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Coherent anti-Stokes Raman scattering microscopy driving the future of loaded mesoporous silica imaging

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ABSTRACT

This study reports the use of variants of coherent anti-Stokes Raman scattering (CARS) microscopy as a novel method for improved physicochemical characterization of drug-loaded silica particles. Ordered mesoporous silica is a biomaterial that can be loaded to carry a number of biochemicals, including poorly water-soluble drugs, by allowing the incorporation of drug into nanometer-sized pores. In this work, the loading of two poorly water-soluble model drugs, itraconazole and griseofulvin, in MCM-41 silica microparticles is characterized qualitatively, using the novel approach of CARS microscopy, which has advantages over other analytical approaches used to date and is non-destructive, rapid, label free, confocal and has chemical and physical specificity. The study investigated the effect of two solvent-based loading methods, namely immersion and rotary evaporation, and microparticle size on the three-dimensional (3-D) distribution of the two loaded drugs. Additionally, hyperspectral CARS microscopy was used to confirm the amorphous nature of the loaded drugs. *Z*-stacked CARS microscopy suggested that the drug, but not the loading method or particle size range, affected 3-D drug distribution. Hyperspectral CARS confirmed that the drug loaded in the MCM-41 silica microparticles was in an amorphous form. The results show that CARS microscopy and hyperspectral CARS microscopy can be used to provide further insights into the structural nature of loaded mesoporous silica microparticles as biomaterials.

1. Introduction

Poorly water soluble drugs are an ever increasing problem in drug development, with estimates suggesting that ~50% of all drugs under development are affected [1]. A large area of research is devoted to increasing solubility and dissolution rate profiles for poorly water-soluble drugs. The amorphous form is a promising approach to increasing the apparent solubility and dissolution rate of many drugs. However, as the amorphous form is thermodynamically a high-energy system, drugs in this form may crystallize. Attempts have been made to stabilize the amorphous form using, for example, solid dispersions [2,3], co-amorphous formulations [4] and drug-loaded mesoporous silica [5,6] and silicon [7,8].

Mesoporous materials contain nanosized pores between 2 and 50 nm [9–11], allowing the loading of a number of biologically relevant chemicals, including drug molecules inside the pores. Incorporation of drug into the pores can be performed using solvent deposition methods [5,8,12], mechanical activation methods [13,14] or vapor-phase mediated mass transfer [11]. The solvent deposition method is based on dissolving the drug into an organic solvent at a high concentration and mixing the solvent with the mesoporous silica, allowing the drug to migrate through diffusion into the pores of the mesoporous silica particles. This process is followed by a solvent removal step, where the excess solvent is removed, leaving the remaining drug loaded in the mesoporous silica.

Characterization of drug-loaded mesoporous silica has had some analytical challenges. Techniques such X-ray powder diffraction [12,15,16], Fourier transform infrared spectroscopy [12,15,17], differential scanning calorimetry [12,15,18,19] and thermogravimetric analysis (TGA) [12,15,19,20] are commonly used to determine the solid-state form of the drug and the extent of the loading process in the loaded mesoporous silica. Other techniques

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such as Raman spectroscopy [21] and X-ray photoelectron spectroscopy (XPS) [21,22] have also been used in the study of drug-loaded mesoporous silica or silicon samples. Vanea and Simon [21] combined Raman spectroscopy and XPS to study zinc containing silica microparticles loaded with insulin. They confirmed the loading of insulin using XPS and determined the biologically active conformation with Raman spectroscopy. However, these techniques do not provide any spatial information about the distribution of the drug within the mesoporous silica or, indeed, whether any drug is present on the surface or as separate particles. Therefore, there is a need for spatially resolved analysis to provide information on both drug distribution and solid state form.

Time-of-flight secondary-ion mass spectroscopy (ToF-SIMS) is a spatially resolved surface-sensitive technique, which has been used to investigate the penetration of proteins into silicon wafers [23]. As ToF-SIMS is only sensitive to the first monolayer of a sample, it is not suitable for completely non-destructive imaging of the three-dimensional (3-D) distribution of drug-loaded silica microparticles [24]. Limnell et al. [25] published the first use of Raman spectroscopic mapping to map the distribution of indomethacin loaded in SBA-15 and MCM-41 silica. Additionally, they used partial least squares analysis of the Raman spectra and were able to determine the solid-state form of the loaded drug. Spontaneous Raman spectroscopic imaging has a large drawback of being a slow imaging method with image acquisition speed of ~30 s for an area of 100 μm^2, depending on the material being imaged [25]. In addition, the axial resolution of the technique is usually in the order of several micrometers, which can limit the ability to obtain spatially resolved information in three dimensions.

Coherent Raman techniques such as coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering can image up to video rate speeds, allowing fast image acquisition. A summary of the CARS microscopy technique is provided elsewhere [26]. Briefly, CARS microscopy is a non-linear optical imaging technique, which provides rapid chemically selective imaging of different drugs and solid-state forms of drugs based on spectral differences in their Raman spectra. As CARS is non-linear, it is inherently confocal with diffraction-limited resolution in three dimensions.

CARS microscopy has recently gained interest in imaging pharmaceutical formulations. Some of the early work involved using CARS to image the composition of dodecane emulsions [27]. Windbergs et al. [28] and Jurna et al. [29] performed CARS on lipid-based oral dosage forms, where they imaged the distribution of the model drug theophylline and monitored theophylline real-time release during dissolution using a flow-through cell setup. Fussell et al. [30] extended this dissolution concept by building an intrinsic flow-through dissolution setup, which allowed correlation of surface solid-state changes occurring during dissolution with changes in drug dissolution rate.

This work demonstrates the use of CARS microscopy as an analytical tool to image drug distribution within drug-loaded ordered mesoporous silica. CARS microscopy is shown to be capable of chemically selective imaging the 3-D distribution of the model drugs griseofulvin and itraconazole, loaded in ordered mesoporous MCM-41 silica microparticles. The loading of the drugs was studied using two different solvent deposition methods (immersion and rotary evaporation) in two different silica particle sizes (63–90 μm and 100–125 μm), with the aim of identifying any differences in drug distribution based on different loading methods or particle size. Additionally, hyperspectral CARS microscopy is also shown to be suitable for evaluating the physical form of the drug loaded in the microparticles.

2. Materials and methods

2.1. Materials

Synthesis of the mesoporous MCM-41 material was carried out in a 300 ml autoclave, as described elsewhere [31,32], using fumed silica (SiO_2, 99.9%), tetramethylammonium silicate ($((CH_3)_4N(OH)\cdot 2SiO_2$, 99.99%), sodium silicate ($Na_2O_7Si_3$, SiO_2, 27%), ethyltrimethylammonium bromide ($CH_3(CH_2)_{15}N(CH_3)_3Br$, 99%) (all from Sigma–Aldrich, USA) and distilled water. The autoclave was kept in a large oven, and the synthesis of MCM-41 was performed at 100 °C. The autoclave was then taken out of the oven and quenched. Mesoporous silica MCM-41 was filtered and washed with distilled water. Drying of the sample was carried out at 110 °C for 12 h and calcined at 550 °C for 10 h. The resulting MCM-41 has an average pore size of 3.4 nm with narrow size distribution; usually within 2–3 nm and the surface area is ~0.79 $cm^3\ g^{-1}$ [32].

The synthesized mesoporous silica materials were ground using a ball mill, and the resulting fine powder was passed through mesh test sieves (63 and 90 μm; 100 and 125 μm) using a sieve shaker apparatus (Fritsch GmbH, Idar-Oberstein, Germany) to achieve particle size fractions of 63–90 and 100–125 μm.

Crystalline itraconazole (Orion Pharma, Finland) and griseofulvin form I (Sigma–Aldrich, USA) with Cambridge Structural Database reference codes of GRISFL and TEHZIP, respectively, were used as received. Amorphous itraconazole and griseofulvin were prepared by quench cooling of the melt and analyzed immediately after preparation.

2.2. Methods

2.2.1. Drug loading using immersion method

The mesoporous silica particles were loaded using an approach similar to that described by Limnell et al. [16]. Solutions of griseofulvin (40 mg ml^{-1}) and itraconazole (235.5 mg ml^{-1}) dissolved in dichloromethane were prepared. Weighed samples (20 mg) of mesoporous MCM-41 (63–90 and 100–125 μm) were added to the drug solutions and stirred for 90 min. After stirring, the samples were centrifuged for 4 min at 8000 rpm, and the supernatant was removed. The samples were washed with 500 μl of water and centrifuged for 4 min at 8000 rpm. The samples were then dried in a vacuum oven at 40 °C for 2 h.

2.2.2. Drug loading using rotary evaporation

The mesoporous silica microparticles were loaded using an approach similar to that described by Limnell et al. [16]. Solutions of 10 mg ml^{-1} (griseofulvin) and 10 mg ml^{-1} (itraconazole) dissolved in dichloromethane were prepared. Weighed samples (20 mg) of mesoporous MCM-41 (63–90 or 100–125 μm) were added to the solutions and shaken for 90 min. The samples were then evaporated using the Rotavap (Büchi, Switzerland) with the water bath set at 32 °C. The samples were dried in a vacuum oven at 40 °C for 2 h.

2.2.3. TGA

The drug payloads loaded in the mesoporous MCM-41 were studied with TGA. TGA was performed on a TGA-7 instrument (PerkinElmer, Waltham, MA, USA) with a heating rate of 10 °C min^{-1} under a N_2 gas purge of 40 ml min^{-1}. Alumel, nickel, perkalloy and iron were used to calibrate the TG temperature scale. Approximately 3–5 mg of sample was used for analysis.