Analusis, 1982, v. *10*, n° 1, p. 23 à 30

Routine ^{15}N analysis on small samples by emission spectrometry

L. LEMASSON (*), J. PAGES (*) et G. GUIRAUD (**)
avec la collaboration technique de Ch. MAROL (**)

(**) *Département de Biologie, Service de Radioagronomie, Centre d'Études Nucléaires de Cadarache, B.P. 1, 13115 Saint-Paul-lez-Durance*

RÉSUMÉ

Analyse de routine de ^{15}N sur de petits échantillonnages par spectrométrie d'émission.

L'article décrit une procédure de routine pour la détermination de ^{15}N sur un spectromètre d'émission-Sopra GS 1. Les échantillons, contenant de 10 à 50 µg N, sont traités par micro-Dumas dans des ampoules à décharge scellées, en Pyrex. Les produits secondaires de combustion sont piégés par l'azote liquide.

Les résultats sont étalonnés par rapport à la spectroscopie de masse dans la gamme 0-30 % atomes ^{15}N en excès. Le coefficient de variation est d'environ 1 % et est dû, surtout, aux incertitudes de lecture des spectres.

Les possibilités de la méthode ont été essayées sur diverses substances : phytoplancton, matière organique végétale, sols et insectes.

SUMMARY

The paper describes a routine procedure for ^{15}N determination with a Sopra GS 1 optical emission spectrometer. Samples containing 10 to 50 g N are processed by micro-Dumas in sealed Pyrex discharge tubes. Combustion by-products are trapped in liquid nitrogen.

Results are calibrated against mass spectrometry in the 0-30 % ^{15}N atomic excess. The coefficient of variation is approximately 1 % and is mainly due to spectrum reading uncertainties.

Possibilities of the method have been tested on various materials : phytoplankton, plant organic matter, soils and insects.

INTRODUCTION

Isotopes are now widely used as tracers in various fields of biology. One of the only usable isotopes of nitrogen is ^{15}N, which is stable. Such stable isotopes are somewhat less easily quantified than the radioactive ones. The classical, and widely used, apparatus employed is the mass spectrometer (M.S.). An alternate method is optical spectrometry (O.S.), based on the fact that the emission wavelength of a gaseous molecule is slightly shifted as the mass of the molecule varies. One of the first routine methods for ^{15}N determination was described by Hoch and Weisser [1]. The ulterior developments of relatively routine methods were rapid [2 to 4]. Reviews of ^{15}N determination methods have been published by Fiedler and Proksch [5] and, more recently, by Middelboe [6].

The O.S. method has been shown by several authors to compare competitively with M.S. [7 to 9]. Most of the studies reported have been carried out with Jasco or Statron optical spectrometers. The aim of this paper is to describe the routine use of a new O.S. model for assaying low quantities of nitrogen.

The transformation of bound nitrogen to gaseous N_2 necessary for O.S. can be achieved by two methods. The nitrogenous compounds may be mineralized to NH_4^+ by the Kjeldahl process, then oxidized to N_2 by the Rittenberg procedure. Another pathway is based on the Dumas process ; the compounds are oxidized by CuO and subsequently reduced to N_2 by Cu. Several variations of the Dumas method have been described where successive samples travel along a common circuit, either in a discontinuous manner [10, 11] or as a continuous stream [8]. We present here the results obtained on various materials by a micro-Dumas method carried out in sealed individual glass tubes, a compared to the results given in parallel by M.S.

MATERIAL AND METHODS

We shall describe here the routine method employed as defined from our various trials. Some more particular points will be discussed in a later section.

The general principle of the method is as follows : each sample, containing 15 to 50 µg N, is processed in a sealed, evacuated, glass discharge tube. A modified Dumas method is used to transform organic nitrogen into N_2. The combustion by-products are trapped by liquid nitrogen inside the electrodeless discharge tube. A high-frequency (HF) excitation induces the discharge, the light of which is analyzed by spectrometry.

A. Mass spectrometer

The results obtained by O.S. were compared to those given on aliquots of the samples by M.S., which is consi-

(*) Adresse actuelle : Antenne ORSTOM, Station INRA, avenue de Corzent, 74203 Thonon-les-Bains.

Manuscrit reçu le 22 janvier 1981 ; accepté le 11 février 1981.

dered as the reference method. We used a Varian GD 150 mass spectrometer. The samples were treated by Kjeldahl mineralization and Rittenberg process [12].

B. Optical spectrometry

The spectrometer used here is a GS 1 (Sopra, France). Its operating principle has been described elsewhere [9]. The salient point is the use of a fixed concave diffraction grating. The scanning of the spectrum is achieved through the rotation of a quartz parallel plate.

The electrodeless discharge tubes are prepared from Pyrex tubing (8 mm o.d. × 25 cm), thoroughly cleaned and sealed at one end. After introduction of the samples (see below), these tubes are evacuated.

The vacuum circuit is simple. A two-stage rotary pump maintains a vacuum of 10^{-3} torr. No secundary vacuum source is needed. A manifold of stainless steel accomodates four discharge tubes. It may be isolated from the pump and a liquid nitrogen trap during the connection of a new set of discharge tubes. A Pirani gauge allows to check the vacuum during the various operations.

C. Operating procedure

The samples contain between 15 and 50 µg N. They are dried (60 °C) and ground if necessary. An appropriate amount is strewn on a glass-fiber filter (Whatman GF/C, 25 mm). About 20 mg of finely ground Cuprox(R) (Coleman) is added and mixed with the sample. The filter is tightly rolled and introduced in a clean discharge tube. No reducing agent (Cu) need to be added. Some bulky powdered samples (soils) may not be amenable to this procedure. They are mixed with Cuprox and the resulting powder is poured down the discharge tube. A wad of glass wool confines the powder to the bottom of the tube.

All the above-mentioned procedure has to be carried out with the utmost care to prevent any contamination of the sample. All glassware should be meticulously cleaned.

Once the sample has been introduced in the tube and pushed down to the closed end, the discharge tube is connected to the vacuum line by a short length of vacuum rubber tubing. After the evacuation is started on a series of tubes, these are heated in small electrical ovens made in the laboratory. This heating (550 °C, 10 min.) ensures degassing of the inner walls ; the sample must not be heated. After cooling, and under an active vacuum of 10^{-3} torr, the discharge tubes are sealed to a length of about 20 cm.

Combustion of the samples is carried out at 550 °C during one hour. The ulterior measurement may be made as soon as the tubes have cooled. Conversely the discharge tubes may be kept at least for one year.

The discharge tube under measurement is clamped vertically between the excitation electrodes and its lower end is immersed in liquid nitrogen (LN), in order to trap the combustion by-products (mainly CO_2 and H_2O). The high-frequency generator is switched on ; an auxiliary high-frequency (H.F.) pulse from a Tesla coil (Edwards ST 200 K) may be necessary to initiate the discharge. The emission wavelength of the three possible molecules of N_2 are 297.7 nm for $^{14}N^{14}N$, 298.3 nm for $^{14}N^{15}N$ and 298.9 nm for $^{15}N^{15}N$ *(fig. 1)*. We shall abbreviate the designation of the molecules as (28), (29) and (30).

The spectrum is scanned back and forth between 297.3 and 299.3 nm. Appropriate amplification of the photomultiplier signal is chosen so as to obtain the maximum deflection of the recorder *(fig. 1)*. A good spectrum should present a low and smooth base line at 299.3 nm. Some of

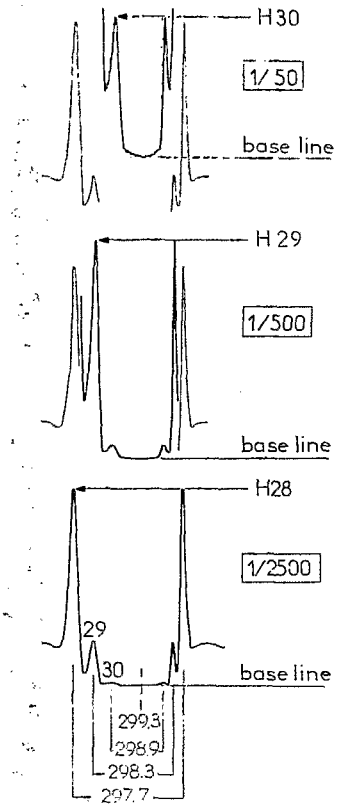

FIG. 1. — *Typical recordings of spectrum scan at three amplification factors.* In routine work the amplification is changed during one scanning cycle. The spectrum is scanned back and forth. Wavelengths are given in nm.

FIG. 1. — *Exemple d'enregistrement de spectre, à trois facteurs d'amplification.* En routine, l'amplification est changée pendant un même cycle de balayage. Le spectre est balayé dans les deux sens. Les longueurs d'onde sont données en nm.

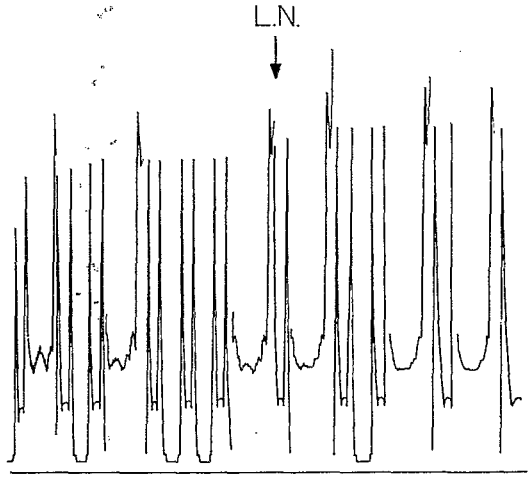

FIG. 2. — *Modification of a spectrum by progressive trapping of the combustion by-products during refrigeration.* The arrow indicates a supplementary addition of liquid nitrogen. The « bottom » of the spectrum around 299.3 nm becomes smoother. Each scanning cycle lasts one min.

FIG. 2. — *Modification d'un spectre par piégeage progressif des produits secondaires de combustion, pendant la réfrigération.* La flèche indique un apport supplémentaire d'azote liquide. Le « fond » du spectre, autour de 299,3 nm devient plus lisse. Chaque cycle de balayage dure 1 min.

the combustion by-products emit in this region ; their trapping by L.N. is indicated by the smoothing-out of the base line region *(fig. 2)*.

D. Calculation

For each type of N_2 molecule, we measure the height, H, of the corresponding peak above the base line. Taking into acount the amplification factor, the peak ratios are calculated :

$$R = H(29)/H(28) \qquad R' = H(30)/H(29) .$$

The percentage, T, of ^{15}N atoms is then calculated by one of the two equations

$$T = 100.R/(2 + R) \qquad T' = 200.R'/(2\,R' + 1) .$$

For less than 10 % ^{15}N only T can be calculated with a satisfactory precision. Above 10 % ^{15}N, both T and T′ can be calculated.

RESULTS AND DISCUSSION

A. Methods

A.1. *Vacuum circuitry*

During their evacuation the discharge tubes are connected to the vacuum line by lengths of rubber tubing after applying a dab of vacuum grease. More sophisticated connections have been described, such as conical joints [13] or O-rings [14]. The use of rubber tubing is cheaper, especially since the connections are often seared during sealing, and must be replaced after some ten uses. We could find no proof of contamination by the vacuum grease [15].

The use of an oil diffusion pump as a secundary vacuum source did not improve the results. The vacuum circuit is thus simplified and less sensitive to possible accidental leaks.

A.2. *Sample preparation and sources of pollution*

Pyrex glass is cheap but has some inconvenient features. Its optical transmission is low in the 300 nm region [5, 9]. A further drawback is that adsorption of N_2 is higher than that of quartz [16, 15] and errors of up to 5 % might be made [5]. Degassing the walls of the tubes appears necessary.

The discharge of a Tesla coil has been used [5]. We tried sweeping the whole length of several tubes with such a discharge for 10 min. while evacuating the tubes. No significant difference could be seen between treated and untreated tubes (Wilcoxon test).

The discharge tubes can be heated with a torch [17] but this is time consuming and the walls of the tubes can be easily melted down. Middelboe [14] has described the use of a heating wire and this solution was adopted. Properly regulated ovens can function unattended at a precise (± 10 °C) temperature.

Degassing by heat seems to have variable effects, depending on the quantity of sample nitrogen. Heating had no significant effect with samples containing 40 µg N (Wilcoxon test). With smaller samples, the effect was more obvious : maintaining samples under HF for 30 min. causes a lowering of the value of T. This variation was most pronounced for small samples in non-degassed tubes *(tabl. I)*. A similar lowering of T was observed in non-degassed tubes after repeated HF stimulation or repeated ignition.

Some unsatisfactory results led to suspecting a contamination by the glass-fiber filters. To test this possibility some filters were cleaned in sulfo-chromic mixture and rinsed in distilled water ; filters from the same package were used without any processing. The results *(tabl. II)* show no significant difference (Student-Newman-Keuls test ; $P < 0.05$). The filters obviously carry a negligeable amount of nitrogen, at least compared with the 20 to 40 µg N of the samples.

A previous series of tubes was made from glass tubing cleaned with a laboratory detergent and handled, as were the samples, with disposable gloves. With samples of 4 to 10 µg N at T = 10 % atoms, the results were 10 to 30 % too low.

TABLE I. — *Effect of degassing by heat.*

Prolonged HF excitation leads to a variation ΔT of T. A decrease of T % (minus values) is often observed in untreated tubes, and is more conspicuous with low N amounts.

TABLEAU I. — *Effet du dégazage à chaud.*

Une excitation prolongée par HF entraîne une variation ΔT de T (en % atomes). Une décroissance de T (valeurs négatives) est fréquente sur les tubes non dégazés et d'autant plus marquée que la quantité de N de l'échantillon est faible.

N amount (µg)	ΔT	
	untreated	degassed
15	− 0.57	0
20	− 0.14	+ 0.04
30	+ 0.03	+ 0.08
40	− 0.21	− 0.07

TABLE II. — *Comparison between acid-washed and untreated filters.*

The results (in E %) are not significantly different when considering mean values and standard deviation (σ). The coefficient of variation, CV, is given in % ; n is the number of samples. The phytoplankton samples (« phyto ») contained about 38 µg N ; the NO_3^- samples contained 20 µg N.

TABLEAU II. — *Comparaison entre filtres lavés à l'acide et bruts.*

Les valeurs de E ne sont pas significativement différentes. « mean » désigne la moyenne, σ l'écart-type (tous deux en % atomes en excès). CV est le coefficient de variation (en %), n le nombre d'échantillons. Les échantillons de phytoplancton (« phyto ») contenaient environ 38 µg N ; les échantillons NO_3^- contenaient 20 µg N.

	Untreated			Acid washed			n
	mean	σ	CV	mean	σ	CV	
phyto	21.04	0.20	0.9	21.27	0.34	1.6	8
NO_3-N	29.58	0.25	0.9	29.59	0.15	0.5	4

A.3. *Mineralization*

The quantity of added Cuprox had no visible influence. We used this oxidizing agent throughout our trials on several materials. No stronger oxidizer appeared to be needed [18].

The basic Dumas method uses a fragment of Cu as a reducing agent. Omitting Cu had no obvious effect. It can be surmised that the excess Cuprox decomposes and yields the necessary reducing agent.

The ignition temperature was set at 550 °C owing to the properties of the Pyrex glass used in our work. The routine ignition duration was one hour. An ignition of 5 min. gave readable spectra, and correct results, for phytoplankton and NH_4^+. With maize (*Zea mays*) straw, the minimum ignition duration appears to be 15 min.

The trials on ignition duration were made by submitting the same tubes to repeated cycles of combustion and reading. We observed a steady, and very highly significant (*t* test) decrease of T as a function of cumulated combustion time on height phytoplankton samples. This decrease of T is still more highly correlated with the number, C, of ignition and reading cycles :

$$T = 21.17 - 0.68.C \qquad (r = -0.67^{***} ; *** : P < 0.001) .$$

This consistent decrease may stem from an incomplete mineralization by the Dumas process ; low molecular weight substances, more highly labelled [19], would be more readily decomposed. However, since this process was also observed with NH_4^+ samples, a more probable explanation lies in the dilution of the sample by nitrogen adsorbed on the tube walls.

A.4. *Trapping of combustion by-products*

During the combustion and the subsequent reduction of the sample, several compounds are produced. H_2O reduces emission [4]. CO may cause overestimation of T through its emission at 297.6 nm [5]. Free O_2 interferes, although only at high concentrations [5]. All these by-products must be eliminated.

Most authors use CaO [3, 10, 20, 18]. We found that the necessary manipulations are awkward and increase the risks of extraneous pollution. Degassing of the CaO was seldom satisfactory. An alternative to CaO is a molecular sieve, used by several authors [21, 22, 23].

Instead of absorbing the gasses, it is possible to condense them. A cold trap is used in the vacuum system of M.S. [4, 24]. Middelboe [14] utilizes as condenser a portion of the discharge tube, immersed in liquid nitrogen then sealed off. Use of LN (− 196 °C) appreciably lowers the pressure of the remaining N_2 (see below). With small nitrogen amounts, we tried solid-liquid ethanol (− 117 °C) and solid-liquid acetone (− 95 °C). T values were 30 to 50 % too high, probably owing to an incomplete trapping.

B. Sample size range

The discharge can occur only within a rather narrow range of pressure [3]. The final pressure of N_2 in the discharge tube depends on the total N amount, the volume of the discharge tube and the inner temperature. The pressure may also be raised by introducing an inert gas in the tube.

The constriction worked into the discharge tube by some authors [19, 25, 26] heightens teh light emission but enhances heating of the gas [9]. The ensuing widening of the emission bands causes a poorer resolution [5]. Suppressing this constriction lessens the heating and stabilizes the emission.

We have already mentioned that trapping the combustion by-products by LN lowers the temperature, and hence the pressure, of N_2 in the discharge tube. Calculations show that the inner pressure is approximately that of the saturating vapor at 77 K. The tubes used for routine work have an approximate volume of 4.5 cm^3. In such tubes the upper limit of sample size is 60 µg N and the readings are possible only after 10 min. refrigeration. The lower limit lies between 12 and 15 µg N.

Some micro-tubes were also made with a volume of about 0.7 cm^3 (2.5 × 150 mm). Under LN refrigeration these tubes allowed the easy measurement of 5 µg N. The lower limit was not determined.

We used another type of tubes with a volume of 1.5 cm^3 at ambient temperature, the combustion by-products being trapped on CaO. The routine quantity was then 4 µg N, with a lower limit of 1 µg. Using pure nitrogen obtained by Rittenberg conversion, tubes of about 2.5 cm^3 allowed routine measurements on 3 µg N at ambient temperature [9].

When the amount of sample N is low, the pressure can be raised by noble gas addition [16, 17, 20]. The high molecular weight gases (Xe, Kr) prevent N_2 adsorption on the tube walls, the low molecular weight gases (He) sustain the discharge [5]. A few trials were made to verify the possible use of Kr. After heating and degassing, the tubes were filled with 1.2 torr Kr. After 10 min. the tubes were evacuated to 10^{-3} torr and filled to 5 torr with a mixture of (He + 0.2 % Kr). With the customary 4.5 cm^3 tubes under refrigeration the lower limit in sample size was 4 µg N. This gain in range implies an *a priori* decision and somewhat complicates the procedure. It could nonetheless be useful in particular cases.

C. Variability on T

Several authors have described the interpretation of recorded spectra [7, 8, 13]. Ferraris and Proksch [25] showed that there is no calculation method of T utilizable across the whole range of ^{15}N percentage. We use their procedures « 2 » and « 4 », without any further correction.

The variability of T on a sample stems both from the reading error and from sub-sampling error.

In what follows, 95 % confidence intervals (CI_{95}) are given in ^{15}N atoms %. (*n*) represents the number

of separate samples. (CV) is the coefficient of variation in percent.

C.1. *Variability on the readings*

A complete cycle of spectrum scanning lasts one minute. Several successive scannings are generally made for a sample to account for possible variations of the emission, base line and instrumental factors.

In the range $(0.54 < T < 1.0)$ we obtain $CI_{95} = \pm 0.008$ for three successive scannings ($n = 37$). In the range $(1 < T < 2)$ we have $CI_{95} = \pm 0.022$ on three scannings ($n = 17$).

Four samples containing 50 µg N were kept under HF, and their spectra recorded, during one hour. For each sample, 15 spectra were chosen at random for the calculation of T. The values of CV fell between 0.4 % and 1.2 % for T values of 5.6 % and 24.4 %.

C.2. *Variability of the replications*

A glass fiber filter bearing phytoplankton may be cut in two halves, each of the duplicates being being treated separately. A solution may be used to make several replications, with aliquots absorbed on separate filters and processed in parallel.

On such replications the CV was 0.7 to 3.8 % depending on the number of replications and the value of T (*tabl. III*).

The data used for the calibration curve (see below) were gathered across a period of two months by two experimenters working mostly on a set of M.S.-standardized solutions. The CV of these data is around 1 % *(tabl. IV)*.

D. Calibration

The data obtained by O.S. on pure nitrogen with the GS 1 have already been compared to those of

TABLE III. — *Variability of replication analysis.*

T is the % ^{15}N atoms, CI_{95} the confidence interval on T at 0.95 probability. CV is the coefficient of variation (in %). *n* is the number of series.

TABLEAU III. — *Variabilité des répliques.*

T est le % atomes ^{15}N, CI_{95} l'intervalle de confiance de T (probabilité 0,95). CV est le coefficient de variation (en %), *n* est le nombre de séries.

Number of replicates	T-%	*n*	CI_{95} (±)	CV
two	0.54-1.0	31	0.020	1.3
	1.0-2.0	14	0.056	1.9
	20	30	0.369	0.9
three	0.54-1.0	4	0.024	1.5
	1.0-2.0	6	0.084	2.8
	20-30	10	0.403	1.6
four	0.54-2.0	7	0.098	3.8
	20-30	7	0.445	0.9
seven	0.694	1	0.010	0.7

TABLE IV. — *Reproductibility of measurements.*

The data used for calibration are chosen as example. The mass spectrometer data (E_{MS}), in % atom excess, are compared to the % atom, T_{OS}, determined by optical spectrometer. The coefficient of variation, CV, is given in %. *n* is the number of samples.

TABLEAU IV. — *Reproductibilité des mesures.*

Les données utilisées pour la calibration sont ici prises comme exemple. Nous comparons les données de spectrométrie de masse (E_{MS}, en % atomes en excès) aux abondances T_{OS} (en % atomes) déterminées par spectrométrie optique. Le coefficient de variation, CV, est donné en % ; *n* est le nombre d'échantillons.

E_{MS}	T_{OS}	CV	*n*	E_{MS}	T_{OS}	CV	*n*
0	0.541	2.2	7	3.08	3.588	0.2	2
0.102	0.653	0.4	2	5.15	5.608	1.1	9
0.204	0.755	0.2	2	10.34	10.628	0.9	4
0.304	0.844	0.7	2	10.92	11.50	1.2	4
0.614	1.154	0.2	2	20.59	21.00	2.0	21
0.772	1.304	0.3	2	20.72	21.42	0.6	10
1.034	1.559	1.6	3	21.64	21.79	0.8	3
1.54	2.067	1.3	10	28.96	30.07	1.2	20

M.S. [9]. The comparison made here takes into account the supplementary variable of sample preparation by different methods. We compare the values of T, absolute ^{15}N atom percentage, as measured by O.S., to those of isotopic excess, E, above the natural abundance (0.366 %), as determined by M.S. Several kinds of samples were used : standard solutions of NH_4^+ and NO_3^-, phytoplankton cultures and soil organic matter.

We have distinguished several ranges of atomic excess values. The slopes are not significantly different (*t* test) throughout the (0 to 10) range ; the (20 to 30) range must be treated separately. We thus have two distinct calibration lines :

from 0 to 10 % :

$$E_{(MS)} = 1.0243.T_{(OS)} - 0.569$$

with

$$r = 0.99988 \ (n = 46)$$

from 10 to 30 % :

$$E_{(MS)} = 0.9572.T_{(OS)} + 0.143$$

with

$$r = 0.99913 \ (n = 43) .$$

With the technique described, seven samples at natural abundance gave a mean value of T :

$$T_0 = 0.541 \pm 0.024 \ (CI_{95}) .$$

The very dispersion of the data used for the calibration leads to an uncertainty on the calibration curve. The confidence interval of the calibration itself is narrow : around 0.05 % in the 0-10 % range and about 0.2 % in the 10-30 range *(fig. 3)*. For a single measured value of T, the corresponding true value of E can be calculated from the correlation equations. The confidence interval on a single E value is wider than the former : about 0.2 % in the

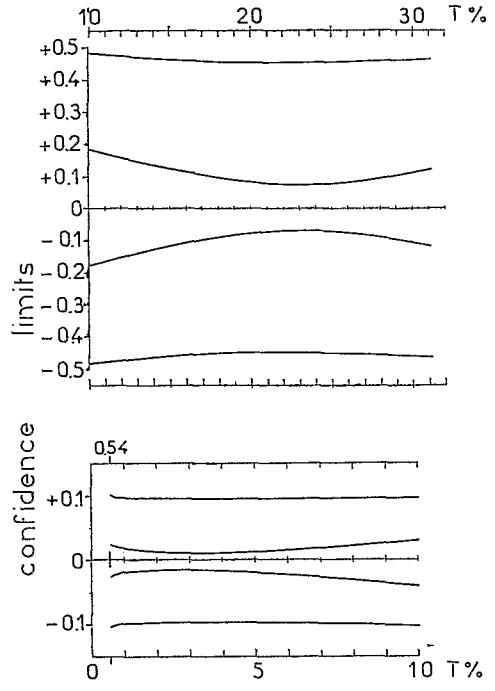

FIG. 3. — *Confidence limits of calibration* (see text). The calibration equations allow to calculate a value of E (in % ^{15}N atoms in excess) from a measured T value (in % ^{15}N atoms). The curves define the upper and lower limits of the confidence interval (in % ^{15}N atoms in excess) around the value of E. The narrower interval corresponds to the calibration, the broader one to an isolated value.

FIG. 3. — *Limites de confiance de la calibration* (voir texte). Pour une valeur de T (% atomes), une valeur de E (% atomes en excès) est définie par les droites de calibration. Les courbes donnent les limites haute et basse de l'intervalle de confiance (en % atomes en excès) autour de cette valeur de E. Le plus petit intervalle correspond à la calibration, le plus large à une valeur isolée.

0-10 % range and about 0.9 % in the 10-30 range *(fig. 3)*.

Our calibration curve is composed of two straight parts. In the various publications, some calibration curves are a straight line in a certain range : 0 to 6 % in [16], 0 to 10 % in [22]. In the 0-30 % range, Keeney and Tedesco [15] obtain a curve with « at least two approximately linear portions » ; the slopes are much higher than unity and tend to decrease with increasing T values. We observe the same trend, although with smaller variations. Keeney and Tedesco [15] explain the departure from linearity by a combination of mechanical and electronic factors. The focusing of the discharge tube has been shown to influence the T value [16] but we do found no such effect.

The various data for the calibration curve show no drift, either in the electronics or in the between-sample runs, across a two month period. We cannot yet evaluate the proper recalibration periodicity.

E. Applications

Our initial interest was centered on the possibilities of using the method in oceanographic and hydrobiological research. During the study itself, the scope was broadened to other fields, especially agricultural research *sensu lato*. Given the available time, none of the following experiments can be taken to represent an independent study in depth of any physiological mechanisms. All trials were considered as mere evidence of the possibilities of the method.

E.1. *Phytoplankton*

Experiments were undertaken with a non-axenic culture of *Phaeodactylum tricornutum* grown on a modified *f*/2 medium [27]. Enrichment amounted to 575 µgat PO_4-P.l^{-1}. Incubations were carried out under natural daylight. Two experiments were made (see below).

Having previously encountered the problem of preserving phytoplankton samples during high-sea cruises, we tried to ascertain the effect of storing labelled phytoplankton. A first series of 14 filters was prepared from a batch culture, half of the filters were kept at 60 °C, the others at ambient (25 °C) temperature. No significant difference was observed after six days (Student-Newman-Keuls test). Another culture yielded eleven filters ; seven of them were treated at once, giving an average value of 20.92 % for T (σ = 0.10 %). The other filters were kept at ambient temperature in short loosely stoppered test tubes in indirect daylight. After 28 days the average T value was 20.92 % (σ = 0.17 %), which is not significantly different from the initial one.

E.1. a) *NH_4-N uptake*

A culture reaching the end of the exponential phase was labelled with 144 µgat NH_4-N.l^{-1} at E = 99.1 %. After various incubation time intervals samples of 5 to 10 ml of the culture (25 to 50 µg N) were filtered and treated by O.S. Parallel samples of 80 to 250 ml were treated by M.S.

The figures obtained by O.S. and M.S. agree well *(fig. 4)*.

E.1. b) *NO_3-N uptake*

A culture near the end of the exponential phase was diluted 1/6 in enriched sea water in order to simulate approximatively an upwelling situation. Aliquots of this dilution were labelled by 30 µgat NO_3-N.l^{-1} at E = 99.1 % after one hour, then after one day, two days and three days. Light and dark incubations were carried out with formaline-killed controls. After a 4-hour incubation all samples were filtered and treated by O.S. ; aliquots of the incubations under light were treated by M.S.

O.S. values are higher than M.S. ones *(tabl. V)*. The Kjeldahl procedure, as carried on in this particular experiment, probably missed an unknown fraction of the intracellular pool of highly labelled NO_3-N.

E.2. *Soils and sediments*

^{15}N-labelled organic matter contained in various

TABLE V. — NO_3-N *uptake by a phytoplankton culture.*

A diluted culture was incubated for up to 3 days (see text). Aliquots were labelled by $^{15}NO_3^-$ in separate 4-hour incubations in transparent and dark bottles. Formaline-killed controls were made. The E ‰ values were determined by O.S. (in duplicates) and M.S.

TABLEAU V. — *Assimilation de* NO_3-N *par une culture de phytoplancton.*

Une culture diluée est incubée jusqu'à 3 jours (voir texte). Chaque jour, des aliquotes sont marquées par une incubation de 4 h *en présence* de $^{15}NO_3^-$ dans des flacons clairs (« light ») et sombres (« dark »). Des témoins formolés (« control ») sont réalisés. Les valeurs de E (‰ atomes en excès) sont déterminées par S.O. (en double) et S.M.

Day		0	1	2	3
E_{MS}	light	0.818	1.16	0.889	0.692
E_{OS}	light	0.966	1.326	1.049	0.760
	dark	0.094	0.088	0.096	0.020
	control	0.089	0.053	0.058	0.024

soils was studied by O.S. *(tabl. VI)*. The previous M.S. analyses agree reasonably with the O.S. values. We have no data on the variability of the M.S. analyses. The wide variation observed with O.S. on soils *1* and *7* stresses the problem of sample size in heterogeneous substances. Soils with a low organic content (soils *3* and *5*) lead to bulky samples ; 2 g of loose soil occupy about a third of the discharge tube. The quantity of oxidizer had to be augmented ; otherwise no particular problem arose for the O.S. treatment of such soils.

$^{15}NH_4$-enriched sediments of marine origin were studied. The spectra were at first anomalous, with a high and irregular signal in the 299.3 to 298.9 nm region. The disturbing substances could not be identified. A 30-min. refrigeration was needed to smooth out the base line region.

E.3. *Plant organic matter*

Maize (*Zea mays*) straw labelled with ^{14}C and ^{15}N was studied on samples of about 1 mg total (dry) weight. The O.S. values had an average of E = 1.57 ‰ (σ = 0.14 ; n = 4). The average M.S. value was E = 1.38 ‰ ; the standard deviation on this value is unknown.

E.4. *Animal organic matter*

Trials were carried out on weevil (*Tribolium confusum*) larvae and adults, fed on flour labelled with nitrate or ammonium. A larva weighing 0.5 to 1 mg (wet weight) may be treated whole. An adult weighing 2 mg must be cut at least in two and the parts treated separately.

TABLE VI. — *Organic matter in soils.*

^{15}N excess was determined in various soil samples by O.S. The individual values and their average are given in ‰ (E_{OS}) with the coefficients of variation CV (in ‰). Previously determined M.S. values (E_{MS} in ‰ atoms) are given for comparison. For soil 7 different M.S. determinations gave different values.

TABLEAU VI. — *Matière organique des sols.*

Divers échantillons de sols ont été étudiés par S.O. Les valeurs individuelles et leur moyenne sont données en ‰ atomes ^{15}N en excès (E_{OS}). Le coefficient de variation, CV, est en ‰. Les valeurs obtenues auparavant par S.M. (E_{MS}, en ‰ atomes ^{15}N en excès) sont données par comparaison. Sur le sol 7, des valeurs très différentes ont été obtenues par S.M.

Soil n°	Weight (mg)	E_{OS}	Mean	CV ‰	EM_S
1	3.0 3.3 2.5 2.5	1.55 1.31 1.41 1.70	1.49	9.9	1.20
2	1.4 1.7 1.8 1.6	0.529 0.612 0.552 0.575	0.567	5.3	0.56
3	2 457	0.624	—	—	0.408
4	4.8 4.7 4.7 4.8	0.33 0.28 0.30 0.28	0.299	7.4	0.260
5	1 513 1 605	0.481 0.495	0.488	1.5	0.338
6	21.8 22.8 25.5 25.0	0.23 0.30 0.26 0.27	0.266	8.7	0.255
7	19.4 25.0 25.5 25.5 23.2 24.0	0.006 0 0.020 0.031 0.030 0.019	0.018	65	0.026 to 0.040
8	162	1.21	—	—	0.958

CONCLUSION

The accuracy of the method is satisfactory over the range of ^{15}N abundance studied (0 to 30 ‰) ; the CV values are comparable to those found by other workers [15]. Since the variability of the readings is of the same magnitude, we may admit that the reading of the spectrum is the main source of uncertainty. The chief problem in the reading lies in defining the base line. Possible variations in light intensity can oblige one to interpolate the height of a peak. These factors are annoying during a visual reading. We feel that they preclude automatic reading without human supervision. Certainly progress should be made in the stabilization of

excitation and in the other components of the apparatus, but with an incidence on the price.

Compared with the generally available mass spectrometers, all optical spectrometers are less bulky ; they may be put in service instantaneously and their handling is quickly learned. Furthermore both their price and their running costs are low. We feel that the apparatus used here presents all these features to a high degree.

We have seen that the minimum quantity of N which can be analyzed is about 15 µg with the described routine set-up. A reduced volume allows to extend the quantity range down to about 2 µg. This is still higher than the minimum quantities quoted in the literature : 0.2 µg [20] to 0.5 µg [28]. The small size of the sample has been stressed by several authors [3, 6, 10, 21] ; the oceanographic applications, among others, are simplified, considering that concentrations of 10 µg $N.l^{-1}$ are frequent. An insect weighing 2 mg may be dissected. Nonetheless we feel that working with samples smaller than 5 µg N may be risky, as the presence of extraneous substances would have a deplorable effect at this concentration level.

The problem of the representativity of a sample has been raised [14, 29] ; any material becomes heterogeneous at a given scale, even in the marine environment [30, 31]. Decreasing the sample size complicates further the problem.

Working with sealed individual discharge tubes suppresses the risks of cross-contamination. The complete treatment of a sample takes an average of 20 min. of actual work, of which an average of 10 min. is devoted to readings and calculations.

The method described was worked out with the constant aim of simplification. Our experience of field work has prompted us to try and streamline both apparatus and methods. A « field » trial is necessary. Two points await further development. The first one is the replacement of liquid nitrogen by an autonomous refrigeration system. The second, and more important, point is the determination of the total nitrogen quantity in the sample. A method has been described [29] whereby the N_2 pressure is measured. Such a measurement destroys the sample but would suppress the now obligatory use of a parallel determination of total nitrogen by Kjeldahl process or with a CHN analyser.

BIBLIOGRAPHIE

[1] HOCH (M.), WEISSER (H. R.). — *Helv. Chim. Acta*, 1950, *33*, 2128.
[2] MEIER (G.), MULLER (G.). — *Isotopenpraxis*, 1965, *1*, 53.
[3] GOLEB (J. A.), MIDDELBOE (V.). — *Anal. Chim. Acta*, 1968, *43*, 229.
[4] LEICKNAM (J. P.), MIDDELBOE (V.), PROKSCH (G.). — *Anal. Chim. Acta*, 1968, *40*, 487.
[5] FIEDLER (R.), PROKSCH (G.). — *Anal. Chim. Acta*, 1975, *78*, 1.
[6] MIDDELBOE (V.). — *Kgl. Vet.-og Landbohojskole. Arsskr.*, 1980, 1-22.
[7] PERSCHKE (H.), KEROE (E. A.), PROKSCH (G.), MUEHL (A.). — *Anal. Chim. Acta*, 1971, *53*, 459.
[8] GOULDEN (J. D. S.), SALTER (D. N.). — *Analyst*, 1979, *104*, 756.
[9] GUIRAUD (G.), FARDEAU (J. C.). — *Analusis*, 1980, *8*, 148.
[10] FIEDLER (R.), PROKSCH (G.). — *Plant Soil*, 1972, *36*, 371.
[11] SLAWYK (G.), MACISAAC (J. J.), DUGDALE (R. C.). — *Limnol. Oceanorg.*, 1976, *21*, 149.
[12] GUIRAUD (G.), PACCHIANI (L.). — *Analusis*, 1973, *2*, 282.
[13] LLOYD-JONES (C. P.), HUDD (G. A.), HILL-COTTINGHAM (D. G.). — *Analyst*, 1974, *99*, 580.
[14] MIDDELBOE (V.). — *Appl. Spectrosc.*, 1974, *28*, 274.
[15] KEENEY (D. R.), TEDESCO (M. J.). — *Anal. Chim. Acta*, 1973, *65*, 19.
[16] MEYER (G. W.), MC CASLIN (B. D.), GAST (R. G.). — *Soil Sci.*, 1974, *117*, 378.
[17] YONEYAMA (T.), KUMAZAWA (K.). — *Plant Cell Physiol.*, 1974, *15*, 655.
[18] FIEDLER (R.), PROKSCH (G.). — *Anal. Chim. Acta*, 1972, *60*, 277.
[19] STORCH (T. A.), SAUNDERS (G. W.). — *Limnol. Oceanogr.*, 1978, *23*, 112.
[20] MUHAMMAD (S.), KUMAZAWA (K.). — *Z. Pflanzenernaehr. Bodenkd.*, 1976, *5*, 529.
[21] FAUST (H.), REINHARDT (R.). — *Isotopenpraxis*, 1975, *11*, 321.
[22] BLACKBURN (T. H.). — *Appl. Environm. Microbiol.*, 1979, *37*, 760.
[23] PHEIFFER MADSEN (P.). — *Water Res.*, 1979, *13*, 461.
[24] PORTER (L. K.), O'DEAN (W. A.). — *Anal. Chem.*, 1977, *49*, 514.
[25] FERRARIS (M. M.), PROKSCH (G.). — *Anal. Chim. Acta*, 1972, *59*, 177.
[26] KANAZAWA (S.), YONEYAMA (T.). — *Soil Sci. Plant Nutr.*, 1976, *22*, 489.
[27] GUILLARD (R. R.), RYTHER (J. H.). — *Can. J. Microbiol.*, 1962, *8*, 229.
[28] MARTIN (F.), MAUDINAS (B.), CHEMARDIN (M.), GADAL (P.). — *Int. J. Appl. Radiat. Isot.* (sous presse).
[29] FIEDLER (R.), PROKSCH (G.), KOEPF (A.). — *Anal. Chim. Acta*, 1973, *63*, 435.
[30] PLATT (T.). — *J. Exp. Mar. Biol. Ecol.*, 1975, *18*, 99.
[31] PAGES (J.), LEMASSON (L.), DUFOUR (P.). — *Cah. ORSTOM, sér. Hydrobiol.* (sous presse).

ANALUSIS

CHIMIE ANALYTIQUE - METHODES PHYSIQUES D'ANALYSE
COMPOSITION DE LA MATIÈRE

Revue publiée
avec la collaboration scientifique et technique :
- du Groupe de Chimie Analytique de la Société de Chimie Industrielle,
- du Groupement pour l'Avancement des Méthodes spectroscopiques et physicochimiques d'Analyse (G.A.M.S.)
- et de la Division de Chimie Analytique et Chimie des Solutions de la Société Chimique de France.

SOMMAIRE

N° 1 Janvier 1982

Volume 10

(*Contents*, p. III)

Mémoires

Les sommaires d'*Analusis* sont reproduits dans les Current Contents Physical and Chemical Sciences.