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Nonlinear Optical Imaging for Sensitive Detection of Crystals in Bulk Amorphous Powders

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Abstract

The primary aim of this study was to evaluate the utility of second-order nonlinear imaging of chiral crystals (SONICC) to quantify crystallinity in drug–polymer blends, including solid dispersions. Second harmonic generation (SHG) can potentially exhibit scaling with crystallinity between linear and quadratic depending on the nature of the source, and thus, it is important to determine the response of pharmaceutical powders. Physical mixtures containing different proportions of crystalline naproxen and hydroxyl propyl methyl cellulose acetate succinate (HPMCAS) were prepared by blending and a dispersion was produced by solvent evaporation. A custom-built SONICC instrument was used to characterize the SHG intensity as a function of the crystalline drug fraction in the various samples. Powder X-ray diffraction (PXRD) and Raman spectroscopy were used as complementary methods known to exhibit linear scaling. SONICC was able to detect crystalline drug even in the presence of 99.9 wt % HPMCAS in the binary mixtures. The calibration curve revealed a linear dynamic range with a R^2 value of 0.99 spanning the range from 0.1 to 100 wt % naproxen with a root mean square error of prediction of 2.7%. Using the calibration curve, the errors in the validation samples were in the range of 5%–10%. Analysis of a 75 wt % HPMCAS–naproxen solid dispersion with SONICC revealed the presence of crystallites at an earlier time point than could be detected with PXRD and Raman spectroscopy. In addition, results from the crystallization kinetics experiment using SONICC were in good agreement with Raman spectroscopy and PXRD. In conclusion, SONICC has been found to be a sensitive technique for detecting low levels (0.1% or lower) of crystallinity, even in the presence of large quantities of a polymer.

Keywords

second harmonic generation; second-order nonlinear imaging of chiral crystals; Crystallinity; solid dispersion; powder X-ray diffractometry; Raman spectroscopy; amorphous

INTRODUCTION

It is well recognized that pharmaceutical materials can exist in different solid forms.^{1,2} The form chosen for development must have acceptable physiochemical properties and be robust

enough to survive the manufacturing and storage process without compromising the quality of the end product. Thermodynamically stable crystalline forms are usually preferred for development from a long-term stability perspective. However, recently, metastable forms (e.g., amorphous solids) are increasingly under investigation as alternatives to improve the apparent solubility, dissolution, and the potential bioavailability of poorly soluble material.^{3,4} When amorphous forms are considered, their development is often hampered by their physical instability (i.e., the tendency to crystallize) and by the lack of characterization tools available to detect changes in crystallinity and particularly low levels of crystallinity.⁵ Sensitive detection of crystallinity in predominantly amorphous samples is important because even low levels of crystals can act as nucleation centers accelerating the crystallization process during both storage and dissolution, which in turn negate the advantages associated with using the amorphous form of the solid.^{6–8} The addition of polymeric crystallization inhibitors to overcome the challenges with the physical stability of amorphous drugs, forming solid dispersions, is a common formulation approach.^{9–13} However, for solid dispersions, detection of early stage crystallization is challenging for numerous reasons, in particular because of the diluting effect of the polymer (which results in a low overall amount of crystallizing material per unit volume) as well as issues with the polymer interfering with the analytical technique.

Several complementary techniques are often necessary to provide complete physiochemical characterization of pharmaceutical materials. These include, but are not limited to, differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), microscopic techniques, Raman, infrared spectroscopy, and solid-state nuclear magnetic resonance (ssNMR).^{2,14–17} However, most of these techniques are unable to detect crystallinity below approximately 1%. In the case of ssNMR, the technique is sensitive, but the time frame for the experiment can routinely be on the order of hours. Therefore, changes in crystallinity can occur during the time course of the measurement. In the case of DSC, the sample may undergo changes during the experimental run due to the temperature effects. Techniques such as transmission electron microscopy (TEM) or scanning electron microscopy (SEM) can, in principle, detect the presence of small crystallites; however, they require sample preparation steps, and there are difficulties in using TEM with powdered samples, and both methods only probe small fields of view that may miss trace crystallites under sampling. In the pharmaceutical industry, powders are common and hence there is a need for an analytical technique that is quick, reliable, needs little sample preparation, and can simultaneously provide microscopic and macroscopic information on crystallinity in powder samples.

In the current study, second-order nonlinear optical imaging of chiral crystals (SONICC) is explored as a sensitive and selective alternative for the detection of crystallization in bulk amorphous powders. Second harmonic generation (SHG), the underlying mechanism of SONICC, describes the nonlinear optical interaction between light and matter whereby light with certain energy interacts with a noncentrosymmetric material to produce light with twice the energy. The second-order nonlinear optical process has been of some interest in the pharmaceutical sciences for protein screening and chiral crystallization.^{18–20} There have been limited studies focused on exploring the utility of SHG for characterization of pharmaceutical solids.^{21–25} Rades and coworkers^{21–23} used SHG to distinguish and quantify polymorphism with the limit of detection calculated to be <5%. In addition, the researchers also used SHG to quantify crystallinity in binary mixtures of polyvinylpyrrolidone (PVP) with enalapril maleate and ranitidine hydrochloride with high sensitivity. Early detection and quantification of crystals from single component amorphous systems has been investigated by our group.^{24,25} In a previous study, the ability of SONICC to detect crystallites of 90 nm size in an optically transparent medium was demonstrated.²⁴ In another study, the loss of crystallinity of griseofulvin powdered samples upon cryogenic milling was

followed using SONICC and PXRD.²⁵ The crystallinity values determined with SONICC measurements compared favorably with those obtained from PXRD. However, SHG was successful in detecting trace amount of crystals, as low as 0.04%, well below the detection limits of PXRD.

The reported studies in the literature have focused on either the pure active pharmaceutical ingredients (API) or binary mixtures of polymer and API. However, the utility of SONICC for detection and quantification of crystallization from solid dispersions has not been explored. Most significantly, the scaling behavior of SHG with crystallinity has not been definitively established in solid dispersions. Preliminary experimental evidence in pure component powders suggests linear scaling (i.e., the integrated SHG intensity is directly proportional to crystallinity) based on comparisons with PXRD at a relatively small number of percent crystallinity values. Interestingly, this linear scaling is not necessarily expected for SHG. In thin ordered films, SHG efficiency scales quadratically with number density in the film by nature of the coherence of the interaction. Similarly, the SHG efficiency of pristine single-crystal scales quadratically with the crystal volume. Therefore, a linear scaling with percent crystallinity would imply incoherent combination of the SHG activities within API microcrystals. Although linear scaling with the number density of identical particles is well established, previous measurements utilized unfocused or gently focused light such that the signal arises from the collective interactions of many particles. It is not clear that this linear scaling should still hold under conditions in which the focal volume is on the order of the crystal size (~1 μm).

In the current study, SONICC was used to detect and quantify the SHG produced from micro crystallites in physical mixtures of naproxen and hydroxyl propyl methyl cellulose acetate succinate (HPMCAS) as well as a naproxen–HPMCAS solid dispersion. Raman spectroscopy and PXRD were used as reference methods with known linear scaling to quantify the SONICC measurements and experimentally assess the scaling behavior of SHG with crystallinity in solid dispersions. A brief introduction to nonlinear optics is provided below. A more detailed description of nonlinear optics is available elsewhere.^{26–28}

NONLINEAR OPTICS THEORY

When an electromagnetic (EM) wave interacts with a medium, a counteracting electric field is produced. The electric field itself can induce dipole moment changes in the medium, which now can act as a source for a secondary electric field. The counteracting electric field that results from the EM wave can be expressed in terms of a polarization vector, $\mathbf{P}$. In the case of linear optics, this polarization vector is linearly proportional to the applied electric field (E):

$$P^{(1)}(\omega) = \chi^{(1)}(-\omega; \omega) E(\omega) \quad (1)$$

where χ is the susceptibility of the medium and ω is the frequency of the EM wave. In other words, if light interacts with a medium, the resultant interaction is a linear function of the first power of the input electric field E .

In the presence of an intense optical field (e.g., from a focused ultrafast laser beam), nonlinear optical interactions between light and matter can occur. In the limit of weak intermolecular interactions, the total frequency-dependent polarization induced in the medium is approximated by the sum of all interactions (linear and nonlinear) given by the vector sum of all the induced molecular polarizations arising from both linear and nonlinear interactions.

The above equation can be expanded to show the relationship between the power of the applied electric field strength E , and its polarization response, where the power indicates the relationship between the polarization vector and the electric field strength. Each term in the series can be written in a general form.²⁸

$$P = P^{(1)} + P^{(2)} + P^{(3)} + \dots \quad (2)$$

$$P^{(2)}(\omega_{\text{sum}}) = \chi^{(2)}(-\omega_{\text{sum}}; \omega_1, \omega_2) : E(\omega_1) E(\omega_2) \quad (3)$$

$$\chi_{IJK} \alpha N_0 \left[\sum_{ijk} \langle R_{I_i} R_{J_j} R_{K_k} \rangle \beta_{ijk} \right] \quad (4)$$

$P^{(1)}$ represents the first order or the linear term as in Eq. 1 for an induced polarization at the same frequency as ω . $P^{(2)}$ represents the second-order nonlinear term, that is, the secondary wave induced at a new frequency $\omega_{\text{sum}} = \omega_1 + \omega_2$ can be induced by two incident waves of frequency ω_1 and ω_2 . These input frequencies can be positive in sign (e.g., sum frequency generation), negative (e.g., difference frequency, in which ω_2 is negative), or zero (e.g., Pockels effect, in which $\omega_2 = 0$). $P^{(3)}$ represents the third-order nonlinearity where the induced polarization can have a frequency $\omega_{\text{sum}} = \omega_1 + \omega_2 + \omega_3$, which collectively describes a of nonlinear optical interactions depending on the frequencies selected. I, J, K are Cartesian coordinates, N is the number density, β is the number element and R is a rotation matrix with its respective Cartesian coordinates.

A special case of this nonlinear optical process is SHG, corresponding to Eq. 3 when $\omega_1 = \omega_2 = \omega$ such that $\omega_{\text{sum}} = 2\omega$. SHG can only arise in certain classes of systems lacking inversion symmetry, including surfaces and the majority of space groups for chiral crystals (i.e., a crystal comprised of a unit cell with a nonsuperimposable mirror image). This requirement arises because the net SHG signal is a coherent sum of the interactions of all the molecules in the medium. Therefore, the net signal obtained is a function of the magnitude and the orientation of the molecule. In an isotropic system with equal probability for all molecular orientations, the net effect is destructive interference with no coherent SHG. Consequently, SHG has an advantage in terms of its high sensitivity to crystallinity because amorphous materials, liquids, solutions, and centrosymmetric crystals do not generate a net coherent SHG signal.

$$I_{c,\text{total}}^{2\omega} = |E_a^{2\omega} + E_a^{2\omega}|^2 \quad (5)$$

$$I_{i,\text{total}}^{2\omega} = |E_a^{2\omega}|^2 + |E_a^{2\omega}|^2 \quad (6)$$

Several qualitative models can be constructed for describing the anticipated scaling behavior of SHG with crystal size. If we consider two sources of SHG a and b (e.g., from two unit cells), two limiting behaviors exhibit different scaling trends with the number density of sources. From Eq. 5, the coherent SHG intensity from a pristine sample scales with the square of the coherently summed fields (i.e., identical phase relative to the driving field), and increases with the square of the number density of sources. If sufficient disorder is present within the sample such that the two sources are no longer phase coherent, a statistically random phase shift between many sources results in a signal scaling linearly

with the number density of sources. However, the coherence length along the beam propagation axis for epi-detected SHG is approximately 100 nm, beyond which increases in crystal thickness can only reduce the net SHG intensity.²⁴ Consequently, pristine lattice growth would be expected to produce oscillations in the SHG intensity with increasing crystal thickness with a period approximately equal to the coherence length. Because the net SHG would still increase with crystal cross-sectional area, the overall net scaling would be with the volume to the 4/3 (i.e., quadratically with the area and independent of the thickness). Finally, imperfections within a lattice over distances >100 nm or the presence of polycrystallinity could result in significant generation of incoherent SHG, in which each local domain effectively acts as a separate independent SHG-active source. In this case, the persistence length of order within the lattice becomes a defining parameter. Once the coherence is lost (i.e., for crystals larger than the single-crystal persistence length), the scaling behavior would be expected to be linear. Consequently, the scaling behavior of SHG may depend sensitively on the nature of the crystals formed and is not necessarily immediately obvious in studies of the crystallinity evolved in solid dispersions.

EXPERIMENTAL

Naproxen–HPMCAS Standard Curve and Solid Dispersion

To prepare the physical mixture standard curve, different proportions of the HPMCAS (Shin-Etsu AQOAT, Type AS-MF, Shin-Etsu chemicals, Niigata, Japan) and crystalline naproxen free base (Sigma–Aldrich, St. Louis, Missouri) were milled using a cryogenic mill (6750 freezer mill; SPEX SamplePrep, Metuchen, New Jersey). An appropriate amount of the sample was weighed and milled at a frequency of 8 Hz. The samples were equilibrated at liquid nitrogen temperatures for 5 min before milling. Each cycle consisted of milling for 2 min followed by a cooling interval of 1 min between the two cycles. The milled samples thus obtained were allowed to equilibrate at ambient temperature before they were transferred to a dry nitrogen purged glove box to minimize the effect of moisture during handling. The samples were sieved to isolate particles having a size <105 µm. Several drug–polymer mixtures in the range of 0%–100% concentrations of naproxen were chosen. Samples were prepared in triplicate. An independent prediction set containing three concentrations of crystalline naproxen–HPMCAS different from the calibration sample values was also milled to check the validity of the standard curve. Solid dispersion samples containing 75 wt % HPMCAS were prepared by rotary evaporation. The drug and the polymer were dissolved in a 1:1 (ratio by weight) mixture of dichloromethane (Mallinckrodt Baker Inc., Paris, Kentucky) and ethanol (Aaper Alcohol and Chemical Company, Shelbyville, Kentucky). The samples obtained by rotary evaporation were then stored under vacuum overnight followed by milling using a cryogenic mill and sieved to generate particles <105 µm. The solid dispersion samples were stored at 60°C and taken out at specific time intervals to follow the kinetics of crystallization using PXRD, Raman spectroscopy, and SONICC. To quantify the crystallinity of the solid dispersion sample using PXRD and Raman spectroscopy, standards containing solid dispersion and crystalline drug were prepared. An appropriate amount of crystalline naproxen was cryomilled along with the solid dispersion to obtain 75 wt % polymer–25 wt % naproxen blends containing different proportions of crystalline and amorphous naproxen. Different crystalline to amorphous naproxen ratios were prepared and analyzed using PXRD and Raman spectroscopy. In the case of SONICC, the signal from the solid dispersion was normalized against physical mixture containing 75 wt % HPMCAS and 25 wt % crystalline naproxen. In addition to the prediction samples, a partially crystallized solid dispersion sample was spiked with a known amount of crystalline material to test the ability of SONICC to recover crystallinity in a sample.

Second-Order Nonlinear Optical Imaging of Chiral Crystals

In these experiments, SHG images were acquired using a custom-built system that performed beam scanning with a resonant vibrating mirror (Electro-Optical Products, New York City, New York, ~8 kHz) to direct the beam along the fast-axis scan, and a Cambridge galvanometer for slow-axis scanning. A single-image frame, scanning with 1000 averages per line, was completed in 25 s. The Z-axis scanning was performed using OptiScan™ motorized focus control (Prior Scientific, Rockland, MA, USA) with a 20 μm step size. The images were acquired with a resolution of $4 \times 4 \times 20 \mu\text{m}^3$. The incident beam was generated from an ultrafast Spectra-Physics Mai Tai, 100 fs, 80 MHz, 20 mW average power at 800 nm during imaging, focused onto the sample using a 10 $\times$ objective (Nikon 0.3 NA) (Fig. 1). The depth-of-field and spot size were measured to be approximately 20 and 2 μm , respectively. The use of relatively low magnification allowed measurements over a large field of view, whereas the low laser power of 20 mW was selected to ensure a linear dynamic range for all percent crystallinity measurements during image acquisition. The SHG signal was collected with dichroic mirrors with narrow band-pass filters (Chroma HQ400/20 m-2p, Bellows Falls, VT, USA) centered around 400 nm used to reject the incident beam. The SHG signals were detected by photomultiplier tubes (Burke, XP 2920PC, Lancaster, PA, USA) with photon counting electronics (Becker-Hickl PMS 400A, Berlin, Germany), with the relative collection efficiencies of the detectors calibrated using the two-photon excited fluorescence from Chroma standard slides. SHG images were analyzed using ImageJ (available at <http://rsb.info.nih.gov/ij/>; developed by Wayne Rasband, National Institutes of Health, Bethesda, Maryland). In each SONICC measurement, approximately 1–2 mg of powder was sandwiched between a microscope glass slide and a cover glass with 150 μm spacers inserted to maintain a uniform packing of the powder sample. Three different fields-of-view were imaged for each sample to obtain a reasonable statistical representation for the crystallinity estimate. Backward propagated SHG was used to estimate the crystal fraction within powders.

Determination of the Relative Crystallinity by Epi-Detected SONICC

Epi-detected SHG was used to estimate the crystal fraction within powders. The average SHG intensity integrated over the field of view for each sample was normalized against the average SHG intensity of 100% crystalline naproxen to estimate the relative crystallinity for the powder blends. The average background noise (near-zero value) obtained from pure HPMCAS was subtracted from each measurement. In the case of the 75 wt % HPMCAS–naproxen solid dispersion sample, a physical mixture containing 75 wt % polymer and 25 wt % crystalline drug was used to normalize the SHG intensity.

Powder X-Ray Diffraction

X-ray diffraction data were obtained using Cu-K α radiation with a Shimadzu XRD-6000 powder diffractometer (Shimadzu Scientific Instruments, Columbia, Maryland) operating at 40 kV and 30 mV. For each sample, the X-ray measurements were conducted over a scan range of 5°–35° 2 θ at a scan rate of 4°/min with a step size of 0.04° and the sample was placed in an aluminum sample holder. The [1 1 1] peak of a Si-standard sample between 28.423° and 28.463° was used as the external standard.

For the data analysis of the naproxen–HPMCAS calibration blends, orthogonal projections to latent structures (OPLS) calibration model was constructed using SIMCA P+ V.12 (Umetrics Inc., Kinnelon, New Jersey) to determine the crystallinity. The data were mean centered, that is, each data point was subtracted by the average value of that variable. A model that adequately described the percent crystallinity of naproxen from the naproxen–HPMCAS physical blends could be constructed, with a R^2 value of 0.98 and Q^2 value of

0.97. The resultant calibration model yielded a root mean square error of estimation (RMSEE) of 4.10.

Raman Spectroscopy

Raman spectra were obtained using a RamanRxn1 –785 Raman spectrometer (Kaiser Optical System, Inc., Ann Arbor, Michigan) operating at an excitation wavelength of 785 nm. The spectra were collected using a fiber optic MR probe attached to noncontact optics (Kaiser Optical System, Inc.). The Raman spectrometer was controlled using Holograms software (version 4.0; Kaiser Optical System, Inc.). Spectra from six to 10 different regions of the powder bed were measured to minimize subsampling and the average of these readings was used for determining percent crystallinity. The experiments were performed in triplicate and the average value of three different experiments is reported. For the data analysis of the naproxen–HPMCAS calibration blends, an OPLS calibration model was constructed using SIMCA P+ V.12 (Umetrics Inc.) to determine the crystallinity. The data were mean centered and the 3120–500 cm^{-1} spectral range was used to build the model and, to minimize the effect of fluctuations on spectral intensity; a standard normal variate correction on mean centered data was performed. A logarithmic transformation was performed and a model that adequately described the percent crystallinity of naproxen from the physical blends could be constructed, with R^2 value of 0.99 and Q^2 value of 0.99. The resultant calibration model yielded a RMSEE of 2.22.

RESULTS

In the current study, we have evaluated the scaling behavior and detection limits of SONICC for quantification of crystallinity in both polymer–drug physical blends and in solid dispersions using Raman spectroscopy and PXRD as comparator techniques. Figure 2 shows the SONICC images, PXRD patterns, and Raman spectra for a 75 wt % HPMCAS–25 wt % crystalline naproxen physical mixture compared with that obtained from a nominally amorphous solid dispersion prepared at the same drug–polymer ratio. The 800–1000 cm^{-1} region of the Raman spectra can be used to qualitatively identify the presence of crystalline drug. In the case of the solid dispersion, the Raman spectrum shows a broad peak around 856 cm^{-1} , whereas a peak can be seen at 863 cm^{-1} in the presence of crystalline material. On the basis of the PXRD pattern and Raman spectrum, the dispersion appears to be amorphous. However, the SONICC images clearly reveal that immediately after preparation, the dispersion does in fact still contain a significant fraction of crystalline naproxen. This presence of crystalline material is readily apparent, even though the drug is heavily diluted by the polymer, which most likely explains the lack of detection of the crystalline material with the PXRD or Raman measurements.

To better understand the relative performance and scaling of SONICC compared with the other analytical techniques, standard curves were constructed by monitoring the response of the technique to samples containing different ratios of crystalline naproxen and HPMCAS. The predicted naproxen concentration as measured from the SHG intensity in the physical mixture as a function of the actual concentration of the naproxen is shown in Figure 3. The data points indicate that SHG intensity varies linearly with naproxen concentration and that the presence of HPMCAS does not appear to interfere with the signal.

To provide additional support for this conjecture, 3D images of pure crystalline and the powder blend containing 0.1% naproxen–HPMCAS sample were constructed from Z-slices of SONICC images as shown in Figure 4. In the case of crystalline naproxen, SHG activity is seen over the entire field of view. For the low naproxen concentration sample, the physical mixture shows discrete regions of SHG activity in the powder bed even though 99.9% of the sample consists of HPMCAS, clearly indicating that HPMCAS does not

interfere with the SHG signal and that miniscule levels of crystallinity can be readily detected. Experiment time to acquire each 3D SONICC image depends on resolution of images, and signal strength. Acquisition time per frame can vary from 10 s (for an estimated 200 averages per line) to several minutes (for several thousand averages per line). In this particular study, 3D images were acquired for 3 min.

The calibration curve demonstrates only a small extent of scatter as seen from the narrow width of the prediction bands with larger standard deviation at higher naproxen concentration. This insensitivity to scatter is further seen in the goodness of fit ($R^2 = 0.998$) and slope value (0.98). The ability of the SON-ICC instrument to detect crystals at lower concentration with good accuracy is shown by the plot of actual versus predicted data in the concentration range 0%–25% naproxen (Fig. 3 insert). The R^2 value and slope of the line has values of 0.99 and 0.92, respectively. The detection limit of SONICC for crystallinity in the physical mixture can be estimated from the intensity in a single voxel producing a signal to background greater than 3 within the total volume probed in the field of view for sample containing no naproxen. The SHG intensity of a single voxel ($4 \times 4 \times 20 \mu\text{m}^3$) can have a broad distribution because of the variations in size and orientation of the crystals. For a signal to background of 3 within a single voxel, the mean detection limits for naproxen in HPMCAS will be given by the ratio of that calculated signal for no naproxen sample to the signal generated by the 100% crystalline powder. For naproxen–HPMCAS under the conditions in which these data were recorded, the mean detection limits correspond to $1 \times 10^{-3}\%$ (i.e., 10 parts per million) crystallinity.

Raman spectroscopy and PXRD were used as the reference methods to quantify crystallinity and determine the experimental scaling of the SHG measurements. The calibration curves for the naproxen–HPMCAS calibration sample obtained from the PXRD and Raman measurements are shown in Figures 5a and 5b, respectively. In the case of PXRD, samples containing less than 5 wt % naproxen did not show detectable peaks indicative of the presence of crystalline naproxen. Therefore, a full pattern fit employing the OPLS method was used. Compared with the SONICC data, the prediction bands showed a larger scatter indicating a larger error in predicting percent crystallinity. In the case of Raman measurements, a full pattern fit with the spectral region $3120\text{--}500 \text{ cm}^{-1}$ was used. The prediction bands showed a smaller error in predicting percent crystallinity compared with the SONICC calibration curve. The R^2 and RMSEE values for PXRD and Raman measurements, as compared with SONICC are summarized in Table 1.

To validate the calibration curve, a prediction set at three different concentrations (different from the calibration values) of naproxen–HPMCAS was prepared and the ability of a given technique to predict the crystallinity based on the calibration curve was evaluated. As shown in Table 2, the value of crystallinity determined for the prediction set correlates well with the theoretical crystallinity values. The predicted values from SHG show about 10% error at low percent crystallinity with the error being closer to 5% at higher concentrations of naproxen. In a separate experiment, the ability of SONICC to show linear additivity of crystallinity was tested by adding a known amount of crystalline naproxen to a partially crystalline solid dispersion sample. The initial crystallinity of the partially crystallized sample as measured by SONICC was 22.2%. Upon addition of a known amount of crystalline naproxen to this sample, SONICC showed a measured crystallinity value of 39.1%. The theoretical value of crystallinity based on spiking the sample with known quantity of crystalline naproxen yielded a value of 41.1%. Therefore, the SONICC experiment demonstrated a recovery of approximately 93% of the crystallinity from the spiked solid dispersion sample, indicating the linear additivity of the initial crystallinity and the added crystalline material.

In addition to physical blends of naproxen and HPMCAS, real time quantitative measurement of crystallinity in a solid dispersion system was also undertaken using SONICC with Raman spectroscopy and PXRD as the reference methods. Figure 6 shows the SONICC image and the corresponding PXRD image and Raman spectra of a 25% naproxen–HPMCAS solid dispersion sample maintained isothermally at 60°C to progress crystallization. The sample did not show detectable peaks indicative of long-range order until the 2 h time point based on the PXRD data. In the case of the Raman data, the initial time point also does not indicate the presence of crystalline naproxen. One of the representative regions, 800–900 cm^{-1} is shown in Figure 6. With increases in crystallinity a shoulder arises at the 2 h time point at 863 cm^{-1} that develops into a peak as the sample crystallizes with time. On the basis of the RMSEE of the calibration models for the dispersions (5% and 7.4% for PXRD and Raman spectroscopy, respectively), the crystallinity could not be estimated for either technique until the 2 h time point. Figure 7 shows the evolution of crystallinity from this time point. For quantitative measurements using SONICC, the intensity values of the samples were normalized using the 75 wt % HPMCAS–25 wt % crystalline naproxen physical mixture as the reference. In the presence of the high polymer concentration (75 wt %), there is incomplete crystallization over 48 h storage at 60°C. The isothermal crystallization kinetics measurements from SONICC are in good agreement with those from Raman spectroscopy and PXRD, with the crystallinity estimates falling in between the values determined with the other techniques.

DISCUSSION

From the systematic comparisons with both Raman and PXRD, it is clear that SHG scales linearly over the range of percent crystallinity in these measurements of solid dispersions. These results agree with previous comparatively limited studies supporting linear scaling of SHG with crystallinity in powders.²⁴ Consequently, it is reasonable to expect linear scaling behavior to emerge as the general trend rather than the exception in API crystallization studies.

Importantly, it was found that nonlinear optical imaging provides detection limits for crystallinity in these solid dispersions that are substantially lower than PXRD and Raman. Spectroscopic techniques and PXRD are commonly used methods to detect and quantify crystallinity. For example, Black and Lovering²⁹ showed good correlation between infrared spectroscopy and PXRD for estimation of degree of crystallinity. Standard laboratory X-ray instruments generally have a detection limit of around 5%–7% for organic crystalline material unless a powerful source such as synchrotron radiation is used for illumination.³⁰ Moreover, accurate quantification of crystallinity in amorphous solid dispersions often requires calibration samples prepared from mixtures of the amorphous dispersion, combined with the crystalline drug.³¹ This requirement is because the diffuse scattering pattern of the dispersion is not necessarily the sum of the scattering halo of the pure amorphous solid and the pure amorphous polymer.³² This factor, combined with the difficulty of detecting the early stages of crystallization in polymer-rich dispersions due to the low overall mass of drug present, limits the utility of PXRD for detecting low levels of crystallinity in dispersions. One of the main advantages of using the SONICC method is that the method is insensitive to amorphous materials, which leads to a near-zero background contribution from amorphous polymers and results in the highly sensitive detection of low levels of chiral crystals. The images from SONICC clearly show the presence of residual crystals below the detection limit of PXRD (Fig. 2).

The SONICC signal is attributed to trace concentrations of naproxen crystals, since over time the sample developed peaks in PXRD characteristic of naproxen. These results are in agreement with an earlier study in which griseofulvin samples, when cryomilled beyond a

certain time period (90 min), showed residual crystallites by SONICC even when the sample appeared X-ray amorphous.²⁵ Therefore, it is clear that SONICC has significantly lower detection limits for crystallinity than laboratory PXRD measurements, particularly in the presence of polymers. Another widely used technique, Raman spectroscopy, has relatively high sensitivity with detection limits as low as 1% in the case of a pure drug in the absence of polymer.¹⁴ However, interpretation of the results from Raman spectra of dispersions is generally not simple because the presence of polymer can interfere with the signal from the drug. Therefore, chemometric tools have to be utilized to extract information about crystallinity of the solid dispersion samples. Similar to Raman spectra, the SHG signal is sensitive to particle size. It is important to ensure that calibration standards match the particle size range of the actual samples. However, SONICC does have the advantage over both Raman spectroscopy and PXRD that the crystallinity values can be determined by using the pure 100% crystalline material or a crystalline-polymer physical blend as the standard without the need for complex chemometric analysis or calibration samples containing different ratios of amorphous and crystalline drug. In terms of a direct comparison of the techniques, it is apparent from Table 1 that the crystallinity values predicted by SONICC for the validation samples closely match those generated by the Raman spectroscopy and PXRD methods. Moreover, the predicted crystallinity values using SONICC show approximately 10% error at the lowest concentration and 5% or less error for the other validation concentrations tested, which is comparable with the other techniques. Overall, the predicted crystallinity values are very close to the theoretical values considering the fact that there are undoubtedly errors associated with preparation of the samples. Moreover, the ability of SONICC to recover greater than 90% crystallinity in a spiked solid dispersion sample helps to further validate this method in terms of its utility for investigating crystallinity in solid dispersions.

The high sensitivity of SONICC for detection of crystallinity relative to the other techniques is further highlighted by the ability to detect crystals even in the presence of 99.9% polymer. This observation is in agreement with the work of Rades and coworkers,²¹ who showed that for PVP-enalapril maleate form II mixtures, the ensemble-averaged limit of detection was calculated to be 0.12% (1.2 parts per thousand). By using femtosecond laser sources and optimizing imaging instrumentation targeting this application, SONICC has significantly improved on those detection limits.³³ The estimated limit of detection of 10 ppm crystallinity calculated for naproxen-HPMCAS in this study is well below the detection limit of solid-state characterization techniques commonly employed to study crystallization. The LOD is higher than that detected for crystallization from amorphous films of griseofulvin (on the order of parts per trillion) because of the nonzero background from the polymer.²⁴ The results from the kinetics experiment highlight the potential utility of SONICC as an online monitoring tool. The kinetics of crystallization from SONICC matches reasonably well with the results obtained from PXRD and Raman and this extent of discrepancy between techniques is common.^{16,34,35}

Moreover, there can be differences with crystallinity values with SONICC measurements associated with differences in size of the crystallites in the solid dispersion as compared with that of crystalline drug in the standard, a factor which also influences the other techniques used in this study. Larger crystalline domains lead to increased SHG intensity, because of greater SHG conversion efficiency, peaking when crystalline domains are on the order of their coherence lengths.³⁶ The other factor that may contribute to the uncertainty in estimated crystallinity by SONICC is the assumption of uniformly distributed orientations of crystallites. Similar to preferred orientation effects in PXRD, the intensity of SHG depends on orientations of crystallites relative to the reference frame of measurement. As a result, the uniform orientation assumption might not be held for a very sparse crystallite sample such as early kinetics of crystallization. Finally, we note that the minimum detectable crystal size

is on the order of 100 nm^{24} ; therefore, if crystal nuclei below this size are present, these are unlikely to be detected with SONICC.

The use of SONICC to detect crystallinity has other advantages than those discussed above, including the availability of microscopic crystallinity information along with the macroscopic overview. Microscopic instruments such as SEM and TEM may be suitable to detect crystals smaller than those seen by SONICC at relatively high number densities. However, SONICC has the advantage that it requires no sample preparation and unlike TEM, the sample field of view does not need to be thin and transparent. Films, solutions, and powder samples can all be analyzed with virtually no sample preparation. Furthermore, SONICC has the advantage of enabling detection of the rare, isolated trace crystals that might be missed by TEM given the relatively low volumes sampled. In contrast to many thermal analytical techniques, SONICC is a nondestructive method. In addition, data collection times are short: of the order of seconds to minutes.

Second-order nonlinear imaging of chiral crystals also has a number of limitations that need to be considered. First, it is not applicable to all compounds, only those that crystallize into an SHG-active noncentrosymmetric space group, which includes the large majority of chiral compounds, but routinely does not arise for achiral targets. Second, with the current instrumental setup, chemical or structural information (beyond an assessment of % crystallinity) is not directly available. As such, SONICC is arguably best used as a complement to conventional analyses, rather than as a replacement. Third, the presence of crystalline chiral excipients other than the molecule of interest will potentially interfere with the measurement in the current instrumental set up.

In spite of these limitations, SONICC is a promising technique that is highly complementary to the currently available solid-state characterization tools to detect crystallinity with low detection limits. This capability would be especially advantageous in monitoring minute levels of crystallinity in amorphous solid dispersions at low drug loadings, where it may be otherwise challenging to optimize polymer choice and processing conditions without undergoing long storage periods to enable crystallinity to be detected with less-sensitive techniques.

CONCLUSIONS

Second-order nonlinear imaging of chiral crystals, which exploits the phenomenon of SHG, has been found to enable sensitive quantification of the extent of crystalline material present in drug-polymer physical blends and solid dispersions. A linear correlation was observed between the SONICC signal and the extent of crystallinity measured independently by powder XRD and by Raman spectroscopy across the entire calibration range, helping to resolve a fundamental scaling ambiguity. The evolution of crystallinity from initially amorphous solid dispersions was also evaluated with this method. SONICC was found to provide both microscopic and macroscopic information, as well as the ability to detect the early stages of crystallization when the mass fraction of crystalline material is low and crystalline domains are of submicron dimensions. SONICC is well suited for the characterization of amorphous solid dispersions prepared with chiral compounds, and can be used as an advanced analytical tool to aid in enhancing the mechanistic understanding of crystallization processes.

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Figure 1.
Representative figure of the SONICC instrument setup.

Figure 2.

PXRD, SONICC images and Raman spectra of the 75 wt % HPMCAS–25 wt % naproxen solid dispersion, immediately after production, and the corresponding physical mixture containing 25 wt % crystalline naproxen. SONICC images clearly show the presence of residual crystals in the solid dispersion that are not detected by PXRD and Raman spectroscopy (* indicates peak associated with crystalline naproxen in the Raman spectrum).

Figure 3. Calibration curve confirming a linear relationship between the SONICC integrated intensity and the crystalline composition in the standard (0%–100% naproxen range). Insert shows linear relationship for the range of 0%–25% naproxen. The dashed lines show the 95% upper and lower prediction bands.

Figure 4. Three-dimensional SONICC images of pure (100%) crystalline (left) and 0.1% crystalline naproxen physically blended with 99.9% HPMCAS powder (middle). The average SHG intensity over a particular crystallite is also shown (right).

Figure 5.

(a) Calibration curve confirming a linear relationship between the PXR response and the crystalline composition in the standard is shown on the left. (b) Calibration curve confirming a linear relationship between the Raman response and the crystalline composition in the standard is shown on the right. The dashed lines show the 95% upper and lower prediction bands.

Figure 6. Change in crystallinity of a 75 wt % HPMCAS–25 wt % naproxen solid dispersion sample monitored at 60°C using SONICC (right) and PXRD (left) and Raman spectroscopy (below).

Figure 7.
Change in crystallinity of 75 wt % HPMCAS–25 wt % naproxen solid dispersion as measured by Raman spectroscopy (▲), SONICC (○), and PXRD (■).

Table 1

Slope and Goodness of Fit for Naproxen-HPMCAS Calibration Curve Calculated Using Both PXRD and SONICC

Technique	Calibration Range	Slope	R^2	RMSEE
SHG	0–100	0.98	0.998	2.68
SHG	0–25	0.941	0.996	1.90
PXRD	0–100	0.985	0.981	4.10
Raman	0–100	0.996	0.998	2.22

Table 2

Crystallinity Values of the Prediction Set

Actual Crystallinity (%)	Predicted Crystallinity (%)		
	SHG	PXRD	Raman
3.5	3.86	-	3.74
18	18.95	16.70	19.92
83	86.53	85.73	88.75