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TG/MS CAPILLARY INTERFACE
Applications to determination of residual moisture in BCG vaccine and other freeze-dried biological products

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Abstract

A TA Instruments Thermal Analysis System (TG) has been interfaced to the Hewlett Packard 5972 quadrupole mass spectrometer. An OSS-2 variable outlet splitter was plumbed between the TG and 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) used to elucidate the TG transitions attributable to residual moisture in freeze-dried biological products. Moisture specifications must be met in order to insure product stability throughout the approved shelf life. TG/MS results are discussed for BCG Vaccine, BCG Live (Intravesical) and U. S. Standard Antihemophilic Factor. Karl Fischer and TG/MS moisture results are compared.

Keywords: lyophilization, residual moisture, TG/MS interface

Introduction

Thermogravimetry/mass spectrometry (TG/MS) elucidates the transitions attributable to residual moisture in freeze-dried biological products [1]. Previous TG/MS data for residual moisture in freeze-dried products was collected by the continuous monitoring method of Chiu and Beattie [2] using a DuPont 990 thermal analysis system interfaced with a glass tee to a DuPont 21–104 mass spectrometer and the methodology of May *et al.* [3] which interfaced a DuPont 1090 Thermal Analysis System to a Hewlett Packard 5995B quadrupole mass spectrometer.

This paper describes a TA Instruments Thermal Analysis System interfaced to the Hewlett Packard 5972 Mass Selective Detector which is equipped with a hyperbolic quadrupole mass filter and vapor diffusion high vacuum pump and applications to the continuous monitoring of the ion abundances of mass peaks $m/e=18$ (water) and $m/e=44$ (carbon dioxide) for the determination of residual moisture in freeze-dried products. This interface utilizes an effluent splitter to reduce the flow into the Mass Selective Detector. The splitter vents a portion of the sample from the TG effluent to the atmosphere.

Samples in this study include freeze-dried BCG Vaccine, BCG Live (Intravesical) and U.S. Standard Antihemophilic Factor (Factor VIII Concentrate) Lot A. Both BCG Vaccine and BCG Live (Intravesical) are made from a culture of an attenuated strain (Bacillus Calmette-Guerin) of living bacillus Mycobacterium bovis

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[4]. The bacilli are lyophilized and are viable upon reconstitution. BCG Vaccine has been used to immunize against tuberculosis (TB) since 1921. Over 2 billion people have been immunized and it is currently an officially recommended vaccine in approximately 180 countries and territories. BCG Live (Intravesical) is used in treatment of carcinoma of the urinary bladder [5, 6]. It promotes a local inflammatory reaction with infiltration in the urinary bladder. The local inflammatory effects are associated with an apparent elimination or reduction of superficial cancerous lesions of the urinary bladder. The exact mechanism is unknown. U.S. Standard Antihemophilic Factor (Factor VIII Concentrate) Lot A is used as a control sample for residual moisture. Antihemophilic Factor (Human) [7] is a stable dried concentrate of Antihemophilic Factor (AHF) to be used in the treatment of Hemophilia A (classical Hemophilia). Hemophilia A is a hereditary disorder of blood coagulation associated with a deficiency of Antihemophilic Factor (Factor VIII), a constituent of normal plasma needed for blood clotting. AHF is highly effective in arresting bleeding due to deficiency of Factor VIII. Whenever needed, AHF can be used to increase the Factor VIII levels of patients to normal or near normal values without overloading their circulatory system.

The accurate determination of residual moisture in these freeze-dried products is necessary to insure compliance with regulatory moisture limits and to ensure the stability and potency of a freeze-dried biological product during its dating period [8]. Moisture results from the TG/MS data are correlated with Karl Fischer residual moisture results for these samples. Less sample is used for analysis by the TG/MS method than by the Karl Fischer method. This is an advantage when sample size per vial is only a few milligrams. The TG/MS method is able to measure vial to vial variations in moisture content for products that contain 5 to 10 mg of freeze-dried sample per vial. A sample size of 15 to 20 mg is usually necessary for an accurate Karl Fischer determination.

Experimental

Samples and control materials

Samples of BCG Vaccine were obtained from Organon Teknika (Durham, North Carolina).

Samples of BCG Live (Intravesical) were obtained from Connaught Laboratories Limited (Toronto, Canada).

Samples of U.S. standard Antihemophilic Factor Lot A (Factor VIII) were obtained from the Center for Biologics Evaluation and Research, U.S. Food and Drug Administration (Bethesda, Maryland).

Methods

Thermogravimetry/mass spectrometry (TG/MS) interface

Figure 1 is a schematic diagram of the interface. The TA Instruments TGA 51 Thermogravimetric Analyzer was interfaced to the Hewlett-Packard 5972 Series

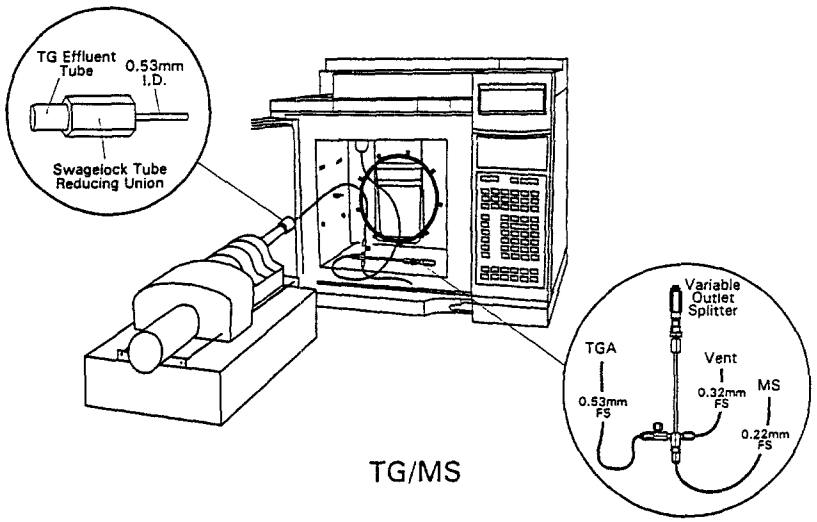

Fig. 1 Schematic of the TG/MS interface configuration with an enlargement of the splitter interface and the TG effluent tube

Mass Selective Detector equipped with a hyperbolic guadrupole mass filter and vapor diffusion high vacuum pump. The Thermogravimetric Analyzer's (TG) effluent tube was modified to terminate in a straight 1/4 inch O.D. glass tube. A 1/4–1/16 tube reducing union (Swagelok) was used to connect the TG effluent tube to a 0.53 mm I.D. fused silica capillary tube. An OSS-2 variable outlet splitter (Scientific Glass Engineering Pty. Ltd., Ringwood, Australia) was plumbed between the TG and the mass spectrometer. The splitter vents a portion of the sample from the TG effluent to the atmosphere. All of the O-rings and connections in the TG were tightened to provide a seal against excessive amounts of oxygen and nitrogen from entering the system. This capillary 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. 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 the 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 the sample is easily calculated. The OSS-2 variable outlet splitter capillary interface replaces a DuPont 1090/Hewlett Packard 5995B TG/MS interface which utilized a jet separator interface [3].

Thermogravimetry (TG) and thermogravimetry/mass spectrometry (TG/MS)

Thermogravimetric measurements are carried out with the electrobalance (Thermal Analyst 51, TA Instruments, Wilmington, Delaware, USA) in a Plexiglas glove box at a low humidity maintained by phosphorus pentoxide and monitored by

a portable hygrometer (Bacharach, Pittsburgh, Pennsylvania, USA). The quartz tubes surrounding the sample and balance counter weights are painted with gold paint (Engelhard Industries, East Newark, New Jersey, USA) to minimize the effects of static electricity on sample handling and the balance in the dry box. Wires connect the gold layer to the TG ground. Pulverized sample ranging from approximately 5 to 11 mg are placed on the TG pan for analysis. TG curves are collected by the Thermal Analyst 2200 (TA Instruments) with IBM Personal System 12 Color Display. The IBM Laser Printer 10 by LexMark prints out the thermograms. In the TG profile method sample looses weight as the furnace temperature increased from room temperature to 600°C at a programmed heating rate of 10 or 20°C min^{-1}. The mass of the residual moisture is taken as the difference of the initial sample weight and the sample weight at constant weight (usually the first horizontal plateau of the thermogram after the initial weight loss or the beginning of the evolution of carbon dioxide as indicated by the TG/MS data). The temperature varies with each product type. The ratio of the lost residual moisture weight to the initial sample weight multiplied by one hundred is taken as the percentage residual moisture in the sample. Sodium tartrate dihydrate is used as a standard. The sodium tartrate waters of hydration are measured thermogravimetrically and are accurately determined.

Karl Fischer

Coulometric Karl Fischer measurements are conducted in a Plexiglas glove box which is operated in a chemical fume hood. A low relative humidity is maintained in the dry box with phosphorus pentoxide. The relative humidity is monitored by a portable hygrometer (Bacharach Instruments, Pittsburgh, Pennsylvania, USA). The Karl Fischer instrument (Aquatest 8 Coulometric Moisture Analysis System, Photovolt, Indianapolis, Indiana, USA) is placed on top of the dry box to minimize corrosion of its electrical wiring. Custom elongated wires (Photovolt) connect the titration vessel inside the dry box to the instrument outside through rubber stoppered ports in the Plexiglas. Samples are vortexed to render the freeze-dried cake into a powder; vials are scraped free of labels and glue. The vial is placed inside the dry box at approximately 15 to 20% relative humidity and the vacuum is released by quickly opening then closing the stopper. The vial is weighed by a four-place Mettler balance and then placed back into the dry box. Approximately 20 to 30 mg of sample is poured into the pyridine containing vessel for titration after a zero microgram of water background reading is obtained by the instrument. After the sample is stirred in the vessel for (the most frequently used) 1.5 min (or another optimized time) the sample is titrated for moisture content. The instrument read-out indicates the micrograms water in the sample. The vial is reweighed to determine the exact amount of sample delivered for titration. The micrograms of water (converted to milligrams) determined by the coulometric titrator divided by the milligrams of sample multiplied by 100 yield the percent water in the sample. Standards used for this method included sodium tartrate dihydrate or known amounts of water carefully and accurately delivered into the titration solution with a microsyringe.

Results and discussion

Typical TG and MS operating conditions are listed in Table 1. Figure 2 shows the TG/MS data obtained for BCG Vaccine. The TG curve itself does not display a clearly defined mass loss transition that could be attributed to residual moisture.

Table 1 TG/MS operating conditions

TG:	TA Instruments TGA 51 Thermogravimetric Analyzer and Thermal Analyst 2200 with IBM Laser Printer 10 (LEXMARK).
	Heating rate: 10 or 20°C min^{-1}
	Atmosphere: helium
	Initial temperature: room temperature (~22°C)
	Final temperature: ~600°C
MS:	Hewlett Packard 5972C quadrupole mass spectrometer
	Electron ionization (e. i.) mass spectra were recorded under the following conditions:
	Ionization potential (fixed): 70 eV
	Temperature: transfer line: 180°C
	MS temperature: ~125°C
	The ion optics were tuned at mass 69, 219 and 502 using PFTBA (perfluorotributylamine) by the instrument Autotune program.
	The MS peak detection threshold was set at 150 linear counts.
	Spectra were recorded in the scan mode saving all spectra. EM voltage, emission current and other parameters were as set by Autotune.

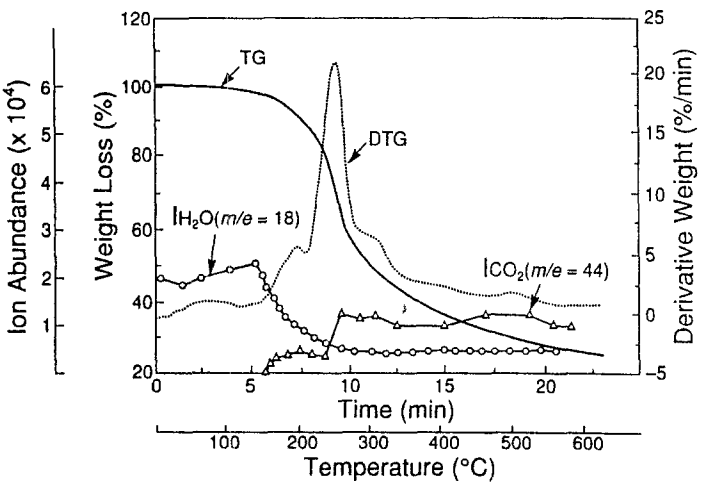

Fig. 2 TG, DTG, and mass spectral ion abundances for water ($m/e=18$) and carbon dioxide ($m/e=44$) *vs.* time and temperature for BCG Vaccine

The presence of carbon dioxide (shown by the ion abundance of $m/e=44$) coinciding with the evolution of water after 140°C would indicate that the water evolved resulted from sample decomposition. Since carbon dioxide is not evolved during the evolution of the first water peak, the TG mass loss attributed to residual moisture is indicated by the ion abundance for water ending at approximately 140°C. The loss in mass of BCG Vaccine up to 140°C is 1.28%. This TG/MS residual moisture result is in close agreement with the residual moisture results obtained by the Karl Fischer method, 1.22% (Table 2).

Table 2 TG/MS and Karl Fischer residual moisture data for BCG Vaccine, BCG Live Intravesical and U. S. Standard Antihemophilic Factor (Factor VIII) Lot A

Sample	% Residual moisture[1]		Relative error[2]/
	TG/MS	Karl Fischer method	%
BCG Vacine	1.28±0.28[3]	1.22±0.08	4.5
BCG Live Intravesical	2.74±0	2.71±0.33[4]	1.1
U. S. Standard AHF Lot A	1.05±0.16[5]	1.03±0.29[6]	1.9

[1] Arithmetic mean and standard deviation of two determinations unless otherwise indicated; [2] Relative error from the Karl Fischer value; [3] 4 determinations; [4] 9 determinations; [5] 3 determinations; [6] 15 determinations

Figure 3 shows the TG/MS data for BCG, Live Intravesical. Again the TG curve does not display a clearly defined mass loss transition that could be attributed to residual moisture. In the TG/MS data the ion abundances of mass peaks $m/e=18$ and $m/e=44$ show that the evolution of water from sample decomposition begins after 175°C. The DTG curve also indicates the break at 175°C. A moisture result of

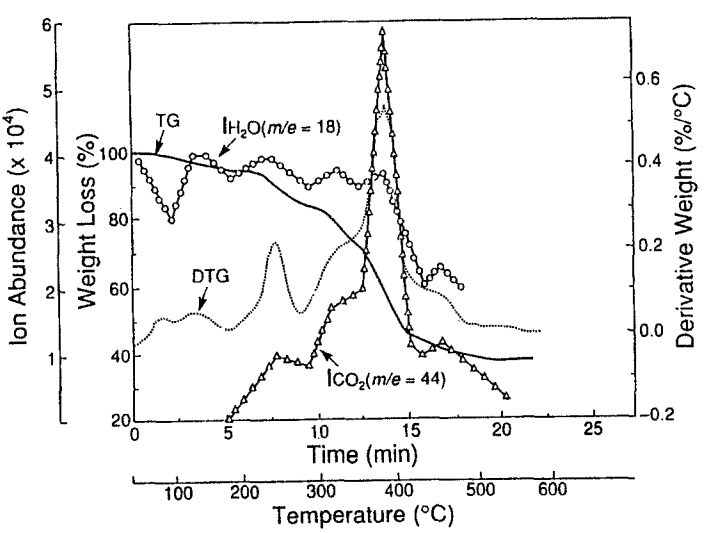

Fig. 3 TG, DTG, and mass spectral ion abundances for water ($m/e=18$) and carbon dioxide ($m/e=44$) *vs.* time and temperature for BCG Live (Intravesical)

2.74% was calculated for the BCG Live (Intravesical) using the transition indicated by the TG/MS data for residual moisture ending at 175°C. The moisture result obtained by the Karl Fischer method was 2.71% (Table 2).

Figure 4 shows moisture results for U.S. Standard Antihemophilic Factor Lot A. In the TG/MS data the ion abundances for mass peaks $m/e=18$ and $m/e=44$ show that the evolution of water from sample decomposition begins after 100°C for this product. The TG/MS moisture result calculated from the TG curve was 1.05%. The moisture result obtained by the Karl Fischer method was 1.03% (Table 2). Relative error of 1.9% indicates good agreement.

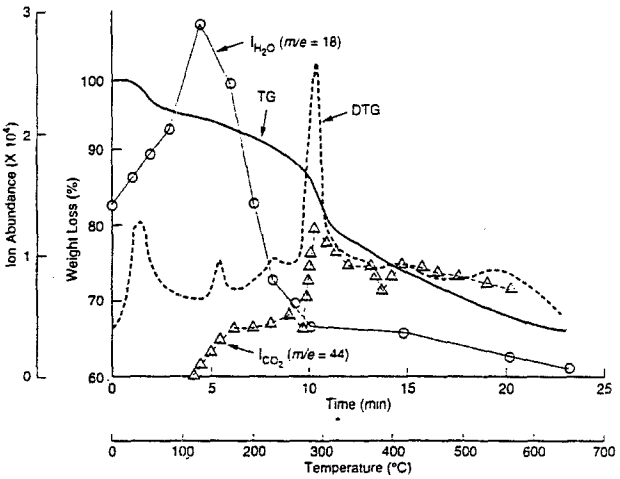

Fig. 4 TG, DTG, and mass spectral ion abundances for water ($m/e=18$) and carbon dioxide ($m/e=44$) *vs.* time and temperature for U.S. Standard Antihemophilic Factor Lot A

The *t*-test [9] was used to determine whether the Karl Fischer and TG/MS residual moisture data listed in Table 2 were significantly different. There was no significant difference found for the TG/MS and Karl Fischer moisture data listed for each sample in Table 2.

The data indicate that this TG/MS data can be used to verify the TG transition due to residual moisture in freeze-dried biological products that yield complex TG curves with no definite plateau attributable to residual moisture evolution. The ion abundances of MS mass peaks $m/e=18$ for water and $m/e=44$ for carbon dioxide indicate the difference between evolution of residual moisture and moisture associated with the thermal decomposition of the product. Karl Fischer residual moisture results are in good agreement (Table 2) with the residual moisture results obtained by the TG/MS method.

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