

Appendix 6

Standard Operating Procedure

for the USGS Reston, Virginia Environmental Organic
Geochemistry Laboratory

Instrumental Analysis for Total Organic Carbon and Total
Nitrogen in Sediments

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Contents

1. Application	3
a. Tested concentration range	3
b. Sensitivity	3
c. Detection limits	3
d. Interferences	4
e. Analysis rate	5
2. Chemistry	5
3. Apparatus	5
a. Instrumentation	5
b. Parameters	6
c. Hardware/glassware	7
d. Chemicals	7
4. Standards	7
a. Calibration standards	8
b. Standard reference material	8
5. Procedures	8
a. Sample preparation	8
b. Instrumental analysis	9
6. Calculations	10
7. QA/QC Considerations	11
a. Certified reference material	11
b. Blanks	11
c. Calibration	11
d. Duplicates	11
8. Health, Safety, and Waste-Disposal Information	11
a. Personal protection	11
b. Electrical hazards	11
c. Chemical hazards	11
d. Gas cylinder handling	12
9. References	12

Instrumental Analysis for Total Organic Carbon and Total Nitrogen in Sediments

1. **Application:** This is a description of procedures used for determination of total organic carbon (TOC) and total nitrogen (TN) in marine sediments.
- a. **Tested concentration range.** Based on data collected by LACSD (1992), the expected concentration range for TOC in Palos Verdes Shelf sediments is 0.3-13.4 %; for TN it is 0.032-1.03%.
- b. **Sensitivity.** Following is a tabulation of peak areas obtained near the detection limit. Each sample consisted of a weighed amount of combusted (450 °C overnight) ball-milled sand.

Table 1. Peak areas for sediments (pre-combusted sand) near the method detection limit.

Sample	Weight (mg)	Peak Area (uV)	Organic Carbon (%)
1	43.815	1737	0.0068
2	41.057	817	0.0034
3	49.292	228	0.0008
4	33.532	251	0.0013
5	46.829	58	0.002
6	44.592	252	0.001
7	46.819	535	0.002

- c. **Detection limits.** The Method Detection Limit (MDL) has been determined according to procedures described in 40 CFR Part 136 (USEPA, 1992). An initial estimate of the detection limit was derived from the instrument detection limit (0.001 %) given in the Carlo Erba Elemental Analyzer EA 1108 instruction manual. The MDL determination was based on the analysis of 7 replicate samples consisting of ball-milled sand. The samples were prepared as follows. The sand was combusted in a muffle furnace at 450 °C overnight. Following combustion, the sand was allowed to cool to room temperature. Aliquots of the sand (~40 mg) were then transferred with a clean stainless steel microscopula to

3.5-mm x 5-mm silver capsules and weighed on a Sartorius MC5 microbalance. The silver capsules rested in wells of a metal sample holder throughout the weighing. The weights of each sample were recorded in a laboratory notebook. Following the weighing of each sample, the silver capsules were placed into the wells of a Teflon™ block specially machined to accommodate the capsules. The block was then placed inside a glass desiccator along with a small petri dish containing 50 mL of fresh concentrated HCl. The samples were exposed to HCl vapor for 24 hours at room temperature, then removed (while remaining in the Teflon™ block) and heated in an oven for 2 hours at 50 °C to drive off residual HCl and water. [Note: The acidification part of the procedure described here differs from that given in section 5.a. because the MDL determination was done at an earlier time. Subsequent to the MDL determination experiment, slight changes to the acidification procedure, now reflected in the description given in section 5.a., were tested and implemented for routine sample analysis. Our data show that these procedures yield identical results.] The silver capsules were then placed into 5-mm x 12-mm tin capsules. The tin capsules were closed, folded into cube-shaped parcels and transferred to the autosampler tray of the Carlo Erba Analyzer. Details of the instrumental analytical methods are given below. The raw data for these analyses are given in Table 1. The detection limit is computed using the following equation:

$$MDL = t_{(n-1, 1-\alpha=0.99)}(S) \quad (1)$$

where: MDL = the method detection limit (%),
 $t_{(n-1, 1-\alpha=0.99)}$ = the student's t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. (The t value for 7 replicates = 3.143).
 (S) = standard deviation of the replicate analyses (%).

The calculated (blank-corrected) method detection limits for this procedure are 0.0071% (TOC) and 0.0027 % (TN).

- d. Interferences.** A possible interference may occur when there is a buildup of residual materials from the sample vessels in the combustion tube (ash). A buildup of residuals in the tube can result in incomplete combustion of a sample when the surface of the combustion packing rises above the high temperature zone in the middle of the furnace. In this case, the “flash combustion” that normally results from oxidation of the tin capsule is not achieved, and the high temperatures resulting from this exothermic reaction do not bring about complete combustion of the sample components. Incomplete combustion of the sample results in a type of “carryover” whereby continued combustion of the partially combusted sample is experienced during the combustion of the next sample. In order to avoid these problems, the *combustion reactor tube* is periodically

removed (after ~300 sample runs) from the furnace and cleared of all debris. The *reduction reactor tube* is also serviced at this time.

Other problems can arise when the water filter is exhausted resulting in the transfer of water to the thermal conductivity detector. This problem is avoided by changing the filter on a periodic basis (after ~200 sample runs).

- e. **Analysis rate.** The analysis rate is determined by initial and final sample preparation and instrumental analysis run times. It is possible to complete sample analyses for sediments from one core containing about 20 sections within a week. The first two days include initial sample prep, that is, determining the water content of each section. This involves initial homogenization, weighing, oven drying, reweighing, and grinding of the sediment. During the following two days, the final sample prep is completed. This involves weighing aliquots of each section of the core individually into silver capsules. The silver capsules containing the sample are exposed to acid vapor treatment for 24 hours and are then dried for 2 hours in an oven. Finally, the silver capsules for each sample are enclosed in tin capsules to provide flash combustion during instrumental analysis. Instrumental analysis requires one full day. The analysis time for each sample is approximately 6 minutes.

2. **Chemistry:** The chemical reactions involved are as follows:

Acidification of sediment samples with concentrated HCl: carbonate removal



3. **Apparatus:**

- a. **Instrumentation.** All quantitative analyses for carbon and nitrogen are performed with a Carlo Erba Elemental Analyzer EA 1108. The operation of the elemental analyzer is based on the complete and instantaneous oxidation of the sample by “flash combustion” whereby all organic and inorganic substances are converted into combustion products. The operation of the instrument is described below.

The sample, which is contained in a tin capsule, is introduced into the instrument automatically by an autosampler (AS-200LS). The AS-200LS autosampler tray holds 49 samples. The autosampler operates in the following manner. The sample container drops from the autosampler tray into the cavity of the autosampler slide where it is continuously purged with a constant flow of carrier gas (helium, 100 mL/min). It then drops into the first of two serially connected reaction columns. The first column, the *combustion reactor tube*, is made of transparent quartz and is filled with the catalyst, chromic oxide (Cr_2O_3 ; 8 cm height) over silvered cobaltous-cobaltic oxide ($\text{Co}_3\text{O}_4/\text{Ag}$; 6 cm height). This column is maintained at 1020 °C. The second column, the ‘*reduction*’ *reactor tube*, is also made of transparent quartz and is maintained at 650 °C. It is filled with reduced copper wire (Cu°) and cupric oxide (CuO) layers above and below the copper wire.

After the samples are dropped into the *combustion reactor tube*, the helium stream is temporarily (2 seconds) enriched with pure oxygen. Oxidation of the tin

capsule results in a violent exothermic reaction known as flash combustion. Flash combustion causes oxidation of 85-95% of the sample. Complete oxidation of reduced carbon and sulfur is achieved when the mixture of gases passes over the catalyst layer of chromic oxide. The silvered cobaltous-cobaltic oxide in the *combustion reactor tube* removes SO_x, halogens (except fluorine) and acid halides through chemi-adsorption. Once the combustion gases have passed through the *combustion reactor tube*, they enter the '*reduction reactor tube*'. Here CO and H₂ are oxidized (on the CuO layers) while O₂ is removed and N_xO_y species are reduced to N₂ (on the Cu⁰ layer). At this point, the gas mixture is composed of N₂, CO₂, H₂O and fluorinated gases. The gas mixture is then directed through a water filter (filled with anhydrous magnesium perchlorate) and on to the chromatographic column. Separation of N₂ and CO₂ (in that order) occurs in a 2- meter stainless steel column, 6 mm o.d., 4 mm i.d. packed with Porapak QS (80-100 mesh). Fluorinated gases are chemi-adsorbed to the stationary phase. The column is held at 60 °C. A thermal conductivity detector measures the individual components of the gas mixture (N₂, CO₂) as they elute from the column. The signal produced from the thermal conductivity detector is passed to an A/D converter, and the peaks are integrated by the EAGER 200 data system. The percent composition of carbon and nitrogen is computed automatically by the data system (if a previous calibration has been performed). A chromatogram and an analysis report (containing raw data and calculated values) are then saved to the hard disk as separate electronic files with *.dat and *.xls extensions respectively.

- b. **Parameters.** Table 2 provides a list of the parameters and settings used during the analysis of sediment samples for TOC and TN content.

Table 2. Analytical conditions for TOC, TN determination.

Parameter	Setting
Elements	C (organic), N
Measuring Range	100 %
Instrument Detection Limit	10 ppm
Accuracy	<0.3% absolute
Repeatability	<0.2% absolute
Sample Size of Solids/Liquids in Capsules	0.1 to 100 mg
Analysis time	6 minutes
Detector	Thermal Conductivity
Gas Requirements:	
-He-operating flow rate	100 mL/min

Table 2. Analytical conditions for TOC, TN determination.

-He-standby flow rate	5-10 mL/min
-O ₂	dosing loop of 10 mL, flow rate: 20-25 mL/min
Compressed air pressure	350 kPa
-Oxidation Furnace	1020 °C/1040 °C
-Reduction Furnace	650 °C
-Chromatographic column oven	60 °C
-TCD Filament	190 °C

c. **Hardware/glassware.** The following are used to prepare samples for analysis and for carrying out the instrumental analyses.

- silver capsules (3.5 mm x 5 mm; Alpha Resources, #ASD-2003)
- tin capsules (5 mm x 12 mm; Alpha Resources, #ATD-1010)
- stainless steel forceps (Carlo Erba, #20500500)
- stainless steel microspatula (Carlo Erba, #20500600)
- Teflon™ block with machined capsule wells and reagent well
- glass micro-desiccator (Kimax cylindrical weighing bottle, VWR #16716-067)
- glass petri dishes
- 50-mL beakers
- muffle furnace (Thermolyne Type 30400)
- drying oven (Baxter Scientific Products, DX-41)
- chromatographic column (2 m stainless steel 6 mm o.d., 4 mm i.d. packed with Poropak QS 80-100 mesh)
- quartz combustion & reduction reaction tubes (Costech Analytical, #061121)
- microbalance (Sartorius MC5)
- aluminum weighing pans
- agate mortar & pester (Baxter, #M9041-2)
- Wheaton glass 4-mL vials (National Scientific, #B7800-2)

d. **Chemicals.**

- Concentrated HCl 36.5 – 38% (VWR, #3110-3)
- Copper (II) oxide (Costech Analytical, #011003)
- Reduced copper wire (Costech Analytical, #011013)
- Chromium (III) oxide (Costech Analytical, #011001)
- Silvered cobaltous-cobaltic oxide (Costech Analytical, #011007)
- acetanilide (C₈H₉NO; Costech Analytical, #031040)
- magnesium perchlorate (Costech Analytical, #021022)
- quartz wool (SiO₂; Costech Analytical, #021035)

4. **Standards:**

- a. ***Calibration standards.*** The instrument is calibrated by analyzing varying amounts of acetanilide (elemental composition: 71.09 % carbon and 10.36 % nitrogen). This compound is stored at room temperature in an amber glass bottle. A five-point calibration curve is obtained by weighing approximately 0.5-mg, 1.0-mg, 2.0-mg, 4.0-mg and 10-mg portions of acetanilide into pre-cleaned 3.5-mm x 5-mm silver capsules using a microbalance. The weights are recorded, the silver capsules are closed with forceps, and the capsules are inserted into pre-cleaned 5-mm x 12-mm tin capsules. The tin capsules are closed, folded in cube-shaped parcels and placed into the wells of the Teflon™ block (in order of preparation). A set of calibration standard samples is prepared each day and is stored in a stainless steel desiccating cabinet until it is analyzed.
- b. ***Standard reference material.*** The standard reference material (SRM) is marine sediment (PACS-1) obtained from the National Research Council of Canada (NRCC). The total carbon content of PACS-1 is 3.69 %, and the unofficial reported total organic carbon (TOC) content is 3.56 %.

5. Procedure:

- a. ***Sample preparation.*** Sediment cores were sectioned frozen into 2-cm intervals according to procedures described in Appendix 3. The frozen sections were immediately placed into wide mouth glass jars, sealed with a Teflon™-lined cap and stored in a freezer at -20 °C. When samples are to be prepared for analysis, all of the subsections of a chosen core are removed from the freezer, placed on an aluminum tray and allowed to thaw for 1 hour at room temperature. After the sediments have thawed, the jars are opened, and a precleaned stainless steel spatula is used to manually homogenize the sample. A portion of the sample (about 2-7 wet g) is removed from the jar and transferred to a pre-combusted, pre-weighed aluminum pan. The jars are then tightly resealed and returned to the freezer.

The aluminum pans containing the wet sediment samples are weighed, and the data are recorded in a laboratory notebook. The aluminum pans containing the wet sediment samples are then placed in an oven to dry at 60 °C overnight. The samples are removed from the oven and placed in a desiccator for at least 1 hour. The dried samples are then reweighed to constant weight, and the data are recorded. The water content of sediments from each section of the core is determined using the weights recorded prior to and following oven drying (*cf.*, Appendix 4).

The dried sediment samples are ground to a fine powder using an agate mortar and pestle. The powdered sediment is then transferred to individually labeled glass vials. The samples are stored in the vials at room temperature in a desiccator until analyses are imminent. Final preparation of the sample is as follows. Aliquots of each sample (8-15 mg) are transferred from the individual glass vials and weighed into tared silver capsules using a stainless steel microscoopula and a microbalance. The weights are recorded, and the samples are placed in order of preparation into a Teflon™ block that has wells machined to accept the capsules. The samples are then exposed to HCl vapors for 24 hours to remove inorganic

carbon (Hedges and Stern, 1984) by placing the Teflon™ block in a sealed glass micro-desiccator with a small Teflon™ dish containing 5 mL concentrated HCl. Following acid treatment, the micro-desiccator is uncovered, all acid is removed by pipette, and the micro-desiccator is placed in an oven for 2 hours at 60 °C. This drives off residual HCl and water from the samples. Each silver capsule is then placed into a pre-cleaned 5-mm x 12-mm tin capsule. Using pre-cleaned stainless steel forceps, the silver and tin capsules are sealed and then transferred to the autosampler tray of the Carlo Erba Analyzer.

- b. Instrumental analysis.** Before commencing measurements on a given day all instrument parameters are checked for correct settings. This includes the temperature settings of the furnace, column oven, and detector, the pressure and flow rates of the gases and the time values (see Table 2 for parameter settings).

Following is a step-by-step description of the daily analysis routine:

- (1) **Bypass.** Two ‘bypass’ runs are performed before each day of analysis. The bypass consists of acetanilide (no specific weighed amount is required) in a tin capsule. Flash combustion is verified by viewing the glow during combustion, and the retention times of the analyte combustion products (CO₂, N₂) are determined during the bypass runs.
- (2) **Blank.** The blank consists of an empty silver capsule enclosed and folded into a tin capsule. The blanks are prepared in exactly the same fashion as sediment samples (*i.e.* including acidification). [Blank runs are repeated at intervals throughout a series of analyses with runs preceding and following] The blank provides a means for correcting the sample, standard and the SRM runs for background contributions of carbon and nitrogen and assessing the condition of the instrument. In doing blank corrections, the average peak area of the blanks (for a given element) bracketing a set of runs is subtracted from the respective peak area of the bracketed sample runs.
- (3) **Calibration.** A multipoint calibration curve is obtained with nominal weights of approximately 0.5 mg, 1.0 mg, 2.0 mg, 4.0 mg and 10 mg of acetanilide. The calibration standard samples are prepared without acid treatment. A description of the procedure is found in section 4.a. The slope and correlation coefficient from linear regression of the resulting curve (blank-corrected peak area *vs.* amount in mg -- with forcing through the origin) are calculated in Microsoft Excel. Calibration curves are obtained at the beginning of the day of analyses. [Note: Acetanilide samples in excess of 1.0 mg result in distorted carbon peaks that elute at earlier retention times than normal. The Eager 200 data system will not identify the carbon peak if its detection window has been too narrowly defined. The raw data (*i.e.* peak area) output in the analysis report, however, is unaffected and manual calibrations performed in Microsoft Excel will be accurate.]

- (4) ***Preceding/Following blank.*** See explanation above (2).
This blank is used for correction of calibration standards and the first set of sample/SRM runs.
- (5) ***Sample.*** The sample consists of a weighed amount of dry powdered sediment in a sealed silver capsule enclosed and folded in a tin capsule. Vapor phase acidification is used to remove inorganic carbon. Duplicates are prepared for all samples.
- (6) ***Standard Reference Material.*** The standard reference material (SRM) used in this procedure is a marine sediment (PACS-1) certified by the National Research Council of Canada for total carbon. The SRM run follows each set of five duplicate sample runs (*i.e.*, $n = 10$) and is analyzed periodically throughout the day of analysis to ensure quality control.
- (7) ***Following /preceding blank.*** See explanation above (2).

Repeat steps (5), (6), and (7) with different sample sets until all analyses are complete.

6. **Calculations:** All raw data are stored on the hard disk automatically by the Eager 200 data system in the form of Microsoft Excel files. The raw data (*i.e.* area counts) are transferred from these files to a spreadsheet, and there the calibration and sample runs are corrected for blank contributions. The data are corrected by subtracting the average peak area for the blank runs bracketing a set of sample runs. The five calibration standards make up one set of blank-corrected runs, whereas a second set consists of ten sample runs and an SRM run.

In an initial calibration exercise we found that when using blank-corrected data and forcing the curve through the origin, both carbon and nitrogen calibration curves exhibited well defined linear behavior ($r > 0.999$). At the same time, negative concentrations reported for samples with low but measurable peak areas were eliminated. For this reason, the blank-corrected calibration runs are subjected to linear regression analysis (with forcing through the origin) to arrive at a slope for computation of sample concentrations.

Concentrations of TOC and TN in the samples are computed using the following equation.

$$C_i = \left[\frac{A_i}{m * W} \right] * 100 \quad (3)$$

where: C_i = concentration of analyte in the sample (%),
 A_i = blank-corrected area of the analyte peak in the sample (counts),
 m = slope of calibration curve (counts/g),

W= weight of sample (g).

7. **QA/QC Considerations:** Following is information on the analysis of blanks, SRMs and other QA/QC considerations.

- a. ***Certified reference material.*** As noted above, PACS-1, a marine sediment SRM obtained from the National Research Council of Canada is processed along with every batch of 5 samples (in duplicate, n = 10 runs) as described in the *Southern California Damage Assessment Analytical Chemistry Quality Assurance Plan* (Manen, 1994).
- b. ***Blanks.*** As described above, blanks are processed in the same fashion as sediment samples and were run prior to and following the calibration runs and each batch of 5 samples (in duplicate, n = 10 runs) as described in the *Southern California Damage Assessment Analytical Chemistry Quality Assurance Plan* (Manen, 1994).
- c. ***Calibration.*** A five level multipoint calibration is carried out on each day of analysis as described in the *Southern California Damage Assessment Analytical Chemistry Quality Assurance Plan* (Manen, 1994). No continuing calibration is carried out. Instead, an SRM sample is analyzed at regular intervals to evaluate method performance.
- d. ***Duplicates.*** Each sediment sample is run in duplicate. After the data are tabulated and graphed, outliers are identified. If duplicate runs fail to agree within 3%, a third replicate is run. This is repeated until acceptable agreement among two replicates are obtained as described in the *Southern California Damage Assessment Analytical Chemistry Quality Assurance Plan* (Manen, 1994).

8. **Health, Safety, and Waste-Disposal Information:**

- a. ***Personal protection.*** Safety glasses and protective gloves are recommended whenever reagents or samples are handled. For other precautions and safety procedures, consult the Material Safety Data Sheets (MSDS) for each chemical used. They are on file in the laboratory; <http://www.ilpi.com/msds/#Manufacturers> provides links to MSDSs of most chemical companies.
- b. ***Electrical hazards.*** Electrical systems must conform to the National Electric Code, the National Fire Protection Association Code (NFPA 70-1971), and the American National Standards Institute (ANSI) Code (C1-1971). Consult the U.S. Geological Survey's Safety and Environmental Health Handbook (U.S. Geological Survey, 2002). Shock hazards exist inside the instruments. Only an authorized service representative or an individual with training in electronic repair should remove panels or circuit boards where voltages are greater than 20 V. The instruments require a third-wire protective grounding conductor. Three-to-two wire adapters are unsafe for these instruments.

- c. **Chemical hazards.** Hydrochloric acid is a strong acid used to remove inorganic carbon from sediment samples. Gloves should be worn when handling strong acids. If contact occurs, the affected area should be rinsed thoroughly with water. Hexane, dichloromethane and methanol are solvents used in cleaning glassware and the preparation of clean reagents. Gloves should be worn when handling organic solvents and, whenever possible, manipulations should be conducted in a fume hood. Waste solvents accumulated during rotary evaporation or other cleaning operations should be stored in a capped glass bottle (satellite accumulation point) and arrangements made for its disposal through the USGS Materials Management Office.
- d. **Gas cylinder handling.** Compressed gas cylinders must be handled and stored according to the Safety and Environmental Health Handbook (U.S. Geological survey, 2002). Each cylinder must be 1) carefully inspected when received, 2) securely fastened at all times with an approved chain assembly or belt, 3) capped at all times when not in use, 4) capped when transported, 5) transported only by a properly designed vehicle (hand truck), and 6) stored separately with other full, empty, flammable, or oxidizing tanks of gas, as appropriate.
9. **References:** Following are citations from this SOP along with some additional sources of information (marked in bold) about the procedures that have been described here.

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