International Journal of Pharmaceutics 469 (2014) 197–205

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Low-frequency shift dispersive Raman spectroscopy for the analysis of respirable dosage forms

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ARTICLE INFO

Article history:
Received 21 November 2013
Received in revised form 4 April 2014
Accepted 23 April 2014
Available online 1 May 2014

Keywords:
Low-frequency shift Raman
Calibration
Pharmaceutical formulations
Crystallinity
Amorphous
Polymorphs

ABSTRACT

A high performance Raman system equipped with a CCD (charged coupled device) sensor and recently developed optical filter technology is described. It provides high sensitivity, high resolution, and access to low-frequency vibrations enabling resolution of spectral features due to lattice vibrational modes and internal vibrational modes, greatly improving the ability to detect small changes due to variations in the three dimensional molecular arrangement, e.g., during loss of crystallinity. Applications to solid state analysis, such as solid phase identification and differentiation of glycopyrronium bromide and formoterol fumarate in pharmaceutical powders, and identification of active pharmaceutical ingredients, e.g., salmeterol xinafoate, fluticasone propionate, mometasone furoate, and salbutamol sulphate, as well as excipients, e.g., amino acids, in different formulations, are presented. For the first time, low-frequency shift Raman spectra of mannitol polymorphs were measured and used for solid phase identification. Unambiguous identification of two similar bronchodilator metered dose inhalers, Ventolin® HFA and Airomir®, was accomplished. The low-frequency shift Raman signals can be used for the analysis of crystallinity of small samples (<5 mg) of respiratory dosage forms in a multi-component formulation matrix containing less than 3% by weight of the component of interest.

1. Introduction

Chemical and solid phase identification of particulate solid dosage forms is frequently required during the pharmaceutical development process and for the testing of finished products, including those already on the market. Particulate dosage forms are widely used in oral and parenteral drug delivery. Powders in the respirable size range are a necessity for drug delivery to the lung. To guarantee the quality of such pharmaceutical products, identity testing of raw materials, and monitoring of chemical and solid phase changes during manufacture, storage, and usage is required (Costantino et al., 1998; Hubert et al., 2011; Zhang et al., 2004).

Identification and verification of finished products are also sometimes needed because of concerns regarding counterfeit and substandard pharmaceutical products in circulation (Deisingh, 2005; Martino et al., 2010; Newton et al., 2006). Pharmaceutical products without active ingredient or having incorrect composition have caused and will continue to cause a severe public health threat (Aldhous, 2005; Kelesidis et al., 2007; Reidenberg and Conner, 2001).

Identification of solid phases is particularly important for inhalable dosage forms. Typical inhalable powders are blends of micronized or spray dried active pharmaceutical ingredients and are often sensitive to environmental conditions, especially moisture (Ahlneck and Zografi, 1990; Hancock and Zografi, 1994). The interactions between drugs and excipients can also affect the potency of the medicine (Jackson et al., 2000; Masuda et al., 2012). Due to the high specific surface area of respirable aerosol particles and the frequently partially or fully amorphous nature of the components, respirable powders may undergo solid phase transformations.

Various phase transitions between different solid forms can occur (Willart and Descamps, 2008; Wunderlich, 1999). Of primary concern for respirable dosage forms are amorphous to crystalline and polymorphic inter-conversions. It has been demonstrated that different solid phases of excipients and active pharmaceutical ingredients in respirable formulations can have different pharmaceutical and therapeutic performance (Hancock and Zografi, 1997; Huang and Tong, 2004; Vippagunta et al., 2001). Even a small fraction of crystalline–amorphous inter-conversions can have a

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http://dx.doi.org/10.1016/j.ijpharm.2014.04.058
0378-5173/

significant impact on the drug performance (York, 1983). Therefore, quality testing should include detection of the solid phase of critical formulation components.

Many analytical techniques sensitive to solid phase transitions of pharmaceutical products have been described (Newman and Byrn, 2003), including calorimetry (Clas et al., 1999; O'Neill and Gaisford, 2011), spectroscopy (Brittain et al., 1993; Bugay, 2001; Heinz et al., 2009), X-ray diffraction (Brittain, 2001), and vapor sorption (Mackin et al., 2002), etc. Usually, these testing techniques are used together with other methods to comprehensively characterize pharmaceutical products both statically and dynamically (Hickey et al., 2007a,b; Newman and Byrn, 2003). Among these techniques, terahertz spectroscopy has been gaining popularity for pharmaceutical applications (Zeitler et al., 2007). Terahertz spectroscopy uses far-infrared radiation to induce intermolecular low energy vibrations in the solid of interest. It is a fast, non-destructive technique that has been successfully applied to the analysis of solid phases, especially crystallinity and polymorphism (Strachan et al., 2004). In comparison to traditional Raman technology, terahertz spectroscopy uses lower power and shorter recording time, minimizing sample loading effects (Beard et al., 2002; Heinz et al., 2009). Also, using radiation in the far-infrared region avoids interference from fluorescence which can be problematic in Raman spectroscopy with visible excitation. However, the current terahertz spectroscopy instruments can only cover the spectral range of about 2–130 cm^{-1} (Taday and Newnham, 2004), i.e., cannot access information from transitions with larger energy difference, e.g., most intramolecular vibrations.

Raman spectroscopy has been proven to be an appropriate method for counterfeit tablet testing (de Veij et al., 2007; Witkowski, 2005). It has several advantages in this context: (1) simple or no sample preparation is required; (2) non-invasive analysis; (3) applicable to aqueous systems or systems with varying water content (Fini, 2004; Vankeirsbilck et al., 2002; Wartewig and Neubert, 2005).

Raman spectroscopy has the potential to identify most pharmaceutical excipients and active pharmaceutical ingredients, because organic molecules are generally Raman active. Also, it is sensitive to the changes in the molecular packing in the solid phase of the measured molecules. Raman spectra of organic compounds mainly reflect two classes of transitions between different energy states: Firstly, internal vibrational modes that are caused by different types of intramolecular vibrations or rotations, creating a unique fingerprint for each type of molecule in the sample. The energy associated with these intramolecular vibrations is relatively high, giving rise to Raman lines that are significantly shifted from the laser frequency, i.e., the excitation frequency. Expressed in wavenumber shift, the internal modes most commonly used for compound identification appear between 150 and 1800 cm^{-1}, the so-called fingerprint region. Some information about the solid phase of the sample can be derived from the fingerprint region, because intramolecular vibrations are often affected by the local chemical environment of the molecules, i.e., the relationship to neighboring molecules. Secondly, external vibrational modes, which are also known as lattice vibrational modes or phonon modes, are caused by intermolecular vibrations (Hedoux et al., 2011b). Analysis of intermolecular vibrations provides a direct measure of crystal structure and level of disorder. Hence, it is a powerful technique for analyzing solid phases. As intermolecular forces are weaker than intramolecular forces, Raman lines associated with external vibrational modes typically appear in the low frequency shift region of less than 150 cm^{-1}. Different solid phases of the same substance are due to differences in the three dimensional arrangements of molecules, which in the Raman spectrum are manifested with higher contrast in intermolecular vibrational bands than intramolecular motions.

Assignments of low-wavenumber Raman signals of active pharmaceutical ingredients have been studied with the assistance of quantum mechanical calculation based on density function theory (Ayala, 2007). A discussion about the feasibility of applying low-frequency Raman spectroscopy to analyze crystalline–amorphous transition has been performed on indomethacin (Hedoux et al., 2009). A precise and low-frequency shift Raman-based method of determining small fractions of a crystallized component within an amorphous matrix was also introduced. Low-frequency shift ($<100\ cm^{-1}$) Raman mapping of polymorph was introduced by Hubert and shown to be capable of precise analysis of local solid phase transitions within a pharmaceutical tablet (Hubert et al., 2011). Low-frequency shift Raman spectroscopy (50–450 cm^{-1}) has also been used to study the mechanism of pressure- and heat-induced denaturation of lysozyme (Hedoux et al., 2011c). To our knowledge, low frequency shift Raman spectroscopy has not been applied to respirable pharmaceutical dosage forms.

Respirable particles or their substructures typically have dimensions on the order of the excitation and scattered wavelengths involved in Raman scattering. This causes inhomogeneous internal radiation fields in the particles and may lead to frequency dependent distortion of the spectra, including morphology-dependent resonance effects (Davis and Schweiger, 2002). This phenomenon has also been discussed extensively in the literature (Aardahl et al., 1996; Schweiger, 1990, 1991). Resonance effects depend on particle size, refractive index, and wavelengths and are difficult to predict for arbitrarily shaped particles, but they tend to average out when measuring a large number of particles with different particle size (Chan et al., 1991; Vehring et al., 1998). Hence, in the work presented here, instead of analyzing individual particles we have chosen to use a small powder sample mass to reduce the effects of particle size and shape. Consequently, the information derived from these measurements is representative of the bulk powder, not of individual particles or particle substructures, e.g., surface or core.

Most Raman instruments used for pharmaceutical applications are either Fourier transform Raman systems or single-stage dispersive Raman systems. Neither of them is capable of measuring frequency shifts that are very close to the laser line. This limitation is particularly severe for respirable particles which scatter the excitation wavelength very strongly due to their particulate nature. Hence, additional laser line rejection filters are generally necessary in this case. The rejection filters used in traditional dispersive Raman systems have a relatively large band width, which leads to rejection of the Raman signals in the low-frequency shift range together with the laser line (Chen et al., 2012; Vehring, 2005). The difficulties of detecting low-frequency signals have limited its application. Monochromators are commonly used as filters to reject the excitation frequency, but this configuration reduces instrumental efficiency. Usually, low frequency shift Raman spectra are recorded using double (Deschamps et al., 2012; Hedoux et al., 2011a) or triple (Ayala, 2007; Ivanda et al., 2007) monochromators. In such cases, Raman signals as close as 10 cm^{-1} to the elastically scattered light could be recorded. Recently, however, ultra-narrow band laser rejection filters have become available that allow measurements closer than 10 cm^{-1} to the laser line (Moser and Havermeyer, 2009). In the work presented here, we have combined ultra narrow band notch filters with traditional dispersive Raman setup to test the feasibility of this approach for the solid state analysis of respirable powders.

2. Materials and methods

2.1. Materials

Most of the samples measured in this study are active ingredients of respirable dosage forms indicated for asthma and chronic obstructive pulmonary disease (COPD). Crystalline raw