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Use of low-frequency Raman spectroscopy and chemometrics for the quantification of crystallinity in amorphous griseofulvin tablets

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ABSTRACT

Low-frequency Raman spectroscopy, which directly probes phonon lattice modes of crystal structures, has much unexplored potential for sensitive qualitative and quantitative analysis of crystallinity in drugs and excipients. In this study, the level of crystallinity in tablets containing amorphous drug is quantified using low-frequency Raman spectroscopy in concert with chemometrics for the first time. Importantly, these data are directly compared to simultaneously obtained mid-frequency Raman spectra, as well as to FT-Raman data, which is commonly used for such quantification. Griseofulvin was used as a model drug. The PLS model using FT-Raman spectroscopy gave a root mean squared error of prediction (RMSEP) of 0.65%. The PLS models of the low- and mid-frequency regions using a charge coupled device (CCD) based Raman system with 785 nm excitation gave an RMSEP = 1.2% when using the low-frequency region (5–120 cm^{-1}) and RMSEP = 1.4% for the mid-range frequencies (520–1740 cm^{-1}). The recrystallization profiles determined using the various Raman techniques and their associated models were similar. The FT-Raman and the low frequency Raman systems were able to detect and quantify crystallinity in stored amorphous samples earlier than the mid-frequency 785 nm Raman system. Overall, this study suggests that low-frequency Raman spectroscopy has at least equally good performance compared to mid-frequency Raman for quantitative analysis of crystallinity in the pharmaceutical setting. More generally, the much stronger Raman scattering in the low-frequency region combined with the intrinsic spectral differences between amorphous and crystalline materials may prove advantageous for some analyses.

1. Introduction

Recent developments in computational drug discovery and design have led to the generation of a large number of new chemical entities [1]. However, only a small fraction of these new chemical entities will be successfully translated into therapeutically effective medicines. One of the major reasons for the translation failure is the poor water solubility of the majority of these compounds [1–3]. This presents a challenge to formulate them into oral formulations as they have low oral bioavailability. Many strategies have been developed to overcome this formulation challenge and these include formulating poorly water soluble drugs into salts, using cosolvents, particle size reduction and formulating them in the amorphous form [1–4].

There is a growing research interest in the area of amorphous formulations as drugs in the amorphous form have a higher apparent solubility than their crystalline counterparts [4,5] and may lead to enhanced dissolution rate and thus greater bioavailability. However, the major drawback of amorphous formulations is the tendency of the drug to revert back to the crystalline form [4,6,7], thus negating the solubility advantage and this may lead to poor product performance. In order to ensure the quality of amorphous formulations, it is crucial to detect and quantify low levels of crystallinity in amorphous formulations during processing and storage.

There are a number of analytical tools that are available to detect and quantify crystallinity in amorphous formulations. These analytical tools can be generally categorized into the particulate or

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molecular level tools according to the level that they probe. The particulate level tools, which include X-ray powder diffractometry (XRPD) and differential scanning calorimetry (DSC), provide information regarding the properties of the individual solid particles. Molecular level tools, on the other hand, specifically probe the inter- and intra-molecular interactions and these include vibrational spectroscopy (e.g. infrared and Raman spectroscopies) and solid-state nuclear magnetic resonance (ss-NMR) spectroscopy [8–10].

Raman spectroscopy may be used to qualitatively and quantitatively characterize active pharmaceutical ingredients (APIs) in formulations. There are a number of advantages in using this technique. Firstly APIs are generally π-conjugated systems whereas excipients are σ-bonded. Raman spectral intensities are related to polarizability changes ($\partial\alpha/\partial q$) and these are generally larger for π-conjugated systems [11]. Secondly, water, which is present in many formulations, can strongly interfere with infrared spectroscopy (including near, mid and far infrared variants) because water is a strong infrared absorber, however the Raman scattering of water is very weak and this water signal does not compromise the spectral analysis. Thirdly, as a spectroscopic technique, it is non-destructive and data may be obtained with minimal sample preparation. However, Raman spectroscopy also has a number of challenges; in many samples fluorescence can swamp the Raman signal rendering useful analysis impossible. This has been ameliorated through the use of longer excitation wavelength sources. A second issue can be that of sample burning or coatings compromising analysis of the bulk sample. This second types of difficulty have been addressed using Raman techniques that use low powers and sample large volumes. This includes transmission Raman [12], spatially offset Raman [13,14] and wide angle illumination methods [15]. The advent of fibre-optic probes has been used for in-line assessment over larger sample regions [16,17].

Raman spectroscopy was first reported to be used to quantify crystalline content in amorphous pharmaceutical materials by Taylor and Zografi [18]. This report successfully used a univariate analysis method involving the intensity of a single peak to quantify the crystalline content in amorphous indomethacin powder. Univariate analysis is routinely used in pharmaceutical settings due to simplicity of the concept and the calculations. However, this approach is not appropriate for drugs in which the Raman spectra of the amorphous and crystalline forms have overlapping peaks as the presence of at least one non-overlapping peak is the prerequisite to the use of this analysis for quantification purpose [16]. In order to overcome this problem, several groups have successfully used multivariate analysis in combination with Raman spectroscopy to quantify crystallinity in amorphous systems [19–21]. Multivariate analysis uses a spectral window, often containing many peaks, to quantify crystallinity and is more powerful and sensitive than univariate analysis. The multivariate analysis technique that is most commonly used for quantification of crystallinity is partial least squares (PLS) regression [16,22].

One way in which crystallization may be observed and quantified is to examine the low frequency vibrational spectral region. This has been pioneered for absorption methods using terahertz spectroscopy [23,24]. Transitions in the low wavenumber (10–200 cm^{-1}) region, sometimes called the terahertz region, can originate from the phonon lattice modes of the crystalline structure [25]. Amorphous materials, having no long range order, give a smooth featureless spectral response; whereas, crystals give sharp spectral features [24].

Low-frequency Raman spectroscopy was used in the 1970s to examine various materials including APIs [26]. These experiments used visible excitation sources with scanning spectrographs and photomultiplier tube detection. These were very effective at measuring Raman shifts down to 10 cm^{-1}, but were slow and required relatively high laser powers as the experimental throughput was poor. The advent of CCD, single-stage spectrograph experiments, allowed for much better signal detection, but relies on the use of notch filters to remove Rayleigh scattering. Until recently, such filters were generally not that effective at Raman shift values below 150 cm^{-1}. However the use of volume Bragg gratings means that a high throughput sensitive CCD-based Raman spectrometer can now detect down to 10 cm^{-1} from the laser line with excellent Rayleigh scattering rejection (10^8). It is, thus, now possible to easily and quickly measure low frequency Raman spectra.

Like terahertz spectroscopy, low-frequency Raman spectroscopy probes the lattice vibrations of a crystalline structure and provides information regarding the long-range order of the system [27]. In a similar fashion to terahertz absorption, spectra of crystalline materials exhibit phonon peaks in the low-frequency Raman region. Amorphous materials, on the other hand, give rise to a characteristic broad peak, known as the Boson peak, with no distinct sharp peaks in the low-frequency Raman region [27]. The distinct spectral features between the different solid state forms of a drug in the low-frequency Raman region enable them to be used to characterize and quantify solid state forms [27–29].

The use of low-frequency Raman spectroscopy in the area of pharmaceutical research to characterize solid state forms is relatively unexplored. At present, there are only a couple of studies that have utilised low-frequency Raman spectroscopy to characterize and quantify pharmaceutical solid state forms. Low-frequency Raman spectroscopy was employed in a polymorphism study to investigate the enantiotropic system of caffeine and other polymorphs [28–30]. The stable form of caffeine (form I) has different spectral features from the metastable form II in the low-frequency Raman region [28]. However, these two forms were not distinguishable in the mid-frequency Raman region [28]. Low-frequency Raman spectroscopy was later used by the same group to investigate the polymorphic transformation of caffeine during compression and grinding in a subsequent report [29]. Hédoux et al. also successfully characterized and quantified low levels of crystallinity in amorphous indomethacin powder [27]. It was found that the low-frequency Raman region was able to detect the onset of recrystallization in amorphous indomethacin earlier than the mid-frequency Raman region [27]. This study utilized univariate analysis to quantify the crystalline content of indomethacin. In a comprehensive study of respirable dosage forms, Wang et al. showed that the low-frequency region of the Raman spectra gave intense bands for a number of crystalline drugs, including salbutamol sulphate, glycopyrronium bromide and fluticasone propionate [31]. They also characterized the amorphous and crystalline forms of these drugs and determined that the low-frequency region offered a more sensitive probe of composition and drug from than the conventional mid-wavenumber range (500–1700 cm^{-1}).

In this study we compare two Raman-based experiments; firstly we use a conventional FT-Raman spectrometer and secondly we use a 785 nm-based Raman experiment with a CCD detector in which both mid-frequency and low-frequency spectra are collected simultaneously. In this way we can compare the low-frequency data to a conventional experiment and to mid-range data from the same 785 nm-based experiment. These data are used to build composition models to detect and quantify crystallization at different temperatures.

Griseofulvin, which has antifungal properties, is categorized as a class 2 drug under the Biopharmaceutics Classification System (BCS), indicating that it has low aqueous solubility but high permeability. There are three reported crystalline polymorphic forms of griseofulvin: the stable form I and the metastable forms