Time and wavelength resolved spectroscopy of turbid media using light continuum generated in a crystal fiber

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Time-Resolved NIR Spectroscopy for Quantitative Analysis of Intact Pharmaceutical Tablets

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Near-infrared (NIR) spectroscopy is a useful technique for quantitative measurements of intact tablets, but it suffers from limitations due to the fact that changes in the physical properties of a sample strongly affect the recorded spectrum. In this work, time-resolved transmission NIR spectroscopy was utilized to conduct quantitative measurements of intact tablets. The technique enables separation of the absorption properties of the sample from the scattering properties and can therefore handle changes of the physical parameters of the samples in a better way than conventional NIR transmission spectroscopy. The experiments were conducted using a pulsed Ti:sapphire laser coupled into a nonlinear photonic crystal fiber as light source. The light transmitted through the sample was measured by a time-resolving streak camera. A comparison of the results from the time-resolved technique with the results from conventional transmission NIR spectroscopy was made using tablets containing different concentrations of iron oxide and manufactured with different thicknesses. A PLS model made with data from the time-resolved technique predicted samples 5 times better than a PLS model made data from the conventional NIR transmission technique. Furthermore, an improvement to predict samples with physical properties outside those included in the calibration set was demonstrated.

In recent years, the application of transmission near-infrared (NIR) measurements of intact tablets has emerged as a useful technique for quantitative measurements.[1,2] It is faster than the traditionally used chromatographic methods, and it does not have the problems with subsampling that the reflectance NIR measurements have.[3] The ability to make transmission measurements of intact tablets relies on the relatively low absorption in this wavelength region. The main absorption bands in the NIR region are vibrational overtones and combination bands of hydrogen bonds, having their fundamental absorptions in the mid-infrared region. The transition probability of the overtones is much lower than that for the fundamental transitions, which makes the measured absorbance in a solid pharmaceutical sample in this region low, especially in the wavelength range 800−1400 nm, where the water absorption is low. Multiple wavelengths together with multivariate analysis are regularly employed in order to make quantitative calibrations, since the broad and often overlapping absorption bands often cannot be coupled to one specific component in the tablet. Previously, multiple linear regression was the preferred choice, but the development of new multivariate tools has made the partial least squares (PLS) the most frequently used calibration method for NIR spectroscopy today.[4] In addition to NIR spectroscopy, other thechniques, such as Raman spectroscopy, have proven useful for pharmaceutical analysis. However, Raman spectroscopy also suffers from limitations, in particular the small sampling volume associated with Raman spectroscopy of solids.[5,6]

Light scattering in intact tablets is due to the many microcavities causing rapid spatial changes in refractive index within the tablet. The scattering properties of a tablet are thus very dependent on the manufacturing process, e.g., on the compression force, grain size, etc. The scattering in a tablet is ∼3−4 orders of magnitude larger than the absorption, which leads to very long optical path lengths. For example, a transmission measurement through a 3.5-mm-thick tablet results in a mean optical path length of 20−30 cm.[7] There is, however, a huge span in effective path lengths in such a measurement. Some photons have path lengths as short as a few millimeters, while others have bounced back and forth within the tablet for as long as a meter before they can escape and reach the detector. From this it can be understood that variations in tablet density, radius, and thickness also affect the measurement and that small changes in the physical parameters during the manufacturing process can affect the NIR transmission signal more than the concentration variations to be measured.

Several data pretreatment methods have been developed to try to compensate for the scattering in the tablets, and in that way improve the quantitative calibrations. The standard normal

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deviate,[8] multiplicative scattering correction,[9] and orthogonal signal correction[10] are all examples of pretreatment methods used to enhance the ability to construct accurate PLS calibrations. Despite the many efforts, it has still been proven hard to incorporate tablets from different batches or tablets manufactured under different conditions into the same quantitative calibration model with a good result.

Similar problems, to accurately measure the absorption if the scattering is not well known, are faced in another field, biomedical optics. In that field, both diagnostic measurements and laser treatment dosimetry depend critically on the optical properties of tissue. It is thus important to be able to measure those. Several methods to measure these properties have been developed, including the integrating sphere,[11] spatially resolved,[12] and time-resolved measurements.[13]

Techniques to measure the optical properties developed primarly for medical applications have been used in some pharmaceutical applications. Scattering and absorption properties have been measured in order to calculate the effective sample size in diffuse reflectance NIR spectroscopy of powders[14,15] as well as for particle size analysis.[16] Measurements of the optical properties have also been used to make quantitative measurements of pharmaceutical powder blend homogeneity.[17]

When using time-resolved measurements, the optical properties of a sample can be assessed by analyzing the broadening of a short light pulse after the passage through the sample. Several methods and models have been developed to extract the optical measurements from such measurements at discrete wavelengths.[18,19] These models show that a change in the scattering coefficient mainly alters the peak position, while a change in the absorption coefficient will alter the final slope of the time dispersion curve.

In our previous work, we investigated the basic capabilities of time-resolved NIR spectroscopy as an analytical tool for spectroscopic analysis.[7] In this work, we report on measurements performed using a novel instrument for time-resolved broadband NIR spectroscopy. The results demonstrate the capability to separate absorption from scattering properties of pharmaceutical tablets using time-resolved spectroscopy and thereby allow recordings of pure absorption spectra. Improved quantitative assessments are thus possible and also for samples with scattering properties different from those covered by the calibration samples.

Table 1. Summary of the Batch ID of the Tablets Used in This Work

	concentration iron oxide (%)			
weight (mg)	0,1	0,08	0,065	0,05
300	A1	B1	C1	D1
400	A2	B2	C2	D2
500	A3	B3	C3	D3

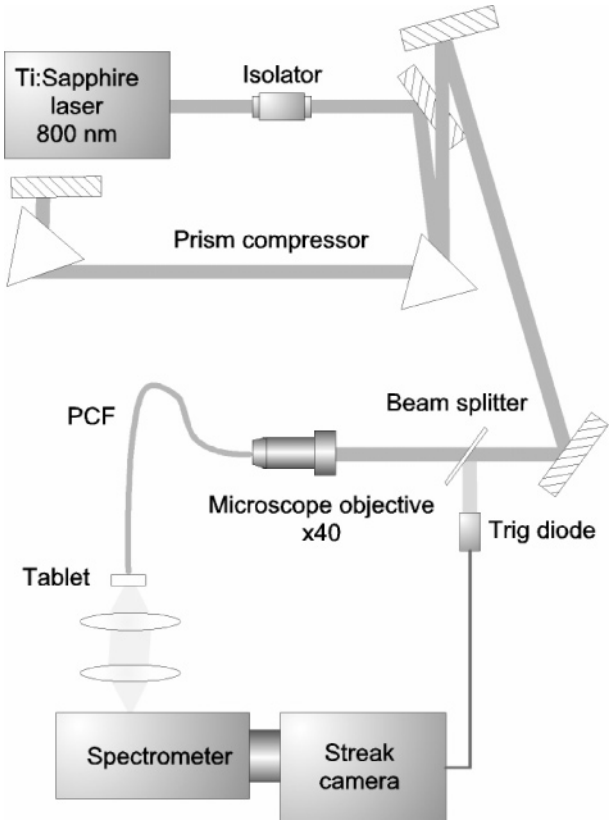

Figure 1. Overview of the instrumentation for time-resolved NIR measurements.

EXPERIMENTAL SECTION

Samples. The tablets used in this work consisted of red iron oxide and microcrystalline cellulose (MCC). The MCC and iron oxide were weighed and mixed in a mortar. The powder blend was pressed into tablets with a diameter of 9 mm using a manual tablet press. The compression force was held constant for all tablets. The tablet set consisted of four concentration levels (batches A−D) and three tablet thicknesses (batches 1−3). A total number of 27 tablets were produced with the characteristics given in Table 1. One sample from batch D2 was destroyed during the reference analysis and is therefore taken out of all evaluations.

Time-Resolved Measurements. The experimental arrangement used in the study is depicted in Figure 1.

The Ar ion laser-pumped mode-locked Ti:sapphire laser produced pulses shorter than 100 fs at a repetition rate of 80 MHz. The wavelength of the laser light was near 800 nm, and the energy of each pulse was 4 nJ. The light was focused into the PCF using

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a standard ×40 microscope objective lens with a numeric aperture of 0.65. An optical isolator was used between the laser and the optics, to prevent optical feedback into the laser due to reflections. A prism compressor was also used in the setup to compensate for the time dispersion caused by the different optical components used. The PCF (Crystal Fibre A/S) was 0.5 m long with a core diameter of 2 μm, manufactured to have zero dispersion at 760 nm. The dispersion properties of the fiber combined with core diameter results in a high peak power of the light through the entire fiber, yielding a widely spectrally broadened light emission due to nonlinear effects. The main broadening effects in the PCF are identified to be self-phase modulation and stimulated Raman scattering.[20] As a result of this, light pulses with almost the same temporal width as the laser, and a spectral width spanning from 400 nm to at least 1200 nm was accessible. The light distribution was not flat though, but modulated with peaks with high intensities surrounded by wavelength regions with low intensities. To increase the signal-to-noise ratio, three measurements were made on each sample. The PCF was slightly realigned between each measurement in order to flatten the average light distribution in the range 860−1150 nm. This provides a good signal-to-noise ratio in the entire wavelength range. The output end of the PCF was put 2 mm from the face of the tablet held into place by an circular iris holder. The spot size on the tablet was ~2 mm. The light from the backside of the tablet was imaged onto the 250-μm slit of an imaging spectrometer (Chromex, model 250 IS), coupled to a streak camera (Hamamatsu, model C5680). The system measures a 600-nm broad wavelength region with a spectral resolution of 5 nm. The streak camera operated in synchro scan mode, allowing all light pulses to be collected. A small portion of the laser light was redirected by a beam splitter onto a photodiode that triggered the streak camera sweep. The system had a total temporal range of 2.1 ns with resolution of 4.5 ps. The instrumental response function was in the range of 30 ps when averaging over 50 s.

Conventional Transmission NIR Measurements. The conventional transmission NIR spectra were measured with a NIRSystems 6500 monochromator, with a NIRSystems InTact Multi-Tab Analyzer presenting the samples. The spectra were collected in the range of 600−1900 nm, but as in previous reports, the range 800-1350 nm was used when building calibration models.

Reference Analysis. For the reference analysis, the samples were heated in beakers with 40 mL of HNO_3 (70%) for 60 min and then transferred into 100-mL flasks and diluted to volume with water. Calibration samples were made using the same method, using concentrations between 1 and 7 ppm iron oxide. The samples were then analyzed using a Perkin-Elmer 3300 atomic absorption spectrometer. By analyzing reference samples, the relative error introduced by the method was measured to be smaller than 2%.

Evaluation of Time-Resolved Measurements. The evaluation of the data was based on the shape of the time dispersion curves, and it was made entirely independent for the different wavelengths. No white light correction was therefore necessary.

To combine the three measurements in the best possible way, optimizing the signal-to-noise ratio, a threshold procedure was employed. The signal level for an individual measurement had to exceed this threshold to be included in the analysis. The threshold was set so that all wavelengths from 860 to 1150 nm was covered in at least one of the three measurements. The wavelength range was chosen to incorporate important absorption features of both constituents. The final slopes of the time-resolved signals were calculated for each individual wavelength, one at the time. The time dispersion curve for each wavelength was first normalized to have intensities between zero and one and then logarithmic transformed. A line was fitted to a 210-ps-long region 105 ps from the peak; see Figure 2. The slopes calculated at the different wavelengths were extracted, plotted as a function of wavelength, and used in the analysis.

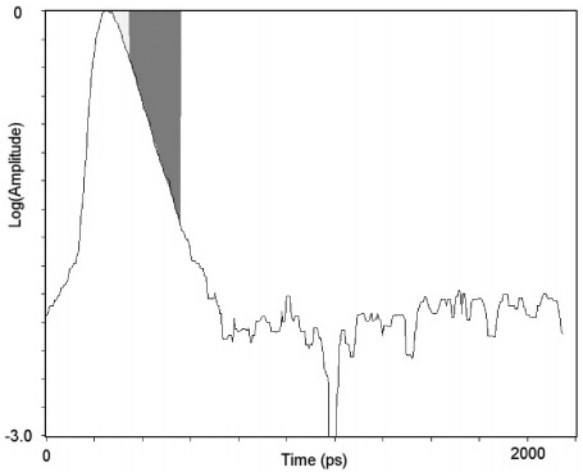

Figure 2. Evaluation of the time-resolved measurements was made by fitting a line to the final slope of the time dispersion curve for each individual wavelength. The fit was made in the shaded region of the curve.

Multivariate Analysis. All multivariate models were made in Simca-P 10.0 (Umetrics AB, Umeå, Sweden). All spectra were mean centered before calculations, and the number of principal components selected in the models were as many as Simca-P 10.0 found suitable. The program uses the cross-validated predicted fraction for both X and Y to find the optimal number of PLSCs. The software found the optimal number of principal components to be three for all models in this work.

RESULTS AND DISCUSSION

Design of Technical Device. The introduction of a photonic crystal fiber and a synchroscan streak camera unit into the instrumentation extended the measurement capability further out into the NIR region. It is important to reach the second overtone region starting at 1100 nm in order collect good absorption data from pharmaceutical samples, and the measurements in this work reached wavelengths as high as 1150 nm with a sufficient signal-to-noise ratio. The instrumentation is a complex laboratory system that is not easily adapted to simple applications.

The simple, but effective evaluation scheme used in this work had the big advantage of evaluating an intrinsic property at each wavelength independently. The evaluation was therefore independent of both the spectral distribution of the light source and the intensity of the light and why no normalization or white light correction was needed. The evaluation scheme had the limitation of extracting only the absorption properties, but new and more

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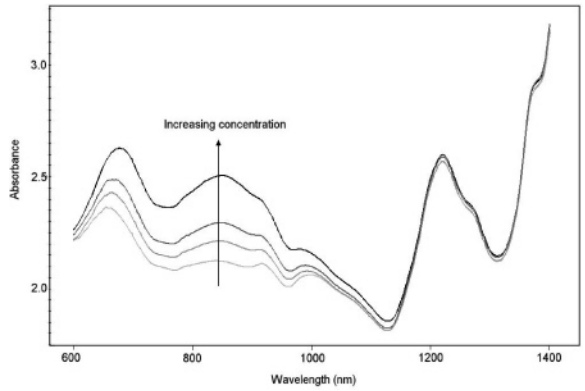

Figure 3. Conventional transmission NIR data from measurements of tablets with the same thickness but with different concentrations of iron oxide. The tablets all weighed 400 mg and contained 0.05, 0.065, 0.08, and 0.1% iron oxide, respectively.

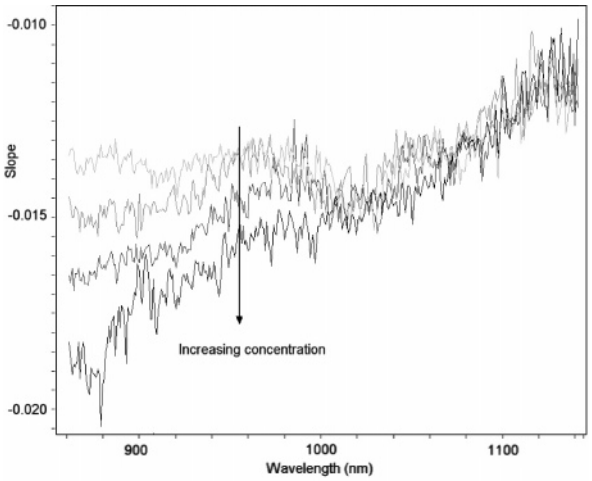

Figure 4. Decay slope versus wavelength from measurements of tablets with the same thickness, but with different concentrations of iron oxide. The tablets all weighed 400 mg and contained 0.05, 0.065, 0.08, and 0.1% iron oxide, respectively.

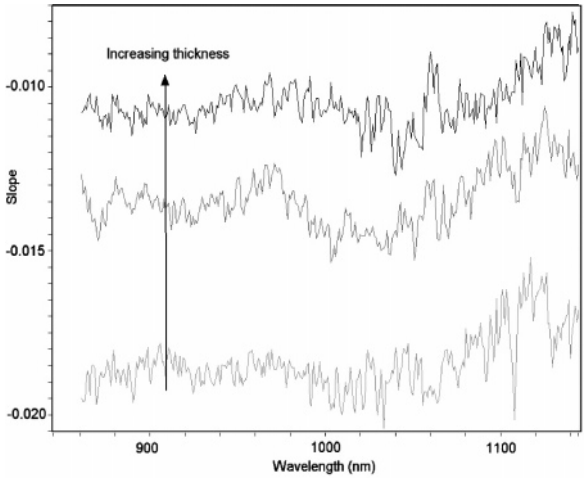

Figure 5. Decay slope versus wavelength from measurements of tablets with the same concentration of iron oxide, but with different thicknesses. The tablets contained 0.05% iron oxide and weighed 300, 400, and 500 mg, respectively.

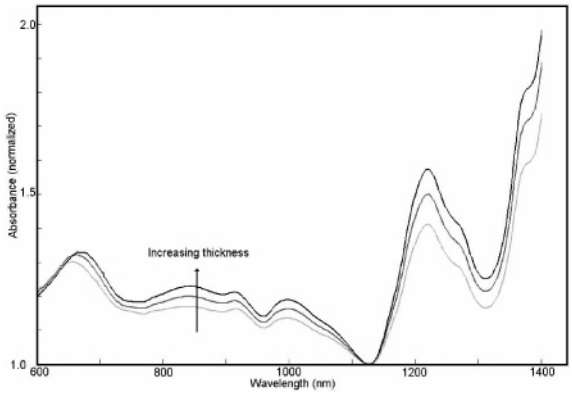

Figure 6. Conventional transmission NIR data from measurements of tablets with the same concentration of iron oxide but with different thicknesses. The tablets contained 0.05% iron oxide and weighed 300, 400, and 500 mg, respectively.

complex evaluation tools that allow the extraction of both the absorption and scattering properties of the sample are under development and will further enhance the technique.

Mechanistic Basis. Results from the conventional transmission NIR measurements are shown in Figure 3. The figure shows absorption spectra from four tablets with the same thickness but with different concentrations of iron oxide. The spectra exhibit a distinct absorption peak from MCC around 1200 nm. The main absorption band from iron oxide is prominent in the lowest wavelength region, below 1000 nm. The spectra from the different tablets exhibit different attenuation in this wavelength region, with the highest concentration of iron oxide having the highest attenuation. Figure 4 shows the extracted slope coefficients as a function of wavelength from time-resolved measurements of the same four tablets. The measurements were made in the wavelength region 860−1150 nm. The slope spectra are relatively noisy, partly due to the low signal in the time-resolved measurement, but also due to the fact that these spectra are evaluated from the slope of a curve rather than a plot of the signal level itself. This type of analysis is very sensitive to the noise in the original signal. When the absorption of a sample is high, the final slope of the time dispersion curve is steeper than the final slope of the time dispersion curve from a sample with low absorption. This is clearly seen in the figure as a steeper slope in the wavelength region below 1000 nm for tablets with higher concentration of iron oxide. Comparing the time-resolved slope coefficients with the conventional transmission NIR measurement yields clear similarities. The slope spectra are noisier than the conventional NIR spectra, but both show differences between the tablets in the region 850−1000 nm where the iron oxide absorbs the light.

Figure 5 shows the slopes as a function of wavelength from measurements of tablets with the same nominal iron oxide concentration but with different thicknesses. The slope spectra are noisy, and the change in thickness between the tablets introduces an offset in the slope values. Unlike conventional transmission NIR measurements on tablets with different thicknesses, see Figure 6, where the longer path length of the light will introduce not only an offset of the absorption profile but also an increased contrast, the differences in shape between the slope spectra are smaller than the noise level. This is because the slope in the slope spectra is proportional to the path length-independent absorptivity, while the conventional NIR spectra are proportional to the total attenuation, which is strongly dependent on the optical path length.

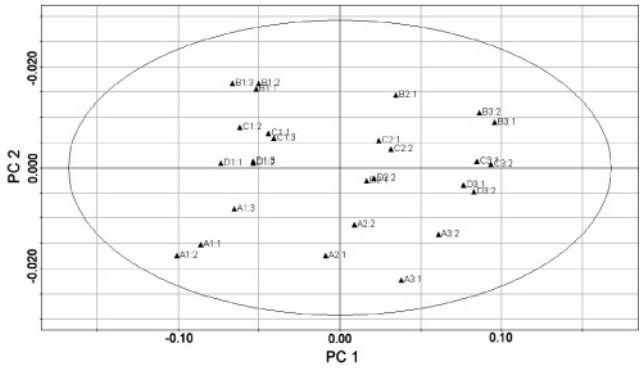

Figure 7. Score plot of a PCA model made from all 26 samples using the slopes calculated from the time-resolved measurements. The scores are from the first two principal components of the PCA model.

Assay of Chemical Content. A PCA model was made from all 26 samples using the slopes calculated from the time-resolved measurements. The wavelength range 850−1160 nm was used in the calculations. A score plot of the first two components was constructed to display the main contribution to the spectral information, see Figure 7. It was found that the samples are spread in two almost orthogonal directions. Principal component 1 divides the samples into the three different thickness groups, while principal component 2 spread the samples according to their concentration of iron oxide.

A PLS model was constructed by using 12 of the samples. This PLS model was used to predict the iron oxide concentration of the other samples. By doing this for the two different techniques, a quantitative comparison was done. The 12 calibration samples included samples from all thickness groups and all concentration levels.

The models were based on three PLS components and the RMSEP values for the conventional NIR measurements was 0.0080% (m/m) iron oxide, compared to 0.0019% (m/m) iron oxide for the time-resolved measurements. The relative prediction error in the time-resolved calibration was on the order of 2.5%, compared to 12% for the conventional NIR calibration. The latter number may seem high for a NIR method, but it is explained by the fact that the calibration model contains only 12 samples that span a wide range of thicknesses and concentrations of iron oxide. To achieve a good predictive ability of a PLS model based on conventional NIR spectra of samples with such divergent physical properties, far more than 12 samples are needed.

To compare the robustness of the two measurement techniques, calibration models were build by using all samples from two of the thickness groups and using the samples from the third thickness group as a prediction set.

The RMSEP values were in this case 0.0026% (m/m) iron oxide for the time-resolved data and 0.011% (m/m) iron oxide for the conventional NIR data. The relative error for the time-resolved data was in this case 3.5% compared to 15% for the conventional NIR data. Again, the time-resolved method is superior to the conventional NIR method and allows the analysis to be extrapolated outside the calibration range of the PLS model.

The fact that the PCA model yields two orthogonal components and that PLS calibrations are linear over a wide range and are even valid outside the calibration range support the conclusion that pure chemical information can be attained with time-resolved spectroscopy. By using the slope of the time-resolved curves, information attributed directly to light absorption and with no relation to light scattering can be attained. So far the analytical precision is limited by the determination of the slope. There should be a significant potential for improvements by using other data evaluation schemes.

CONCLUSIONS

In this work, we have used a newly developed instrumentation for time-resolved transmission broadband NIR spectroscopy to separate the absorption from the scattering of the samples. The time-resolved measurements were used to make quantitative assessments of intact tablets with different thicknesses, and the results were then compared with conventional transmission NIR spectroscopy.

The comparison shows that the time-resolved technique is better at handling physical variations of the tablets, in this case different thicknesses. Although the thickest tablets were more than 50% thicker than the thinnest, the relative prediction error was only 2.5% in a PLS model incorporating slope spectra from tablets with different thicknesses, compared to 12% for a PLS model based on the conventional transmission NIR technique. The slope spectra also showed better potential than the conventional NIR technique when predicting samples from a thickness group not included in the PLS calibration.

The time-resolved technique seems promising, for analysis of solid samples with varying physical properties, which would make the samples difficult to analyze with conventional NIR techniques. The future work will be focused on increasing the signal-to-noise ratio in the measurements and enabling measurements on real pharmaceutical tablets. Other evaluation schemes will also be developed, to get a better understanding of the interaction between the light and the sample.

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