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Time-Resolved NIR/Vis Spectroscopy for Analysis of Solids: Pharmaceutical Tablets

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Time-resolved spectroscopy in the visible and near-infrared (NIR) regions was used in a feasibility study for analysis of solid pharmaceuticals. The objective of the experiments was to study the interaction of light with pharmaceutical solids and to investigate the usefulness of the method as an analytical tool for spectroscopic analysis. In these experiments, a pulsed Ti:sapphire laser and white light generation in water was utilized to form a pulsed light source in the visible/NIR region. The light was focused onto the surface of tablets, and the transmitted light was detected by a time-resolving streak camera. Two types of measurements were performed. First, a spectrometer was put in front of the streak camera for spectral resolution. Secondly, the signal originating from different locations of the sample was collected. Time-resolved and wavelength/spatially resolved data were generated and compared for a number of different samples. The most striking result from the experiments is that the typical optical path length through a 3.5-mm-thick tablet is about 20–25 cm. This indicates very strong multiple scattering in these samples. Monte Carlo simulations and comparison with experimental data support very high scattering coefficients on the order of 500 cm^{-1}. Furthermore, the data evaluation shows that photons with a particular propagation time through the sample contain a higher chemical contrast than other propagation times or than steady-state information. In conclusion, time-resolved NIR spectroscopy yields more information about solid pharmaceutical samples than conventional steady-state spectroscopy.

Index Headings: Time-resolved spectroscopy; Near-infrared; Transmission; Diffuse reflectance.

INTRODUCTION

Near-infrared (NIR) spectroscopy[1,2] has been shown to be a highly useful tool for analysis of pharmaceuticals.[3–6] NIR analysis has been used for many diverse applications in the pharmaceutical industry, such as for qualitative and quantitative analysis of powders, pellets, whole tablets, tablets in their blister packages, freeze-dried products, etc. Among the advantages of NIR analysis, it can be mentioned that NIR is nondestructive, fast, and can be performed remotely through optical fibers—factors that make NIR analysis particularly useful for process analysis.[7] In addition, spectroscopic analysis of solids may offer probing of solid state properties such as crystallinity and sample density, parameters that are entirely lost by chromatography and other wet-chemistry methods. The discussion here is confined to pharmaceutical tablets; however, the method reported in this paper is applicable for other types of turbid samples.

Advances in NIR analysis have been substantial in instrumental development as well as in the development of data evaluation tools, mainly based on multivariate analysis. The instrumental development includes the use of different detection principles such as the Fourier transform spectrometer, the diode array detector, and the acousto-optic tunable filter approach.[8] Instrumental developments also include improvements in the sample presentation, such as fiber-optic probes and the use of transmission geometry rather than the traditional reflectance geometry.[9–12] Comparing the two geometries, transmission NIR has shown better precision than reflectance due to the fact that the entire tablet volume is probed by the light in the case of transmission geometry.[11,12]

There are, however, also some drawbacks to spectroscopic techniques in general and to NIR spectroscopy in particular. Perhaps the most important drawback stems from the fact that NIR analysis of turbid media does not strictly follow the Beer–Lambert law. In other words, the reflected or transmitted intensity is not a simple function of the absorptivity of the sample, but is also affected by multiple scattering of light in the sample volume. In fact, since in the red/NIR optical region scattering is more prominent than absorption, variations in scattering properties of a sample may alter a measured signal more than a corresponding change in absorption properties. The light scattering properties of the sample matrix are strongly affected by its physical parameters, such as tablet hardness and porosity. The more micro-cavities and variations in the refractive index of the matrix, the more prominent the light scattering will be, resulting in wavelength-dependent attenuation. Other physical parameters of relevance for tablets are the height and diameter. Small changes in the tablet diameter, shape, or thickness have strong effects on the transmission NIR spectrum. Similarly, engravings and scores on the tablet surface will have a strong effect on the reflectance NIR spectrum. The fact that NIR analysis is perhaps more sensitive to physical parameters than to chemical content limits the utility of the technique. The most common way to deal with these problems is the use of numerical transformations and spectral pretreatment before analyzing the spectroscopic data. In this context, chemometrics has evolved as a very powerful tool to extract minute variations from complex spectral data sets.

Another limitation of NIR spectroscopy of pharmaceuticals is that a strong light scattering in the matrix results in varying path lengths through the tablet. The path length is critical to how effectively the absorption will

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attenuate the signal. In a situation where the chemical content is evaluated from the recorded transmission spectrum, chemometric methods are frequently used to extract the absorption properties of interest. It is thus extremely important that this evaluation algorithm is trained using representative tablets. The quality of NIR measurements is clearly limited by small variations in the physical properties of the tablets.

To be able to measure and evaluate the effects of both absorption and scattering and to obtain a better understanding of the light propagation and attenuation in the sample, more sophisticated measurement techniques are required. Such experimental tools have been developed mainly for studying biological tissue and can roughly be divided into time- or frequency-resolved and spatially resolved techniques.[13] Using one of these general approaches, the optical properties of the sample can be elucidated. Hence, time- or spatially resolved spectroscopy have been used to map, for instance, human breast,[14] brain,[15] and skin[16] tissues, as well as green leaves from plants.[17] The optical properties of pharmaceutical solids have also been measured, e.g., in connection with determination of absorption coefficients for powders[18] and effective sample size.[19] One interesting feature found in all these studies was the strong domination of scattering over absorption for some but not all wavelength regions. The optical properties of a turbid sample can be described by the absorption coefficient μ_a, the scattering coefficient μ_s, and the scattering anisotropy g. Together, these three parameters characterize the interaction of light with the sample. Often, μ_s and g are represented as the reduced scattering coefficient $\mu_s' = (1 - g)\mu_s$. In order to model the light propagation in the sample, either hard models, such as diffusion theory, or numerical methods, such as Monte Carlo modeling, are utilized and fitted against the experimental data. In addition, chemometric tools are often used in connection with numerical fitting algorithms for a better characterization of the sample.

In this paper we have explored the use of time-resolved transmission spectroscopy as an analytical tool for pharmaceutical tablets. Both wavelength-resolved and spatially resolved data have been obtained at a high time resolution by using ultra-short pulsed lasers and a streak camera. The main objectives of the investigation were to estimate the degree of light scattering in this type of tablet and to evaluate whether this more sophisticated measurement technique can improve NIR spectroscopy. The experiments were mainly carried out in the visible rather than in the NIR optical region, although the aim is to extend future measurements further into the NIR region, where characteristic absorption features may enhance the analytical quantitative information. The obtained data are discussed in relation to conventional NIR spectroscopy to indicate the potential advantages of time-resolved spectroscopy over steady-state techniques.

MATERIALS AND METHODS

Optical Set-Up. A system for ultra-short white light illumination and picosecond (ps) time-scale detection was utilized to measure the photon propagation time through the sample tablets. The general-purpose light source was a table-top tera-watt laser system located at the Lund La-

ser Centre.[20] It used an Ar-ion laser pumping a Ti:sapphire laser as a master oscillator and one or two Nd:YAG laser-pumped amplifier stages. The Ti:sapphire laser was passively mode-locked at a frequency of 76 MHz to yield 100 femtosecond (fs) pulses at a wavelength near 800 nm. Before amplification, the pulses were sent to a pulse stretcher consisting of two gratings yielding different path lengths for different wavelengths within the line profile, creating chirped pulses. After pulse stretching, the pulses had a pulse length of about 260 ps. A Pockels cell was used to select pulses for the amplifier stages at a repetition rate of 10 Hz. The amplifier stages employed Q-switched Nd:YAG laser pumped Ti:sapphire crystals. After amplification in two stages the pulse energy could be as high as 450 mJ. The amplified pulses were sent to a compressor, where another pair of gratings was used to compress the pulses back to about 200 fs. The compressor had a transmission efficiency of about 50%. These transmission studies required substantially lower pulse energies than those available, and a maximum of 50 mJ was utilized.

The experimental set-up used for the transmission studies is shown in Fig. 1. In the case of the wavelength-dispersed measurements, the light pulse train from the laser system was focused into a water-containing cell using a 15-cm-focal-length lens (L1) for white light generation. Self-phase modulation of the refractive index was induced, resulting in a spectral profile ranging from the UV to the NIR region. As a result, laser pulses of 200 fs duration were accessible at all the desired wavelengths. The light from the white light generator was made parallel with an achromatic camera lens (L2). The light was focused with a 100-mm camera lens (L3) onto the sample, which was fixed in an iris holder allowing light to pass through the sample. The average light power used at the sample surface was approximately 1 mW and the size of the laser spot on the sample was approximately 1 mm. The transmitted light was imaged onto the 120-μm entrance slit of a spectrometer (Oriel, Model 77480) using two 50-mm camera lenses (L4 and L5). The spectrometer was equipped with one of two different gratings optimized for the blue or NIR optical region. The output from the spectrometer was imaged onto a streak camera (Hamamatsu, C1587) using a pair of camera lenses (L6 and L7) providing an overall magnification of either 1 or 0.4. In the case of spatially resolved measurements, the white light generator was removed and the light transmitted through the sample was directly imaged onto the streak camera without passing though the spectrometer. The streak camera received a trigger signal from a photodiode close to the Ti:sapphire laser. The trigger signal was preamplified, stabilized by a constant fraction discriminator, and delayed in a delay generator before it was sent to the streak camera control unit. The signal from the streak camera was fed to a PC and the time-dispersed transmission curves were stored and displayed on the monitor. The signal from up to 100 laser pulses was integrated to allow a sufficient signal-to-noise ratio. The time resolution of the streak camera was about 20 ps and the time window was about 2 ns. The optical set-up allowed a fraction of the excitation light to be split off and sent directly to the spectrometer slit in order to have a fixed time reference in the recorded time-resolved spec-
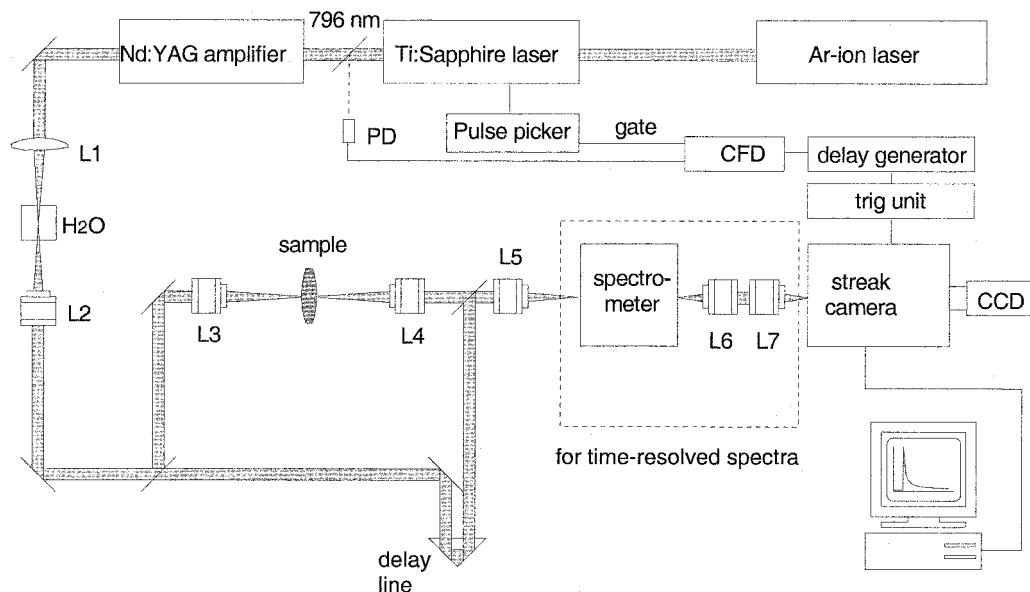
FIG. 1. Experimental set-up for time-resolved transmission of tablets. *L1*: lens, *L2–L7*: achromate camera lenses, *PD*: photo diode, *CFD*: constant fraction discriminator.

tra. Using the reference pulse it is possible to partly correct for the electronic time jitter that limits the time resolution.

Samples. *Model Samples.* Solutions of varying concentrations of intralipid were used as model substances. Different amounts of ink were added to the intralipid solution in order to obtain different absorption coefficients for the sample solutions. Detailed experimental conditions are given elsewhere.[21]

Tablets. The manufacturing and experimental design has been described in detail previously[12] and will only be described here in part. The tablets consisted of film-coated pellets (AstraZeneca R&D Mölndal) and excipients, and were manufactured according to an experimental de-

sign in which several tablet parameters were varied. The most abundant component in these tablets was microcrystalline cellulose. The heights of the tablets were 3.5 and 4 mm and the diameter was 9.0 mm. The tablets were stored at room temperature until the measurements were taken.

Data Evaluation. The transmission data obtained from the streak camera were primarily displayed as 3D matrices. In Fig. 2, a data matrix from a spatially resolved measurement is shown. The transmitted intensity is shown as a function of photon migration time (abscissa) and position of light exiting the sample (ordinate). The main information comes from the light area in the center of the image. The smaller white areas in the left part of

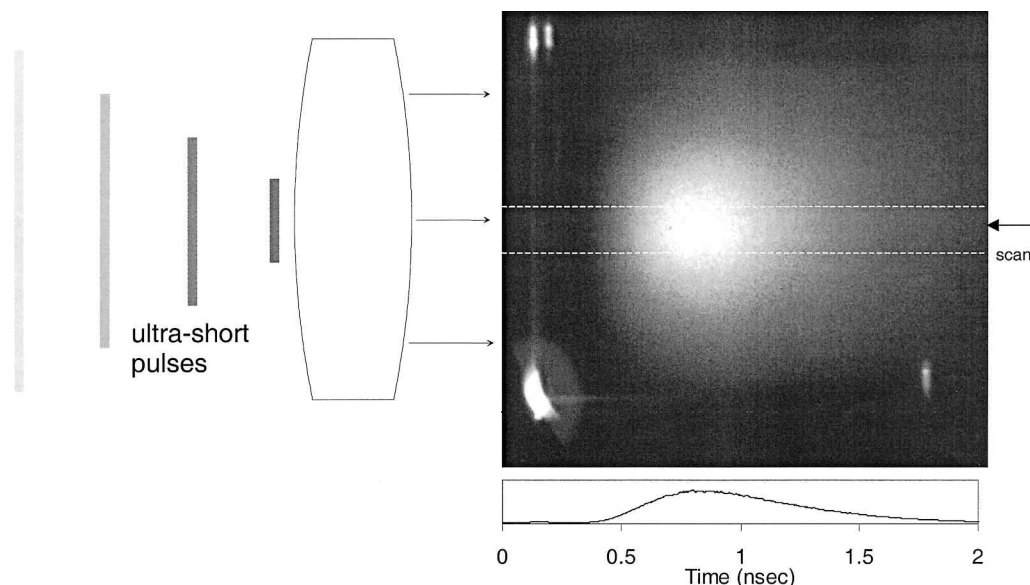FIG. 2. Time-resolved transmission of a tablet using 200 fs pulses at 790 nm. The transmitted intensity is displayed as a function of time (abscissa) and position on tablet (ordinate). Below the picture is a scan across the picture at the position indicated between the dashed lines. The bright spots to the far left in the picture originate from light leaking beside the tablet, thus with very little time retardation.
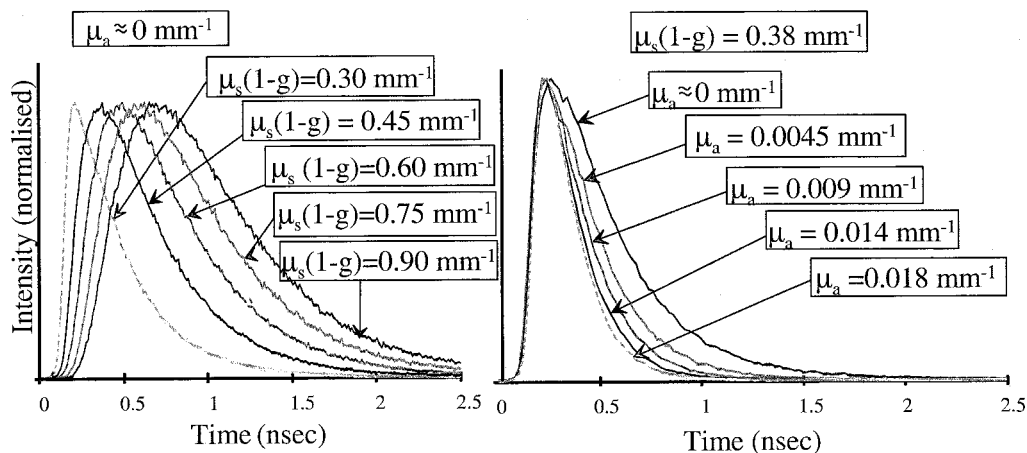
FIG. 3. Time-resolved transmission spectra of solutions of Intralipid and ink. The Intralipid concentration was varied with zero ink concentration (left graph) and held constant for varying concentrations of ink (right graph).

the image are due to light leakage between the sample holder and the sample. A horizontal scan through the image (between the dashed lines) results in a time profile at a given position on the sample, as is shown below the image in Fig. 2 for the center of the sample. In the same way, vertical scans through the image may be extracted (not shown). These are consequently interpreted as time-gated one-dimensional images (or spectra, if the spectrometer is used).

Monte Carlo Simulations. Monte Carlo simulations were utilized to model the light transport through the tablets. One of the most used and best validated implementations of Monte Carlo simulations of photon transport in multilayered tissues (MCML)[22] was used. The routine was altered for this application to allow a cylindrical geometry mimicking a tablet for which the radius and thickness were changed.

RESULTS AND DISCUSSION

Absorption and Scattering in Turbid Media. Time-resolved transmission experiments were performed on pharmaceutical tablets to roughly estimate the optics of

the tablets. In addition to the streak camera measurements reported here, some measurements on tissue phantoms are discussed at this point in order to improve the understanding of the experimental data. The time-resolved transmission data of tissue phantoms were recorded with a time-correlated single-photon counting technique at 640 nm rather than with the streak camera and have been described in detail previously.[21] The tissue phantom consisted of a series of Intralipid water solutions with ink added as an absorber. By varying the particle concentration of the Intralipid and the ink concentration, both the scattering coefficient and absorption coefficient were varied. In Fig. 3 (left graph) it is observed that by increasing μ_s' the transmission profile is shifted towards longer times. On the other hand, when μ_a is increased (right graph) merely the late slope of the profile is affected, leaving the peak position almost unchanged. For very high μ_s' the profiles approach a symmetric diffusion profile, whereas for high μ_a the curves collapse to Beer–Lambert's law. Hence, from the analysis of time-resolved transmission profiles, it is possible to qualitatively understand the optical paths of the samples. An interesting observation from Fig. 3 is that at early gate times changes in μ_a have a very small effect on the measured light intensity, whereas μ_s' affects the early transmitted light to a much higher degree.

In Fig. 4, time-resolved transmission profiles extracted from the image in Fig. 2 are shown. The different time profiles correspond to horizontal scans through the center (region marked between dashed lines), the mid-upper, and the upper part of the data image. These in turn, can be interpreted as light exiting through the center part, the off-center part, and close to the margin of the back side of the tablet. The light was focused onto the central part of the front side of the tablet as illustrated in Fig. 2. A most striking observation is the high mean photon propagation time, approximately 1 ns. Assuming a refractive index of 1.4, this corresponds to a mean optical path length of more than 200 mm for a tablet with a thickness of 3.5 mm. This indicates extraordinarily strong light scattering, also supported by the symmetric diffusion-like time profile. Monte Carlo simulations were performed for a tablet-shaped object and compared with the experimen-
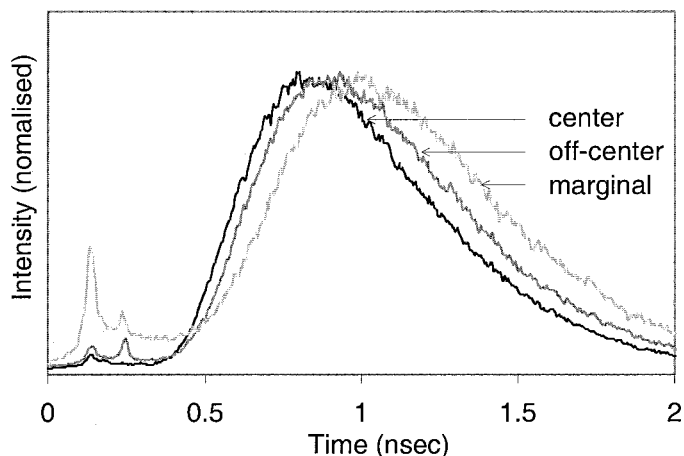

FIG. 4. Time-resolved transmission of a 3.5-mm-thick tablet using 200 fs pulses at 790 nm. The profiles correspond to light exiting the tablet at the center, 2 mm off the center, and close to the margin of the tablet, respectively. The curves are autoscaled to the same maximum intensity.
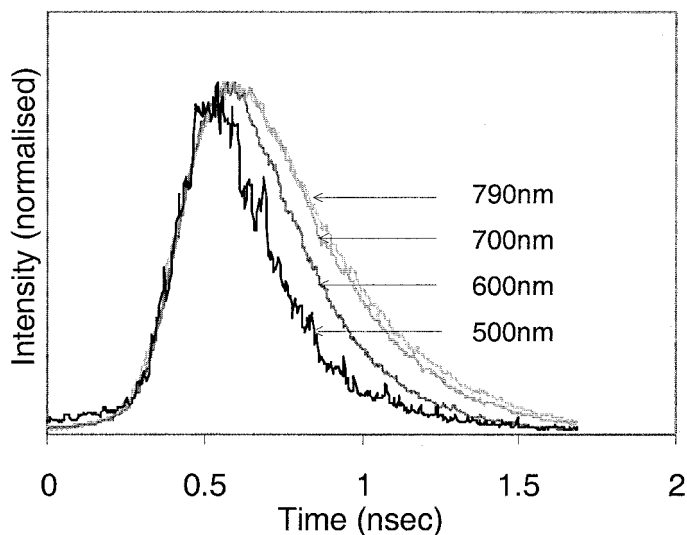
FIG. 5. Time-resolved transmission profiles of a tablet using ultra-short white light pulses. The profiles were extracted from the raw data matrix at 500, 600, 700, and 790 nm, respectively. The curves are autoscaled to the same maximum intensity.
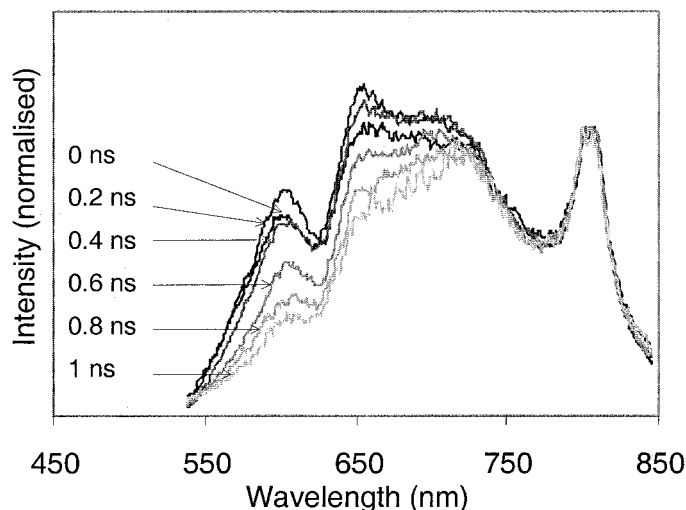

FIG. 6. Time-resolved transmission spectra; data extracted from same data matrix as the data in Fig. 5. The extracted spectra correspond to gating times indicated in the figure. Note that all spectra are uncorrected for the spectral instrument function. Each set of spectra is normalized at local maximum intensity around 800 nm.

tal data in order to estimate the optical properties of the sample. The flat cylinder used in the simulation had a diameter of 9 mm and a thickness of 3 mm. The best fit between the experimental data and the simulated was found when μ_s' was set to 500 cm^{-1} and μ_a was set to 0.01 cm^{-1}.

Comparing the three scans, it is noted that the light exiting from the margin of the tablet is shifted in time in relation to the centrally transmitted light. It can also be observed that the marginal profile is broadened compared to the center profile. With Fig. 3 in mind, these observations indicate that the light exiting further away from the center exhibit more light scattering than the central rays. This is consistent with a longer average path length for light recorded close to the margin as compared to that detected in the center.

Time-resolved data were collected with the spectrometer attached to the streak camera, enabling spectral in addition to temporal resolution. Thus, the data matrix here has a spectral rather than a lateral dimension. In these experiments, light from the center portion of the tablet backside was collected. In Fig. 5, a number of time profiles extracted from the data matrix at various wavelengths are shown. Note that the experiment was performed with a slightly modified optical set-up and the time scale is shifted compared to that in Fig. 4. As can be observed in Fig. 5, the time profile is more narrow for shorter wavelengths. In addition, the peak appears at slightly shorter times at shorter wavelengths. This suggests that the light transmission is more dominated by absorption at shorter wavelengths than at longer wavelengths. The effect of light scattering appears to be less significant since the peak intensity only shows a small shift upon the changing wavelength. In Fig. 6, the same data is displayed as time-gated spectra, i.e., transmission spectra for a number of different photon propagation times. The spectra are normalized to the same intensity to better illustrate the spectral differences. Comparing the spectra at different propagation times, it is again shown that at shorter wavelengths the light is attenuated more rapidly. The spectral features in Fig. 6 are not of particular importance in this study and originate not only from the sample but also from the detector response function and the spectrum of the white light source (including, e.g., inverse Raman effects[23]).

Photon Migration in Tablets. A series of measurements were performed using tablets with three different contents of the active substance. The experimental data was evaluated as shown in Fig. 7. In the lower graph (**e**), an example of a time profile is shown. In the upper four graphs (**a–d**), time-gated spectra are shown, one graph for each of four gate times analyzed. These gate times are approximately indicated in the time profile in the lower graph. In each of the four upper graphs, spectra from each of the three tablets are shown. In prompt light (**a**), light that has passed the tablet in a straight forward manner, very little difference between the curves can be observed: the three spectra have virtually the same shape (spectra autoscaled). However, for the spectra obtained at 0.25 ns photon propagation time (**b**), a substantial contrast between the 43-mg and the 52-mg tablets is observed. The behavior is similar at longer times (**c**, **d**), however, with a gradual loss of contrast as the gate time is increased. The important conclusion from this experiment is that the spectral contrast is different for different photon propagation times, and there appears to exist an optimal gating time where the highest contrast between tablets of different strengths is attained. Thus, by separating the spectral information in time, a higher analytical selectivity can be reached compared with the steady-state case of traditional transmission (NIR) spectroscopy.

It is interesting to compare the spectra in Fig. 7 in view of the effects shown in Fig. 3. As was discussed above, the absorption properties of the sample do not affect the early transmitted light, but the importance of absorption increases at longer times. For the tablet spectra in Fig. 7, it is observed that the most reliable spectral differences are found at medium propagation times. If the spectral
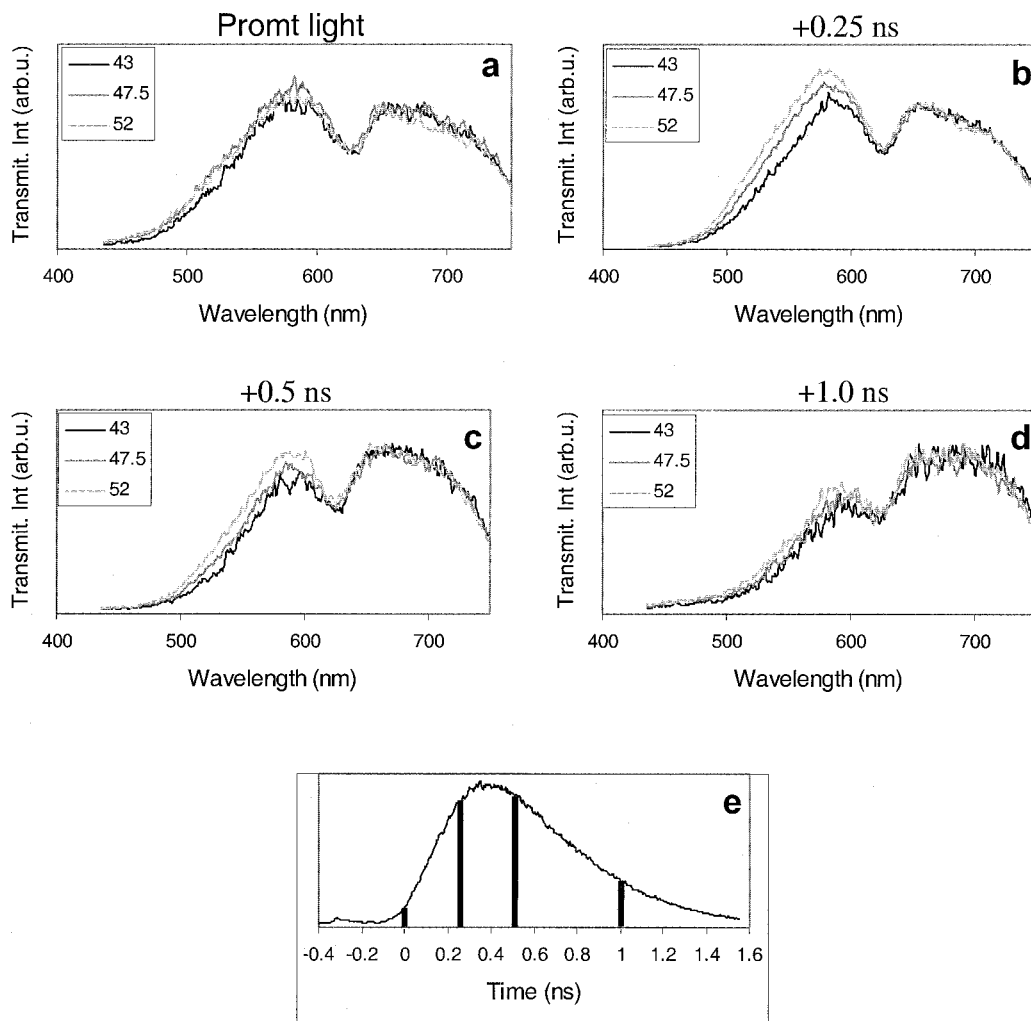
FIG. 7. Time-gated transmission spectra (a–d) at gate times indicated in the time profile (e). In each graph spectra from 3 tablets of different tablet strengths are shown. Each set of spectra is normalized at local maximum intensity around 650 nm.

differences were due only to absorption, Fig. 3 predicts that the contrast would increase with increased gate time, which is obviously not the case here. This suggests that not only the absorption but to a great extent also the scattering is important for the analysis of these samples.

Implications for NIR Spectroscopy. The main advantage of time-resolved spectroscopy is that the spectral contributions from both absorption and scattering effects can be experimentally determined. An attractive possibility should be to develop analytical methods for tablets of different shapes, thickness, and granular structure, based solely on their absorption characteristics, something that is impossible with today's NIR instruments. Also, calibration transfer between different systems should be simplified with a technique capable of measuring pure absorption features.

Future developments of time-resolved spectroscopy for quantitative analysis of solids, such as tablets, powders, etc., have to include both instrumental improvements to push the technique further into the NIR spectral region and development of new data evaluation techniques. The white light generation can be made much more compact and can be further optimized by harder focusing of the laser light and by using other nonlinear media, such as

sapphire or photonic bandgap fibers. The collection optics can be improved by using optics coated for the desired wavelength region and in particular a more efficient spectrometer. The data collection can also be improved by increasing the repetition rate of the laser up to the kHz region for better data averaging. The main challenge in extending further into the NIR region is the lack of efficient photo cathode materials available for streak tubes; the quantum yield of the S1 material drops two orders of magnitude going from 800 to 1200 nm. Measurements in the 800–1200 nm spectral region were performed in addition to the experiments reported above. Results comparable to the above data matrices were obtained, however, with poor signal-to-noise ratios, preventing any meaningful evaluation.

An alternative approach is to use a different technique. Phase modulation spectroscopy is an attractive alternative technique in which the phase of the measured light is compared with the phase of the light source, a simple NIR-emitting lamp. From the phase difference, the photon propagation times can be calculated to yield the same information as with time-resolved techniques. The major advantage of this technique is the simplicity and low cost of the optical set-up. A possible disadvantage is the lim-

ited time resolution. Spatially resolved methods may also be an alternative to time-resolved techniques. The benefit of spatially resolved techniques is a much simplified experimental set-up; however, a homogeneous sample is required. Future work will also include improvements of the data evaluation. Three-way analysis such as the PARAFAC method[24] appears to be a useful alternative to Monte Carlo calculations. A more extensive study of simulation of photon propagation through tablets is currently being performed.

CONCLUSION

Time-resolved transmission measurements were performed on pharmaceutical tablets in a feasibility study to investigate the usefulness of the general technique for analytical work. The results show that the light scattering of the samples is very high, leading to mean optical path lengths of 20–25 cm. Monte Carlo simulations suggest a reduced scattering coefficient in the range of 500 cm^{-1} for a typical tablet. The spectral characteristics were found to depend on the delay of the detector gate time. For light with a long propagation time through the sample matrix, the spectrum was found to peak at higher wavelengths in relation to the early light. This was due to a greater attenuation of the blue/green light. Gated transmission spectra were recorded for tablets with different contents of the active substance, and it was found that the contrast between tablets was higher for some photon propagation times than for others. In particular, for these samples short times yielded no contrast, whereas medium times exhibit high spectral contrast. Thus, by gating the light transmission in time, a higher analytical contrast can be achieved than with conventional transmission spectroscopy. The technique seems promising and future work will be focused on extending the spectral range further into the NIR spectral region.

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