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Chemical Imaging of Oral Solid Dosage Forms and Changes upon Dissolution Using Coherent Anti-Stokes Raman Scattering Microscopy

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Dissolution testing is a crucial part of pharmaceutical dosage form investigations and is generally performed by analyzing the concentration of the released drug in a defined volume of flowing dissolution medium. As solid-state properties of the components affect dissolution behavior to a large and sometimes even unpredictable extent there is a strong need for monitoring and especially visualizing solid-state properties during dissolution testing. In this study coherent anti-Stokes Raman scattering (CARS) microscopy was used to visualize the solid-state properties of lipid-based oral dosage forms containing the model drug theophylline anhydrate during dissolution in real time. The drug release from the dosage form matrix was monitored with a spatial resolution of about 1.5 µm. In addition, as theophylline anhydrate tends to form the less soluble monohydrate during dissolution, CARS microscopy allowed the solid-state transformation of the drug to be spatially visualized. The results obtained by CARS microscopy revealed that the method used to combine lipid and active ingredient into a sustained release dosage form can influence the physicochemical behavior of the drug during dissolution. In this case, formation of theophylline monohydrate on the surface was visualized during dissolution with tablets compressed from powdered mixtures but not with solid lipid extrudates.

The dissolution behavior of drugs is a critical quality attribute for oral solid dosage forms since, in almost all cases, their therapeutic efficacy depends on this very behavior. A combination of chemical and physical properties of both the solid dosage form and the dissolution medium determine the drug dissolution behavior. Important properties of the solid dosage form include the apparent solubility of the drug and the other components, particle size, and drug distribution. These characteristics and hence the dissolution rate change during drug dissolution.

Dissolution testing is universally used in the development, production, and quality assurance of oral solid dosage forms. During such testing, the dosage form is immersed in a flowing aqueous medium and the concentration of the released drug in the medium is measured at defined time intervals using techniques such as UV spectroscopy or HPLC. Although valuable, such analysis provides no direct information on the changing dosage form phenomena, and hence the dissolution behavior of drugs cannot be completely understood with such analysis alone. Therefore, there is a need to monitor the changing chemical and physical properties of dosage forms during dissolution.

Initial attempts to characterize dosage form changes involved the bulk characterization of samples ex situ, with for example X-ray diffraction. Recently, Raman spectroscopy has been used to detect solid-state transformations during dissolution in situ. However, since drug release from dosage forms is largely dependent on spatial phenomena, it is obviously pertinent to obtain spatially resolved information. Spatially resolved analysis of oral dosage forms has experienced a surge of interest within the past decade, in part due to much advanced analytical technology. Scanning electron microscopy has been used to characterize dosage form morphology changes after dissolution testing, and X-ray powder diffraction has been used to depth-profile dissolution related phase transformations. Methods exhibiting chemical selectivity that are suitable for imaging dosage forms include near-infrared (NIR), mid-infrared (IR), terahertz and Raman imaging, as well as imaging based on secondary ion mass spectrometry. While these methods are all appropriate for imaging physical and/or chemical aspects of pharmaceutical solids, the technique of coherent anti-Stokes Raman scattering (CARS) has several distinct applications because it is a sensitive, rapid, selective, and spatially resolved technique. This paper describes an initial attempt at using CARS microscopy to analyze the solid-state behavior of a model drug theophylline anhydrate during dissolution in real time.

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chemical changes of dosage forms after dissolution testing ex situ, they all exhibit serious shortcomings with respect to in situ imaging. Demands for in situ analysis include an absence of analysis-related dosage form destruction, a sampling setup that does not interfere with the dissolution medium flow, an ability to obtain data in the presence of dissolution media, and sufficient temporal resolution. Secondary ion mass spectrometry cannot be used for sample materials in a dissolution medium. With NIR, IR, and terahertz imaging, the radiation used with these techniques is strongly absorbed by water which severely limits their use in aqueous environments. For IR imaging, this problem has been circumvented by the use of an attenuated total reflectance (ATR) setup, whereby an ATR crystal is interfaced with both a tablet and dissolution medium in a flow through cell dissolution testing setup. However, the requirement for intimate contact between the dosage form and the ATR crystal severely limits sampling setup flexibility making analysis of different kinds of solid dosage forms problematic. Furthermore, commonly observed particle sizes in oral solid dosage forms may be below the spatial resolution of this technique. Raman imaging has potential for in situ analysis of solid dosage forms dissolution due to its lack of water sensitivity and relatively high spatial resolution (up to about 1 µm), but potential disadvantages include a typically longer data acquisition time (minutes or hours for 512 by 512 pixels) and possible interference from fluorescence.

Coherent anti-Stokes Raman scattering microscopy is a chemically selective imaging method that appears to fulfill all the requirements listed above for chemically selective in situ analysis of dosage forms during dissolution testing. The method is based on two laser beams where one is tunable in wavelength. The two laser beams are colinearly overlapped and focused into the sample of interest. If the wavelength difference between the two input laser beams coincides with a Raman active vibrational mode, an anti-Stokes wavelength (blue-shifted compared to the input wavelengths) is created. When there are differences in the vibrational spectra of the molecules in the sample, chemically selective imaging is possible with this technique with submicrometer resolution in three dimensions. Different configurations and a more complete description can be found in reviews about the technique.

![Raman spectra of the powdered substances and water](image1.png)

Figure 1. Raman spectra of the powdered substances and water: (a) spectra of water (green), tripalmitin (black), theophylline anhydrate (red), and theophylline monohydrate (blue) and (b) highlighted region of the spectrum.

![Distribution of lipid (red) and drug (green) in solid dosage forms](image2.png)

Figure 2. Distribution of lipid (red) and drug (green) in solid dosage forms: (a) tablet of tripalmitin/theophylline anhydrate, (b) tablet of tripalmitin/theophylline monohydrate, (c) tablet of extrudates of tripalmitin/theophylline anhydrate, and (d) extrudate of tripalmitin/theophylline anhydrate.

films as well as living cells and tissues. Additionally, the technique has recently been used to monitor drug distribution and release from polymer films used in stents. In a subsequent study, drug crystallization during preparation of films was observed for stent coating materials. To the best of our knowledge, oral dosage forms and their physicochemical changes during dissolution have not previously been visualized with the use of CARS microscopy.

In these experiments, CARS microscopy was combined with a purpose-built flow-through dissolution cell to visualize physicochemical changes in oral dosage forms during dissolution. The flow-through cell allows the solid dosage form to be fixed in a constant fluid-flow bed which is covered by a microscope cover glass facing the objective of the microscope. The dissolution medium is continuously pumped through the cell surrounding the sample. The two laser beams (of suitable wavelength to coincide with the selected vibrational stretch) are focused onto the sample in the flow cell, creating the anti-Stokes signal. With CARS it is possible to achieve temporally and spatially resolved visualization of the distribution and the solid-state properties of the sample.

Lipid-based oral dosage forms exhibiting particulate drug dispersion were used as samples. Lipid-based formulations have shown promise as controlled release oral dosage forms as they can effectively control the release rate of drugs, they are physiological and therefore nontoxic, they mask the unpleasant taste of drugs, and no organic solvents are required during dosage form preparation. In these studies different lipid-based oral dosage forms were investigated. Physical powder mixtures of lipid and drug were compressed and compared to extruded matrices consisting of the same components. In addition, the extruded matrices were compressed to tablets. The aim of the study was to combine CARS microscopy with a suitable flow-through cell setup as a means to gaining deeper understanding of the physicochemical behavior of oral dosage forms during dissolution.

**EXPERIMENTAL SECTION**

**Materials.** Powdered theophylline anhydrate (BASF, Ludwigshafen, Germany) was used as received. Theophylline monohydrate was obtained by recrystallization of theophylline anhydrate from deionized water. Tripalmitin (Dynasan 116), a pure powdered monoacid triglyceride, provided by Sasol (Witten, Germany) was used as received. The solid state structure of all materials was

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Figure 3. Solid dosage forms consisting of lipid (red) and drug (green) after different immersion times in dissolution medium: (a–c) tablet of tripalmitin/theophylline monohydrate, (d–f) tablet of tripalmitin/theophylline anhydrate, and (g–i) tablet of extrudates of tripalmitin/theophylline anhydrate.
verified using X-ray powder diffraction as detailed in a previous publication.25

Preparation of Tablets. powdered tripalmitin was weighed in a 50/50% (w/w) mixture with either theophylline anhydrate or with theophylline monohydrate powder and blended in a Turbula mixer (Willi A. Bachofen AG, Basel, Switzerland) for 15 min. The powder mixture (designated as “physical mixture” in this study) was compressed in a tabletting machine (Korsch EK 0, Erweka Apparatebau, Berlin, Germany) using flat-faced punches (diameter 9 mm). Tablets were also compressed using extrudates instead of powder mixtures.

Preparation of Extrudates. The powdered tripalmitin and theophylline anhydrate were combined to form a 50/50% (w/w) mixture and blended for 15 min at 25 rpm in a laboratory mixer. The samples were irradiated by a Kr ion Laser (coherent, Innova 90K, Santa Clara, CA) of 30 mW at 647.1 nm and focused by a 20×0.5 NA objective lens.

CARS. The CARS setup consisted of a coherent Paladin Nd: YAG laser and an APE Levante Emerald optical parametric oscillator (OPO). In this setup, the fundamental (1064 nm, 80 MHz, >15 ps) of the laser is used as Stokes, whereas the signal from the OPO (tunable between 700—1000 nm and spectral width of 0.2 nm) is used as the pump and probe. The beams are scanned over the sample by galvano mirrors (Olympus Fluoview 300, IX71) and focused by a 20×0.5 NA objective lens. Both beams have a power of several tens of milliwatts at the sample. Because of the highly scattering samples, the forward generated CARS signal is collected in the backward direction.22 The collected signal is filtered and detected by a photomultiplier tube. All images are 512×512 pixels over the full field of view and were obtained in 2 s. Images at different wavelengths require tuning of the OPO but no realignment of the optics. Different images are collected consecutively. For the dissolution testing, the tripalmitin matrix was imaged before and after dissolution to verify the absence of change in the matrix material. During the dissolution, only the theophylline was imaged real time.

Dissolution Testing. The dissolution flow-through cell consisted of a Teflon chamber in which two metal bars were used to fix the dosage form in the middle of a flowing dissolution medium. A hose pump circulated the medium through the chamber via suitable conduits at a constant rate of 5 mL/min. On one side, the cell was equipped with a thin microscope cover glass that was transparent to the incident and scattered radiation. The dissolution medium was purified water, and the measurements were conducted at room temperature. Such a setup allowed the solid-state properties of a solid oral dosage form to be visualized in situ with an appropriate medium flow for dissolution testing of oral solid dosage forms.26

RESULTS AND DISCUSSION

Determination of Suitable Vibrational Bands for Component-Specific Imaging. Raman spectra were recorded of all powdered substances to determine suitable vibrational bands for component-resolved analysis using CARS. Bands that were largely resolved for each component but within a limited spectral range were required. The spectra are shown in Figure 1. Figure 1a depicts the comparison of the three powdered substances, with a region with large spectral differences between the components.


highlighted and displayed in Figure 1b. From 1600 to 1800 cm$^{-1}$, the peaks at 1687 (theophylline monohydrate), 1707 (theophylline anhydrate), and 1728 cm$^{-1}$ (tripalmitin) have been assigned to C=O stretching. All three substances exhibit CH stretching between 2700 and 3200 cm$^{-1}$. In tripalmitin, the CH stretching associated with the aldehyde function occurs at 2715 cm$^{-1}$ while CH$_2$ and CH$_3$ symmetric stretching correspond to peaks at 2850 and 2880 cm$^{-1}$, respectively. Theophylline exhibits CH$_3$ antisymmetric stretching at 2961 (theophylline monohydrate) and 2967 cm$^{-1}$ (theophylline anhydrate) and CH stretching associated with the imidazole ring at 3109 (theophylline monohydrate) and at 3123 cm$^{-1}$ (theophylline anhydrate). For component-specific analysis, the 1600–1800 cm$^{-1}$ region with the three components exhibiting highly distinguishable peaks seemed to be favorable. Unfortunately water gives a strong CARS response in the corresponding anti-Stokes region at about 1650 cm$^{-1}$, which precluded the use of this region. In the 2600–3200 cm$^{-1}$ region, tripalmitin and theophylline can be selectively imaged using the peaks at 2880 (tripalmitin) and 3109 cm$^{-1}$ (theophylline). CARS spectra and Raman spectra are not identical but uniquely related to each other. The spectra of the pure samples will be very similar to the Raman spectra due to the relatively weak nonresonant signal from the sample itself. The CARS spectra of the samples dissolved in water will change slightly, due to the mixing of the nonresonant water signal with the resonant signal. This causes the peak positions to shift a few wavenumbers down. On the basis of the Raman spectra, it is clear that a distinction between theophylline anhydrate and monohydrate was not possible with CARS in this region. Nevertheless, the peak at 3109 cm$^{-1}$ can be used to image both forms of theophylline. Around 2880 cm$^{-1}$, the CARS intensity for tripalmitin exceeds the intensity for the theophylline (both monohydrate and anhydrate). The images obtained at 2880 cm$^{-1}$ can thus be used to represent the (square of the) tripalmitin density distribution. At 3109 cm$^{-1}$, the CARS intensity for the monohydrate exceeds that for tripalmitin. The intensity for the monohydrate is closer to that for the tripalmitin so that the precise ratio is strongly influenced by the amount of nonresonant background. From the images it was clear that the monohydrate exceeded the tripalmitin by a factor larger than 3, based on areas that could be identified to contain only one of the constituents. The images at different wavenumbers can be related to each other by picking a spot that can be seen to contain pure tripalmitin and scaling the intensity in the 3109 cm$^{-1}$ image to reflect the correct ratio. Furthermore, the phase of the tripalmitin signal is between 70 and 90 degrees separated from the signal of the theophylline (monohydrate or anhydrate) so that the total signal from a combination of the substances (the absolute square of the combined amplitude) is almost equal to the addition of the absolute square of both images. The tripalmitin image, once correctly scaled, can thus be subtracted from the other image to obtain an almost pure image, and this procedure is possible even in regions that contain signals from both substances. For a more precise analysis, the CARS amplitude and phase can be detected locally using heterodyne detection to extract the relative components. This paper focuses on the qualitative description; further work will include detailed quantitative measurements. All images in this paper are based on CARS signals only.

**Drug Distribution in Different Lipid-Based Oral Dosage Forms.** Tablets and extrudates were analyzed using CARS microscopy to visualize the distribution of lipid and drug in the dosage forms. Figure 2 depicts false-color images of the surface of the dosage forms with a spatial resolution of about 1.5 μm. The images show that the theophylline drug particles (signal at 3109 cm$^{-1}$, encoded in green) are randomly distributed at the surface of the lipid matrix (signal at 2880 cm$^{-1}$, encoded in red). A mix of both signals would appear yellow, and since this is not observed, the substances do not mix at this resolution. The comparison of parts a and b of Figure 2 reveals that theophylline monohydrate is characterized by thin needles whereas the anhydrate exhibits anisometric particles. Figure 2c depicts the image of a tablet compressed from extrudates. In this image, the drug particles represent a smaller proportion of the surface signal than is the case for the unextruded samples, which may be

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attributed to a depletion of drug adjacent to the extruder barrel wall during processing.\(^3\) The images of the extrudate itself are depicted in Figure 2d. In the extruded matrixes, the drug particles are homogenously distributed.

**Monitoring Drug Release and Solid-State Transformations during Dissolution Testing.** The lipid-based dosage forms were imaged after immersion in 500 mL of purified water and compared with the images of the dosage forms before dissolution. After 30 and 180 min of immersion, they were removed and imaged using CARS microscopy to investigate drug release and solid-state transformations (Figure 3). The release of the drug from a tablet consisting of a physical mixture of tripalmitin and theophylline monohydrate (Figure 3a–c) is evident from a gradual loss of green color representing the drug. After 30 min of immersion, the drug was still visible whereas after 180 min no drug was evident on the surface of the tablet. Instead, pores in the lipid matrix where drug needles were located are represented as dark areas in the false-color image. The lipid (red color) remained after dissolution, demonstrating that the matrix stays completely intact during dissolution. It can thus be concluded that the release of the drug is purely diffusion controlled with the drug initially dissolving at the surface of the matrix and then diffusing through the resulting pores in the matrix.

Different phenomena were observed in the tablet consisting of tripalmitin and theophylline anhydrate (Figure 3d–f). Figure 3d depicts the tablet surface before immersion with the anhydrous theophylline clearly visible as dispersed particles in the lipid matrix. After 30 min immersion in water, the surface of the tablet was completely green and fine needles could be observed (Figure 3e). This can be attributed to the solution-mediated formation of theophylline monohydrate, a phenomenon which has previously been observed upon immersion of the anhydrate in water.\(^3,\)\(^3,\)\(^3\) In these studies, monohydrate growth in water has always been associated with needle-like morphology. After 180 min of immersion in water, the green color had completely disappeared and the red color of the lipid was once more visible, suggesting that the theophylline monohydrate had completely dissolved. The dissolution process of theophylline anhydrate in this dosage form can therefore be subdivided in several stages. First, the theophylline anhydrate dissolves and, with the monohydrate being less soluble than the anhydrate, a supersaturated solution with respect to theophylline monohydrate is created. This is followed by a transformation phase in which the monohydrate crystallizes.\(^2\) Afterward, dissolution of the two forms takes place.

To investigate the influence of the extrusion process on the release behavior of the drug, a tablet was compressed from tripalmitin and theophylline anhydrate extrudates and subjected to the same dissolution study. For this tablet (Figure 3g–i), release of the anhydrate particles was observed but, strikingly, no monohydrate needle formation on the tablet surface was observed. These observations correlated with results obtained on the same preparations in the same setup using in situ Raman spectroscopy (the Raman spectroscopy setup used has been published\(^2\)), where monohydrate formation was observed for the compressed powder mixtures but not for the extrudate (data not shown).

With the CARS setup, additional studies were conducted using the uncompressed extrudates made of tripalmitin and theophylline anhydrate (results depicted in Figure 4a–d) and in these unmodified extrudate samples there was also no evidence of theophylline monohydrate formation during the dissolution of the anhydrate particles. A scan in the pores of the extrudate at a depth of 50 μm was performed (Figure 4d) to observe physicochemical changes within the matrix in addition to at the surface. At this depth a very small monohydrate needles can be found inside the pores. The reason for such different solid-state behavior of the extruded and extruded samples needs further investigation. However, in an attempt to understand such behavior, one can look at the mechanism of crystallization of the monohydrate. The mechanism of conversion is believed to be solution-mediated. First, nucleation from solution must occur followed by crystal growth. Nucleation typically occurs in the presence of a suitable surface and supersaturated solution with respect to the monohydrate, and crystal growth is dictated by the degree of supersaturation. The outer surface of the extrudate may be a poor substrate for nucleation since it is very smooth. In contrast, the surface of the compressed powder mixtures is likely to be rougher. With regard to supersaturation, as already stated it appears that the extrusion process reduces the drug exposure at the surface of the extrudate and hence of the surface of the tablet consisting of extrudates, which may mean that the solution adjacent to the surface is less supersaturated with respect to the monohydrate inhibiting its nucleation and crystal growth. Within the pores, the rougher surface which is left after the release of drug particles in the pores may promote nucleation. In addition, the diffusion within the small pores is likely to be very slow, and therefore through greater supersaturation in these regions crystal formation is more likely to occur. We plan to investigate this issue in future work.

**In Situ Imaging of Solid-State Transformations.** To monitor the solid-state transformation in real time, tablets consisting of the physical mixture of tripalmitin and theophylline anhydrate were placed directly on the microscope stage in a small container mounted on a thin glass slide which was filled with purified water so that a thin water layer was located between the sample and the microscope objective. Figure 5a–f depicts several frames of the recorded images. In real time, the transformation from theophylline anhydrate to monohydrate could be visualized for the first time on a tablet surface (see Video S-1 in the Supporting Information).

**In Situ Monitoring of Solid-State Characteristics during Dissolution Testing.** In this part of the study, experiments with the dissolution flow-through cell, which provides a pharmaceutically relevant dissolution setup for oral dosage forms, were performed. It was possible to obtain a good CARS signal intensity from the tablet through the flowing dissolution medium. Figure 6 depicts images recorded during the dissolution testing with a tablet consisting of the physical mixture of tripalmitin and theophylline monohydrate. The release of the drug from the matrix could be visualized in real time. Unfortunately focal drift and wavelength drift prohibited a reliable quantitative analysis for these images at this time.

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CONCLUSIONS

CARS microscopy was used to visualize the spatial distribution of different components in oral pharmaceutical dosage forms and, by combining the method with a suitable flow-through cell, drug release and physicochemical changes during dissolution testing were monitored in real time. As solid-state properties of pharmaceutical dosage forms affect the dissolution behavior, the visualization of solid-state changes with this method can expand the knowledge about dissolution mechanisms. Such knowledge will help lead to tailor-made dissolution profiles by manufacturing of pharmaceutical dosage forms that exhibit desirable physicochemical properties during dissolution. In the case of solid lipid extrudates, the drug and lipid distribution on the surface of the solid dosage form was rapidly visualized with a spatial resolution of about 1.5 µm every 2 s. CARS was used to monitor the loss of the model drug theophylline from the lipid matrix as well as solution-mediated solid-state transformations (from theophylline anhydrate to the monohydrate form) on the surface of tablets in real time. Solid lipid extrusion prevented theophylline hydrate formation, which was clearly observed with CARS microscopy. On the basis of these results, CARS microscopy may be a valuable characterization method in the future development of different kinds of oral solid dosage forms.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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