Nondestructive Near-Infrared Analysis of Intact Tablets for Determination of Degradation Products

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Abstract □ Near-infrared spectrometry was used in this study to examine intact aspirin tablets in order to demonstrate the usefulness of the technique as a nondestructive method of quality control. Unique sampling optics were used to simultaneously illuminate the entire surface of the tablets, including the top, bottom, and side. Changes in individual tablet spectra were correlated to (*a*) the time that the tablets spent in a hydrator, (*b*) the mass of water absorbed by the tablets, and (*c*) the mass of salicylic acid formed by base-catalyzed hydrolysis of acetylsalicylic acid. A prediction equation for each of these three parameters was constructed using near-infrared spectral reflectance values obtained from intact tablets. Prediction errors were low for (*a*) the time that tablets spent in the hydrator (±19 h over a period of 168 h), (*b*) the mass of water absorbed (±0.04% of tablet mass), and (*c*) the mass of salicylic acid formed (±0.04% of tablet mass).

The application of near-infrared spectrometry to the solution of pharmaceutical problems is becoming increasingly popular. Advances in multivariate analytical techniques and computer hardware, combined with the availability of modern near-infrared instruments with signal-to-noise ratios that can approach 10^6, allow pharmaceutical scientists to perform near-infrared reflectance experiments that would have been difficult or impossible a short time ago. Near-infrared spectrometry has been applied to the study of pharmaceutical raw materials,[1] packaging materials, particle-size determination, and polymorphism,[2] optical isomers,[3] moisture determinations, and quantitative and qualitative tests of dosage forms.[4] In 1987 and 1988, use of near-infrared spectrometry in the detection of tampering with intact capsules and tablets was reported.[5,6] The use of near-infrared spectrometry in determining purity levels of USP-grade aspirin powder was reported in 1989.[7]

Careful use of sampling optics and critical analysis should permit virtually any tablet to be analyzed noninvasively and nondestructively for identity, homogeneity, moisture content, and presence of degradation products using near-infrared spectrometry. As a spectrometric technique, near-infrared analysis is fast (20-s analyses are currently reported as typical), simple to perform (intact tablets are placed in a holder, scanned, and then removed), and relatively inexpensive. The nondestructive character of the method suggests its use as a zero-defect quality assurance technique, or its application to long-term stability studies where the ability to continuously monitor the same sample over time is desirable.

The near-infrared method of analysis saves time and materials in comparison with the standard U.S.P. methods of analyzing aspirin tablets for identity and salicylic acid content.[8] There are two U.S.P. methods of identification for aspirin tablets. In the first test, one tablet is crushed and boiled in water for 5 min, and then the solution is cooled. A ferric chloride test solution is then added to the tablet solution to produce a violet-red color. In the second test, a quantity of powdered tablets equivalent to 500 mg of aspirin is shaken with chloroform and then centrifuged. The supernatant is evaporated and the residue is dispersed with potassium bromide. The infrared absorption spectrum of the dispersion exhibits maxima only at the same wavelengths as that of a similar preparation of U.S.P. aspirin.

The U.S.P. chemical assay for salicylic acid involves high-performance liquid chromatography. At least 20 aspirin tablets are weighed and finely powdered before weighing an amount of powder equal to 100 mg of aspirin. The powder is dissolved, diluted, and subjected to assay by HPLC. The percentage of salicylic acid is calculated and cannot be >0.3% (1.11 mg for a tablet weighing 370 mg) for tablets that are uncoated and contain no buffers.

In contrast, the 701-wavelength near-infrared scan of each intact aspirin tablet (reflectance values obtained every 2 nm from 1100 to 2500 nm) takes ~90 s and enables tablet identification and resolution of salicylic acid content. Scanning from 1500 to 1700 nm and from 1850 to 2300 nm would still include the important spectral data and decrease the time required for analysis.

A unique system of sampling optics has been designed for tablets to separate specular reflectance (which contains predominantly information about the spectrum of the light source) from the diffuse reflectance (which contains more information about the spectrum of the sample). This near-infrared sampling system is employed in the current study to monitor the decomposition of aspirin, and could be readily adapted to monitor other products as well. This paper presents the use of near-infrared spectrometry, principal-component regression, and a novel method, the Bootstrap Error-Adjusted Single-sample Technique, to determine the moisture content and presence of degradation product in individual intact aspirin tablets.

Experimental Section

Materials—Near-infrared diffuse-reflectance spectra were obtained from aspirin tablets (Interamerican Foods, Cincinnati, OH) and salicylic acid crystals (Sigma, St. Louis, MO). The near-infrared spectrum of distilled water was obtained by a transmission measurement. Tetrabutyl ammonium phosphate (Eastman Kodak, Rochester, NY), monosodium phosphate monohydrate (Fisher, Pittsburgh, PA), ammonium hydroxide (Fisher), and methanol (HPLC grade, Fisher) were used for the reference HPLC analysis of salicylic acid in aspirin.

Instrumentation—Tablet masses were determined with a Cahn 29 Automatic Electrobalance (Cahn, Cerritos, CA). Tablets dissolved for HPLC analysis were ground to a fine state in a glass mortar and then dissolved in 400 mL of 0.1 M HCl using a magnetic stirring plate (Thermolyne, Dubuque, IA). The near-infrared spectrophotometer was an InfraAlyzer 500 (Bran+Luebbe, Elmsford, NY). The spectrophotometer was operated with an IBM PS/2 model 50 computer (International Business Machines, Armonk, NY), and was capable of scanning the spectral range between 1100 and 2500 nm in 2-nm increments with a 10-nm bandpass. Data analysis was performed using a MicroVAX II computer (Digital Equipment, Maynard, MA). The IBM computer ran IDAS data-collection software (Bran+Luebbe), while the MicroVAX ran software written by the

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authors in Speakeasy IV Epsilon (Speakeasy Computing, Chicago, IL).

A hydrator was constructed to control the conditions under which the tablets decomposed. Tablets were exposed to moisture or pH 9.0 ammonium hydroxide solution by placing each tablet in an uncapped scintillation vial and supporting the vials over the liquid inside a closed glass container (hydrator) with ground glass seals. When tablets were removed from the hydrator, the scintillation vials were capped until the moment of weighing or analysis.

The equipment used for HPLC analysis of salicylic acid included a Spectroflow 773 absorbance detector (ABI Analytical, Ramsey, NJ), a 3390A integrator (Hewlett-Packard, Avondale, PA), a C-18 column (Analytical Sciences, Santa Clara, CA), a model 6000 A chromatography pump, and a 712 Wisp autoinjector (Waters Associates, Milford, MA). The mobile phase was 27:73 methanol:water, 0.15 M NaH_2PO_4, 5 mM tetrabutylammonium phosphate (Sigma) at pH 6.0. The pH–rate profile for the hydrolysis of aspirin (not shown) demonstrated that a mobile phase pH of ~2.3 would minimize the hydrolysis of aspirin after injection onto the chromatographic system. However, a system that was running and available to us in the college used a mobile phase of pH 6.0 for separation of peaks from biological samples. The half-life of aspirin at pH 6.0 is >5 days. Retention times for aspirin were ~5 min. No attempt was made to subtract any salicylic acid formed during analysis from the amount formed in the hydrator for tablets involved in this study. The mass of salicylic acid detected in eight control tablets (no exposure to moisture or base) averaged 0.264 mg and the range of masses was 0.119 mg. The prediction equation for determination of salicylic acid by near-infrared spectrometry would improve only slightly if aspirin hydrolysis during analysis was ruled out by adjusting mobile phase pH.

Figure 1 is a drawing of the sampling optics that were used to examine intact tablets in the InfraAlyzer 500 spectrophotometer. This optical system was suspended beneath a gold-coated integrating sphere containing lead-sulfide detectors. A window in the integrating sphere opened onto the tablet and main body of the sampling optics. A nickel-chromium wire was used to support individual tablets over a pair of reflective cones. The main body was machined from solid aluminum. The primary cone in the main body (opening upward toward the tablet) was a 90° right-circular cone with a polished curved surface. The secondary cone (labeled polished insert in Figure 1) was a smaller 90° cone oriented in the opposite direction to the first. The sampling optics were designed to simultaneously illuminate the top, bottom, and side of each tablet, concentrating the available light on the tablet and maximizing the signal-to-noise ratio without brightly illuminating one small spot on the tablet (which might inordinately emphasize a small tablet inhomogeneity).

Mathematical Analysis—Principal-component regression (PCR) was employed on near-infrared spectral data because PCR offers certain advantages over other quantitative methods.[9] Transformation of spectral data to principal axes simplified the selection of variables for regression and permitted variables to be added or deleted from the regression (calibration) equation without changing the coefficients of the remaining variables. Spectral variations (from sources other than changing analyte values) could be removed easily from the calibration by this method. Calibration equations developed through PCR can be used with either filter- or monochromator-based near-infrared instruments. Plotting the elements of the transformation matrix (the matrix that described how to move from wavelength-space to principal-axis space and back) versus wavelength provided approximate spectra of the analytes, effectively separated from the bulk of the sample matrix.

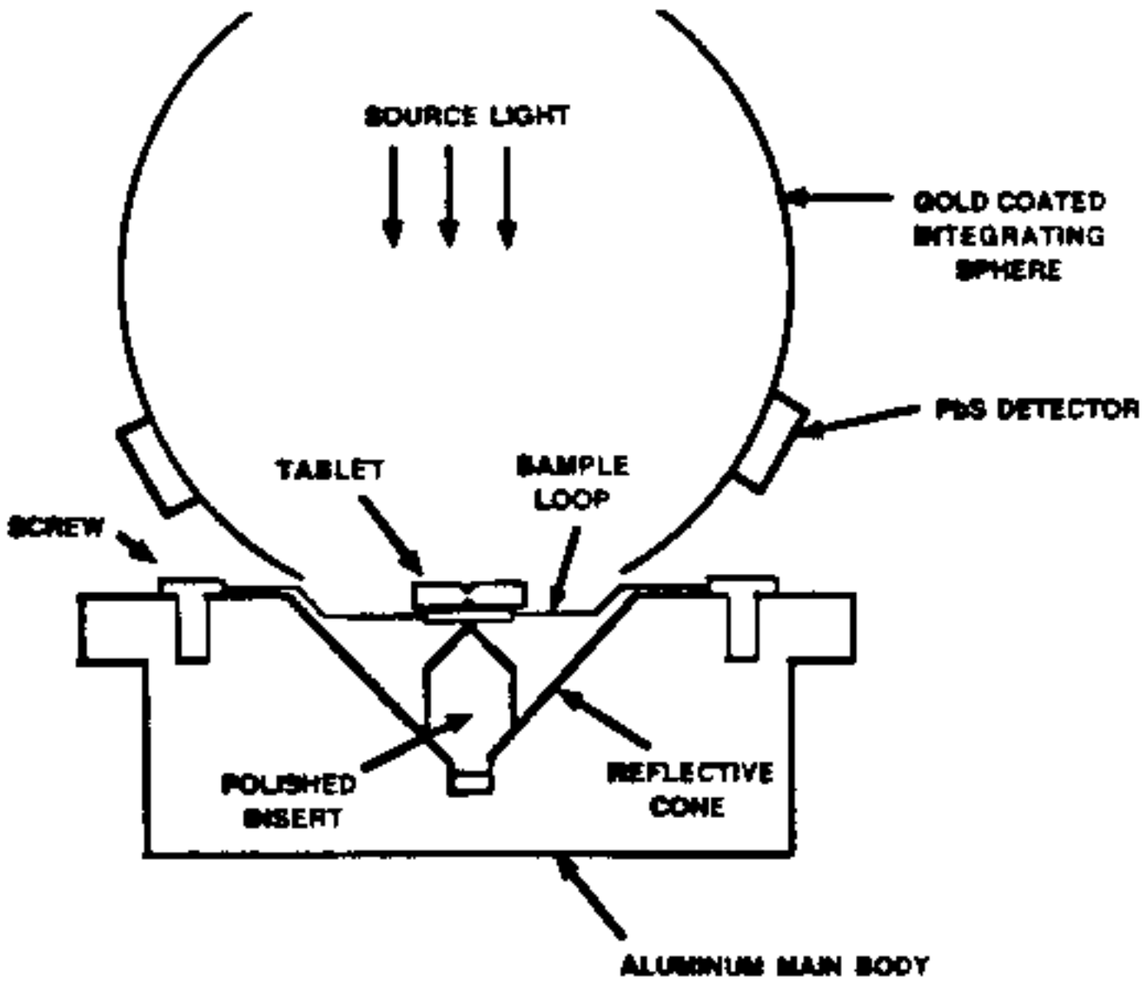

Figure 1—Cross-section of optical sampling system for tablets. The device is mounted beneath a gold integrating sphere that has a quartz window in its bottom. The quartz window is positioned above the tablet.

The Bootstrap Error-Adjusted Single-sample Technique (BEAST) represents a new type of analytical procedure, designed to operate in the high-speed parallel or vector mode required of pattern-recognition involving thousands of samples.[10] The BEAST was used to provide both quantitative and qualitative analyses on intact products. The BEAST began by treating each wavelength in a spectrum as a dimension in hyperspace. A spectrum recorded using 701 wavelengths became a single point in a 701-dimensional hyperspace, translated from the origin along each axis by an amount that corresponded to the magnitude of the signal observed at each wavelength. Samples with similar spectra mapped into clusters of points in similar regions of hyperspace, with larger cluster sizes corresponding to samples with greater intrinsic variability.

The BEAST developed a discrete estimate of the total sample population using a small set of known samples (e.g., tablets). A point estimate of the center of this tablet population was also calculated. When a new tablet was analyzed, its spectrum was projected into the same hyperspace as the known tablets. A vector was then formed in hyperspace to connect the center of the population estimate to the new sample spectral point. A hypercylinder was formed about this vector to contain a number of estimated-population spectral points. The density of these points in both directions along the central axis of the hypercylinder was used to construct an asymmetric nonparametric confidence interval. The use of a central 68% confidence interval produced BEAST distances analogous to standard deviations.

The BEAST standard deviations were used to identify sample constituents. When a new sample spectrum projected to a point within three standard deviations of the center of a cluster of spectral points from a known substance or product, the new sample was considered to be a sample of the known material. The known product was either a pure substance or a mixture of components. When the new sample contained different substances or components in concentrations that differed from the known product, the new sample spectral point was displaced from the known spectral cluster. The magnitude of this displacement increased as the differences between the new sample and the set of known samples increased. Furthermore, the direction of displacement of the new sample point corresponded to the spectra of the constituents responsible for the displacement. The technique was thus useful in both quantitative and qualitative analysis.

Spectral Processing—Several different mathematical procedures were also implemented in the Speakeasy programming language to preprocess the spectral data. The first of these procedures was a median-based filtering procedure that was applied to the raw spectral data. This procedure required three scans of each sample, thus creating three reflectance values at each recorded wavelength. The filtering process retained only the median value at each wavelength and eliminated the upper and lower values. The process of taking medians at each wavelength eliminated "spikes" in the spectra that arose as a result of a high gain setting on the PbS detector preamplifier. The high gain occasionally caused the analogue-to-digital converter in the spectrophotometer to oscillate. Nevertheless, the high-gain setting increased overall spectral reproducibility, albeit at the expense of an occasional noise "spike".

A digital smoothing program was applied to the median spectral data. Smoothing was done in the wavelength domain using a 10-nm moving average.

After smoothing, transformation of the spectra to principal axes was performed. This transformation required the spectra (collected at 701 wavelengths) to be projected as points in a 701-dimensional space. As was the case with the BEAST, the coordinates along each axis represented the magnitude of the signal observed at each

wavelength. Transformation to principal axes was useful as a preprocessing treatment for spectra before analysis using the BEAST because transformation to principal axes simplified the 701-dimensional space by removing the wavelengths that added only noise information. Spectra originally recorded at 701 wavelengths and described by points in a 701-dimensional space were often expressed through transformation to principal axes as points in a space of as few as four or five dimensions.

The principal-axis transformation process was effectively a two-step procedure. The first step involved the translation of the Cartesian coordinate system defined in wavelength space to the center of a spectral cluster. The second step was the rotation of the Cartesian coordinate system to describe, as nearly as possible, all of the variations present in the spectral cluster. The coordinate system remained rectangular throughout the process and was moved rigidly from one position to another. The rotation step decomposed the spectral variation into orthogonal (or independent) components. In the process of calculating each principal axis, the perpendicular distances between the spectral data points and each axis were minimized. The first principal axis was defined to describe the largest amount of spectral variation; this variation was then effectively subtracted from the data cluster. The second principal component was defined in a manner similar to the first, except that during the rotation to the second principal axis (the second largest axis of variation), the second axis was kept perpendicular to the first. This orthogonality condition forced each component to account for the maximum spectral variation remaining in the cluster, independent of the variation of the preceding components. In most cases, only a small number of iterations of this process[4-6] were required before the rotation-subtraction process was describing merely random spectral noise. At this point, further principal axes were ignored. The transformation process ultimately produced spectral coordinates that were expressed in a reduced wavelength space. The principal-axis transformation process decreased the problems created by many noise sources and by the highly correlated nature of near-infrared wavelength data.

Multiple least-squares procedures were applied to the spectra in the reduced (principal-component) space to calculate coefficients of a hyperplane equation. The regression procedure fitted a hyperplane to an (n + 1)-dimensional space that described the spectral wavelengths and the concentration of the analyte of interest (which was generally water or salicylic acid). The regression coefficients (coefficients of the hyperplane equation in principal-axis space) did not change when components (axes or pseudowavelengths) were added or deleted, so coefficients corresponding to noise sources were simply dropped from the regression equation to increase the resistance of the method to spectral noise.

Cross validation was employed to verify that the hyperplane produced by the modeling process was actually the best one to use. A cross validation program took a set of samples (the training set) and fit the best hyperplane to these samples using reference analyte values obtained by weighing or HPLC. A standard error of estimate (SEE) was calculated from these training samples. The validation step was accomplished using samples that were not incorporated in the training set. The analyte values of the validation samples were predicted by a calibration equation developed on the training set, and a new standard error, called the standard error of prediction (SEP), was calculated. The goal of the modeling process was to minimize the SEE and SEP. When the SEE and SEP were minimal and approximately the same, the pattern-recognition process was said to have produced a valid pattern-recognition model.

Results

Figure 2 shows the spectra of water, aspirin, and salicylic acid. The water was scanned in a disposable liquid microcell that uses a concave microscope slide and glass coverslip to hold the sample.[11] In this cell, liquids are placed in the cavity and covered with the coverslip to prevent evaporation. To collect spectra, the slide is placed over an aluminum reflecting cone similar to the one used in this experiment, but using a secondary reflective insert with a 135° apical angle. The salicylic acid crystals were scanned in a traditional powder cup that compacts powders or crystals against a quartz window for near-infrared illumination. The aspirin spectrum

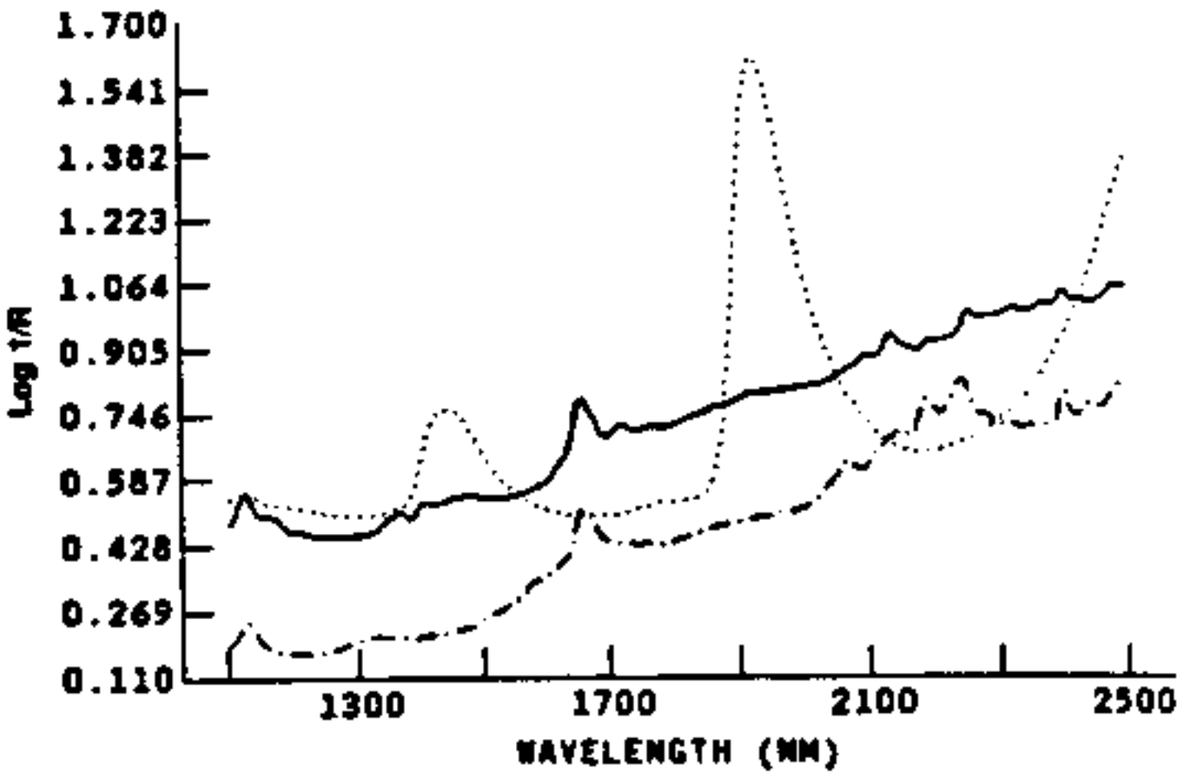

Figure 2—Near-infrared spectra of water (dotted line), aspirin tablet (solid line), and salicylic acid crystals (dot-dashed line).

is a result of scanning an aspirin tablet in the tablet holder that has been described.

Three experiments were performed. Changes in near-infrared spectra were correlated to the time that aspirin tablets spent in a hydrator, the mass of water absorbed by aspirin tablets, and the mass of salicylic acid formed by base-catalyzed hydrolysis. Figure 3 depicts typical aspirin tablet spectra for fresh tablets and tablets that have spent time in the hydrator. Figure 3 is taken from the experiment where the mass of salicylic acid formed by hydrolysis was determined spectroscopically; it shows the spectra of six tablets before exposure to basic conditions and the spectra of those same tablets after exposure to basic conditions (spectra taken at 24, 24, 48, 84, 92, and 92 h of exposure in the hydrator). The six initial scans can be seen grouped together at 1900 nm and ~0.7 absorbance units. After exposure to base, the baseline is seen first to drop (for two scans) and then to increase (for the remaining four scans). The peaks resulting from water are very visible near 1450 and 1930 nm.

To better visualize the changes taking place, the original spectra have been subtracted from the final spectra to produce the corrected spectra in Figure 4. The water peaks are again seen to increase over time, but two other features are more important. First, and most visible, is the peak arising at ~1680 nm. This peak becomes more prominent as time elapses, apparently because of the production of salicylic acid. However, this peak is not unique to salicylic acid (it also

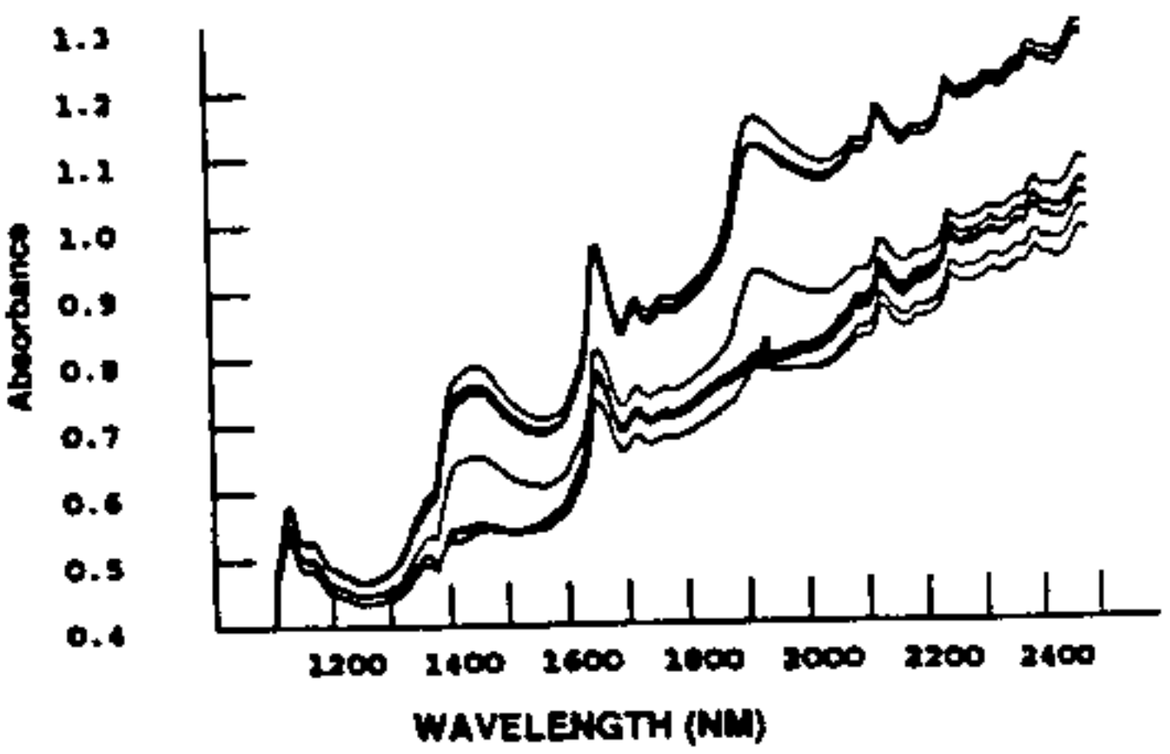

Figure 3—Near-infrared spectra of six aspirin tablets before exposure to aqueous base in the hydrator (ammonium hydroxide, pH = 9) and after exposure to base. The tablet spectra obtained before exposure to base form the tight group of six spectra labeled (a).

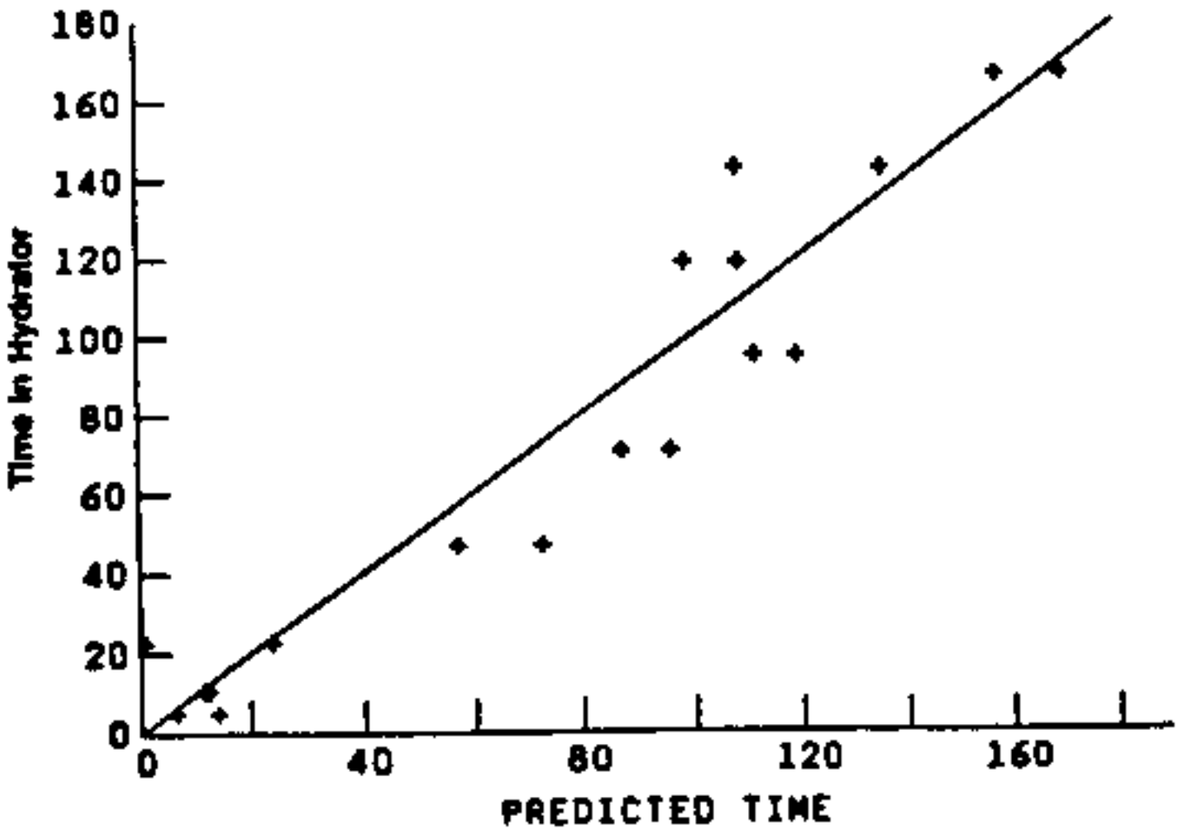

Figure 8—Scatterplot showing the actual time that 18 aspirin tablets spent in the hydrator and the time predicted by analysis of the 18 near-infrared tablet spectra (time is given in hours). The solid line represents a theoretical perfect correlation between the actual time and the predicted time.

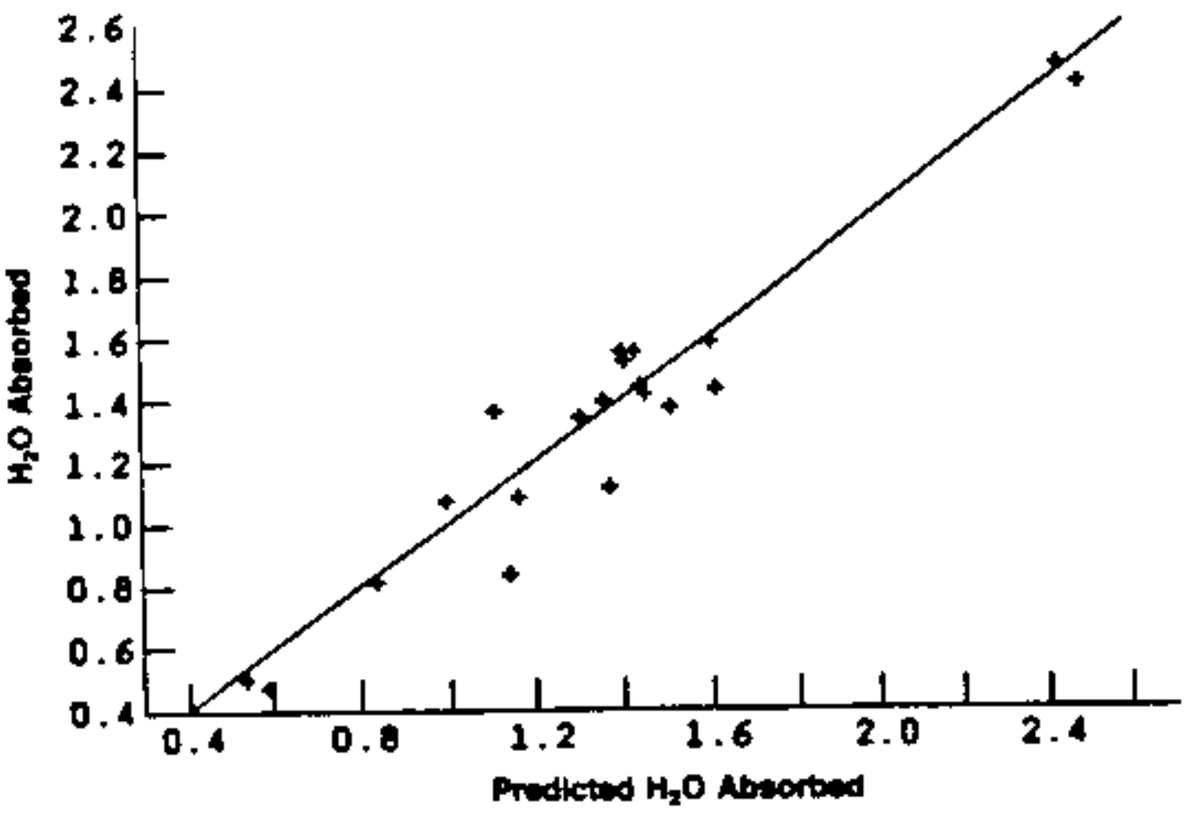

Figure 9—Scatterplot depicting the actual mass of water absorbed by 20 aspirin tablets (after exposure to moisture in the hydrator) and the mass predicted by analysis of the 20 near-infrared tablet spectra (mass is given in milligrams). The line represents a theoretical perfect correlation between the actual mass absorbed and the predicted mass absorbed.

variation in the extent of water absorption in the tablets is apparent in the plot, despite the fact that all of the tablets were in the hydrator for the same amount of time.

Discussion

The mass of salicylic acid formed by hydrolysis in intact aspirin tablets has been determined nondestructively using near-infrared spectrometry with an error of only 144 μg. This error is only 0.04% of the total tablet mass (~400 ppm).

The mass of water actually absorbed by the aspirin tablets was determined by weighing the tablets on a microbalance before and after exposure to moisture in the hydrator. However, once the tablets were removed from the hydrator, the water that they had absorbed began to evaporate. It is possible that the correlation between changes in near-infrared spectra and the mass of water absorbed by the tablets would have been stronger if a reference method superior to weighing had been used. But, the actual mass of water involved was so small that conventional moisture-determination methods, such as Karl Fischer titration, could not be employed.

The near-infrared method involves a process of correlation, and the amount of salicylic acid that is formed by hydrolysis increases with the amount of aqueous base absorbed by the tablets. Therefore, one must investigate (*a*) the possibility that the change in the spectra that correlates to salicylic acid formation is just a correlation to water absorption, and, therefore, (*b*) that the spectral changes shown in Figure 4 result merely from changes in water and acetylsalicylic acid in the tablets. One way to check these possibilities is to examine the spectrum of the vector that forms the principal axis that correlates to salicylic acid, and determine whether this spectrum resembles the spectrum of water, aspirin, or salicylic acid.

Figure 10 displays the spectra of the second column of the principal-axis transformation matrix (which correlates to salicylic acid and water concentrations), salicylic acid crystals, and an aspirin tablet. The salicylic acid spectrum increases gradually from 1450 to 1640 nm, while the aspirin spectrum increases more suddenly and steeply between 1600 and 1640 nm. The two reagent spectra are conspicuously different in this region. The second column of the principal axis transformation matrix (representing the weight or relative importance of each wavelength in construction of the second principal component) is nearly a duplicate of the salicylic acid spectrum. This similarity suggests that the salicylic acid formed by hydrolysis of aspirin significantly changes the spectra of aspirin tablets after exposure to aqueous base, and that the correlation to salicylic acid results from salicylic acid formation and not some correlated process.

Conclusions

Moisture in intact tablets was measured in this study. More importantly, the decomposition of these intact tablets was followed by a regression model that related two principal components of near-infrared spectral data to the mass of the degradation product formed.

Presently, it appears practical to use near-infrared spectrometry to perform identity and content uniformity tests, as well as certain chemical tests (such as testing aspirin for salicylic acid), on samples of intact tablets from every lot produced and recognize a savings in time and expense over the traditional methods of assay. In the near future, using fast parallel-processing computers with near-infrared instru-

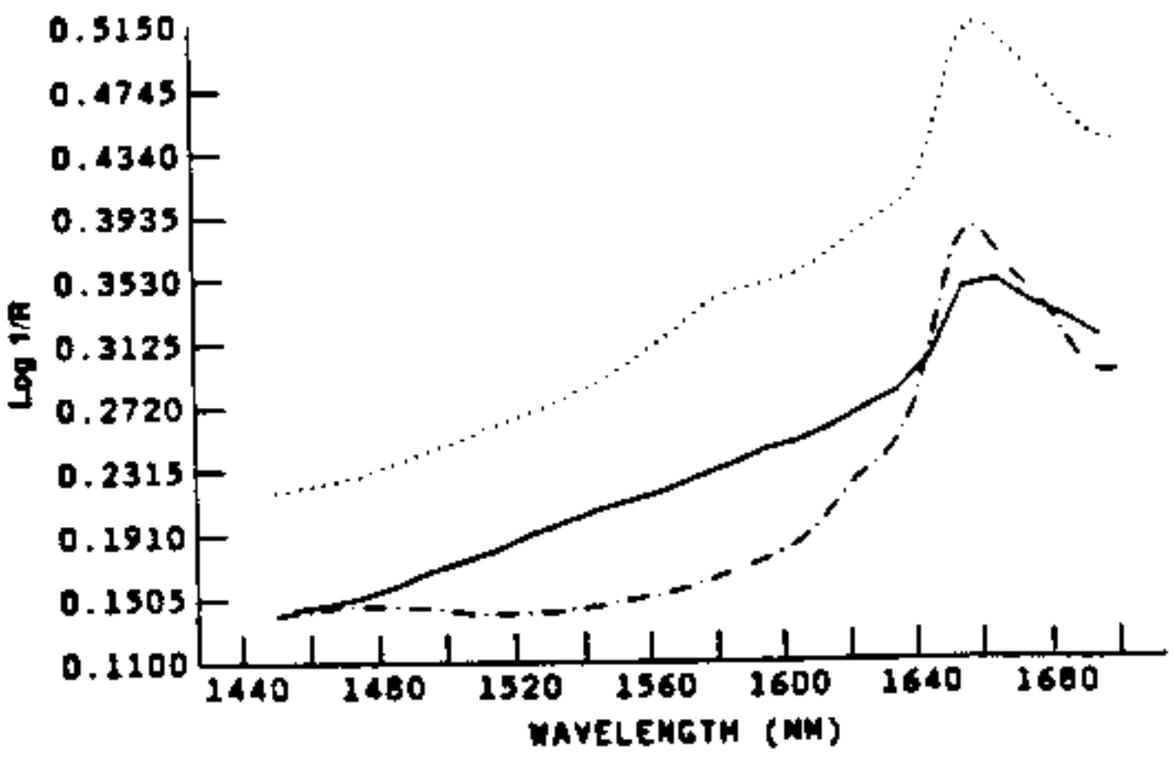

Figure 10—The near-infrared spectrum of an aspirin tablet (dot-dashed line), salicylic acid crystals (solid line), and the elements of the transformation matrix that form the second principal component (dotted line). The second principal component correlates to salicylic acid formed, and the spectrum of its column in the transformation matrix resembles salicylic acid more than it resembles aspirin.

ments and optics specially adapted to the pharmaceutical production line, it should be possible to test every tablet that is produced. Other dosage forms will be susceptible to this technology as well.

References and Notes

1. Gemperline, P. J.; Webber, L. D.; Cox, F. O. *Anal. Chem.* 1989, *61*, 138–144.
2. Gimmett, R.; Luong, A. T. *J. Pharm. Biomed. Anal* 1987, *5*, 205–211.
3. Buchanan, B. R.; Ciurczak, E. W.; Grunke, A. Q.; Honigs, D. E. *Spectroscopy* 1986, *3*, 54–56.
4. Ciurczak, E. W. *Appl. Spectrosc.* 1987, *23*, 47–16.
5. Lodder, R. A.; Hieftje, G. M. *Appl. Spectrosc.* 1988, *42*, 556–558.
6. Lodder, R. A.; Selby, M.; Hieftje, G. M. *Anal. Chem.* 1987, *59*, 1921–1930.
7. Torlini, R. P. *1989 Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy*, Atlanta, GA, March 1989; paper 401.
8. *U.S. Pharmacopeia*, 21st rev.; U.S. Pharmacopeial Convention: Rockville, MD, 1985; p 78.
9. Cowe, I. A.; McNicol, J. W. *Appl. Spectrosc.* 1985, *39*, 257–265.
10. Lodder, R. A.; Hieftje, G. M. *Appl. Spectrosc.* 1988, *42*, 1351–1365.
11. Lodder, R. A.; Hieftje, G. M. *Appl. Spectrosc.* 1988, *42*, 518–519.

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