NITROGEN (Chemiluminescence)

PRINCIPLE

The sample is pyrolyzed at 1100 °C in the presence of oxygen to produce nitric oxide (NO). The NO is further oxidized with ozone to produce nitrogen dioxide in the excited state (NO_2*). The decay of NO_2* from the excited to the ground state produces chemiluminescence in the 650-900 nm range. This signal is detected and quantitated by reference to calibration standards.

SCOPE

This method is applicable to the determination of trace residual nitrogen in aqueous solutions of crude and refined sugars and syrups.

SPECIAL APPARATUS

1. Nitrogen Analyzer: Model 703C, Antek Instruments, Inc., 6005 North Freeway, Houston, TX 77076, or equivalent

2. Sample Injector: Use autosampler Model GC 311H, Dynatech Precision Sampling Corp., P. O. Box 15119, Baton Rouge, LA 70895 (includes interface hardware from Antek Instruments). For manual injection, use a precision sampling syringe, 10 µL capacity.

3. Integrator: A computing integrator with calculating and recording capabilities such as Spectra-Physics Model 4270 compatible with Antek Instruments' software for autosampler control and calculations

4. Digital Data Recorder: Model DC2, Pegasus Data Systems, 236 Lackland Drive, Middlesex, NJ 08846, or equivalent (optional)

5. Autosampler Vials: 1.5 mL measuring 12 mm X 32 mm, with crimp caps

6. Sample Vial Crimper

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REAGENTS

1. Standard Nitrogen Solution, 1000 µg/mL nitrogen:

   Stock Preparation: Dissolve 2.143 g of reagent grade urea in 100 mL of purified water. Transfer quantitatively to a 1 L volumetric flask, dilute to volume with purified water, and mix well.

   Working Standards: Pipet 10 mL of the stock solution into a 1 L volumetric flask, dilute to volume with purified water, and mix to make a 10 µg/mL N solution. Pipet 5, 10, 20, 30, 40 and 50 mL of the 10 µg/mL N solution into separate 100 mL volumetric flasks. Dilute each to volume with purified water, and mix to make 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 µg/mL N solutions, respectively.

2. Oxygen: 99.997% purity research grade in gas cylinder equipped with a regulator capable of delivering 20 psi followed by an in-line gas purifier. Keep away from flame or sparks.

3. Argon: 99.99% purity nitrogen-free research grade in gas cylinder equipped with a regulator capable of delivering 20 psi followed by an in-line gas purifier.

4. Compressed Air: Medical grade purity in gas cylinder equipped with a regulator capable of delivering 60 psi followed by an in-line gas purifier.

PROCEDURE

Instrument Conditioning: Turn on power switch, depress "ON" and "STBY" heater control buttons. Allow pyrolysis tube to equilibrate at 650 °C for 15 mins., then bring to operating temperature. Turn on detector and initiate gas flow.

Operating Conditions: Typical parameters are listed below:

Flow Rate Settings	
Inlet (Argon)	2.5
Inlet (Oxygen)	2.5

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Pyro	4.5
Ozone	1.5
Heater Temperature	1100 °C
Detector Attenuation	1X
Detector Level	HIGH
Detector Baseline	MANUAL
Integrator Attenuation	1024

Allow instrument to equilibrate for 30 mins. Turn on ozone generator and allow to equilibrate another 15 mins. Adjust "ZERO" knob on detector so signal level meter is at zero. Press detector "RESET" button for 2 seconds. Purge by injecting purified water several times until three successive injections give counts within a range of 10 (Note 1). Load integrator program "LIQQUAD."

Sample Preparation: Generally the concentration of the prepared sample solution should not exceed 10% dry substance and should contain 1-5 µg/mL N. Weigh an appropriate amount of sample into a 10 mL volumetric flask and dilute to volume with purified water (Note 2).

Analysis: If using an autosampler, respond to prompts of Antek Instruments' "LIQQUAD" program (Note 3), entering 3 for "INJECTIONS/VIAL" and 10 for "MAXIMUM VARIANCE." Place the six standard solutions in the sample carousel and then arrange the samples such that each sample is followed by two vials containing a standard solution (Note 4). Place two vials of purified water in the sample tray after the last sample vial to purge the system. Enter the dilution factor to enable the computing integrator to calculate the results (Note 5).

When injecting manually, rinse the syringe with the standard solution at least 4 times prior to injection. Inject the appropriate volume of the standard solution and record the counts. Following manual injection, rinse the syringe 4 times with warm tap water allowing air bubbles to scrub the walls of the syringe, and then rinse the syringe twice with purified water. Inject each standard solution 3 times, recording the counts. Inject sample solutions and rinse the syringe as described for standard solutions. Record the counts.

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CALCULATIONS

The computing integrator will provide a printed table of nitrogen results.

If injecting manually:

$$N, \mu g/g = \frac{(\text{Sample, counts})(\text{Standard, N}\,\mu g/mL)(\text{Sam ple dilution volume, mL})}{(\text{Standard, counts})(\text{Sample Wt., g})}$$

To obtain protein, multiply N by 6.25.

NOTES AND PRECAUTIONS

1. A well purged system should give about 600 counts for a water injection, although the counts for the first injection may be higher than 600. Different instruments and water sources may lead to different typical purge counts.

2. Samples may be diluted on a weight-to-weight basis. This approach is useful for viscous syrups which will not mix well in a 10 mL volumetric flask. These samples may be prepared in a glass vial and mixed on a shaker.

3. Suggested integrator software function values:

FUNCTION VALUE

TT = 0.01	TF = T5	TV = 1
TT = 0.02	TF = T5	TV = 0
TT = 1.25	TF = ER	TV = 1
TT = 2.00	TF = SR	TV = 1

These variables represent a time function table in which specific events are preset to occur at particular times during, or after the sample run. TT specifies the time at which the function is to take place during the run. TF specifies a certain function (for example, ER means "end run"). TV is a switch where TV = 1 enables the time function, and TV = 0 disables it. For more information, refer to the Spectra-Physics SP4270 Operators Manual.

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4. Water may be used, but the use of an internal standard will allow maximum recovery of data if a malfunction should occur. Two standard solution vials between each sample vial will minimize the gradual accumulation of sugar solutions in the syringe.

5. Dilution factor equals the dilution volume (normally 10 mL) or dilution weight, g, divided by the sample weight, g. The integrator printout will include a message "Caution: Exceeds Calibrated Range" if the dilution factor is entered. This same message will be printed if the sample concentration exceeds that of the most concentrated standard solution. The dilution factor does not need to be entered for the run to proceed. Final results can be obtained by multiplying the printed data by the dilution factor on a calculator.

METHOD HISTORY

Corn Sugar, Nitrogen (Chemiluminescence) (F-38), Date of Acceptance 3-30-1993.