Application Note

Instrument: CN828

Determination of Carbon and Nitrogen in Plant Tissue

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Introduction

Determination of carbon and nitrogen concentrations in crop plant tissue provides an important diagnostic tool to the grower, giving an indication of nutritional health and nutrient uptake efficiency from the soil, as well as providing an avenue for monitoring high-value, intensively managed crops such as tobacco, cotton, and fruits. Often a combination of carbon and nitrogen determination in both the crop plant tissue and surrounding soil will be used to diagnose and correct any nutritional-related growth issues. Carbon content in soils can represent the presence of organic matter and is used to estimate nitrogen availability from the natural decay of organic materials, especially when using organic fertilizers. Nitrogen is considered an essential macronutrient for plant development, playing a key role in the formation of enzymes and proteins. Testing for total carbon and nitrogen in plant tissue is useful in predicting fertilization needs and making fertilization management decisions for crops.

Instrument Model and Configuration

The LECO CN828 is a combustion carbon and nitrogen determinator that utilizes a pure oxygen environment in a vertical quartz furnace, ensuring complete combustion and superior analyte recovery. A thermoelectric cooler removes moisture from the combustion gases before they are collected in a ballast. The combustion gases equilibrate and mix in the ballast before a representative aliquot (3 cm^3 or 10 cm^3 volume) of the gas is extracted and introduced into a flowing stream of inert gas (Helium or Argon) for analysis. The aliquot gas is carried to a non-dispersive infrared (NDIR) cell for the detection of carbon (as CO_2) and a thermal conductivity cell (TC) for the detection of nitrogen (N_2).

Thermal conductivity detectors work by detecting changes in the thermal conductivity of the analyte gas compared to a reference/carrier gas. The greater the difference between the thermal conductivity of the carrier gas and the analyte gas, the greater sensitivity of the detector. The CN828 supports either the use of helium or argon as the instrument's carrier gas. When used as a carrier gas, helium provides the highest sensitivity, and the best performance at the lower limit of the nitrogen range. The thermal conductivity difference between argon and nitrogen is not as great as the thermal conductivity difference between helium and nitrogen, therefore the detector is inherently less sensitive when using argon as a carrier gas.

The CN828 offers the additional advantage of utilizing either a 10 cm^3 aliquot loop or a 3 cm^3 aliquot loop within the instrument's gas collection and handling system. The 10 cm^3 aliquot loop optimizes the system for the lowest nitrogen range and provides the best precision. The 3 cm^3 aliquot loop extends reagent life expectancy by approximately three-fold when compared to the 10 cm^3 aliquot loop, while providing the lowest cost-per-analysis.

Note: When changing carrier gas type, refer to the 828 Series Operator's Instruction Manual for the procedure on setting the gas flow rate. The aliquot loop size is changed by selecting the desired aliquot loop size in the software's Method Parameters.

Sample Preparation

Samples must be of a uniform consistency to produce suitable results. Reference materials should be prepared as directed by the certificate, prior to analysis.

Note: Carbon and Nitrogen results for plant tissue samples are typically reported on a dry basis in order to avoid a reporting bias due to fluctuations in moisture levels. Therefore, either the material can be dried prior to analysis, or the moisture content can be determined and entered into the software to correct for moisture. Plant tissue samples are typically dried between 80°C and 85 °C for two hours prior to analysis. The dried samples should be stored in a desiccator and must be used for analysis within 24 hours. For plant tissue reference materials, follow the sample drying instructions provided by the certificate.

Accessories

502-186 Tin Foil Cups, commercially available Reagent Grade Sucrose (finely ground), and 501-614 Spatula

Reference Materials

LCRM®, LRM®, NIST, or other suitable reference materials.

*Method Parameters**

Gas Type	Helium or Argon
Furnace Temperature	950 °C
Afterburner Temperature	850 °C
Nominal Mass	1.0000 g
Purge Cycles	3
Ballast Equilibrate Time	10 s
Ballast Not Filled Timeout	300 s
Aliquot Loop Fill Pressure Drop	200 mm Hg
Aliquot Loop Equilibrate Time	6 s
Interleave Analysis	Yes
Sample Drop Detection	Disabled
Dose Loop Size	Large (10 cm^3) or Small (3 cm^3)

*Element Parameters**

	Helium		*Argon*	
Parameter	Carbon	Nitrogen	Carbon	Nitrogen
Integration Delay	4 s	4 s	4 s	4 s
Starting Baseline	15 s	15 s	15 s	15 s
Post Baseline Delay	0 s	14 s	0 s	20 s
Use Comparator	No	No	No	No
Integration Time	18 s	50 s	25 s	65 s
Use Endline	Yes	Yes	Yes	Yes
Endline Delay	15 s	20 s	15 s	20 s
Ending Baseline	15 s	15 s	15 s	15 s
Use Profile Blank	--	--	--	Yes

**Refer to the 828 Series Operator's Instruction Manual for Parameter definitions.*

Burn Profile

Burn Step	*Furnace Flow*	*Time*
1	5.00 L/min	40 s
2	1.00 L/min	30 s
3	5.00 L/min	End

Procedure

1. Prepare the instrument for operation as outlined in the operator's instruction manual.
2. Condition the System.
   a. Select five or more Blank replicates in the Login screen.
   b. Initiate the analysis sequence.
3. Determine Blank.
   a. Select five or more Blank replicates in the Login screen.
   b. Initiate the analysis sequence.
   c. Set the blank following the procedure outlined in the operator's instruction manual.

   Note: The standard deviation of the last five blanks should be less than or equal to 0.001% (10 ppm) for both carbon and nitrogen when utilizing Helium as a carrier gas, and less than or equal to 0.005% (50 ppm) for both carbon and nitrogen when utilizing Argon as a carrier gas. Additional blanks beyond the recommended five may be required in order to achieve the recommended precision.
4. Calibrate/Drift Correct.
   a. Select the desired number of calibration/drift replicates in the Login screen (minimum of five).
   b. Weigh an appropriate mass (~0.1 g to ~0.3 g) of a suitable reference material into a 502-186 Tin Foil Cup and seal the cup in a manner to minimize entrapped atmosphere by twisting the top edges of the foil together.
   c. Enter reference material mass and identification into the Login screen.
   d. Transfer the tin foil cup containing the reference material to the appropriate position in the sample carousel.
   e. Perform steps 4b through 4d a minimum of five times.
   f. Initiate the analysis sequence.
   g. Calibrate or Drift Correct the instrument following the procedure outlined in the operator's instruction manual.
   h. Verify the calibration/drift correction by analyzing an appropriate mass of another/different suitable reference material and confirm that the results are within the acceptable tolerance range.

   Note: Typically, the CN828 can be calibrated using several replicates of a single mass range of a suitable reference material utilizing a linear, force through origin calibration. This is a cost-effective and simple process. A multi-point calibration (fractional mass or multiple calibration materials) may be used to calibrate if desired.
5. Analyze Samples.
   a. Select the desired number of sample replicates in the Login screen.
   b. Weigh ~ 0.25 g of the plant tissue sample into a 502-186 Tin Foil Cup and seal the cup in a manner to minimize entrapped atmosphere by twisting the top edges of the foil together.
   c. Enter sample mass and identification into the Login screen.
   d. Transfer the tin foil cup containing the sample to the appropriate position in the sample carousel.
   e. Perform steps 5b through 5d for each sample to be analyzed.
   f. Initiate the analysis sequence.
6. Atmospheric Blank Determination.

   Note: Some atmosphere may be trapped with the sample when it is encapsulated in the tin foil cup. This may cause biased nitrogen results at low nitrogen concentrations. Therefore, an atmospheric blank should be determined and entered using the following procedure:

   a. Select the desired number of sample replicates in the Login screen (minimum of 3).
   b. Weigh a similar mass (to the mass of the samples being analyzed) of reagent grade sucrose (finely ground) into a 502-186 Tin Foil Cup and seal the cup in a manner to minimize entrapped atmosphere by twisting the top edges of the foil together.
   c. Enter the mass of the sucrose into the Login screen.
   d. Transfer the tin foil cup containing the sucrose to the appropriate position in the sample carousel.
   e. Perform steps 6b through 6d for each sucrose sample to be analyzed.
   f. Initiate the analysis sequence.
   g. The average nitrogen value obtained is considered the atmospheric blank and can be automatically compensated for using the CN828 software**.

   ***Refer to the 828 Series Operator's Instruction Manual for details regarding the setting of the atmospheric blank.*

TYPICAL RESULTS

Data was generated utilizing a linear, full regression calibration for carbon determination, and a linear, force through origin calibration for nitrogen determination, using fractional masses (~0.1 g to ~0.3 g) of 502-896 (Lot 1001) EDTA LCRM (41.00% C, 9.56% N). Plant tissue samples were dried following the drying instructions on the certificate and stored in a desiccator until use.

	Helium 10 cm³			***Helium 3 cm³***			***Argon 10 cm³***			***Argon 3 cm³***		
	Mass (g)	*% C*	*% N*	*Mass (g)*	*% C*	*% N*	*Mass (g)*	*% C*	*% N*	*Mass (g)*	*% C*	*% N*
Tobacco LRM	0.2539	47.12	2.51	0.2553	47.16	2.51	0.2506	47.11	2.50	0.2528	47.18	2.49
LECO 502-082	0.2520	47.13	2.51	0.2546	47.05	2.52	0.2535	47.14	2.50	0.2543	47.21	2.48
Lot: 1018	0.2541	47.14	2.51	0.2535	47.10	2.51	0.2516	47.11	2.49	0.2545	47.25	2.48
% C = 47.27 ± 0.23	0.2527	47.13	2.51	0.2553	47.09	2.52	0.2520	47.13	2.50	0.2549	47.18	2.48
% N = 2.48 ± 0.04	0.2523	47.08	2.51	0.2553	47.07	2.51	0.2522	47.05	2.47	0.2546	47.21	2.48
	Avg =	***47.12***	***2.51***	***Avg =***	***47.10***	***2.52***	***Avg =***	***47.11***	***2.49***	***Avg =***	***47.21***	***2.48***
	s =	***0.02***	***< 0.01***	***s =***	***0.04***	***< 0.01***	***s =***	***0.03***	***0.01***	***s =***	***0.03***	***< 0.01***
Orchard Leaves LCRM	0.2512	49.54	2.31	0.2552	49.60	2.32	0.2522	49.66	2.32	0.2536	49.48	2.31
LECO 502-931	0.2513	49.55	2.31	0.2552	49.69	2.31	0.2530	49.42	2.32	0.2531	49.39	2.27
Lot: 1000	0.2541	49.50	2.28	0.2544	49.58	2.31	0.2530	49.54	2.29	0.2537	49.59	2.36
% C = 49.54 ± 0.22	0.2533	49.57	2.32	0.2539	49.53	2.30	0.2518	49.58	2.31	0.2533	49.58	2.32
% N = 2.31 ± 0.05	0.2529	49.44	2.27	0.2551	49.52	2.28	0.2539	49.39	2.26	0.2551	49.32	2.28
	Avg =	***49.52***	***2.30***	***Avg =***	***49.58***	***2.31***	***Avg =***	***49.52***	***2.30***	***Avg =***	***49.47***	***2.31***
	s =	***0.05***	***0.02***	***s =***	***0.07***	***0.01***	***s =***	***0.11***	***0.03***	***s =***	***0.12***	***0.03***
Alfalfa LCRM	0.2537	44.22	3.13	0.2530	44.21	3.12	0.2539	44.13	3.12	0.2518	44.18	3.09
LECO 502-983	0.2536	44.17	3.13	0.2534	44.18	3.12	0.2535	44.18	3.12	0.2522	44.15	3.13
Lot: 1000	0.2546	44.22	3.13	0.2557	44.21	3.13	0.2538	44.11	3.10	0.2532	44.20	3.12
% C = 44.10 ± 0.26	0.2544	44.19	3.13	0.2541	44.23	3.12	0.2537	44.13	3.11	0.2546	44.16	3.11
% N = 3.09 ± 0.06	0.2511	44.20	3.13	0.2549	44.18	3.12	0.2546	44.21	3.09	0.2545	44.21	3.08
	Avg =	***44.20***	***3.13***	***Avg =***	***44.20***	***3.12***	***Avg =***	***44.15***	***3.11***	***Avg =***	***44.18***	***3.11***
	s =	***0.02***	***< 0.01***	***s =***	***0.02***	***< 0.01***	***s =***	***0.04***	***0.01***	***s =***	***0.02***	***0.02***

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