Elemental Analysis: CHNS/O determination of marine samples

Authors

Dr. Liliana Krotz and
Dr. Guido Giazzi
Thermo Fisher Scientific,
Milan, Italy

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Goal

Demonstrate the performance of the Thermo Scientific FlashSmart EA for the CHNS/O characterization of marine samples.

Introduction

Carbon, hydrogen, nitrogen and sulfur are basic building blocks that are analyzed for marine science. Fluctuations in the concentration ratio and/or content of carbon, nitrogen and sulfur define the origin of marine samples, the depositional environment and the diagenetic alteration of the source materials.

Nitrogen functions as a limiting nutrient in the oceans. The global carbon cycle and, consequently, atmospheric CO_2 are tightly coupled to the nitrogen cycle. Therefore, changes in the size of wells and fixed nitrogen sources in the oceans can significantly influence global climate. The biological fixation of nitrogen, denitrification and nitrate consumption by phytoplankton are the main biological processes of the global nitrogen cycle. Changes in oceans circulation and nutrient supply, which occur in response to changes in environmental conditions, influence the relative importance and spatial extent of the major nitrogen cycle pathways.

The C/N ratio indicates diagenesis and productivity changes in sediments. Diagenesis can cause a reduction in C/N while productivity changes tend to have relatively constant C/N ratios. Low C/N values occur in poor sediments of organic carbon. These values can be influenced by the tendency of clay minerals to absorb the ammonium ions generated during the degradation of organic matter. The high C/N values compared to the algal values are common in marine sediments rich in organic carbon. These values evidently result from the selective loss of nitrogen as the organic substance settles from the photon area, because the nitrogen-containing proteins are more labile than other components of organic matter such as carbohydrates and lipids.

Sulfur is also an essential component in the marine environment. Sulfur analysis is very important in marine sediments to study the wide range of microbial metabolic strategies.

Therefore, the typical materials studied in marine science are sediments, plankton and algae. Sediment is any particulate matter that can be transported by fluid flow and which eventually is deposited as a layer of solid particles on the bed or bottom of a body of water. Seas, oceans and lakes accumulate sediment over time. Plankton is the diverse collection of organisms that live in large bodies of water and are unable to swim against a current. They provide a crucial source of food to many large aquatic organisms, such as fish and whales. These organisms include bacteria, archaea, algae, protozoa and drifting or floating animals that inhabit the pelagic zone of oceans and seas.

Carbon, nitrogen, hydrogen, sulfur analysis by combustion analysis, and oxygen determination by pyrolysis are commonly used for the characterization of these marine samples and for R&D purposes. Marine samples testing by traditional methods are no longer suitable for routine analysis, due to their time-consuming preparation and the use of environmentally hazardous reagents. For this reason the use of an accurate instrumental analytical technique is required. As there is an increase in the demand for improved sample throughput, reduction of operational costs and minimization of human errors, a simple and automated technique, which allows fast analysis with excellent reproducibility, is the key for efficient element determination.

The Thermo Scientific™ Flash*Smart*™ Elemental Analyzer (Figure 1), based on the dynamic combustion of the sample, provides automatic and simultaneous CHNS determination in a single analysis run and oxygen determination by pyrolysis in a second run. The Flash*Smart* Elemental Analyzer (Figure 1) is equipped with two totally independent furnaces allowing the installation of two analytical circuits, that can be used sequentially and are completely automatic through the Thermo Scientific™ MultiValve Control™ (MVC) Module. Each analytical circuit accepts its own autosampler. The Elemental Analyzer can also determine nitrogen and carbon by a double reactor system: the first reactor is used for combustion and catalytic oxidation of the combustion gases and the second is used to reduce nitrous oxides as N_2. In this way the system copes effortlessly with laboratory requirements such as modularity, accuracy, day to day reproducibility and high sample throughput.

Figure 1. Thermo Scientific Flash*Smart* Elemental Analyzer.

This note presents CHNS/O data on marine sediments, algae and plankton and shows the performance of the Thermo Scientific Flash*Smart* Elemental Analzyer.

Methods

The Elemental Analyzer operates according to the dynamic flash combustion of the sample. Samples are weighed in tin containers and introduced into the combustion reactor via the Thermo Scientific™ MAS Plus Autosampler with oxygen.

For NC determination, after the combustion, the produced gases are carried by helium flow to a second reactor filled with copper. They are then swept through a H_2O trap and onto a GC column. Finally they are detected by a Thermal Conductivity Detector (TCD) (Figure 2).
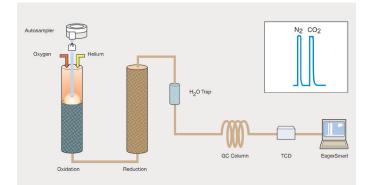
Figure 2. NC configuration.

For CHN or CHNS determination, after the combustion, the resulted gases, are carried by a helium flow to a layer filled with copper, then swept through a GC column that provides the separation of the combustion gases. Finally, they are detected by a Thermal Conductivity Detector (TCD) (Figure 3). For sulfur analysis only, after combustion the resulted gases are carried by a helium flow to a catalyst, a layer filled with copper and a water trap. They are then swept through a GC column that provides the separation of the combustion gases. Finally, they are detected by a Thermal Conductivity Detector (TCD).
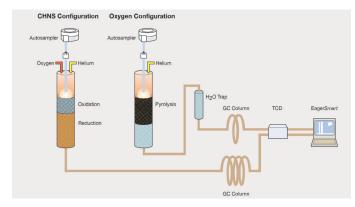

Figure 3. CHNS/O Configuration.

For oxygen determination, the system operates in pyrolysis mode. Samples are weighed in silver containers and introduced into the pyrolysis chamber (right furnace) via the MAS Plus Autosampler. The reactor contains nickel coated carbon maintained at 1060 ˚C. The oxygen in the sample, combined with the carbon, forms carbon monoxide, which is then gas chromatographically separated from other products and detected by the TCD Detector (Figure 3).

A complete report is automatically generated by the Thermo Scientific™ Eager*Smart*™ Data Handling Software and displayed at the end of the analysis.

Results
Several marine samples such sediments, algae and plankton, with a large range of concentrations were analyzed to show the repeatability of the data obtained by the Flash*Smart* Analyzer. Samples were homogenized with a ball mill after being dried (105 ˚C, 2 hours).

Sediment samples were analyzed in NC, NCS, CHN, CHNS and sulfur-only configurations to demonstrate the modularity of the system according to the laboratory requests. For nitrogen and carbon determination, two tests were performed. The instrument was calibrated with 4-6 mg aspartic acid and EDTA using K factor as the calibration method, and with the samples weighed at 50-100 mg. Table 1 shows the NC data obtained analyzing 50-100 mg of samples in duplicate. Table 2 shows the NC data obtained for triplicate analyses of the samples.

Table 1. NC data of sediment samples.

Sample	N%	RSD%	C%	RSD%
1	0.0378 - 0.0362	3.056	4.403 - 4.443	0.639
2	0.0975 - 0.0972	0.218	2.671 - 2.654	0.451
3	0.108 - 0.110	1.297	2.789 - 2.759	0.765
4	0.103 - 0.104	0.683	2.886 - 2.898	0.293
5	0.113 - 0.116	1.852	2.998 - 2.983	0.355
6	0.113 - 0.114	0.623	2.805 - 2.794	0.278
7	0.117 - 0.116	0.607	2.852 - 2.907	1.347
8	0.134 - 0.138	2.079	3.015 - 3.020	0.117
9	0.136 - 0.139	1.543	0.979 - 0.981	0.144
10	0.138 - 0.140	1.017	0.990 - 0.997	0.498
11	0.139 - 0.142	1.510	1.018 - 1.022	0.277
12	0.191- 0.193	0.737	1.986 - 1.966	0.726

Table 2. NC data of sediment samples.

Sample	Weight (mg)	N%	RSD%	C%	RSD%
1	100 - 150	-	-	0.0106 0.0100 0.0102	2.976
2	50 - 100	0.0282 0.0283 0.0281	0.355	0.8419 0.8312 0.8313	0.737
3 (Coastal)	50 - 70	0.0612 0.0622 0.0618	0.815	3.4320 3.3371 3.3570	1.483
4	50 - 100	0.1917 0.1940 0.1917	0.690	1.8368 1.8227 1.8177	0.542
5 (Stream)	15 - 20	0.6427 0.6367 0.6446	0.643	8.9137 8.8485 8.9226	0.455
6 (Lake)	15 - 20	0.7739 0.7922 0.7897	1.2634	12.8951 12.8367 12.7741	0.471
7 (Reef)	10 - 15	0.0109 0.0110 0.0102	4.074	12.0334 11.9596 11.9567	0.363

For NCS determination, three sediments were analyzed. The Elemental Analyzer was calibrated with 2-3 mg BBOT (2,5-Bis (5-tert-butyl-benzoxazol-2-yl) thiophene) using K factor as the calibration method, with the samples weighed at 10-15 mg. Table 3 shows the NCS data obtained for triplicate analyses of the samples.

Table 3. NCS data of sediments.

Sample	N%	RSD%	C%	RSD%	S%	RSD%
1	0.1029 0.1061 0.1055	1.623	0.7460 0.7359 0.7377	0.728	0.2823 0.2871 0.2850	0.845
2	0.0716 0.0735 0.0716	1.519	1.1807 1.1931 1.1770	0.713	0.1507 0.1509 0.1549	1.557
3	0.2891 0.2845 0.2885	0.870	4.2044 4.1995 4.2176	0.223	0.1431 0.1424 0.1414	0.601

With the CHN configuration, seven sediment samples from the Pacific and the Atlantic Ocean were analyzed. The Elemental Analyzer was calibrated with 2-3 mg acetanilide using K factor as the calibration method. Each sample was weighed at 10-15 mg. Table 4 shows the NCS data obtained for duplicate analyses of the samples.

Table 4. CHN data of sediments.

Sample	N%		C%		H%	
A	0.13	0.14	9.20	9.22	0.33	0.33
B	0.03	0.03	6.72	6.72	0.14	0.14
C	0.04	0.04	6.91	6.96	0.16	0.10
D	0.08	0.08	8.46	8.49	0.24	0.25
E	0.06	0.06	8.25	8.30	0.22	0.22
F	0.02	0.02	5.62	5.63	0.11	0.11
G	0.03	0.02	6.47	6.44	0.13	0.17

Additionally, total organic carbon values (TOC) were determined for three sediment samples using the CHNS and NCS configurations. In both configurations, the system was calibrated with 2-3 mg BBOT as standard using K factor as the calibration method. Each sample was weighed at 10-15 mg for CHNS and NCS analysis. For TOC determination each sample was weighed at 5-10 mg. The differentiation of Total Carbon (TC) and Total Organic Carbon (TOC) was performed by sample manipulation prior to analysis in accordance with the Official Italian Method on Soils Analytical Chemistry, Method 248 (Gazzetta Ufficiale). TOC values were determined after removing carbonates by acidification of the sample with HCl 1:1 (Figure 4) using the kit showed in Figure 5. The TC and TOC analyses were performed consecutively using the same analytical conditions of the Elemental Analyzer. Table 5 and 6 show the CHNS, NCS and TOC values of different sediment samples.
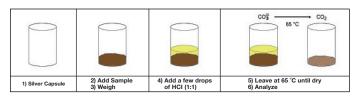

Figure 4. Method for TOC determination.

Figure 5. Kit for Total Organic Carbon (TOC) for solid samples.

Table 5. CHNS and TOC determination of sediment.

N%	RSD%	C%	RSD%	H%	RSD%	S%	RSD%	TOC%	RSD%
0.15		4.58		0.54		0755		2.15	
0.15		4.59		0.53		0.752		2.15	
0.15	0.000	4.59	0.097	0.54	1.555	0.754	0.151	2.15	0.000
0.15		4.59		0.55		0.753		2.15	
0.15		4.59		0.53		0.753		2.15	

Table 6. NCS and TOC determination of sediments.

Sample	N%	RSD%	C%	RSD%	S%	RSD%	TOC%	RSD%
1	0.0664 0.0670 0.0670	0.52	0.7583 0.7635 0.7601	0.35	0.0368 0.0378 0.0371	1.38	0.7486 0.7498 0.7590	0.71
2	0.8943 0.8980 0.8971	0.22	9.8773 9.9216 9.8533	0.35	1.1723 1.1881 1.1859	0.72	8.7689 8.7114 8.8676	0.90

Several sediment samples were analyzed in the sulfur-only configuration. The Elemental Analyzer was calibrated with 1-2 mg BBOT using K factor as the calibration method (Table 7). Each sample was weighed at 5-6 mg. Table 4 shows the sulfur data obtained for duplicate analyses of the samples.

Table 7. Sulfur data of sediment samples.

Sample	S%	RSD%
1	0.0245 - 0.0266	5.812
2	0.0610 - 0.0638	3.173
3	0.0633 – 0.0661	3.060
4	0.0652 – 0.0683	3.284
5	0.0734 – 0.0738	0.384
6	0.0812 – 0.0838	2.228
7	0.0854 - 0.0868	1.150
8	0.0934 - 0.0926	0.608
9	0.1037 - 0.1019	1.238
10	0.1136 - 0.1117	1.193
11	0.1314 - 0.1299	0.812
12	0.1704 - 0.1710	0.249
13	0.1704 - 0.1710	0.249
14	0.2035 - 0.2059	0.829
15	0.2090 - 0.2064	0.885
16	0.2094 - 0.2104	0.337
17	0.2110 - 0.2082	0.945
18	0.2191 - 0.2211	0.643
19	0.2191 - 0.2211	0.642
20	0.2344 - 0.2316	0.850
21	0.2355 - 0.2342	0.391
22	0.2355 - 0.2342	0.391
23	0.2494 - 0.2471	0.655
24	0.3235 - 0.3230	0.109

Algae and plankton samples were analyzed by the FlashSmart Elemental Analyzer in different configurations. An algae sample was analyzed in the CHNS configuration, and the system was calibrated with 2-3 mg BBOT using K factor as calibration method (Table 8). Each sample was weighed at 3-4 mg. Table 5 shows the CHNS data of the algae sample.

Table 8. CHNS data of algae sample.

N%	RSD%	C%	RSD%	H%	RSD%	S%	RSD%
4.465 4.455	0.162	28.754 28.549	0.506	4.749 4.733	0.240	1.027 1.071	2.919

Freeze-dried microalgae were analyzed in the CHNS/O configuration. For CHNS, the Elemental Analyzer was calibrated with 2-3 mg BBOT using K factor as the calibration method. Each sample was weighed at 3-4 mg. For oxygen determination by pyrolysis, the Elemental Analyzer was calibrated with 1-2 mg benzoic acid using K factor as the calibration method (Table 9). Each sample was weighed at 1-2 mg. Table 9 shows the CHNS/O data of the two samples.

Table 9. CHNS/O data of freeze-dried microalgae.

Sample	N%	RSD%	C%	RSD%	H%	RSD%	S%	RSD%	O%	RSD%
A	3.35 3.30 3.31	0.80	19.62 19.48 19.57	0.36	3.94 3.98 3.98	0.58	1.21 1.19 1.18	1.28	21.99 21.86 21.77	0.50
B	6.44 6.42 6.47	0.39	31.87 31.68 31.89	0.36	4.71 4.79 4.77	0.87	1.40 1.37 1.38	1.10	20.41 20.55 20.31	0.59
C	4.17 4.16 4.15	0.24	32.89 32.19 32.58	1.08	5.00 4.98 4.99	0.20	1.38 1.40 1.38	0.83	22.42 22.31 22.44	0.31
D	3.85 3.82 3.81	0.54	25.92 25.88 25.96	0.15	4.74 4.71 4.72	0.32	1.31 1.29 1.31	0.89	22.25 22.34 22.31	0.21
E	7.66 7.68 7.66	0.15	39.33 39.44 39.31	0.18	5.82 5.83 5.87	0.45	1.17 1.15 1.16	0.86	20.65 20.46 20.49	0.50
F	5.36 5.37 5.39	0.28	40.45 40.46 40.47	0.02	6.17 6.14 6.15	0.25	1.11 1.11 1.10	0.52	23.42 23.43 23.38	0.11

Two plankton samples were analyzed in the sulfur-only configuration. The Elemental Analyzer was calibrated with 2-3 mg of BBOT using K factor as the calibration method. Each sample was weighed at 3-4 mg. Table 10 shows the sulfur data obtained.

Table 10. Sulfur data of plankton samples.

Sample	S%	RSD%
Plankton	0.453 0.467 0.461	1.60
Zooplankton	0.323 0.347 0.330	3.65

Conclusions

For automated CHNS/O analysis of marine samples (sediment, algae, plankton) elemental analysis based on the combustion method (Dumas) proved to be a valuable solution, in accordance with Official Methods.

The FlashSmart Elemental Analyzer performs CHNS analysis by combustion and oxygen by pyrolysis determination for the analysis of marine samples, providing excellent reproducibility and no memory effect when changing the type of sample. Complete and accurate detection of the elements is therefore achieved.

As a complete automated CHNS/O solution, the FlashSmart Elemental Analyzer enables analysis of samples at low and high content without matrix effects. The Elemental Analyzer allows CHSN/O measurement without the use of sample digestion or toxic chemicals normally required by traditional methods.

In addition, the dual analytical configuration capability allows:

- Analysis of solids, liquids, viscous samples
- One or two MAS Plus Autosamplers installed on the same system controlled by the EagerSmart Data Handling Software

From the NC configuration:

- Conversion to CHNS, NCS or CHN configuration
- Conversion to S only configuration
- Full control of the workflow by the EagerSmart Data Handling Software.

The FlashSmart EA also meets the requirements of the Official Method EPA (Environmental Protection Agency): Determination of NC in sediments and particulates of estuarine/coastal waters using elemental analysis (Method 440.0, 1997).

Find out more at **thermofisher.com/OEA**

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