Application Note

Instrument: FP828

Determination of Nitrogen/Protein in Beer

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Introduction

Nitrogen determination in beer is utilized to calculate the protein concentration using a nitrogen protein conversion factor. The protein content of beer is not only an important criterion in evaluating the quality of beer but also is an important parameter utilized to monitor process quality during production. The protein contained in beer is primarily water-soluble proteins from the grains used in the malting and brewing process. Control and measurement of protein throughout the brewing process is important in order to ensure the survival, growth, and productivity of the yeast utilized to convert sugars to ethanol and carbon dioxide. The yeast depends on a variety of conditions including the availability of amino groups derived from enzymatic hydrolysis of protein during the brewing process.

Instrument Model and Configuration

The LECO FP828 is a combustion nitrogen/protein 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 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 FP828 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 FP828 offers the additional advantage of utilizing either a 10 cm^3 aliquot dose loop or a 3 cm^3 aliquot dose loop within the instrument's gas collection and handling system. The 10 cm^3 aliquot dose loop optimizes the system for the lowest nitrogen range and provides the best precision. The 3 cm^3 aliquot dose loop extends reagent life expectancy by approximately three-fold when compared to the 10 cm^3 aliquot dose 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. When using the FP828 Performance model, the aliquot dose loop size is changed by selecting the desired aliquot dose loop size in the software's Method Parameters. When using the FP828 Base model, the desired dose loop is installed by the operator.

Sample Preparation

Beer samples should be prepared according to official AOAC Method 920.49: Beer - Preparation of Sample. The AOAC method states that beer should be degassed at room temperature. Reference materials should be prepared as directed by the certificate prior to analysis. Glycine solutions should be prepared using the procedure found on the last page of this document.

Accessories

502-825 Large Tin Capsules, and disposable pipettes.

Note: When using 502-825 Large Tin Capsules, LECO recommends that the 614-961-110 porous reticulated crucible be replaced every 150 analyses to avoid excessive ash buildup.

Reference Materials

Calibration should be performed using glycine solutions prepared using the procedure found on the last page of this document. Verification can be performed using appropriate concentrations of a glycine solution and/or 502-602 Ammonium Solution Standard (0.1% N).

*Method Parameters**

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
Dose Loop Size**	Large (10 cm^3) or Small (3 cm^3)
Interleave Analysis	Yes
Sample Drop Detection	Disabled

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

***Due to the low levels of nitrogen in this sample matrix, a 10 cm^3 dose loop is recommended when analyzing samples using the FP828 Base Model.*

Element Parameters*

Parameter	Helium 10 cm^3 & 3 cm^3 Nitrogen	Argon 10 cm^3 Nitrogen
Integration Delay	4 s	4 s
Starting Baseline	15 s	15 s
Post Baseline Delay	14 s	20 s
Use Comparator	No	No
Integration Time	50 s	65 s
Use Endline	Yes	Yes
Endline Delay	20 s	20 s
Ending Baseline	15 s	15 s
Use Profile Blank	--	Yes

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

Burn Profile

Performance Model

Burn Step	Furnace Flow	Time
1	5.00 L/min	End

Base Model

Burn Step	Furnace Flow	Time
1	High	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 nitrogen when utilizing Helium as a carrier gas, and less than or equal to 0.005% (50 ppm) for 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. Using a pipette, weigh ~0.75 g of an appropriate concentration of glycine solution into a 502-825 Large Tin Capsule. Leave the capsule open so that atmosphere can be purged from the capsule when in the purge chamber.
   c. Enter reference material mass and identification into the Login screen.
   d. Transfer the tin capsule 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 by analyzing ~0.75 g of a different concentration of glycine solution or ammonium solution, following steps 4b through 4f, and confirm that the results are within the acceptable tolerance range.
5. Analyze Samples.
   a. Select the desired number of sample replicates in the Login screen.
   b. Using a pipette, weigh ~0.75 g of beer sample into a 502-825 Large Tin Capsule. Leave the capsule open so that atmosphere can be purged from the capsule when in the purge chamber.
   c. Enter sample mass and identification into the Login screen.
   d. Transfer the tin capsule 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.

TYPICAL RESULTS

Data was generated utilizing a linear, force through origin calibration using a 0.2% N glycine solution. The calibration was verified using 502-602 Ammonium Solution (0.1% N), and a 0.05% N glycine solution. All samples were weighed and analyzed at ~0.75 g. A protein factor of 6.25[†] was used for all samples to calculate the protein content.

	10 cm^3 Helium			3 cm^3 Helium			10 cm^3 Argon		
	Mass (g)	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*
American IPA	0.7758	0.143	0.896	0.7605	0.140	0.876	0.7560	0.141	0.881
	0.7520	0.145	0.909	0.7574	0.140	0.874	0.7571	0.146	0.909
	0.7615	0.145	0.906	0.7553	0.140	0.873	0.7537	0.150	0.938
	0.7592	0.146	0.912	0.7460	0.145	0.904	0.7742	0.134	0.838
	0.7561	0.143	0.895	0.7657	0.144	0.900	0.7695	0.149	0.933
	Avg =	***0.145***	***0.904***	***Avg =***	***0.142***	***0.885***	***Avg =***	***0.144***	***0.900***
	s =	***0.001***	***0.008***	***s =***	***0.002***	***0.015***	***s =***	***0.007***	***0.041***
Pilsner	0.7622	0.035	0.219	0.7700	0.031	0.195	0.7434	0.035	0.216
	0.7453	0.035	0.218	0.7413	0.031	0.195	0.7459	0.031	0.191
	0.7501	0.035	0.220	0.7625	0.032	0.198	0.7485	0.040	0.250
	0.7528	0.034	0.213	0.7608	0.034	0.214	0.7478	0.027	0.168
	0.7448	0.034	0.214	0.7646	0.034	0.212	0.7572	0.033	0.208
	Avg =	***0.035***	***0.217***	***Avg =***	***0.032***	***0.203***	***Avg =***	***0.033***	***0.207***
	s =	***< 0.001***	***0.003***	***s =***	***0.002***	***0.010***	***s =***	***0.005***	***0.030***
Porter	0.7560	0.113	0.704	0.7499	0.112	0.702	0.7715	0.119	0.745
	0.7466	0.112	0.703	0.7824	0.111	0.695	0.7460	0.114	0.710
	0.7484	0.113	0.707	0.7716	0.113	0.704	0.7584	0.105	0.659
	0.7695	0.112	0.698	0.7692	0.107	0.669	0.7494	0.113	0.709
	0.7754	0.111	0.694	0.7448	0.111	0.692	0.7641	0.110	0.686
	Avg =	***0.112***	***0.701***	***Avg =***	***0.111***	***0.692***	***Avg =***	***0.112***	***0.702***
	s =	***0.001***	***0.005***	***s =***	***0.002***	***0.014***	***s =***	***0.005***	***0.032***

[†]Protein factor was obtained from the United States Department of Agriculture, Circular No. 183. The choice of protein factor to be used for determining protein content in different materials is the subject of some debate. As a result, if being used for commerce, the value of this conversion factor should be part of the contractual agreement between buyer and seller.

Note: Due to the decreased sensitivity of the TC cell when using argon as a carrier gas, it is recommended that a 10 cm^3 dose loop be utilized when analyzing samples with low nitrogen content if argon is being used as a carrier gas.

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GLYCINE SOLUTION PREPARATION

1. The following formula can be used to make a specific concentration:

$$G = \frac{C}{(0.99^{\dagger\dagger} * 0.18658)}$$

where: C = desired nitrogen concentration as percent
G = grams of glycine powder

Example for 1% solution:

$$G = \frac{1}{(0.99^{\dagger\dagger} * 0.18658)} = 5.414$$

NOTE: A quick reference chart, shown below, shows the grams of glycine powder needed to reach given concentrations.

2. Place a flask on the balance and tare. The flask should be large enough to hold 100 ml (where 100 g = 100 ml).
3. Add the amount of glycine calculated in step 1 and record the mass.
4. Add distilled water until the total mass equals 100 g, then record the mass (W).
5. Seal the flask and mix the contents.
6. To figure the exact concentration:

$$\% \text{ Nitrogen} = \frac{G\,(18.658 * 0.99^{\dagger\dagger})}{W}$$

where: G = mass in grams of glycine recorded in step 3
W = mass in grams of water and glycine powder recorded in step 4

7. If the distilled water is not pure, determining the nitrogen concentration may be necessary.
   a. Analyze five samples of distilled water.
   b. Average the nitrogen content of the five samples (A).
   c. Add this average to % nitrogen calculated for the calibration solution.

Example: To make a calibration solution of approximately 0.3% nitrogen:
where: G = 1.672 g
W = 99.824 g
A = 0.004%

$$\frac{1.672(18.471)}{(99.824)} + 0.004 = 0.313\%\ \text{N}$$

QUICK REFERENCE CONCENTRATION TABLE

Nitrogen Concentration	Grams of Glycine[††]
0.10%	0.541
0.30%	1.624
0.50%	2.707
0.75%	4.060
1.00%	5.414

[††]*Assuming 99.0% purity of glycine powder.*