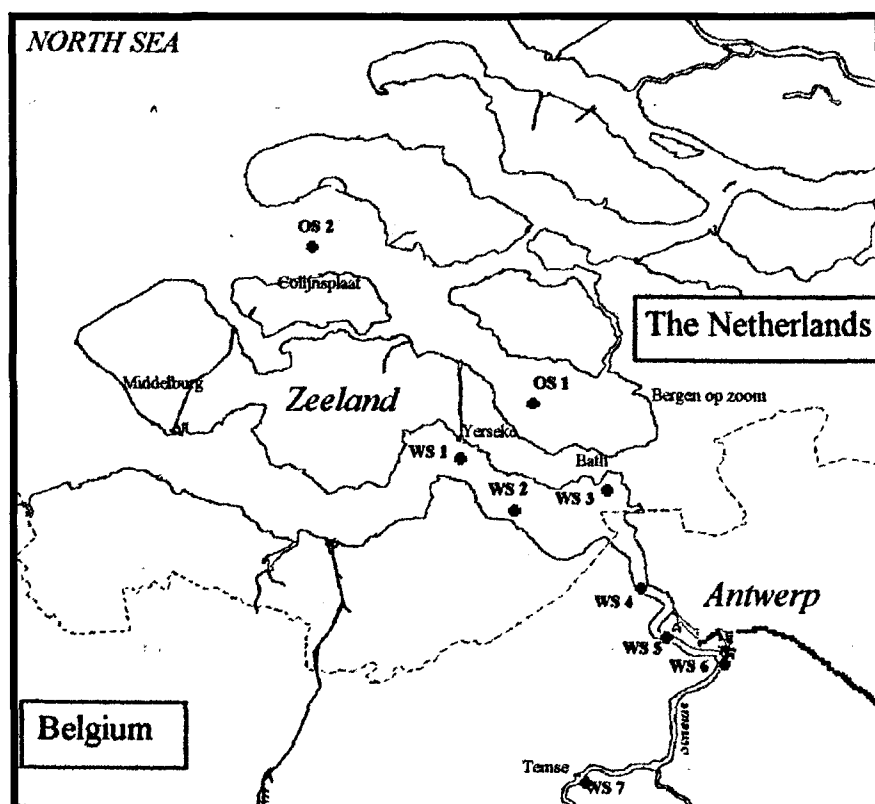


Figure 6

Location of sampling stations in the Western Scheldt (WS) and the Eastern Scheldt (OS).



Figure 7

Inorganic nitrogen and total dissolved nitrogen values in the Western Scheldt. Comparison between the three methods. (1) inorganic nitrogen ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ), (2) total nitrogen by UV oxidation, (3) total nitrogen by high temperature oxidation, (4) total nitrogen by reduction.

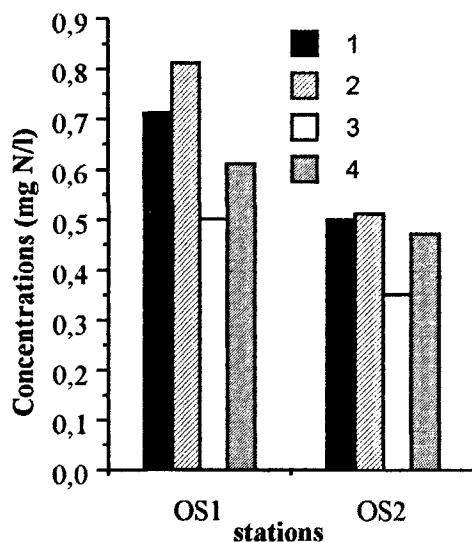
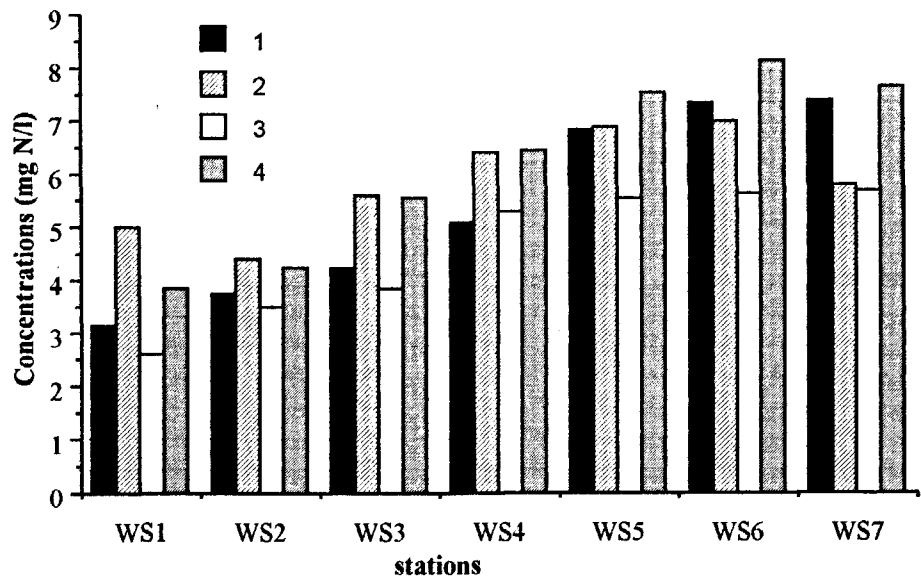


Figure 8

Inorganic nitrogen and total dissolved nitrogen values in the Eastern Scheldt. Comparison between the three methods. (1) inorganic nitrogen ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ), (2) total nitrogen by UV oxidation, (3) total nitrogen by high temperature oxidation, (4) total nitrogen by reduction.

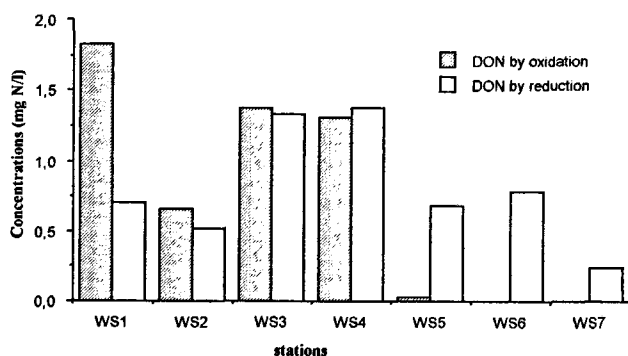


Figure 9

Dissolved organic nitrogen in Western Scheldt stations. Comparison between oxidative and reductive determinations.

rather than a failure of the Antek procedure. The other values were determined immediately after sampling.

Dissolved organic nitrogen is calculated using total and inorganic nitrogen from Table 5, and results are also shown in Figure 9.

Comparing the values obtained by UV plus chemical oxidation and those by the reductive method, DON is similar in stations WS2, WS3 and WS4. The near zero values in polluted stations WS5, WS6 and WS7 may be ascribed to high phosphate concentrations, which interfere in the determination of nitrogen (reduction of  $\text{N-NO}_3^-$  with  $\text{Cd/Cu}$  alloy) by DON oxidation (Olson, 1980). These latter values, calculated by difference, are unrepresentative. Such high phosphate concentrations do not affect the reductive analytical process, with or without catalyst. Measurements with reductive conditions are not limited in this case.

## CONCLUSION

An automatic DON analyser has been constructed with a spiral silver reactor in a tight oven and a spectrophotometric detector. This apparatus may serve as an additional tool for the analysis of dissolved organic matter in different sea-water and fresh-water environments. The equipment, though being simple and connected to basic autoanalyser techniques, requires skilful handling in order to obtain reliable results. Calibration is constantly required during the execution of the analyses, which reduces the chance of failures.

The reductive method exhibits good precision, and appears near absolute in accuracy by virtue of its complete recovery of all tested inorganic and organic nitrogen compounds. In the case of natural sea-water analysis, salt effect can be expected as for other methods; consequently, high salinity samples can slightly alter reductive conditions, perturbing measurements. In heavily polluted waters, such as those near industrial waste-water effluents, the determination of DON in a reductive medium has the additional advantage of not being interfered with high phosphate concentrations over the photo-oxidation method.

Thus, the automated reductive DON analyser, used either with or without catalysts, contributes to a better insight into the reliability of DON concentration in the marine environment, taking the specificity and inconveniences of the various methods into account.

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