REVIEW PAPER

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Insights into solid dosage forms with nonlinear optical imaging

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ABSTRACT

Microscopic chemical and solid-state structures and their changes in solid drugs and dosage forms can profoundly affect pharmaceutical performance and patient safety. Despite this, their detailed spatially-resolved analysis can be difficult or impossible with established analytical technologies. Multimodal non-linear optical imaging presents opportunities for sensitive and specific chemical and solid-state pharmaceutical imaging. Non-linear optical imaging encompasses several nonlinear optical phenomena, including coherent anti-Stokes Raman scattering (CARS), stimulated Raman scattering (SRS), and sum frequency/second harmonic generation (SFG/SHG). Imaging in 3D with (sub)micron resolution is rapid, non-destructive, possible *in situ* in aqueous media, and generally does not require prior sample preparation. This mini-review explores several applications of non-linear optical imaging for solid drug and dosage form analysis.

Introduction

Most medicines are marketed as solid dosage forms comprised of an active pharmaceutical ingredient (API) and excipients. Their optimal therapeutic performance and safety requires understanding of numerous critical quality attributes, such as particle size and morphology, solid-state form, stability and drug release. Characterizing these attributes is comparatively straightforward with individual APIs and excipients, but much more challenging once the components are combined into a dosage form. Traditional pharmaceutical analysis techniques are often not sufficiently sensitive and/or specific for critical quality attribute analyses in solid dosage forms. One emerging analytical approach to help address this issue is nonlinear optical imaging (NLO). NLO offers chemical and solid-state specific imaging of solid dosage forms in a non-destructive, rapid,

and label-free manner, with (sub-)micron 3D resolution, either in dry or aqueous environments. In this mini-review, NLO is briefly introduced in the context of solid dosage forms and some possible related applications are considered.

Brief introduction to nonlinear optics

In general, two or more photons interact in an NLO process to create a new photon of a different frequency carrying information on the physical properties of the medium. The interaction scales nonlinearly with the incident light intensity, implying inherent confocality as any significant nonlinear process can take place only at tight focal point of a laser beam, and requiring the use of high peak power and ultrashort laser pulses in the femtoseconds to nanoseconds regime. In the sum frequency generation (SFG))/second harmonic (SHG) process, the frequency of the generated photon the sum of two incident ones and can only take place in non-centrosymmetric crystal structures, which represents a significant proportion of new small-molecule drug candidates [1]. In coherent Raman scattering, including coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS), vibrational Raman modes of the sample molecules are probed by tuning the frequency difference of two laser beams to match the desired wavenumber. Hence, the technique, with spectra exhibiting both chemical and solid-state specificity, is applicable to many sample types and is perfectly complemented by SFG/SHG. **Table 1** compares the characteristics of NLO microscopy to confocal Raman microscopy (with spontaneous Raman scattering).

Solid dosage form applications

Chemical imaging

Coherent Raman microscopy, including both CARS and SRS, are well suited to qualitative 2D and 3D imaging of component distribution in solid dosage forms such as tablets [2, 3], dry powder inhalation mixtures [4], solid dispersions [5] and microparticles/granules [6–8]. As an example, the distributions of an active ingredient and the excipients at the surface of a tablet are presented in **Figure 1**, together with their SRS spectra. The spectral data was collected in approximately 3 minutes and analyzed with classical least squares to classify the pixel spectra into different components. Quantitative analysis of coherent Raman data may be performed through two general routes: analysis of the images or the coherent Raman spectra. The former is most suited to determine the particle/domain size of different components or quantification in a mixture, for example, as demonstrated by Francis et al (2018) [5]. For the latter, SRS has the advantage over CARS data of a signal intensity that is theoretically linear in the concentration. A review considering the potential, benefits and pitfalls of quantitative SRS has recently been published [9].

Solid-state imaging

Solid-state forms of the API and excipients have been imaged using both SFG/SHG and CARS/

Table 1. Spontaneous Raman and nonlinear optical imaging modalities most relevant to solid dosage form analysis

Microscopy Technique	Confocal spontaneous Raman	Coherent Raman (CARS and SRS)	Second harmonic/Sum frequency generation (SHG/SFG)
Spectral resolution	~1–4 cm^{-1}	~5–20 cm^{-1}	NA
Spatial resolution (lateral)	Variable (sub-micron to 20 mm)	Intrinsically confocal (sub-micron)	Intrinsically confocal (sub-micron)
Aquisition time	Slow, typically hours per image	Fast (video rate if single wavenumber measured)	Fast (video rate)
Chemical information content	High (whole spectrum can be recorded)	Moderate (whole spectrum can be recorded, but it is still technologically challenging)	Low (classifies crystals to symmetric/ non-centrosymmetric)
Challenges	Sample burning, fluorescence interference (sample dependent)	Sample burning, limited fluorescence interference (sample dependent)	Sample burning, limited fluorescence interference (sample dependent)

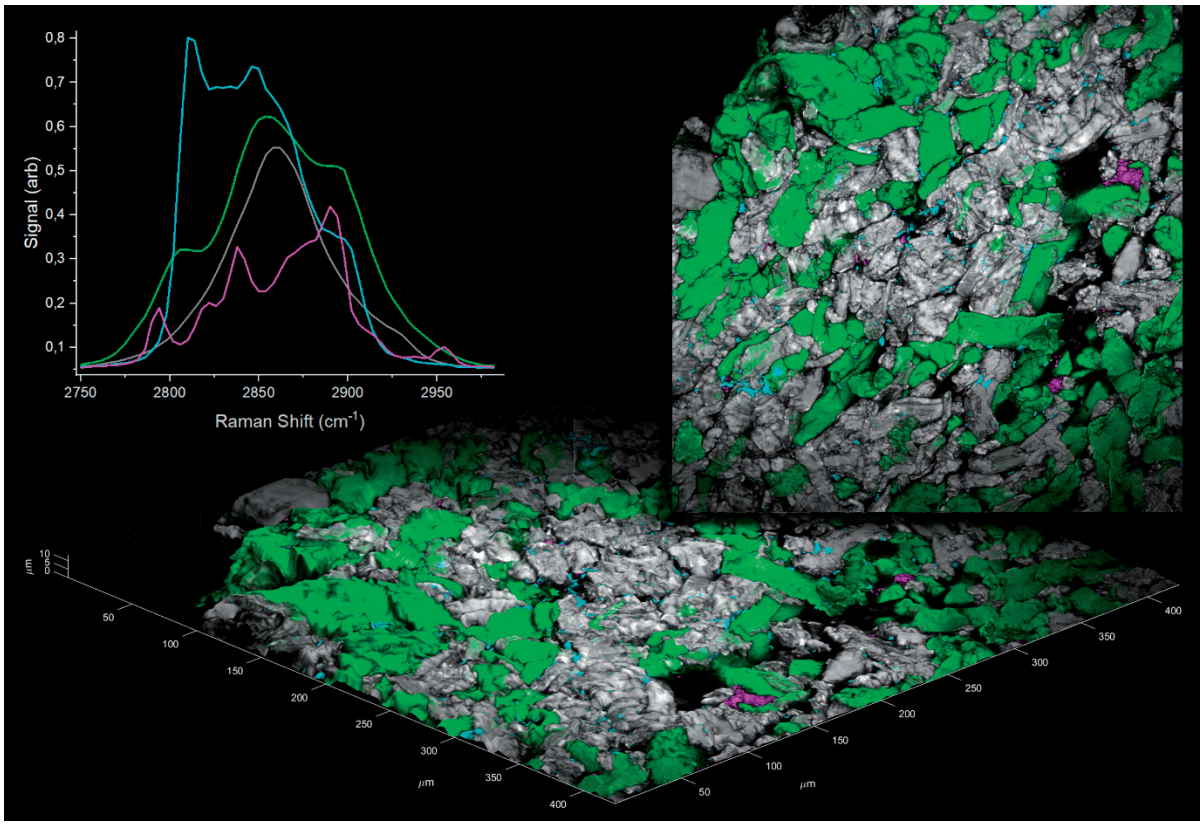

Figure 1. Example of SRS imaging of component distribution at the surface of a pharmaceutical tablet with a low-dose API. Component SRS spectra employed for classical least squares (CLS) analysis (top left), 2D composite projection (top right) and 3D projection (bottom). Green: hypromellose, grey: microcrystalline cellulose, cyan: magnesium stearate, magenta: API.

SRS. SHG imaging, which generally involves technically simpler instrumentation than CARS/SRS, has been used to detect and image crystallinity in otherwise amorphous powders and formulations subjected to different manufacturing processes [10–13]. The technique can be extremely sensitive to trace crystallinity (on the order of parts-per-million). The Simpson laboratory at Purdue University, in particular, has investigated and developed SHG imaging for solid-state pharmaceutical analysis [1, 14–16]. Combining SFG/SHG with CARS/SRS can increase solid-state specificity and confidence in the analyses. For example, simultaneous multimodal CARS and SFG imaging have been used to simultaneously resolve the amorphous form and up to four crystalline forms indomethacin in compacted mixtures [17, 18]. Trace levels of the forms were detectable below the sensitivity limit of x-ray powder diffraction, and infrared and Raman spectroscopies. By virtue of the chemical/solid-state specificity, rapid measurements and sub-micron spatial resolution, NLO imaging is highly suited for detecting trace levels of, for example, solid-state forms of low dose APIs in formulations.

Stability analysis

NLO imaging is well suited to the analysis of (surface) transformations in pure drugs and excipients, as well as in formulations. SHG/SFG and CARS/SRS can be used to detect subtle solid changes at an earlier stage than conventionally applied solid-state analysis methods, and in principle can detect in the parts-per-million range [16–19]. By virtue of both the spectral and spatial resolutions, crystallization of multiple solid-state forms may be efficiently detected in a single sample, especially when multimodal non-linear optical imaging is employed. For example, Novakovic *et al* detected the simultaneous crystallization of amorphous indomethacin into four different polymorphs [18].

Drug release and dissolution

A great advantage of NLO imaging in the context of drug release analysis, is the ability to rap-

idly and non-destructively image formulations *in situ* in aqueous environments. This benefit has allowed, for example, *in situ* non-linear optical imaging of drug release from sustained release formulations [5] and solid-state changes in dissolution media [20, 21]. The speed of NLO imaging (video-rate to minutes) means there is tremendous potential to further explore important and otherwise hard to detect physicochemical phenomena that occur in solid dosage forms and the surrounding media upon drug release.

Conclusions

NLO imaging is well suited to elucidating solid dosage form structure and behaviors. Potentially valuable applications include high-resolution chemical- and solid-state imaging of low-dose formulations, detection of trace- and surface components/changes as a function of production and storage, and *in situ* examination of physicochemical phenomena upon drug release. Increased availability of the technique and awareness of its analytical possibilities and pitfalls, will help its potential to be realized in pharmaceutical industry and academia.

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Conflict of interest statement
The authors declare no conflict of interest.

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