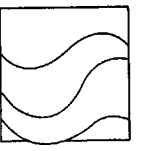

Determination of dissolved organic nitrogen in seawater using Kjeldahl digestion after inorganic nitrogen removal

Dissolved organic nitrogen
Kjeldahl
Seawater
Northeast Atlantic Ocean
Dissolved organic carbon

Azote organique dissous
Kjeldahl
Eau de mer
Océan Atlantique NE
Carbone organique dissous

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ABSTRACT

An update of the Kjeldahl method is presented for the direct determination of dissolved organic nitrogen (DON) in seawater. Dissolved inorganic nitrogen is previously removed: ammonium as NH_3 with NaOH at pH 9.4; and, subsequently, nitrate and nitrite as nitric oxide with $FeSO_4$ in acid medium. The sample is then mineralized to ammonium, which is measured with a Technicon autoanalyzer by the indophenol blue method.

The range of recovery for tested standard compounds is similar to those obtained by high temperature oxidation (HTO) techniques. Direct determination of DON by the method described in this work marks an improvement in precision, in comparison with other methods; the standard deviation obtained for samples of seawater is ± 0.2 $\mu mol.l^{-1}$. The precision of DON measurements is not dependent on dissolved inorganic nitrogen analysis.

For several stations in the Northeast Atlantic Ocean, DON values ranged between 3 and 10 $\mu mol.l^{-1}$.

RÉSUMÉ

Mesure de l'azote organique dissous dans l'eau de mer par la méthode de Kjeldahl, après élimination de l'azote inorganique.

La méthode de Kjeldahl a été modifiée pour réaliser la mesure directe de l'azote organique dissous (NOD) dans l'eau de mer. Les composés inorganiques dissous dans l'échantillon sont préalablement éliminés comme suit: l'ammonium, en rendant le milieu basique avec de la soude; le nitrate et le nitrite, en les transformant en oxyde nitrique par le $FeSO_4$ en milieu acide. L'échantillon est ensuite minéralisé pour transformer le NOD en ammonium dont la concentration est déterminée sur un AutoAnalyseur Technicon.

La récupération des produits standard est identique à celle obtenue par des techniques d'oxydation à haute température. La détermination du NOD par cette méthode est améliorée car elle ne dépend plus de la mesure des concentrations en azote minéral comme dans les autres méthodes. L'écart-type est d'environ $\pm 0{,}2$ $\mu mol.l^{-1}$ pour des échantillons d'eau de mer.

Les concentrations, mesurées par cette technique à plusieurs stations de l'océan Atlantique NE, varient de 3 à 10 $\mu mol.l^{-1}$.

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INTRODUCTION

Despite the fact that dissolved organic nitrogen (DON) is the dominant form of fixed nitrogen in oligotrophic surface waters, it has been historically ignored because it was considered biologically inert. Recent studies have shown that a considerable fraction of this organic pool must be highly or semi-labile (Carlson and Ducklow, 1995), and that the inclusion of DON concentration in the total dissolved nutrient inventory has important intermediate and long-term biochemical implications (Jackson and Williams, 1985).

Measurement of DON is relevant to research concerning: firstly, the role of DON in oceanic nitrogen cycling; secondly, the importance of benthic fluxes of DON in sedimentary nitrogen cycles; and thirdly the role of these fluxes and sources of DON to the oceans (DON subgroup report, 1993).

Dissolved organic matter (DOM), predominantly of *in situ* origin, is produced by: planktonic metabolism (excretion and secretion by phytoplankton and excretion by zooplankton); losses into soluble forms when phytoplankton is grazed; viral attack and spontaneous autolysis of microorganisms; and, ultimately, microbial degradation of particulate organic matter (Copin-Montégut and Avril, 1993; Kirchman *et al.*, 1993).

Knowledge of speciation of DON in seawater and fresh water was reviewed by Walsh (1989). Less than 25% has been identified as dissolved hydrolysable amino acids. Urea can account for as much as 10% of the DON. Amino sugars, nucleic acids, chlorophyll and related pigments, together with amines and vitamins, constitute no more than a few per cent. Thus, 50% or more of DON remains uncharacterized.

Most procedures for the determination of DON show certain similarities. Total dissolved nitrogen (TDN) and dissolved inorganic nitrogen (DIN) need to be measured, DON being calculated by the difference between them. Analytical methods for the determination of DON are based on three different principles: wet oxidation; dry combustion; and high temperature oxidation (Suzuki *et al.*, 1985, Fry *et al.*, 1996). The wet oxidation method involves UV photo-oxidation, persulphate digestion or a combination of the two. High temperature oxidation involves high temperature combustion at 1100 °C (HTC) and high temperature catalytic oxidation at 680 °C (HTCO). Dry combustion involves analyses in elemental analyzers and sealed tube combustion (STC).

The precision of DON determination is often hampered because it involves establishment of the difference between two larger numbers, TDN and DIN, especially in bottom water. Small relative errors in TDN or DIN measurements lead to large relative errors in the DON determination. This analytical constraint will be overcome with a technique which quantitatively removes DIN from a sample before TDN analysis. Possibilities for this involve the utilization of an ion retardation column to separate inorganic forms from DON (Bronk and Glibert, 1991), Devarda and the procedures of Cox (1980) and Garside (1982). But these techniques will probably require modification to maximize the removal of DIN, to ensure minimal loss of DON and to minimize contamination (DON subgroup report, 1993).

The conventional Kjeldahl method determines the sum of DON and ammonium. A sample of filtered seawater is concentrated with excess sulphuric acid (digestion mixture: SeO, sulphuric acid and sulphates), and organic nitrogen is converted to ammonia by Kjeldahl digestion. The residue is dissolved in water, neutralized and determined (Hansen and Grasshoff., 1983; Strickland and Parsons, 1968).

The conventional Kjeldahl method has been used by Robinson and Wirth (1934*a, b*) and Mober and Fleming (1934) to analyse unfiltered seawater samples from Puget Sound and southern Californian coastal waters, respectively. This method involves a loss of organic nitrogen as a result of nitrate and nitrite interference. This is especially important in deep waters with low DON and high NO_3^-. In a later modification, ammonium, nitrate and nitrite were eliminated before digestion (Fraga, 1959); this method was employed for Indian Ocean waters (Fraga, 1966, 1969), and for the Cariaco Trench (Fraga and Ballester, 1966) with good results.

In the present work, a method for the direct determination of DON in seawater samples is proposed. In the improvement, checking and updating of Fragas method, we distinguish the following stages:

1. Ammonium is removed by boiling, at pH 9.4 buffered by the magnesium in seawater.

2. Nitrate and nitrite are removal as nitric oxide by reduction with ferrous ions.

3. The sample is digested with sulphuric acid.

4. Ammonium is co-distilled with water vapour and concentration in the distillate is determined by the indophenol blue procedure.

The time required for the determination of six samples is about 3.5 h. First, the six samples in each group are digested simultaneously (30 min); next, the samples are co-distilled (15 min per sample); and finally they are measured on a Technicon AAII system (5 min per sample). The amount of time involved in the two first stages is also necessary to concentrate the sample, therefore the digested time is the same when DIN is not removed.

MATERIAL AND METHODS

Reagents

1. Milli-Q water. This should be used for preparing solutions, for the determination of blanks and for standardization.

2. Sodium hydroxide, 0.5 mol.l^{-1}. Dissolve 20 g of sodium hydroxide pellets in one litre of water.

3. Sulphuric acid-Fe (II) sulphate. Dissolve 2 g of $FeSO_4$ 7 H_2O in 310 ml of water and add 225 ml of concentrated sulphuric acid (ammonium-free).

4. Sodium hydroxide, 33 %. Dissolve 250 g of sodium hydroxide pellets in 520 ml of water and add 4 ml of ethanol.

5. Hydrochloric acid solution, 10^{-3} M. Dilute 41 µl of concentrated hydrochloric acid (ammonium free) in 500 ml of water.

6. Malachita green indicator, 0.1 % in water.

For the determination of ammonia on the Technicon autoanalyzer, the following reagents are used: Phenol reagent; DTT reagent; and Nitroprusside. Ammonium was analysed by segmented flow analysis according to Hansen and Grasshoff (1983).

Sample collection

Samples were collected with 5-litre PVC Niskin bottles. Samples of DON were drawn into one-litre polyethylene containers, after rinsing the bottles three times. Immediately after sampling, they were filtered through Whatman GF/F filters. Filtration of samples was performed in an all-glass filtering system. The filtrate was collected, after rinsing the bottles, in 250 ml polyethylene bottles and analysed immediately or frozen until analysis.

The one-litre and 250 ml bottles as well as the filtering system were thoroughly washed with diluted sodium hypochloride, 0.1 mol.l^{-1} hydrochloric acid and, finally, with Milli-Q water.

Procedure

A 100 ml sample was introduced into a 300 ml Pyrex Kjeldahl flask. One millilitre of NaOH 0.5 mol.l^{-1} was added; the solution was boiled until the sample was reduced by one-half, and for approximately ten minutes in order to eliminate ammonium. Next, we added 10 ml of sulphuric acid-Fe(II) sulphate reagent and two 5 mm boiling glass-balls. The sample was concentrated until white sulphuric acid fumes appeared. The critical moment of the analysis occurs just before their appearance: at this point, if the boiling balls have not been added or the flame is of low intensity, violent splashes will be produced, spoiling the analysis. This can be avoided by slightly shaking the sample. Heating must be continued for forty minutes in order to mineralize the sample by gentle boiling. The best procedure for sample heating is to use a narrow flame, affecting only that part of the flask which contains liquid.

The residue, when cooled, is diluted with Milli-Q water and transferred to the distillation device. There, 20 ml of NaOH 33 % is added and the ammonia is co-distilled with water vapour until 20 ml of distillate (over 5 ml of 10^{-3} mol.l^{-1} HCl) have accumulated. The distillate weight is measured with a precision of ±0.001 g.

Ammonium concentration in the distillate is determined on a Technicon AAII SFA system. We used a 15 mm flowcell, with a range of 1-50 µmol.l^{-1} NH_4^+. The determination was made at 630 nm. The precision for ammonium is ±0.05 µmol.l^{-1} Calibration was done with a standard solution of NH_4Cl, in the range between 5-40 µmol.l^{-1}. Dilute solutions were made daily from a primary standard stored at 4 °C in the dark.

RESULTS ANS DISCUSSION

Analytical conditions

We distinguish four stages for DON determination: i) ammonium elimination; ii) nitrate and nitrite elimination with DON mineralization; iii) ammonium co-distillation; and iv) measurement.

Ammonium elimination

The addition of NaOH to seawater precipitates magnesium hydroxide which buffers the pH at about 9.4, and ammonium is removed by boiling. Ammonium elimination at this pH is slow, but amide hydrolysis is avoided. For a fixed pH, the removal of ammonium depends on the volume of water, while amide hydrolysis depends on the duration of boiling. Optimum conditions involve reduction of the sample to half of its initial volume in about 10 min. Experiments with NH_4Cl in seawater have shown that 98 % of the ammonium initially present is eliminated. For seawater with 5 µmol.l^{-1} ammonium, 0.1 µmol.l^{-1} would remain, this concentration being below the sensitivity of the method. We also investigate the possible loss of organic nitrogen when ammonium is removal. Two types of compounds were tested: volatile bases and amides. Amide nitrogen is not lost even in compounds which are easily hydrolysable, such as urea and guanidine, or in amides like asparagine and sulphanilamide. Volatile bases suffered a considerable loss, but their amount in seawater is very low. Methylamines at the nanomolar levels have been commonly found in non-polluted natural waters (PML annual report 1994-1995).

Nitrate and nitrite elimination, DON mineralization

The addition of $FeSO_4$ is necessary to recover 100 % of organic nitrogen because nitrate and nitrite in seawater cause losses of organic nitrogen (Tab. 1). Nitrite reacts with amino groups in acid medium to form N_2 which is lost when heated. Experiments were performed using three aminoacid solutions adding $NaNO_3$ or $NaNO_2$. For all the experiments, the amount of organic nitrogen found is greater if $FeSO_4$ is used.

Decomposition of nitrate with $FeSO_4$ follows the reaction:

$$3\,Fe^{+2} + NO_3^- + 4\,H^+ \rightarrow 3\,Fe^{+3} + NO + 2\,H_2O$$

The amount of $FeSO_4$ necessary for the total elimination of nitrate and nitrite is determined by using a glycine solution with sodium nitrate. The amount of $FeSO_4$ in the reagent was varied (Tab. 2). An increase in the ratio Fe^{+2}/NO_3^- produces an increase in the recovery of DON. When the ratio Fe^{+2}/NO_3^- is 4, 100 % of DON is recovered. We used a reagent 10-fold the amount of $FeSO_4$ necessary for the elimination of the greatest expected amount of nitrate.

Table 1

Fe(II)-sulphate influence on losses of DON caused by nitrate and nitrite.

	Nitrogen (μM)	$NaNO_2$ (μM)	$NaNO_3$ (μM)	% Nitrogen recovered without Fe^{+2}	with Fe^{+2}
Glycine	497.0	288			101.0
Glycine	323.5		456	85.2	100.6
Tryptophan	42.7	161		39.1	99.2
Tryptophan	42.7		119	74.3	98.7
Lysine dihydrochloride	37.7	57		93.2	100.4
Lysine dihydrochloride	37.7		48	95.0	99.9

Table 2

Test of the amount of $FeSO_4$ necessary for quantitative recovery of DON.

Glycine (μM)	$NaNO_3$ (μM)	$FeSO_4$ (μM)	Fe^{+2}/NO_3^-	%N recovery
248.5	456	18	0.04	73.5
248.5	456	182	0.40	77.8
248.5	456	911	2.00	95.4
248.5	456	1823	4.00	101.0

At this stage, first NO_3^- and NO_2^- were eliminated, and then the seawater was mineralized to convert DON into ammonium sulphate. An adequate amount of concentrated H_2SO_4 in the 100 ml seawater sample is essential in order to regulate the boiling point of the mixture. With an acid volume less than 4 ml, losses of ammonium sulphate occurred; and with acid volumes greater than 5 ml, mineralization was not completed in 40 min. We used 4.5 ml of acid in 10 ml of reagent. The salinity range of seawater for which this volume of sulphuric acid is suitable is 30-38. For seawater with salinity between 24 and 30, a 125 ml seawater sample must be used.

The time necessary to mineralize the sample completely is 40 min. This can be decreased by using a catalyst, but results are less precise and the blank is higher. Experiments were performed, varying the mineralization time. The percentage of organic nitrogen found is shown in Table 3.

Co-distillation of ammonia from the mineralization solution

The distilled volume required for ammonium to be recovered quantitatively depends on the steam distillation device, but usually amounts to 15 ml. In our experiments, 20 ml were collected in 50 ml glass borosilicate bottles, over 5 ml of 10^{-3} mol.l^{-1} HCl. The distillate volume is measured exactly by weight.

At this stage, contamination must be carefully controlled. Attention must be paid to the following conditions: a) the steam generator water must be kept at pH 1, using malachite green as indicator (1 ml/100 ml water); b) the steam distillation device must be washed by distilling Milli-Q water with NaOH reagent daily, before the samples are distilled; and c) it is also necessary to replace the steam generator water daily, in order to avoid laboratory contamination.

Measurement

Ammonium concentration is determined with a Technicon Autoanalyzer AAII SFA system. Every day, immediately after co-distillation of ammonium, the ammonium concentration was determined.

System blank

Total blank includes contributions from: the water used; the reagents; possible contamination in the course of handling.

Blank measurement was done with Milli-Q water (100 ml) and calcinated NaCl (3 g), treated as a sample. Salt concentration influences the mineralization temperature of the sample. Therefore, a concentration of NaCl, 0.5 mol.l^{-1}, similar to that of seawater, was used. Several blank tests were made everyday. The average blank was subtracted from the values of analysed samples daily.

The preparation of reagents calls for special care in order to avoid NH_4^+ contamination. In general, acids contain a maximum of 0.0002% w/v of NH_4^+-N. Experiments were performed with several trademarks of acids and that with the least NH_4^+ was retained (Riedel-de Haën, n° 30743).

Table 3

Test of necessary mineralization time for quantitative recovery of DON.

		Time (min) 15	20	30	35	40
	Nitrogen (μM)			N% found		
Animal protein extracts	664.5	96.2		98.6		100.0
Lysine	37.7		75.8	88.6	93.5	100.7

The quality of the sulphuric acid is of utmost importance in this procedure (Hansen and Grasshoff, 1983). Sulphuric acid-Fe(II) sulphate reagent was prepared every one or two weeks and tested by distilling 5 ml with 20 ml of NaOH 33 %. The ammonium concentration of these distillates must be not greater than 1 $\mu mol.l^{-1}$. Hydrochloric acid and the water used were also tested. The ammonium concentration of the total blank was about 2 $\mu mol.l^{-1}$.

Before seawater samples co-distilled, the device was washed and several blank tests made.

Recovery

Recoveries of some standard compounds are shown in Table 4 and compared to recoveries obtained with other methods (data were taken from DON subgroup report, 1993). Standard compound solutions were prepared by standard addition to seawater. In addition, several solutions were prepared with NaCl blank water; the recoveries were the same. Each standard was tested several times and the standard deviation (SD) for all samples was ≈ 0.2 $\mu mol.l^{-1}$. These results indicate that most common biochemical and most of the identified major constituents of seawater DON are accurately measured by the proposed method. This technique allows us to obtain recoveries greater than 98 % for amino acids, urea and amides. For nicotinic acid, one of the most difficult compounds to mineralize by the Kjeldahl method, the recovery was 95 %. For refractory compounds, notably sulphathiazole, caffeine and antipyrine, the recovery was 94, 91 and 73 %, respectively. It should be pointed out that several compounds considered refractory are not difficult to oxidize, but N_2 is released from the N–N or N=N bonds. For methylamine, the low recovery of 55 % was due to loss in the first stage of the process (ammonium elimination of the sample).

This method eliminates the ammonium initially present during the first stage, when NaOH 0.5 $mol.l^{-1}$ is added. Experiments with NH_4Cl were done without this step, and the recovery of DON was 100 %.

Reproducibility and precision of the method

Several measurements of reproducibility were made: i) blanks; ii) seawater samples (A); and iii) organic nitrogen standards, such as sulphanilamide solution (sulphanilamide addition to NaCl blank water) (B).

Table 4

Recovery of standard compounds by Kjeldahl compared with UV[1], $S_2O_8^1$, HTO[1] and HTCO[1] methods. ([1]Data are taken from DON subgroup report, 1993).

	Kjeldahl N($\mu mol.l^{-1}$)	%	UV %	S_2O_8 %	HTO %	HTCO %
Inorganics						
Ammonium	205	0	98	≥96	100	60, 100
Sodium nitrite	288	0			101	98
Sodium nitrate	466	0	100		100	90
Amine, amino acids and proteins						
Methylamine hydrochloride	32	55				
Arginine hydrochloride	264	100				
Lysine dihydrochloride	38	100				
Proline	66	99	99		92	
Arginine	10	99				
Tryptophan	43	99	92	108	98	113
Glycine	323	100	96	100	100	≥90
Protein extract	664	100				
Amides						
Sulphanilamide	11	100				
Urea	314	98	75, 98	100	101	90
Asparagine	292	100				
Guanidine carbonate	81	99				
Thiourea	90	87	88	85, 96		≥90
Nitrogen heterocyclics						
Caffeine	75	91		20, 103		≥90
Sulphathiazole	65	94	86		102	47, 65
Nicotinic acid	11	95	99	100	100	98
N-N bond						
Antipyrine	103	73	51	30	98	90

Blanks, seawater and standard organic nitrogen results are presented in Table 5. The value of blank was subtracted from the values of sulphanilamide and seawater shown. Every day, two or three blanks were done before seawater samples. The standard deviation (SD) of blank measurements was 0.1 $\mu mol.l^{-1}$. We have used one sample of seawater and another of sulphanilamide (11.04 $\mu mol.l^{-1}$). Each day during one week, measurements were done. The solution of sulphanilamide was refrigerated for storage. The seawater samples were kept in a 25-litre dark plastic container. The average SD amounted to 0.22 and 0.17 $\mu mol.l^{-1}$ for A and B, respectively. Reproducibility can be expressed as a coefficient of variation (CV). For DON concentrations ranging between 4 and 12 $\mu mol.l^{-1}$and SD: 0.2, the CV varies from 1.8 to 5.5%. We always analyse duplicate seawater samples.

Comparison with other methods

The range of recoveries for tested compounds is similar to or better than those obtained by other authors (Table 4: data were taken from DON subgroup report, 1993), both by wet chemical and HTO (Walsh, 1989) and by HTCO techniques (Maita and Yanada, 1990; Fiztwater and Martin, 1993). HTO and HTCO techniques give quantitative recovery of nearly all standard compounds, although they give variable results for sulphathiazole. Wet chemical techniques (persulfate oxidation, UV photo-oxidation) give greater than 90% recoveries for amino acids and humic acids; between 80-90% for many nucleotides and other compounds containing more than one nitrogen atom in a ring; but low recoveries ($\approx$75%) for urea, by the UV method (Walsh, 1989) and compounds containing more than one nitrogen atom in a ring; antipyrine (30 and 50% by S_2O_8 and UV, respectively) and caffeine (28 or 103% by S_2O_8).

Table 5

Test of the reproducibility of the method for blanks, one sample of seawater[1] and other of sulphanilamide[1, 2] (units: $\mu mol.l^{-1}$; [1] the blank is substrated from seawater and sulphanilamide samples; [2] N: 11.04 $\mu mol.l^{-1}$).

1	Blank	n = 6	M = 2.0	σ = 0.08
2	Blank	n = 4	M = 2.1	σ = 0.08
3	Blank	n = 3	M = 1.9	σ = 0.01
1	Surface seawater	n = 7	M = 5.51	σ = 0.33
2	Surface seawater	n = 4	M = 5.60	σ = 0.07
3	Surface seawater	n = 5	M = 5.58	σ = 0.18
4	Surface seawater	n = 7	M = 5.48	σ_m = 0.29
		m = 5.54	(σ = 0.06)	σ_m = 0.22
1	Sulphanilamide	n = 3	M = 11.02	σ = 0.22
2	Sulphanilamide	n = 4	M = 11.05	σ = 0.10
3	Sulphanilamide	n = 4	M = 11.07	σ = 0.29
4	Sulphanilamide	n = 3	M = 11.00	σ = 0.05
		m = 11.03	(σ = 0.03)	σ_m = 0.17

(M = mean; n = n° samples; m = mean of M; σ_m = mean of σ).

DON values calculated by measuring TDN and then subtracting an independently determined DIN concentration contain any inaccuracy that may be introduced by either or both measurements. This is especially dramatic in the deep ocean where DIN accounts for most of the measured TDN. As an example of how the DIN gradient influences the precision of DON measurements, Hedges *et al.* (1993) in an intercomparison exercise, calculated a precision of 8.1, 48.4 and 33.9%, respectively for DON in surface, mid and deep seawater samples.

Direct determination of DON by the method described in the present work allows improved precision, DON values not being dependent on accurate DIN analysis. In addition, duplicate sample determination seems advisable, according to the standard deviation values obtained. The elimination of ammonium (which makes it possible to reduce the typical contamination problem) and of NO_3^- and of NO_2^- (which permits determination of deep seawater samples) seems to constitute the main advantage of this method, although it is a tedious one, the time required for six determinations amounting to about $3^{1/2}$ hours. Therefore, in the case of a small number of samples, this method is useful and an adequate tool to check other methods.

Application of the present method to the analysis of seawater samples in the Northeast Atlantic

As an example of DON vertical profiles obtained by this method, Figure 1 and Table 6 show data from stations sampled in the Northeast Atlantic Ocean. Vertical profiles

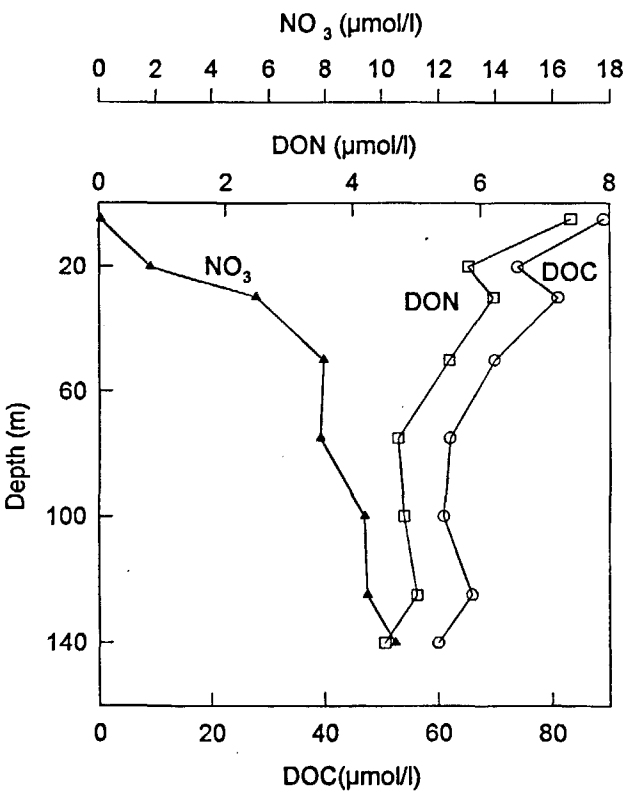

Figure 1

Vertical profile of DON, DOC and NO_3^- at station 1 (42° 7.8' N, 9° 7.5' W) in the Northeast Atlantic Ocean off NW Spain.

Table 6

Mean values with 95 % confidence limits of DON, DOC, NO_3^- and C/N (DOC/DON) in the Northeast Atlantic (units: μmol.l^{-1}).

Depth	DOC		DON		NO_3^-		C/N
(m)	M	n	M	n	M	n	M
10	90 ± (10)	35	6.8 ± (0.4)	21	0.1 ± (0.0)	58	13.1 ± (2.4)
30	88 ± (6)	59	6.6 ± (0.3)	19	0.4 ± (0.0)	50	13.4 ± (1.4)
50	77 ± (6)	51	6.0 ± (0.3)	21	1.0 ± (0.0)	46	13.0 ± (1.6)
75	72 ± (6)	36	5.8 ± (0.4)	13	3.3 ± (0.2)	40	12.6 ± (2.0)
110	72 ± (6)	23	5.3 ± (0.4)	8	5.3 ± (0.3)	35	13.7 ± (2.3)
200	74 ± (6)	17	5.2 ± (0.4)	10	8.3 ± (0.4)	40	14.7 ± (2.1)
405	67 ± (8)	9	5.4 ± (0.8)	4	11.5 ± (0.5)	45	12.5 ± (3.2)
940	60 ± (4)	27					
2200	69 ± (6)	19					
	Average M: 77.6		Average M: 5.9				Average M: 13.3
	SD: 7.8		SD: 0.6				SD: 0.7

(Depth = mean of depth interval; n = n° samples; M = mean).

of corresponding DOC (dissolved organic carbon), analysed by us with an Shimadzu TOC-5000 and NO_3^- analysed on a Technicon AAII SFA system, are also shown. (All the results obtained for DOC, DON and other variables measured in the Northeast Atlantic Ocean will be shown in future works, in preparation).

Figure 1 shows the vertical profile at Station 1, sampled on the continental shelf of the Iberian Peninsula, off the Ría de Vigo, in September 1994. The station, 148 m depth, is situated at 42° 7.8′ N, 9° 7.5′ W.

Table 6 shows mean values of DON, DOC and NO_3^- at several stations sampled on the continental slope and shelf waters of the NW Iberian Peninsula on May 1993, during cruise Morena 1. These stations are located between 40 and 43° N and between the coast and 11° W along legs perpendicular to the western Iberian coast.

The vertical distribution of DON and DOC for Station 1 has one maximum at the surface (7.3 μmol.l^{-1} DON; 88 μmol.l^{-1} DOC), with values becoming uniformly lower with depth (minimum: 4.5 μmol.l^{-1} DON; 60 μmol.l^{-1} DOC). DOM (dissolved organic matter) values decrease with depth almost uniformly (Table 6) in the Northeast Atlantic. The average DOM values have one maximum at the surface (90 μmol.l^{-1} DOC; 6.8 μmol.l^{-1} DON). DON values ranged between 3 and 10 μmol.l^{-1} on the continental slope and shelf of the Iberian Peninsula.

NO_3^- values permit comparison of the inorganic/organic levels of seawater tested. C/N molar ratios were in the range 12-15 for all the samples.

We have not found DON concentrations in the Northeast Atlantic approaching the high values found by Suzuki *et al.* (1985) in the Western Pacific. Our DON values for these stations are in the same range as those recently reported by several authors (Hansell, 1993; Walsh, 1989; Maita and Yanada, 1990).

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