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Communication

Preliminary Assessment of Solid Crystal Suspensions using Nonlinearmicroscopy

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Abstract: A few years ago Solid Crystal Suspensions (SCS), made via hot-melt-extrusion, were proposed as sufficient technique to increase the bioavailability of poorly soluble drugs of biopharmaceutic classification system (BCS) Class II. The drug particle size and the dispersity of these systems are crucial properties because they determine the drug release.

The nonlinear imaging techniques, Coherent Anti Stokes Raman Scattering (CARS) and Sum Frequency Generation (SFG) microscopy, were used to characterize SCS in order to understand the dissolution behaviour of different formulations. CARS microscopy is based on the detection of molecular vibrations similar to Raman spectroscopy[1-2]. The advantage in comparison with conventional Raman spectroscopy is the detection of a single vibrational resonance allowing for much faster imaging with CARS.

The setup consisted of a laser and an optical parametric oscillator. The beams were scanned over the sample by galvano-mirrors and focused by an objective lens into the sample. Compared to the incident beam the generated CARS signal was shifted to a higher frequency. When the sample contained non-centrosymmetric structures the SFG signal was created. The generated signal was created simultaneously with the CARS signal in the sample and detected on a different detector. The SCS consisted of a drug (griseofulvin) and a matrix (mannitol). The matrix was detected with CARS microscopy and the drug with SHG microscopy offering high contrast between the compounds.

The nonlinear microscopy images of the SCS showed a homogeneous distribution of the drug particles. There were no agglomerates and the drug distribution in the centre was almost the

same as at the surface of the objects. The drug particle size was also investigated and similar results to the ones in laser diffraction were found.
The combination of the two nonlinear microscopy techniques, CARS and SFG was identified as a promising tool to characterize drug distribution and drug particle size in this SCS.

Keywords: solid crystal suspension, hot melt extrusion, nonlinear microscopy, coherent anti stokes raman, sum frequency generation

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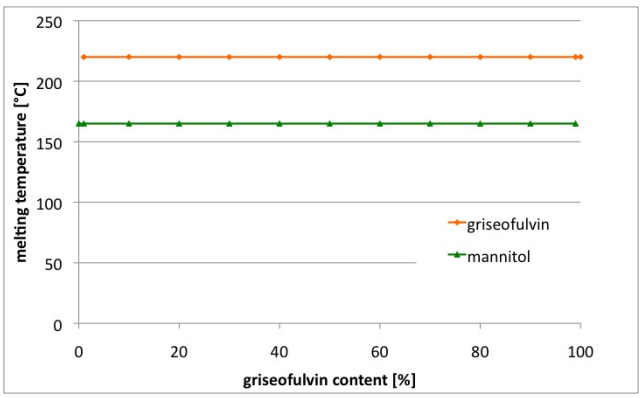
1. Introduction

The low solubility of drugs is still challenging in pharmaceutical development because these drugs often show low bioavailability. Particle size reduction is one possible opportunity to increase the bioavailability of those drugs owning low solubility and high permeability (BCS Class II). One new concept in this respect are Solid Crystal Suspensions (SCS) where the drug is milled in molten carrier via hot melt extrusion[3,4]. The final product is in the crystalline state, therefore physical stability issues are not likely.

The drug particle size, size distribution and dispersity were identified as crucial product properties, which affect the drug release as well as the bioavailability. Therefore an imaging technique was considered as a sufficient tool for characterization. Since SCS contains drug as well as carrier the imaging technique should detect both components simultaneously.

In this study griseofulvin was used as model drug and mannitol as carrier because several investigations were previously performed using this system. Furthermore, this system seems to be particular sufficient because there is no molecular interaction between the drug and the carrier that was shown by the phase diagram (Fig. 1).

Figure 1. Phase diagram of griseofulvin and mannitol



A particular property of these system are the two polymorphic states of the carrier. Mannitol can exist in the alpha and beta modification. Therefore both had to be considered by the imaging. Initially several imaging technologies were considered: Near infrared microscopy as a spacial resolution of about 30µm [5] that was too high since drug particles of less than 10µm were expected from SCS. X-Ray-Tomography did not seem to be applicable because the densities of mannitol and griseofulvin are

quite similar[6]. Confocal fluorescence microscopy was considered as insensitive for mannitol therefore it is not possible to differentiate between carrier and pores. Magnetic resonance imaging has also a poor special resolution while Raman mapping could be difficult due to the fluorescence of griseofulvin[7].

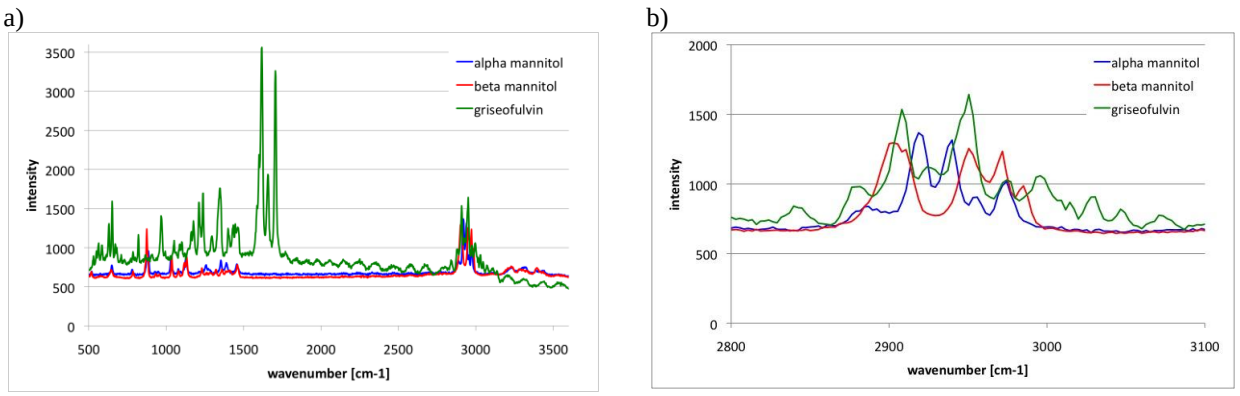
Based on this nonlinear microscopy was identified as the most promising technology to characterize solid crystal suspensions.

2. Results and Discussion

2.1. Experimental Setup

The drug (griseofulvin) and the carrier (mannitol) should be investigated independent of each other with nonlinear microscopy. Therefore first investigations dealt with the identification of different frequencies that allow a specific detection of the components. For that reason Raman spectra of each single component were recorded (Fig. 2a and 2b).

Figure 2. (a) Raman spectra of the SCS components, **(b)** high frequency regime of the Raman spectra

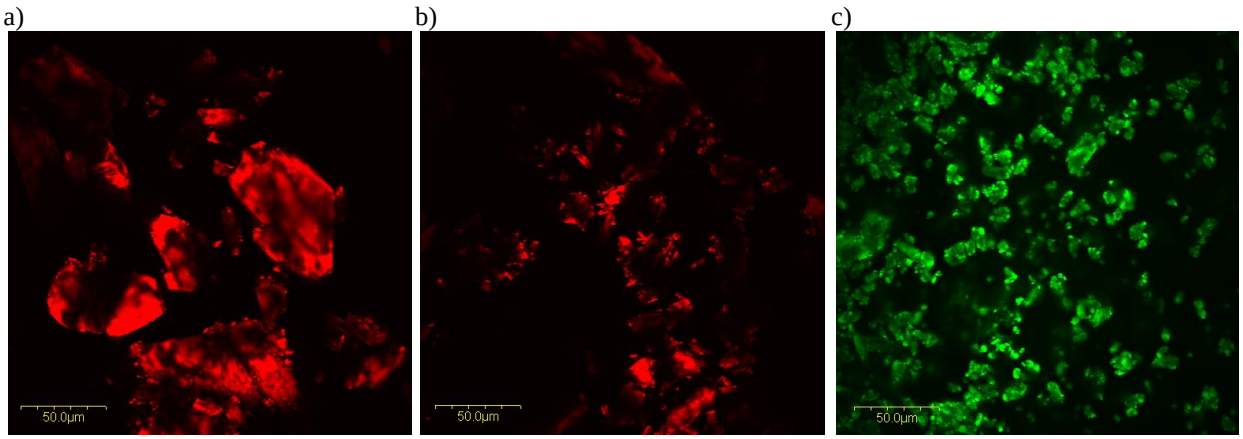


The griseofulvin only has an isolated peak in the middle frequency regime, but CARS in this setup was limited to frequencies in the high frequency regime.

The detailed spectrum of the high frequency regime shows two strong peaks for the alpha mannitol. Both of these partially overlap with the griseofulvin signal and the beta mannitol modification. CARS spectra are derived from the same resonances as the Raman spectra but since they are composed of the coherent addition of a non-resonant part and the resonant part, they show Fano-lineshapes with a red-shifted peak before the peak Raman position and a dip after the resonance[8,9]. At 2910 cm^{-1}, the two types of mannitol showed a reasonable CARS response whereas the griseofulvin showed a deep dip due to the interference of several resonances so that both mannitol modifications could be detected without interference from the griseofulvin (Fig. 2).

In Fig. 3 are images of powdered components. The alpha modification shows higher intensity than the beta modification. In this study no differentiation between the modifications was considered.

Figure 3. CARS-image (2910 cm^{-1}) of **(a)** alpha-mannitol powder, **(b)** beta-mannitol powder and **(c)** SFG-image of pure griseofulvin

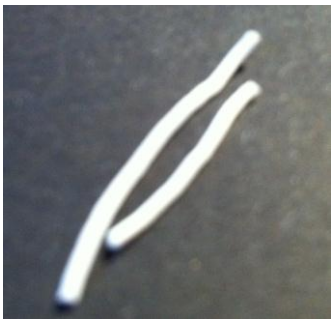


Because griseofulvin is largely undetected by CARS at 2910cm^{-1}, SFG was used to image the griseofulvin separately.

2.2. Characterization of Extrudate

The drug distribution was investigated analysing multiple cross sections. Therefore extrudates with 10 and 50% (w/w) drug load were considered (Fig.4).

Figure 4. SCS - extrudate



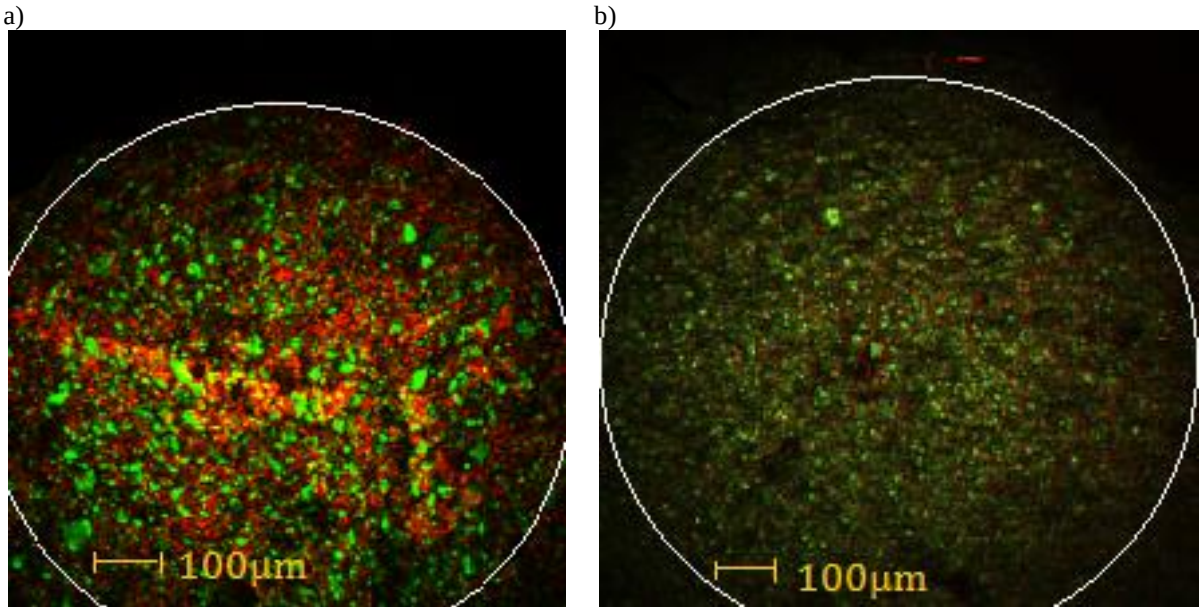
A smaller drug particle size of the formulation containing 50% drug was observed (Fig.5). This could be explained by a higher solid fraction during the extrusion because just the carrier is able to melt in extrusion. The higher solid fraction could increase the viscosity and the shear stress of the melt. The higher specific energy during extrusion (Tab.1) indicated this behaviour. The higher shear stress could increase the dispersive mixing during extrusion.

Table 1. Specific energy in extrusion

drug load	mechanical energy
10%	84 J/g
50%	332 J/g

The drug distribution in extrudates with high drug load was quite homogeneous with respect to the cross section. There seem to be no differences between the surface and the center of the extrudate. The drug particles in extrudates with lower drug load are more concentrated in the center of the extrudate. Also the particles in the centre are larger than in the outer layer. This can be explained by the lower mechanical energy during extrusion. Higher energy affects smaller particles that are easier to disperse, caused by less segregation.

Figure 5. CARS/SFG-images of 10% **(a)** and 50% **(b)** drug load

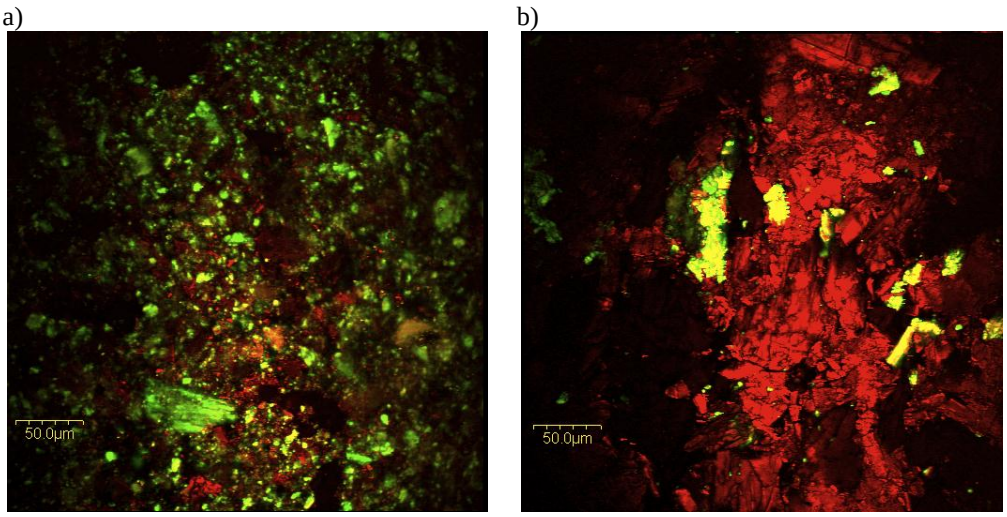


2.3. Characterization of Tablets

Figure 6. SCS-tablet

The SCS extrudates were ground to coarse particles with a median particle size of 130µm and combined with silicified microcrystalline cellulose. Tablets that are more common in administration to the patient than extrudate were made (Fig. 6). A physical mixture from drug, carrier and silicified microcrystalline cellulose were also tableted and served as comparison. Both tablets were characterized by using CARS and SFG microscopy. Several parts of the tablet surface and cross section were investigated and representative images were given. The drug particles in the tablets containing SCS are smaller and more homogeneous distributed than in the tablets containing a physical mixture (Fig. 7). This was attributed to the extrusion process were the drug particles were milled and mixed with the excipient in a dispersive and distributive manner. The smaller drug particle size of SCS was confirmed with laser diffraction measurement using the ground extrudate.

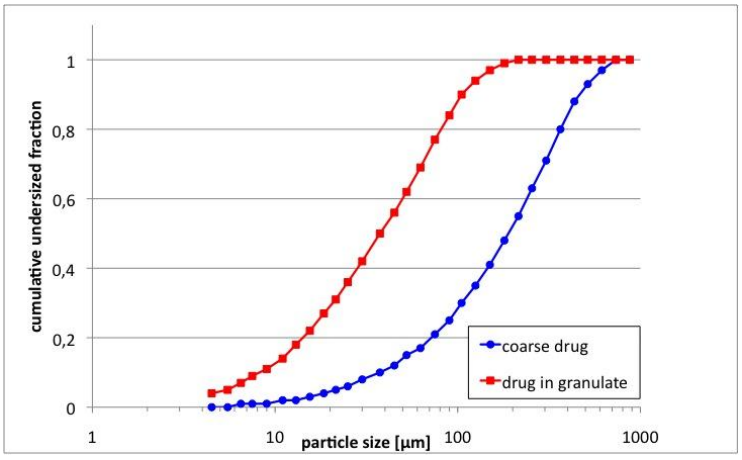
Figure 7. CARS/SGH-images of tablets containing **(a)** the grinded extrudate and **(b)** the physical mixture



Remarkable is the subjectively lower drug content in the physical mixture that could not be explained at this point. Two different possibilities reasons were identified. On one hand there might be a certain drug gradient in the tablet based on segregation during filling of the tablet die. On the other hand some large particle could look less even if they have the same total volume like a large number of small particles.

Based on this there is a need to evaluate nonlinear microscopy images quantitatively. Further investigations are going to deal with this issue.

Figure 8. Particle size by laser diffraction measurement



3. Experimental Section

3.1. Materials

The SCS consist of grisefulvin (Hawkins, Minneapolis, MN, USA) and mannitol (Pearlitol 50 C, Roquette, Lestrem, France). Additionally, silicified microcrystalline cellulose (Prosolv HD 90, JRS,

Rosenberg, Germany) was used in tabletting. All substances were pharmacopoeia grades and used as received.

3.2. Raman spectroscopy

Raman spectra of pure substances were recorded on a 1600 pixel CCD camera (Newton DU-970N, Andor Technology, Belfast, Northern Ireland). The samples were irradiated by a Kr ion Laser (coherent, Innova 90K, Santa Clara, CA) at 647.1 nm of 30 mW and focused by 40x 0.65NA microscope objective lens.

3.1. Microscope setup

CARS: The CARS setup was reported previously[10,11] and 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 Stokesand 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 into the sample. Both beams have a power of several tens of milliwatts at the sample. Because of the highly scattering samples, the forward generated CARS signal can be collected in the backward direction. 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. Due to the relatively high intensity the sum frequency of the fundamental and the OPO-signal signal can be observed. Sum Frequency Generation (SFG) is a non-resonant process that only generates a signal for materials that lack a center of inversion, such as chiral or non-centro-symmetric crystalline structures. The SFG and CARS can be recorded simultaneously on separate detectors.

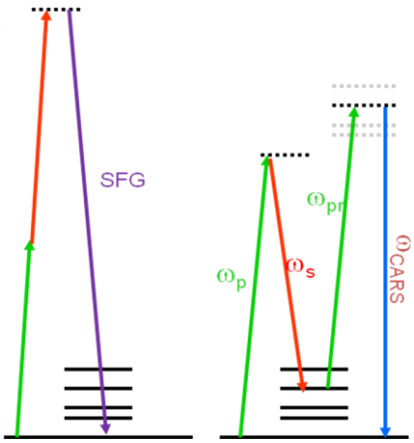


Figure 9. Energy diagrams for the two process used in the imaging. Left: Sum frequency Generation between the 1064nm and the OPO-signal. Right: CARS

3.1. Particle size by laser diffraction

The drug size was determined by a laser diffraction analyser (Helos/KF-Magic Sympatec, Claustal-Zellerfeld, Germany) using a liquid dispersing system (Cuevette 50ml) at 600 rpm. 8 mg of extrudate was placed in a saturated aqueous griseofulvin solution in order to strip the mannitol from extrudates. Each extrudate was measured in triplicate and the arithmetic mean of the median particle size was used for data analysis.

4. Conclusions

The nonlinear microscopy, especially the combination of SFG and CARS is suitable to investigate the crucial properties of SCS. The method was applied to extrudates as well as tablets. New insights in the extrusion and tableting process of SCS were made.

The combination of the SFG and CARS microscopy is a promising imaging technique to characterize the particle size and drug distribution in Solid Crystal Suspensions. Further investigations are required to obtain quantitative information from the images.

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