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TG/MS INTERFACE: APPLICATIONS TO THE DETERMINATION OF MOISTURE IN POLYSACCHARIDES AND FREEZE-DRIED BIOLOGICAL PRODUCTS

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ABSTRACT

A DuPont 1090 thermal analysis system has been interfaced to a Hewlett Packard 5995B quadrupole mass spectrometer with a turbo-molecular pumping system. The glass tubing interface connects the quartz combustion tube of the thermogravimetric analyzer (TG) to the jet separator of the mass spectrometer. This interface allows continuous monitoring of the ion intensities of mass peaks $m/e = 18$ (water) and $m/e = 44$ (carbon dioxide) for the determination of residual moisture in freeze-dried biological products such as Giant Ragweed Allergenic Extract and the moisture content of bulk polysaccharides such as Meningococcal Polysaccharides Groups A and C used in vaccine production.

INTRODUCTION

Thermogravimetry/mass spectrometry (TG/MS) has been shown to elucidate the transitions attributable to residual moisture in freeze-dried biological products [1,2]. This earlier TG/MS data for residual moisture in freeze-dried products was determined by the continuous monitoring method of Chiu and Beattie [3]. Chiu and Beattie utilized a DuPont 990 thermal analysis system interfaced with a glass tee to a DuPont 21-104 mass spectrometer. MS scans were taken continuously every 2 min. The 3-l pressure chamber in the heated inlet system of the MS was evacuated at each sampling to remove the residual volatiles of the previous point. The MS scan followed the TG curve closely to provide interpretation of the thermal events.

This paper describes a new TG/MS interface and its application to the continuous monitoring of the ion intensities of mass peaks $m/e = 18$ (water) and $m/e = 44$ (carbon dioxide) for the determination of residual moisture in freeze-dried biological products with moisture contents of less than 1% to approximately 5% and the moisture content of bulk polysaccharides with

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moisture contents of about 5–25%. Mass spectra were taken approximately every minute as the helium gas carrying the thermal decomposition products flowed from the TG through a 10 in. glass interface through the jet separator of the mass spectrometer and past the MS source. TG heating rates from 10 to 20 °C min^{-1} were used depending on the type of sample studied.

This straightforward TG/MS interface utilizes the characteristics of the jet separator to reduce TG effluent pressure (~ 1 atm) to the low pressure necessary for MS operation (10^{-6} torr). The jet separator interface used to connect a gas chromatograph to a mass spectrometer was first introduced by Ryhage and was described for both packed column [4] and capillary column [5] work. The Ryhage jet separator was based on the "molecular separator" designed by Becker [6]. In this device, sample molecules of relatively higher molecular weight (and consequently higher momentum) pass straight through a convergent nozzle under pressure and into another orifice which is very close to the first. The gas emerges from the first nozzle in the form of an expanding jet. The helium carrier gas and molecules of lower molecular weight (and lower momentum) diffuse to the sides of the first constricted nozzle and are pumped away before reaching the second orifice [7]. While discriminating against lower molecular weight species (helium) and eliminating carrier gas, the jet separator increases the relative concentration of thermal decomposition products in the flow.

Previously described techniques for interfacing thermogravimetry to mass spectrometry have involved metering valves, liquid nitrogen traps to condense organic vapors evolved during certain weight-loss steps or successive evacuations of the heated inlet system of the mass spectrometer before each sample is taken. Chiu and Beattie [3,8] and Yuen et al. [9] have summarized methodologies used to interface TG to MS, including direct connection under vacuum and direct connection under reagent gas. Successful interfaces have included metering valves, capillary–orifice connections and other methods. Yuen et al. [9] interfaced a Mettler thermoanalyzer, Model TA-1, with a Hewlett-Packard 5992 quadrupole mass spectrometer through a Varian 951-5100 leak valve. Shushan et al. [10] recently described thermogravimetric analysis combined with sequential mass spectrometry (MS/MS) involving a triple quadrupole tandem mass spectrometer fitted with an atmospheric pressure chemical ionization (APCI) ion source.

Samples in this study include the freeze-dried Giant Ragweed Allergenic Extract and Meningococcal Polysaccharides Groups A and C used in vaccine production. In the case of the freeze-dried Giant Ragweed Allergenic Extract the accurate measurement of a low residual moisture is necessary to ensure the stability and potency of the product during its dating period [11]. Excessive moisture could lead to product degradation. TG methodology has been uniquely applicable to measuring moisture in bulk polysaccharides such as meningococcal polysaccharides [12], 23 types of pneumococcal polysaccharides and Haemophilus b polysaccharide, since

only a few milligrams are available for analysis. Alternate methodologies are not as sensitive and require 15 or more milligrams of sample for a single analysis.

EXPERIMENTAL

Samples and control materials

Freeze-dried Giant Ragweed Allergenic Extract was prepared at the Office of Biologics Research and Review.

Meningococcal Polysaccharides Groups A and C were obtained from U.S. manufacturers (Merck, Sharp and Dohme, Division of Merck and Co., Inc., West Point, PA, U.S.A. and Connaught Laboratories, Inc., Swiftwater, PA, U.S.A.). The polysaccharides were from lots released by the Office of Biologics Research and Review of the Center for Drugs and Biologics.

Interface

Figure 1 is a schematic diagram of the DuPont Model 1090 thermogravimetric analyzer (DuPont Instruments, Wilmington, DE, U.S.A.) and Hewlett-Packard Model 5995B quadrupole mass spectrometer with a turbomolecular pumping system (Hewlett-Packard, Rockville, MD, U.S.A.) configured with the interface. The interface consists of a glass tube of 1/4 in. outer diameter which is 10 in. in length. The glass tube has a right-angle bend on one end with 2 in. of tubing above the bend, which is necessary to

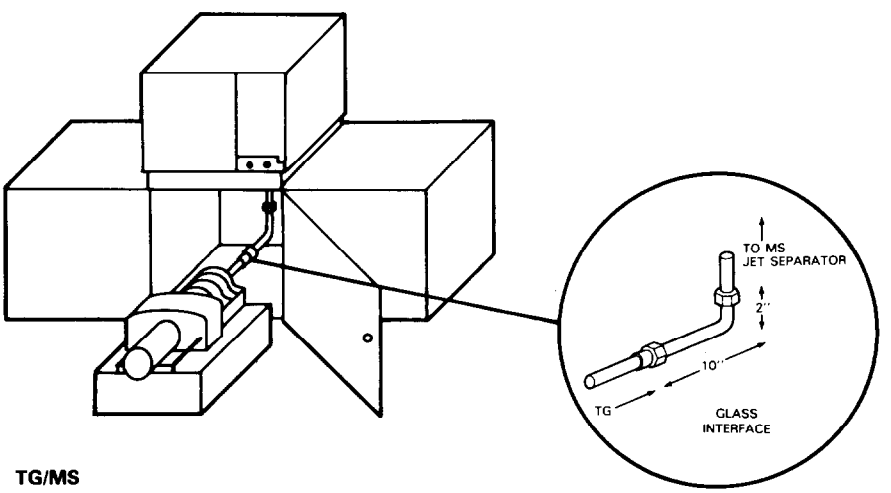

Fig. 1. Schematic of the TG/MS interface configuration with an enlargement of the interface.

connect to the jet separator of the mass spectrometer. The end of the glass tube above the right-angle bend is fitted snugly into a Swagelock connection with a Supeltex M-2A Packed Column Ferrule (Supelco, Inc., Bellefonte, PA, U.S.A.) and connects to the jet separator. When the TG glass interface is not in place, the gas chromatographic column connects to the jet separator. The quartz combustion tube of the thermogravimetric analyzer normally ends in 1/4 in. tubing with a ground glass ball joint. This quartz tube ball joint was removed and replaced with a 2 in. extension of 1/4 in. quartz tubing, so that a Swagelock connection could fit between the TG quartz tube and the glass interface for a tighter seal. The glass interface and extension of the quartz combustion tube were covered with heating tape (Thomas Scientific, Swedesboro, NJ, U.S.A.) to prevent condensation of effluent components on the tube interior. The temperature of the heating tape was regulated at approximately 120°C by a potentiometer. Mass spectra were collected every half minute or minute depending on the sample heating rate.

The O-rings within the TG balance were changed to provide tight seals and prevent excessive amounts of oxygen and nitrogen from entering the system.

RESULTS AND DISCUSSION

Typical TG and MS operating conditions are listed in Table 1.

Figures 2 and 3 show the TG/MS data obtained for Meningococcal Polysaccharides Groups A and C, respectively. Mass spectral relative ion intensities for water and carbon dioxide are distinguishable above background and, when superimposed on the respective TG data, verify the transition due to moisture in the polysaccharide by indicating the difference between the water content of the polysaccharide and the water evolved from thermal decomposition of the polysaccharide which coincides with the evolution of carbon dioxide. With the TG transition due to water verified by the MS data, the water contents of the polysaccharides were calculated to be 17 and 15.5%, respectively.

Figure 4 shows the TG/MS data obtained for a freeze-dried Giant Ragweed Allergenic Extract which contains less than 5% residual moisture. The transition due to residual moisture is not easily distinguished from the thermogravimetric data. When the mass spectral ion intensities of water and carbon dioxide are superimposed on the thermogram, the TG transition due to residual moisture becomes clear and is easily distinguished from water evolved from decomposition whose evolution coincides with the evolution of carbon dioxide. Once the transition due to residual moisture is identified by the TG/MS data the percentage residual moisture in this sample was easily calculated to be 1.5%.

TABLE 1

TG/MS operating conditions

TG DuPont Model 951 thermogravimetric analyzer
DuPont Model 1090 thermal analyzer

Heating rate: 10 or 20 °C min^{-1} [a]
Atmosphere: helium
Initial temperature: room temperature (22 °C)
Final temperature: ~ 400 °C

MS Hewlett-Packard Model 5995B quadrupole mass spectrometer with a turbo-molecular pumping system
Electron ionization (e.i.) mass spectra were recorded under the following conditions:
Ionization potential (fixed): 70 eV
Ionizing current (fixed): 220 μA
Temperature: transfer line (jet separator), 280 °C
source, 200 °C
analyzer, 250 °C
Electron multiplier: 1400 V

The ion optics were tuned at mass 502 using DFTPP (decafluorotriphenylphosphine [b]) The MS peak detection threshold was set at 100.0 linear counts. Spectra were recorded in the peakfinder mode saving all spectra

[a] Heating rate was 10 °C min^{-1}, except for 20 °C min^{-1} used for Meningococcal Polysaccharide Group C.
[b] Ref. 13.

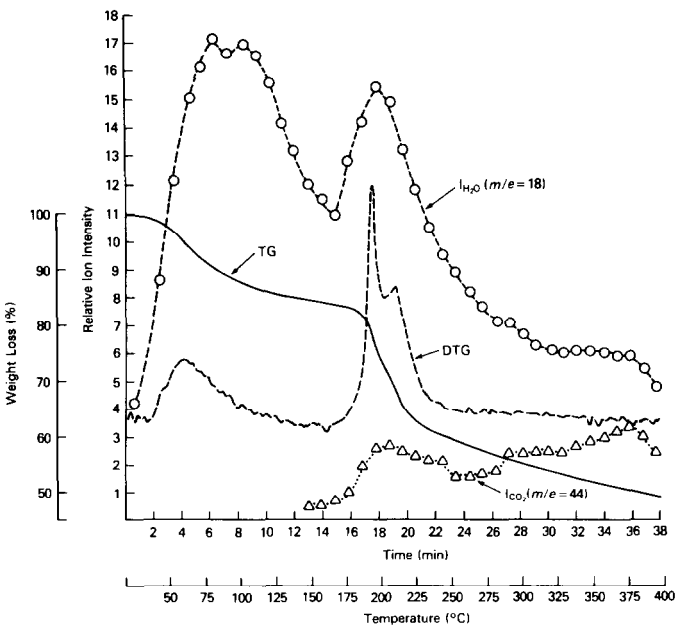

Fig. 2. TG, DTG, and mass spectral ion intensities (I) for water ($m/e = 18$) and carbon dioxide ($m/e = 44$) versus time and temperature for Meningococcal Polysaccharide, Group A.

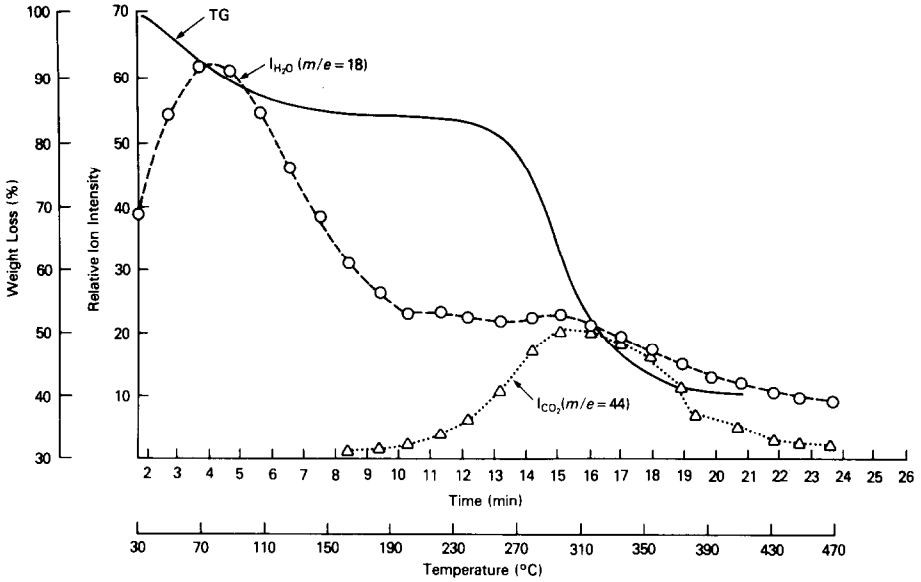

Fig. 3. TG and mass spectral ion intensities (I) for water ($m/e = 18$) and carbon dioxide ($m/e = 44$) versus time and temperature for Meningococcal Polysaccharide, Group C.

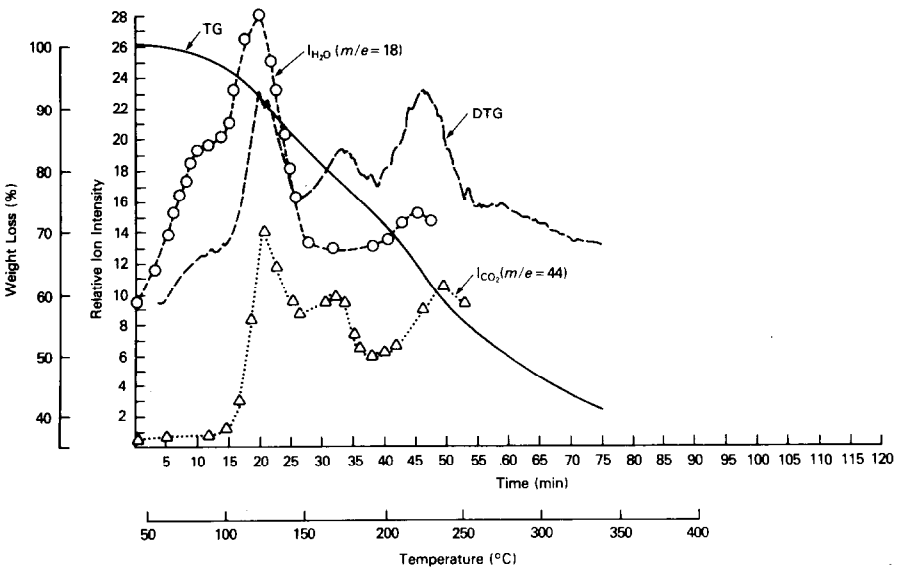

Fig. 4. TG, DTG, and mass spectral ion intensities (I) for water ($m/e = 18$) and carbon dioxide ($m/e = 44$) versus time and temperature for freeze-dried Giant Ragweed Allergenic Extract.

This TG/MS interface produces mass spectral relative ion intensities for water and carbon dioxide that are easily distinguishable above mass spectral background and that can be used in conjunction with TG data for the determination of moisture in samples that contain from 10 to 20% moisture, such as the meningococcal polysaccharides, as well as freeze-dried samples that usually have less than 5% residual moisture, such as the freeze-dried Giant Ragweed Allergenic Extract.

TG/MS data have validated the TG transitions utilized for the determination of moisture in bulk polysaccharides. This TG/MS methodology is especially applicable to measuring moisture in freeze-dried products in which sample is limited and unavailable in the amounts necessary for moisture testing by the coulometric Karl Fischer methodology (approximately 15 mg per test) or the gravimetric method (200 mg). The TG/MS methodology is sensitive enough to provide validation data at this low moisture level.

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