Determination of Moisture in Intact Gelatin Capsules by Near-Infrared Spectrophotometry

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INTRODUCTION

Pharmaceutical application of near-infrared spectrophotometry offers rapid, nondestructive, and noninvasive techniques to analyze samples. The analytical power of near-IR spectrophotometry is derived from the computerized analysis of complex spectra with overlapping bands, and advances in computer algorithms allow increasingly detailed analysis of samples nondestructively. Recent examples of pharmaceutical near-IR spectrophotometry include the analysis of degradation products (1), the detection of tampering in tablets and capsules (2,3) and moisture determination (4). As for moisture, it has been noted that gelatin in capsules undergoes conformational change and crosslinking when stored under high humidity conditions, as demonstrated by etodolac (5), gemfibrozil (6), hydrochlorothiazide (6), and diphenhydramine hydrochloride capsules (6), and by gelatin-coated acetaminophen tablets (7). The crosslinking process in gelatin causes formation of a swollen, rubbery, water insoluble gelatin (pellicle) resulting in reduced *in vitro* dissolution rates of drugs (8,9,10). Apparently this water insoluble gelatin film (pellicle) acts as a barrier, restricting drug release. It has been proposed that the mechanism by which humidity acts is through indirect catalysis of imine formation, which is the first intermediate in most gelatin crosslinking reactions (11). The ϵ-amino functions of the lysine residues from the polypeptide backbone of gelatin have been implicated in the crosslinking reaction, which occurs after prolonged exposure of gelatin capsules to humidity and heat. Several reactions involving imine intermediates have been suggested to take place during the crosslinking process of gelatin (11). The effect of crosslinking in gelatin capsules on *in vivo* bioavailability is being studied (5,7,12), and the determination of moisture levels in intact gelatin capsules using a convenient and noninvasive method, such as near-IR spectrophotometry, is a genuine advantage. The coupling of fast computer algorithms with near-IR spectrophotometers should allow real-time analysis and zero-defect quality control of capsules on pharmaceutical production lines.

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MATERIALS AND METHODS

Materials. Size 0, clear hard gelatin capsules were obtained from Capsugel (Warner-Lambert Company, Greenwood, SC, USA).

Instrumentation. Special data were collected by an InfraAlyzer 500 (Bran + Luebbe, Elmsford, N.Y.) spectrophotometer. Absorbance values were obtained as log(1/R) data from capsules every 4 nm from 1100 to 2500 nm using a 10 nm bandpass. The data were collected with an IBM PS/2 model 50 computer (I.B.M., Armonk, N.Y.). Data analysis was performed on an IBM RS6000 with software written in Speakeasy IV Zeta (Speakeasy Corporation, Chicago, IL).

Procedure. Capsules were scanned in a 90° conical reflector machined from aluminum (3). The use of this reflector allows reflected light to be collected from most of the surface area of the capsule, which helps eliminate errors due to capsule inhomogeneity. A steel wire support was used to achieve reproducible upright vertical capsule positioning within the cone. The optical surface of the reflector was polished with a commercial polishing paste with no residual near-IR spectrum. Capsule masses were obtained with an electronic balance (model B1240, American Scientific Products).

A hydrator was constructed to control the humidity to which the capsules were exposed. The hydrator consisted of a large desiccator in which the desiccant was replaced by water. The capsules were exposed to moisture by placing them inside the hydrator on a flat aluminum wire screen support just above the water. Principal-component regression (PCR) was employed to analyze the spectra of intact capsules (13). In general, water uptake causes an increase in absorbance across the spectrum, with greater increases at 1450 nm and 1940 nm where water has absorbance peaks. These spectral features are complicated by additive and multiplicative baseline shifts that occur as a result of specular reflectance and scattering effects in the capsules, hence the need for PCR instead of simpler calibration methods.

RESULTS

The near-infrared spectra were obtained for forty-five hard gelatin capsules, subjected to varying degrees of hydration. After drying the capsules for five days in a desiccator, the capsules were weighed and placed in the hydrator at various times (0–5 days). Each capsule was removed from the hydrator, scanned in the near-IR spectrometer, placed in a preweighed glass vial and capped. The total elapsed time out of the hydrator was approximately three minutes. The capsules were then weighed again to confirm water uptake by the capsules independently of the spectral method. The increase in mass of the capsules with time spent in the hydrator is shown in figure 1, which shows a large change in the capsule mass upon absorption of water.

Principal component regression (PCR) reduces the dimension of a spectral data set by transforming the absorbance values at each wavelength into a linear combination of the values at all wavelengths based on the contribution to the total variation of the data set. The result is a set of principal components (PCs) in which the first PC contains information from the constituent (or constituents) that contribute most to

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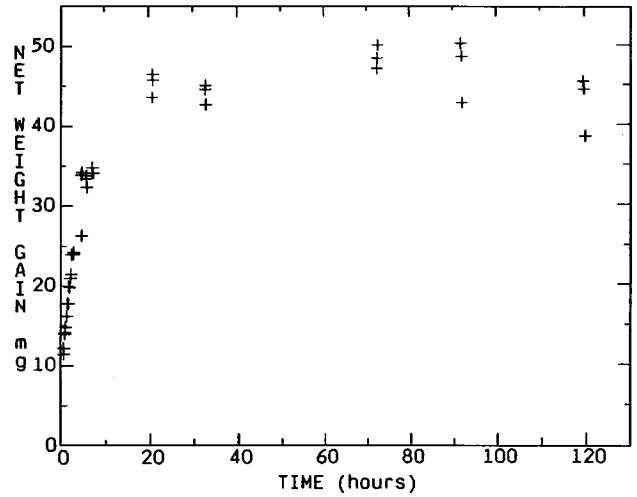

Fig. 1. The mass of the hard gelatin capsules increases on exposure to water. Most of the increase occurs in the first day of exposure to moisture in the hydrator (temperature 22°C, RH = 100%). The net weight gain (on the ordinate) is the difference between the capsule mass at time 0 and time t (on the abscissa).

the total spectral variation of the data. The second PC is orthogonal to the first, and weights most heavily the wavelengths that contribute the most variation to the spectra after calculation (removal) of the first PC. Subsequent PCs describe progressively smaller orthogonal contributions to the total spectral variation. Most of the variation in the spectra (98%) was described by four principal components. Examination of the transformation matrix connecting wavelength and PC hyperspace showed the signal on the first PC to be due to water. (The second PC also heavily weighted wavelengths due to water.)

The first PC of the capsule spectra was plotted against time spent in the hydrator to give figure 2. Figures 1 and 2 show a smooth uptake of water until 20 hours, and then both show large irregular changes. Figures 1 and 2 also show that

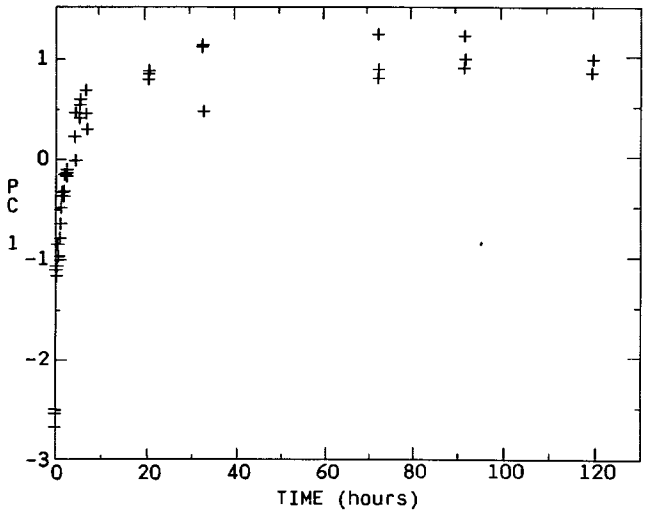

Fig. 2. The first PC of the spectra of the capsules increases in a manner similar to the capsule mass (temperature 22°C, RH = 100%). The PC 1 value on the ordinate is a weighted sum of the near-IR absorbances recorded at all wavelengths.

water actually seems to be lost from the capsules after 100 hours, instead of water uptake simply leveling off due to saturation. Figure 3 presents the results of principal component regression versus the capsule masses using PCs 1 to 4. Figure 3 shows that the near-IR and the gravimetric methods correlate linearly up to 20 hours (SEE = 0.97 mg, SEP = 1.14 mg).

Several possible explanations exist for the unusual behavior after the capsules have spent 20 hours in the hydrator:

1. After 20 hours the formation of degradation products could begin to dominate the variation in the sample set, interfering with the determination of water.

2. The pellicle material could become less hygroscopic after crosslinking and release water. The capsules could become saturated with water by 20 hours and become more likely to lose that water when removed from the hydrator for scanning.

3. The physical deformation of the capsules after spending time in the hydrator could interfere with the analysis; however, this is unlikely because of the nature of the diffuse reflectance holder used in this experiment.

Because unusual mass and spectral changes occur in the capsules after substantial exposure to moisture, PCR or other statistical techniques such as the BEAST (14) should be used on the spectra of capsules to identify those with more than 20 hours exposure to 100% relative humidity (RH). Linear prediction of the extent of hydration by near-IR spectrophotometry is not possible beyond 20 hours exposure to 100% RH.

CONCLUSIONS

Near-IR analysis has shown to be an effective means of measuring water uptake by hard gelatin capsules exposed to 100% humidity for less than 20 hours. It is possible to detect

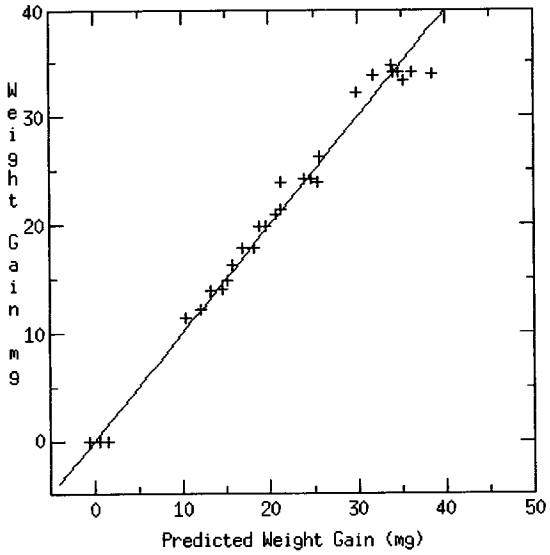

Fig. 3. Near-IR spectra of capsules show a strong correlation to increase in capsule mass with exposure to moisture (temperature 22°C, RH = 100%). The predicted weight gain is calculated by regression of PC values from near-IR spectra against gravimetric values (SEE = 0.97 mg). Cross validation of the regression equation was successful (SEP = 1.14 mg).

spectrophotometrically individual capsules with more than 20 hours of exposure to 100% RH. For example, actual or predicted mass gain of more than 30 mg indicates that a capsule cannot be used in this PCR model. PCR analysis coupled with other statistical methods allows the continuous monitoring of water uptake by capsules. Near-IR spectrophotometry differentiates between water uptake and gelatin degradation. The convenience of near-infrared determination of water uptake in hard gelatin capsules has been demonstrated. Near-IR techniques may allow intact capsules to be analyzed in bulk quantity (when coupled with appropriate hardware) giving vital information regarding capsule dissolution. Such an assay represents a savings in time, effort, and expense over traditional methods of moisture assay, such as Karl Fischer titration.

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