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Report

Near-Infrared Spectroscopic Determination of Residual Moisture in Lyophilized Sucrose Through Intact Glass Vials

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A rapid, noninvasive, and nondestructive method for determining moisture in sealed freeze-drying vials is described. The method, based on near-infrared spectrometry, used a novel fiber-optic diffuse-reflectance probe to make remote reflectance measurements from 1100 to 2500 nm through the bottom of glass vials. The correlation of the method to results obtained by Karl Fischer analysis was good ($r^2 = 0.97$). The moisture content of sucrose, a common cryoprotectant, was determined with an error of 0.27% using a single sample scan.

KEY WORDS: sucrose; fiber-optic; freeze-drying; water.

INTRODUCTION

Lyophilization of pharmaceuticals for parenteral use is generally performed in a final container such as a glass ampule or vial. A low residual moisture content in the dried product is essential to maintain the stability of compounds that are prone to hydrolysis (1,2) as well as to preserve the structural integrity of the dried cake. The drying stage must end at a precise time to ensure low residual moisture content; this is critical in preserving both the physical and chemical properties of the lyophilized product.

The moisture content of lyophilized samples is determined by Karl Fischer titration (3,4), by thermogravimetry, or by gas chromatography (2). Karl Fischer titration is sensitive to a limit of 0.1% (w/w) water, but the method is time-consuming, requires high-purity reagents, and is destructive in the sense that the sample cannot be reused. Thermogravimetric determination not only requires an expensive thermal analysis system but also is time-consuming and destructive and leads at times to erroneous conclusions because many lyophilized samples with extreme hygroscopicity pick up atmospheric moisture during sample handling. Methods based upon gas-chromatographic analysis of the headspace gas in the vials are the most sensitive; however, these involve sampling through the rubber closures and hence are invasive in nature. An ideal method for the determination of moisture content on a routine manufacturing schedule should be noninvasive, nondestructive, and rapid to ensure that time, costly material, and sterility of the product are conserved.

Near-IR spectrometry, a rapid analytical technique, uses the diffuse reflectance of a sample at several wavelengths to determine the sample's composition. Near-IR spectrometry can correct for background and sample-matrix interferences through a computerized modeling process, making ordinarily difficult analyses seem routine (5). Near-IR spectrometry has a number of advantages over other spectrometric methods.

(1) Near-IR analyses are typically rapid. Twenty-second analysis times are often quoted as standard.
(2) Little sample preparation is required. No preparation steps were required in this study. Near-IR spectrometry can be used to analyze liquid and solid samples with equal facility.
(3) Near-IR spectrometry is both noninvasive and nondestructive. Near-IR light (approximately 1-eV photon energy) passes through glass vials well and does not cause photodecomposition of samples (6). Near-IR equipment is simple and inexpensive.
(4) Near-IR spectrometry determines of a wide variety of properties; not only sample component identities and concentrations, but also characteristics such as molecular weight, taste, hardness, and even thermodynamic parameters such as heat of formation are determined by near-IR spectrometry (7).

All of the properties described above are determined simultaneously using a single exposure to a light beam. Simultaneous analyses are accomplished by collecting near-IR signals at a number of wavelengths during a single sample scan and analyzing the signals using a number of calibration equations stored in a computer. These calibration equations are obtained through a modeling process that employs a training set of samples to "teach" a computer, in effect, to recognize relationships between diminutive spectral features and sample compositions. Clearly, the training set must be analyzed by an additional, reliable chemical procedure in order to make the training process effective. The result of the training process is a calibration equation that is stored in

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a computer. Many times a single set of spectra will have a number of sets of associated reference values; thus, a number of sample properties can be determined simultaneously from the spectra.

Currently, most near-IR analyses are being conducted for agricultural and food applications. In most near-IR applications the analyte is present at a concentration greater than 1%, and the detection limit for most substances is of the order of 0.1% (8). Agricultural products are commonly analyzed by near-IR spectrometry for constituents such as water, total protein, lipids, and ash. Results of invasive and destructive pharmaceutical moisture analyses in the near-IR region have been demonstrated to be comparable to those from Karl Fischer titration (9). Recently, investigators have begun to perform qualitative and quantitative near-IR analyses on intact pharmaceutical capsules and tablets as well (10).

The objective of this study was the development and testing of a noninvasive, nondestructive, and rapid method of moisture determination in lyophilized samples. Near-IR diffuse-reflectance spectrometry accomplishes this objective by scanning through the bottom of glass freeze-drying vials. Sucrose, a common cryoprotectant, was lyophilized for use as a model sample in this study.

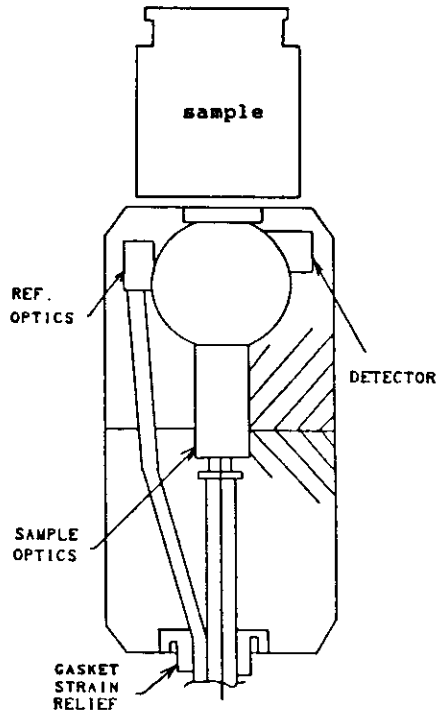

Fig. 1. The sampling end of the fiber-optic probe. Two fiber bundles enter a gold integrating sphere inside the aluminum probe, one carrying a sample beam and one carrying a reference beam. The sample fiber and optics illuminate the bottom of the vial through a sapphire window at the top of the sphere.

MATERIALS AND METHODS

Lyophilization of the Sucrose Solutions. Sucrose (J. T. Baker, Philadelphia) solutions (10%, w/w) were prepared in demineralized water (10 MΩ/cm). After filtering through a Millipore filter (0.5-μm size), 3 ml of the solution was poured into 8-ml borosilicate vials. The vials were semistoppered using rubber closures and were placed in the freeze-dryer (Hull Corp., model X8F12). Two lots of sucrose vials were lyophilized. In each lot the vials were frozen to −40°C. After approximately 8 hr at this temperature, the pressure in the drying chamber was reduced to less than 80 mTorr and sublimation was initiated. After 18 hr of drying, the shelf temperature was increased to 0°C and drying was continued for another 10–16 hr (for one lot) and 48 hr (for the remaining lot). With the help of a remotely controlled upward movement of the shelves in the drying chamber, the vials were closed at various time intervals in order to obtain lyophilized sucrose product containing a range of moisture content normally expected in a dry product (up to 5%, w/w). Moisture content in the dried cake was determined by Karl Fischer titrations using a Lab Industries Aquameter (model: GCA/Precision Scientific Company). The lower limit for detecting moisture was between 0.20 and 0.25% (w/w).

Equipment. Spectral data were collected using a Bran + Luebbe/Technicon Industrial Systems InfraAlyzer 500 spectrometer system and a fiber-optic diffuse-reflectance probe. Each spectrum was obtained from 1100 to 2500 nm in 2-nm increments. The spectrometer was connected to a MicroVAX II computer system (Digital Equipment Corp., Maynard, MA). Data analysis was conducted on the MicroVAX II using programs written by one of the authors (R.L.) in Speakeasy IV Epsilon (Speakeasy Computing Corp., Chicago, IL).

Figure 1 is a diagram of the fiber-optic diffuse-reflectance probe that was attached to the spectrometer to collect spectral data through sealed glass freeze-dried vials. Sample vials were placed above a window in a gold integrating sphere for spectral data collection. The gold sphere is inside the probe. Near-IR light was directed upward onto the bottom of the vial using an optical fiber at the bottom of the sphere, while a second optical fiber on the left side of the sphere directed light onto the integrating sphere's wall. Spectral data were recorded at the detector as the ratio of the light intensity from one fiber versus that from the other. The "reference" beam directly illuminated the wall of integrating sphere, and the "sample" beam directly illuminated the bottom of the freeze-drying vial through a sapphire window. In this configuration, specular reflectance from the glass and cake surface was directed back toward the sample optical fiber, while diffuse reflectance (which contained the information from light that interacted with the sample cake) was scattered into the integrating sphere. The sample vial was enveloped with two aluminum covers to prevent stray light signals from being recorded. A single spectrum was recorded for each sample vial by simply placing the vial atop the probe, covering the vial, and recording the spectrum. After analysis the intact vials were removed and could be analyzed by other means if desired. Basing the system on a fiber-optic probe enables the remote analysis of intact samples, potentially even inside the freeze-dryer itself.

Data Analysis. Each spectrum, recorded at 701 wavelengths, was expressed as a point in a 701-dimensional space, translated along each dimensional axis by an amount that corresponded to the magnitude of the signal observed at

each wavelength. The sucrose samples, being similar to one another, projected into the 701-dimensional space as a cluster of points in a specific region of the hyperspace. The direction of the displacement of a sample from the center of this cluster identified the cause (i.e., the spectrum) of the displacement, while the distance of the displacement indicated how much material was responsible for the displacement. Principal-component regression was one tool used for interpreting the spectra. Principal-axis transformation (PAT) simplified the spectra by removing the wavelengths that added only "noise" information. Spectra originally collected at 701 wavelengths and described as points in a 701-dimensional space were expressed through PAT as points in a space of as little as three or four dimensions. The regression process then fitted a hyperplane through the spectral data points to describe trends induced by a particular physical or chemical phenomenon (e.g., changing moisture content). The hyperplane was used to predict the concentrations or physical parameters of new samples based upon the near-IR spectrum of each new sample.

The principal-component analysis began with the transformation of the spectra to principal axes. Transformation to principal axes was, in effect, a two-step process that involved (i) the *translation* of the Cartesian coordinate system defined in wavelength-space to the center of the spectral cluster and (ii) the *rotation* of the Cartesian coordinate system to describe the variations present in the spectra. The coordinate system remained rectangular throughout PAT and was moved rigidly from one orientation to another. The rotation step decomposed all of the spectral variation into orthogonal (or independent) components. In the process of calculating each principal axis, the perpendicular distances between the spectral points and the axis were minimized. The first principal axis was defined to describe the largest amount of spectral variation; this variation was then "subtracted" from the spectra. The second principal axis was defined in a similar manner to the first, except that during its rotation to describe the maximum variation remaining after construction of the first axis, the second axis was kept perpendicular to the first. The orthogonality condition effectively resulted in each component's accounting for the maximum variation in the spectra independent of the variation of all of the preceding spectral components. Only a few iterations of axis construction were required before the rotation/subtraction process described spectral variation that was merely random noise. The Scree Test (11) was used for determining the number of spectrally significant components. The test searched for a sharp drop in the amount of variation accounted for by each subsequent component. When this drop was identified, no further axes were considered in the analysis. The net result of the transformation was spectral coordinates expressed in "reduced-wavelength" space. The PAT rotation process reduced problems created by noise and by the highly correlated nature of near-IR wavelength data.

A transformation matrix was prepared by the PAT algorithm. The transformation matrix allowed new wavelength-spectra to be projected into the same space originally defined by the PAT modeling process. Ordinary multiple least-squares techniques were applied to the spectra in the reduced space to calculate coefficients of a hyperplane equation. The regression coefficients did not change when components were added or deleted, therefore component coefficients corresponding to noise sources were simply dropped from the regression.

A cross-validation technique was employed to verify that the hyperplane produced by the modeling process was actually the correct one to use. The cross-validation process started with the training set and fit the best hyperplane to the training-set samples using reference moisture values obtained by Karl Fischer titration as a guide. A standard error of estimate (SEE) was calculated from these training samples. A validation set, composed of samples that were not used in developing the hyperplane equation, was also assembled. The hyperplane equation developed using the training set was applied to the validation set and a new standard error (the standard error of prediction, or SEP) was calculated. When the SEE and the SEP were approximately the same, the pattern-recognition model developed was considered to be valid. The overall goal of the modeling process was the minimization of the SEE and SEP.

RESULTS AND DISCUSSION

The overall range of water concentrations (as determined by Karl Fischer titration) in the freeze-dried sucrose samples was 0.72 to 4.74%. The first lot of vials ranged from 2.67 to 4.74% (w/w) water, while the second lot ranged from 0.72 to 1.20%. Figure 2 shows the spectrum of liquid water in the near-IR region from 1100 to 2500 nm. The peaks attributed to water appear at 1450 and 1930 nm. Figure 3 is the powdered crystalline sucrose spectrum, which is devoid of the 1930-nm peak, while Fig. 4 depicts the spectra of three freeze-dried amorphous sucrose samples [each approximately 4% (w/w) water]. Note the presence of a water peak near 1930 nm in the freeze-dried sucrose spectrum that corresponds to the major peak of water in the near-IR region. An intense band near 2100 nm is typical of sucrose in both the crystalline and the amorphous states (12). In data analysis, each principal axis (or pseudowavelength) is tested for correlation to water. Some axes (in our spectra, numbers 1, 3, and 4) show a relatively significant correlation (as indicated by t statistics) to water concentration. It is interesting to note that analysis of eigenvalues shows that 99% of the spectral variation is on the first principal axis, while 0.08% is

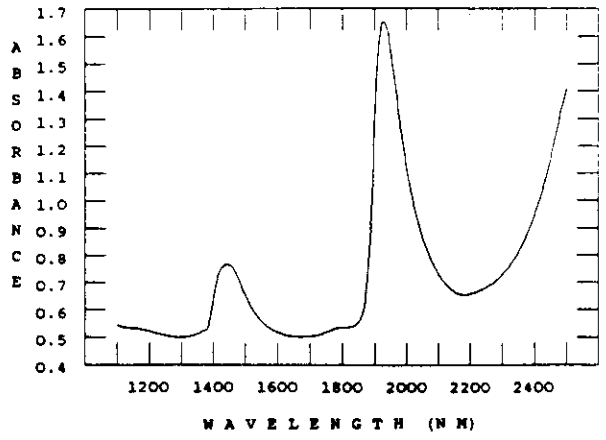

Fig. 2. The near-IR transmission spectrum of liquid water.

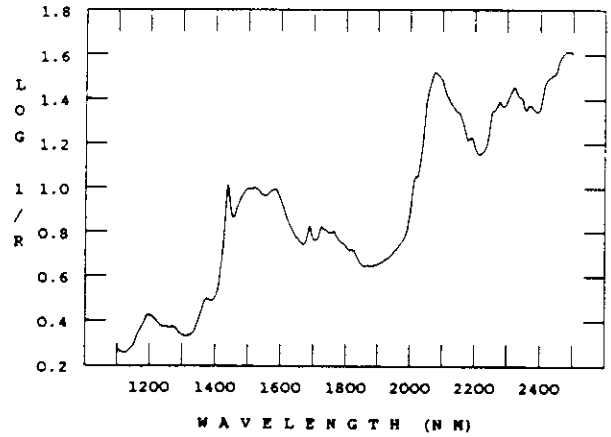

Fig. 3. The near-IR reflectance spectrum of ground crystalline sucrose.

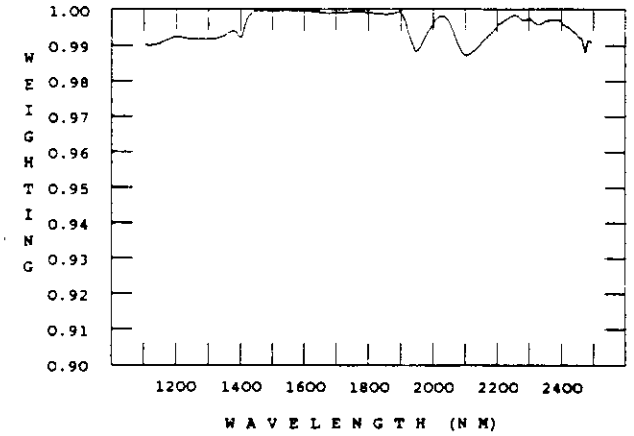

Fig. 5. The weighting spectrum of the largest independent variation in the spectra of freeze-dried sucrose.

on the third principal axis and 0.06% is on the fourth. By the fourth axis a total of 99.98% of the spectral variation has been accounted for by the PAT. In true wavelength-space, nearly 100 wavelengths would show some correlation to water, and all of these wavelengths would be subject to interferences from other phenomena, such as particle-size variations.

An examination of the elements of the transformation matrix corresponding to each principal axis provides an indication of which spectral regions are important to the construction of each principal axis. Figures 5 and 6 describe the importance of each wavelength to the construction of the first and third principal axes, respectively. The abscissa in Figs. 5 and 6 corresponds to the wavelength examined, while the ordinate corresponds to the relative weight applied to the information at the corresponding wavelength by the PAT algorithm. The first principal axis accounts for the vast majority of the spectral variation. Figure 5 shows that almost all wavelengths are used equally in the construction of the first principal axis. However, a closer examination of Fig. 5 shows that two or three regions are not as highly weighted as the rest. These regions correspond to the water band near 1940 nm and the sucrose band around 2100 nm, and to the range of 1100–1300 nm, a region where neither sucrose nor water has a particularly significant absorption signal, but where the spectral noise of the fiber probe is higher. The fact that the first principal axis has relatively high weights on most regions (except where significant sucrose or water variations or noise occurs) means that the first principal axis encompasses the vast majority of the baseline variation typically observed in near-IR spectra.

Figure 4 shows the spectra of three freeze-dried sucrose samples that demonstrate a baseline "bounce" variation. The bouncing effect can be caused by several factors. Smaller particle sizes tend to increase sample reflectance, lowering the baseline. Furthermore, vertical movement of the freeze-dried cake inside the vial may occur. Increasing the distance between the cake and the bottom of the vial allows more reflected light from the sample to miss the window in the integrating sphere on the fiber-optic probe. Thus, increasing the distance between the cake and the bottom of the vial results in a darkening of the sample (raising the baseline). Increased amounts of water also result in baseline shifts due to the relatively high absorption of water throughout the near-IR region (see Fig. 2). In general, higher water concentrations will correspond to higher baselines in sucrose. Fortunately, principal-component analysis provides a

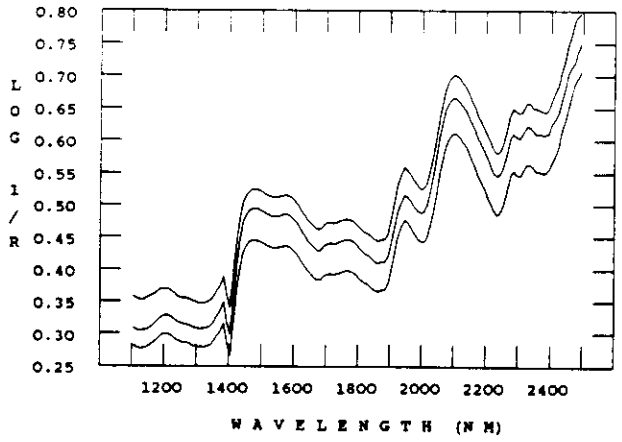

Fig. 4. The near-IR reflectance spectra of three freeze-dried sucrose samples obtained with the fiber-optic probe.

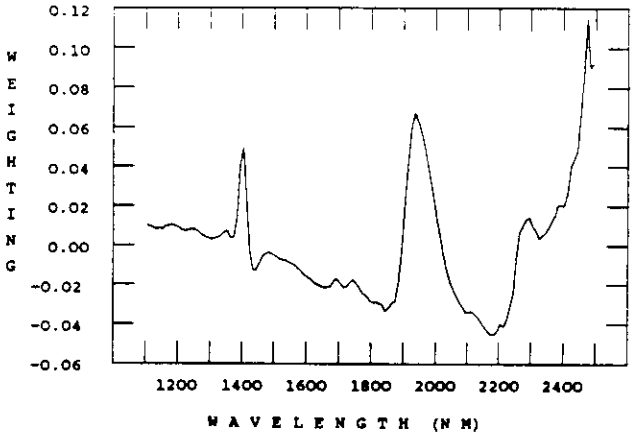

Fig. 6. The weighting spectrum of the third-largest independent source of variation in the spectra of freeze-dried sucrose.

means at least partially to separate, identify, and quantify all of these effects.

Figure 6 is a plot of the relative contribution of each wavelength to the third principal axis. This plot shows that the most significant portion of the contribution to the third principal component comes from the water absorption region near 1940 nm. (The sharp peak near 1400 nm is the result of Wood's anomaly, an effect caused by the orientation of light as it leaves the diffraction grating in the monochromator. A small water peak appears to the right of the anomaly region.) The important item to note is that there is a principal axis on which the primary source of variation is the variation of water content in the samples as monitored by near-IR spectrometry.

Principal-component regression was used to create a quantitative model for the prediction of moisture in sucrose spectra. A calibration equation constructed using 45 training samples produced a standard error of estimate of 0.27% H_2O (r^2 = 0.97). Applying the calibration equation to the spectra of 43 validation samples (samples not used to construct the calibration line) produced a standard error of prediction of 0.27% H_2O. Near-IR diffuse-reflectance spectrometry, using a single spectral scan through the wall of an intact freeze-drying vial, appears capable of determining water concentration in a lyophilized sucrose sample in the range of 0.72–4.74% H_2O with an RSD of 6.7% (see Figs. 7 and 8). Furthermore, Fig. 7 demonstrates that spectra with similar water concentrations can vary significantly in appearance, particularly in their baseline offsets. For this reason, calculation of water concentration from the area under the water peak is not as effective as the PAT procedure employed in this study.

The prediction error of 0.27% for water was achieved with a single scan and without background subtraction of the spectra of the empty vials, without going to great lengths to position the vial reproducibly atop the sampling apparatus, and without disturbing the contents of the vial in any way.

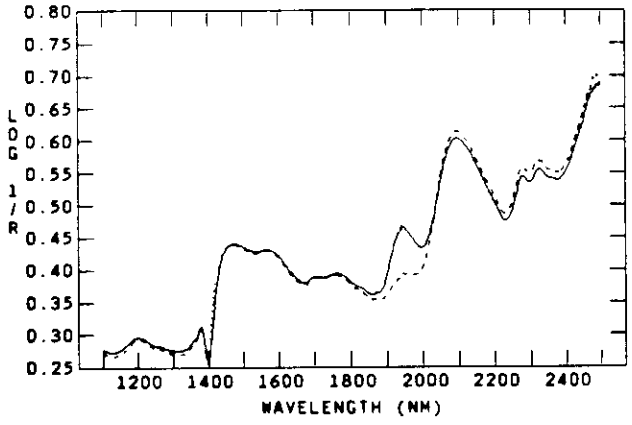

Fig. 7. Representative near-IR spectra of lyophilized sucrose with a moisture concentration of 4.44% (dotted line), 4.49% (solid line), and 0.72% (dashed line) H_2O (w/w), as determined by Karl Fischer titration.

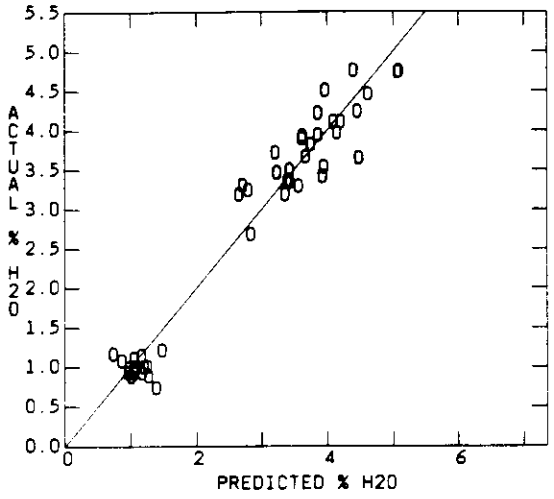

Fig. 8. A calibration line for moisture in freeze-dried sucrose. Validation samples (○) are shown superimposed on the line calculated using training samples.

Approximately 500 μg of sample is enough to perform an analysis. Taking steps to improve the reproducibility of the sucrose image as observed by the detector (such as by averaging several scans of the same vial, or spinning the vial, or filtering the spectra digitally) should improve the performance of this moisture-determination method. The current results demonstrate what can be expected from investigating the contents of the vial with a diffuse-reflectance fiber-optic probe in a situation such as might be encountered inside a freeze-dryer.

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