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THE DETERMINATION OF RESIDUAL MOISTURE IN SEVERAL FREEZE-DRIED VACCINES AND A HONEY BEE VENOM ALLERGENIC EXTRACT BY TG/MS*

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Thermogravimetry/mass spectrometry is used to identify TG transitions corresponding to loss of residual moisture in freeze-dried biological products with complex TG curves. While TG weight losses were recorded, ion intensities of mass peaks $m/e = 18$ (water) and $m/e = 44$ (carbon dioxide) were monitored continuously for Typhoid Vaccine U.S.P., Meningococcal Polysaccharide Vaccine Groups A and C Combined and Honey Bee Venom Allergenic Extract. MS ion intensities indicated the difference between evolution of residual moisture and moisture associated with product thermal decomposition. Residual moisture values calculated from TG weight losses indicated by mass spectral data agreed with Karl Fischer moisture data.

Limits for the residual moisture content of freeze-dried biological products determined by the gravimetric (loss on drying) method are specified in the United States Code of Federal Regulations [1]. Levels of residual moisture should be low so that the potency of the freeze-dried product is not compromised over time either by the promotion of microorganism growth or by other degradation [2]. Residual moisture has been the term used to describe the low level of moisture, usually less than 1% to 5%, remaining after the bulk of the solvent has been removed during the freeze-drying process. In practice, the freeze-dried product contains not only the freeze-dried vaccine material, for example, attenuated measles virus in Measles Virus Vaccine, but other residuals of the manufacture of the product such as media or buffer and any vehicle such as lactose that has been added to the product to facilitate the freeze-drying process. The residual moisture content of freeze-dried biological products was first determined by the gravimetric method [3]. Amperometric Karl Fischer, coulometric Karl Fischer, gas chromatographic and thermo-

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gravimetric (TG) methods have also been used. For samples with uncomplicated TG curves, thermogravimetric results have been shown to correlate with coulometric Karl Fischer results for residual moisture in several types of freeze-dried viral vaccines [4]. Karl Fischer and TG moisture results may be different from the gravimetric moisture result for the same freeze-dried product due to the fact that different types of moisture (physically adsorbed or chemically bound moisture) are being measured. The thermogravimetric method has been used to determine the moisture content of Group A and Group C Meningococcal Polysaccharide bulks at levels of 5 to 25% moisture [5]. In this study thermogravimetry/mass spectrometry (TG/MS) [6, 7] is used to identify the TG transition corresponding to the loss of residual moisture in vaccines that have complex TG curves. Thermogravimetry provides precise heating conditions and weight loss information at specified temperatures, while mass spectrometry identifies volatile compounds evolved during the weight loss process [8]. TG/MS residual moisture results are compared to Karl Fischer residual moisture results for Typhoid Vaccine U.S.P., Meningococcal Polysaccharide Vaccine, Groups A and C Combined, Honey Bee Venom Allergenic Extract and Measles Virus Vaccine Live, Attenuated.

Experimental

Samples and control materials

All of the samples in this study except the Typhoid Vaccine samples were from lots meeting the release requirements of the Office of Biologics Research and Review of the Center for Drugs and Biologics. The samples of Typhoid Vaccine, U.S.P. were suitable for moisture studies. However, Lot A was a lot that was manufactured in 1977 and was outdated. Lot B was an experimental lot that had failed the residual moisture requirement for Typhoid Vaccine, U.S.P. specified in the U.S. license for this product (not more than 1.5%).

Typhoid Vaccine, U.S.P., Acetone Inactivated, Dried was manufactured by Wyeth Laboratories, Marietta, Pennsylvania, U.S.A.

The Meningococcal Polysaccharide Vaccine, Groups A and C Combined (Meningovax — AC) stabilized with lactose was manufactured by Merck, Sharp and Dohme, Division of Merck and Co., Inc., West Point, Pennsylvania, U.S.A.

The Measles Virus Vaccine, Live, Attenuated stabilized with a protein (casein) hydrolysate and sorbitol was manufactured by Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, U.S.A.

The Honey Bee Venom Allergenic Extract was manufactured by Pharmacia AB, Uppsala, Sweden and distributed by Pharmacia Diagnostics, Division of Phar-

macia, Inc., Piscataway, New Jersey, U.S.A. The Honey Bee Venom Allergenic Extract is freeze-dried in 0.06% normal serum albumin and 3.0% mannitol and 0.9% sodium chloride.

Sodium tartrate dihydrate, $Na_2C_4H_4O_6 \cdot 2H_2O$ (Fisher Scientific Company, Fair Lawn, New Jersey, U.S.A.) was used as a control sample [9].

Methods

TG/MS

TG/mass spectral data were obtained from E. I. DuPont de Nemours and Company (Wilmington, Delaware, U.S.A.) under contract.

Mass spectra were taken continuously of the gases (off-gases) exiting from the quartz tube surrounding the sample in the TG oven while the weight loss (TG) and rate of change of weight loss (DTG) scans were recorded for a sample of approximately 6 mg by a DuPont Model 951 TGA interfaced [6, 7] to a DuPont Model 21–104 mass spectrometer (DuPont Instruments, Wilmington, Delaware, U.S.A.). The analyses were performed at a heating rate of 5 deg per min in a flowing helium atmosphere. The sample was heated from room temperature to 400° or higher. The relative ion intensities of mass peaks $m/e = 18$ representing water and $m/e = 44$ representing carbon dioxide were monitored and plotted versus temperature to show the changes in the amounts of water and carbon dioxide in the TG off-gases obtained in relation to the weight loss profiles.

Karl Fischer

Karl Fischer moisture measurements were made with an Aquatest IV (Photovolt Corporation , New York, New York, U.S.A.) coulometric titrator with microprocessor control [4]. In this procedure, iodine generated coulometrically reacts quantitatively with water in a solvent containing pyridine, sulphur dioxide and methanol. The unit was operated in a Plexiglass glove box with anhydrous phosphorus pentoxide used to obtain a low relative humidity in the box.

Thermogravimetric analysis

Thermogravimetric moisture measurements were made with a DuPont Model 951 Thermogravimetric Analyzer (DuPont Instruments, Wilmington, Delaware, U.S.A.) used in conjunction with the temperature programmer and recorder of the DuPont 1090 Thermal Analyzer. The TG balance was enclosed in a Plexiglass glove box containing anhydrous phosphorus pentoxide to maintain a low relative

humidity. The quartz tube of the TG balance which surrounds the sample pan and the tube surrounding the balance counter weights were painted with gold paint (Engelhard Industries, East Newark, NJ, U.S.A.). Wires connected the gold layer to the electrical ground of the TGA. This prevented static charge buildup from interfering with balance operation during analysis within the low humidity glove box [4]. In this procedure, sample weight loss was monitored as the furnace temperature was increased from room temperature (23°) to 400° or higher at a programmed heating rate of 5 deg per min. The weight of the evolved moisture was taken as the difference between the initial sample weight and the sample weight at the temperature indicated by the mass spectral data to be the end of the evolution of residual moisture. The ratio of the weight of the lost residual moisture to the initial sample weight multiplied by one hundred yielded the percentage residual moisture in the sample.

Results and discussion

Figure 1 shows the TG/mass spectrometry data obtained for a Typhoid Vaccine containing primarily *S. typhosa* bacteria, sodium chloride and monobasic sodium phosphate. The TG curve itself does not display a clearly defined weight loss transition that could be attributed to residual moisture. The relative ion intensity of mass peak $m/e = 18$ (water) indicates that one weight loss due to water ends at approximately 150°. The presence of carbon dioxide (shown by the relative ion intensity of $m/e = 44$) coinciding with the evolution of water after 150° would indicate that the water evolved resulted from sample decomposition. Since carbon

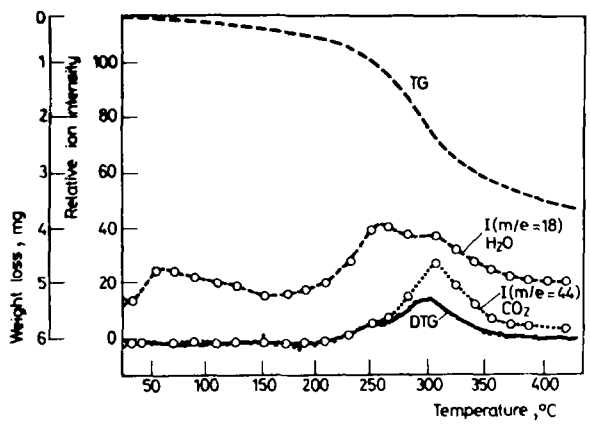

Fig. 1 TG, DTG and mass spectral relative ion intensities (1) for water ($m/e = 18$) and carbon dioxide ($m/e = 44$) versus temperature for Typhoid Vaccine, U.S.P., Acetone Inactivated, Dried

dioxide is not evolved during the evolution of the first water peak, the TG weight loss attributed to residual moisture is indicated by the relative ion intensity for water ending at approximately 150°. The loss in weight of Typhoid Vaccine, Lot A up to 150° is 3.09%. This TG/MS residual moisture result is in close agreement with the residual moisture results obtained by the Karl Fischer method, 3.21% (Table 1). A second Lot B of the same manufacturer's Typhoid Vaccine was also analyzed by TG/MS and the coulometric Karl Fischer method. The residual moisture results obtained by the two methods are listed in Table 1. The results are above the 1.5% residual moisture limit for this product. A future study will establish the correlation between gravimetric, coulometric Karl Fischer and TG/MS moisture results for this freeze-dried Typhoid Vaccine and an acceptable limit for its residual moisture content when the coulometric Karl Fischer or TG/MS methods are used. As was shown earlier for several measles, mumps and rubella viral vaccines [4], coulometric Karl Fischer and TG moisture results may be different from gravimetric moisture

Table 1 TG/MS and Karl Fischer residual moisture data for Typhoid Vaccine, Meningococcal Polysaccharide Vaccine, Groups A & C Combined, Measles Virus Vaccine, Live, Attenuated, Honey Bee Venom Allergenic Extract and the Sodium Tartrate Dihydrate Control

Sample	% Residual moisture		Relative error*, %
	TG/MS method	Karl Fischer method	
Typhoid Vaccine, Lot A	3.09 ± 0.75**	3.21 ± 0.27**	3.74
Typhoid Vaccine, Lot B	3.18 ± 0.86**	3.13 ± 1.37**	1.60
Meningococcal Polysaccharide Vaccine Groups A & C, Combined	0.66 ± 0.19	0.65 ± 0.003	1.54
Honey Bee Venom Allergenic Extract	0.68 ± 0.25	0.70 ± 0.10	2.86
Measles Virus Vaccine Live, Attenuated	1.32 ± 0.86	1.28 ± 0.11	3.13
Sodium Tartrate Dihydrate*** ($Na_2C_4H_4O_6 \cdot 2H_2O$)	15.68 ± 0.21 S	15.49 ± 0.34 SS	1.23

* Relative error of TG/MS value from Karl Fischer value

** Residual moisture limit for this product is 1.5%. The values reported are above the moisture limit for this product. Lot A was outdated; Lot B failed the release requirements for moisture for this product. Limits have not been set for this product by the TG/MS or coulometric Karl Fischer methods.

*** Actual lot water analysis — 15.64% H_2O

S Average of 10 determinations

SS Average of 16 determinations

results for the same lot of freeze-dried product. Presumably, this is due to the fact that the different methods are measuring different types of moisture, i.e., varying amounts or all of physically adsorbed water or chemically bound water.

Figure 2 shows the TG curve and TG/MS data obtained for lactose stabilized Meningococcal Polysaccharide, Groups A and C Combined. Again, the TG curve does not display a clearly defined weight loss transition that could be attributed to residual moisture. The relative ion intensity of mass peak $m/e = 18$ shows three

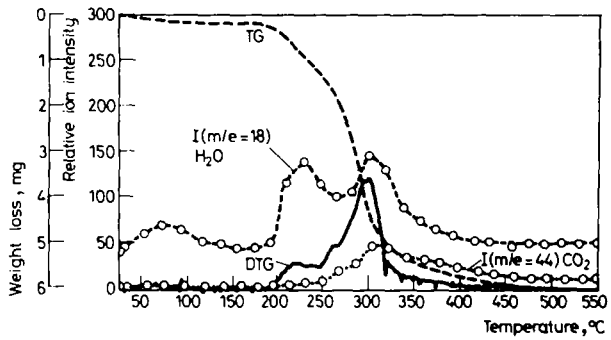

Fig. 2 TG, DTG and mass spectral relative ion intensities (1) for water ($m/e = 18$) and carbon dioxide ($m/e = 44$) versus temperature for Meningococcal Polysaccharide Vaccine, Groups A and C Combined freeze-dried with lactose as a stabilizer

peaks as water is evolved in the course of application of heat to the sample. The appearance of the relative ion intensity of carbon dioxide (mass peak $m/e = 44$) would occur when sample decomposition was taking place via combustion. The ion intensity of carbon dioxide appears after 175° coinciding with a large increase in the relative ion intensity from water which is also a combustion product. Since the evolution of carbon dioxide indicates that the second and third water peaks are due to sample decomposition, and since no carbon dioxide is evolved coinciding with the evolution of the first water peak, the TG weight loss attributed to adsorbed water is indicated by the relative ion intensity for water ending at approximately 175°. Using this as a general pattern TG/MS moisture test results were obtained for another lot of this vaccine. The TG/MS moisture result calculated from the TG curve was 0.66%. The moisture result obtained by the Karl Fischer method was 0.65%.

Figure 3 shows the TG curve and TG/MS data obtained for the Honey Bee Venom Allergenic Extract. The TG curve does not display a clearly defined weight loss transition that could be attributed to residual moisture. In the TG/MS data the ion intensities of mass peaks $m/e = 18$ and $m/e = 44$ show that the evolution of water from sample decomposition begins after 150°. The sample displays less than one

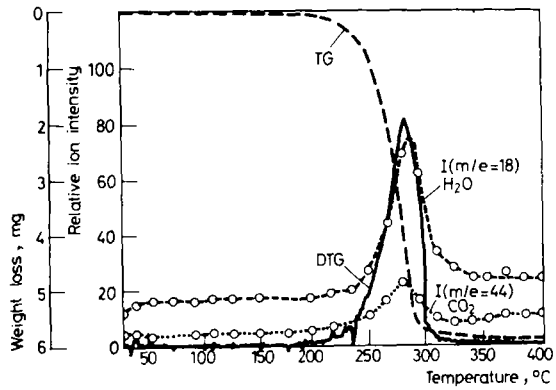

Fig. 3 TG, DTG and mass spectral relative ion intensities (I) for water ($m/e = 18$) and carbon dioxide ($m/e = 44$) versus temperature for a Honey Bee Venom Allergenic Extract freeze-dried in 0.06% normal serum albumin, 3.0% mannitol and 0.9% sodium chloride

percent weight loss up to 150° indicative of a very low residual moisture. The TG/MS moisture result calculated from the TG curve was 0.68%. The moisture result obtained by the Karl Fischer method was 0.70%.

The TG curve and TG/MS data for a Measles Virus Vaccine, Live, Attenuated stabilized with casein and sorbitol have been published previously [4]. The TG curve does not show a clearly defined moisture transition. The ion intensities of mass peaks $m/e = 18$ and $m/e = 44$ show that the evolution of water from sample decomposition begins after 110° for this product. The TG/MS moisture result calculated from the TG curve was 1.32%. A 1.28% residual moisture result was obtained by the Karl Fischer method.

The t-test [10] was used to determine whether the Karl Fischer and TG/MS residual moisture data listed in Table 1 were significantly different. There were no significant differences found for the TG/MS and Karl Fischer moisture data listed for each sample in Table 1.

The data demonstrated that TG/MS 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 intensities of MS mass peaks $m/e = 18$ (water) and $m/e = 44$ (carbon dioxide) indicate the difference between evolution of residual moisture and moisture associated with the thermal decomposition of the product.

The advantage of the TG/MS methodology is that small samples can be analyzed for residual moisture more easily than by the gravimetric method or the Karl Fischer method. The TG/MS technique is especially useful for the measurement of residual moisture in freeze-dried vaccines packaged in single dose containers and final containers that contain only 1 to 4 mg of material (Typhoid Vaccine). The

residual moisture determination by the gravimetric method for such samples involves pooling approximately one hundred individual vials that may contain one or two recoverable milligrams per vial. This procedure is tedious and consumes many vials of sample. The main disadvantages of the Karl Fischer methodology when applied to freeze-dried biological products are that not all biological products are soluble in the Karl Fischer reagents (or indeed any other non-aqueous solvents) and that some freeze-dried biological products have components that interfere with the Karl Fischer reaction and therefore cannot be analyzed. The TG/MS methodology can be used to measure residual moisture in freeze-dried biological products when the Karl Fischer or gravimetric methods are inappropriate.

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References

1 Code of Federal Regulations, 21 CFR 610.13 (a) U.S. Government Printing Office, Washington, D.C., 1984, p. 46.
2 E. B. Seligmann, Jr., Developments in Biological Standardization, 36, S. Karger, Basel, 1977, p. 175.
3 E. B. Seligmann, Jr. and J. F. Farber, Cryobiology, 8 (1971) 138.
4 J. C. May, E. Grim, R. M. Wheeler and J. West, J. Biological Standardization, 10 (1980) 249.
5 K. H. Wong, O. Barrera, A. Sutton, J. May, D. H. Hochstein, J. D. Robbins, J. B. Robbins, P. D. Parkman and E. B. Seligmann, Jr., J. of Biological Standardization, 5 (1977) 197.
6 J. Chiu and A. J. Beattie, Thermochim. Acta, 40 (1980) 251.
7 J. Chiu and A. J. Beattie, Thermochim. Acta, 50 (1981) 49.
8 W. W. Wendlandt, Thermal Methods of Analysis, 2nd Ed., John Wiley & Sons, New York, 1974, p. 345.
9 A. C. Glatz and A. Pinella, Analytical Calorimetry, Vol. 3 (eds. R. Porter and J. Johnson), Plenum Press, New York, 1974, p. 713.
10 H. A. Laitinen, Chemical Analysis, McGraw-Hill, New York, 1960, p. 549.

Zusammenfassung – Thermogravimetrie/Massenspektrometrie wurde zur Identifizierung von TG-Effekten herangezogen, die auf die Abgabe des Restwassers von gefriergetrockneten biologischen Produkten zurückzuführen sind. Für Thyphoid Vaccine U.S.P., Meningococcal Polysaccharide Vaccine Groups A and B combined und Honey Bee Venom allergenic extract wurden gleichzeitig mit der thermogravimetrischen Registrierung des Gewichtsverlustes die Ionenintensitäten der Massenpeaks m/e = 18 (Wasser) und m/e = 44 (Kohlendioxid) kontinuierlich verfolgt. Der Verlauf der

Ionenintensität ermöglicht eine Unterscheidung zwischen Restwasser und dem bei der thermischen Zersetzung des Produktes entstehenden Wasser. Der massenspektrometrisch als solcher erkannte und auf Grund der TG-Kurve quantifizierte Restwassergehalt stimmt mit dem Wert der nach Karl Fischer bestimmten Feuchtigkeit überein.

Резюме — Термогравиметрия и масс-спектрометрия были использованы для идентификации ТГ переходов, соответствующих потере остаточной влаги в биологических веществах, высушенных путем вымораживания. Одновременно с регистрацией потери веса, на мониторную систему выводились ионные интенсивности масс-пиков $m/e = 18$ (вода) и $m/e = 44$ (двуокись углерода). Масс-спектрометрические данные ионных интенсивностей показали различные между выделением остаточной влаги и влагой, выделяющейся в результате термического разложения. Значения остаточной влаги, вычисленные из ТГ кривых потери веса и установленные на основе масс-спектрометрических данных, согласуются с данными, установленными по методу Карла Фишера.