doi:10.2533/chimia.2013.923

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Non-destructive Localization and Identification of Active Pharmaceutical Compounds by Raman Chemical Imaging

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Abstract: Raman spectroscopy is a powerful and non-destructive technique for chemical and structural identification. Based on inelastic scattering of laser light by molecular vibrations, the analysis can be localized on a microscopic area when combined with a microscope. Thus, by moving the sample under the microscope objective and recording a Raman spectrum at each point, a map of the intensity of specific Raman bands can be generated, effectively creating a chemical image of the sample at the microscale. Here, we present an application of this technique for identifying and localizing active pharmaceutical ingredients in a polymer matrix.

Keywords: Active pharmaceutical ingredients (API) · Chemical imaging · Polymer microparticles · Raman spectroscopy

Chemical and physical characterization of active pharmaceutical ingredients (API) is of major importance for the drug development process, particularly for solid-state products. Not only the chemical composition, but also the structure of an API is critical. Polymorphs of a single API can influence its stability, dissolution properties, and therefore bioavailability. Moreover, drugs are in general not sold as single products, the final tablet being a precise formulation of API and excipients, and the spatial distribution of the API inside the formulated tablet is crucial too. For instance in divisible tablets, API should be evenly distributed to avoid wrong dosing. The size of the API particles plays also a role in its galenic properties.[1]

Physical characterization of API can be performed visually, with the help of an optical microscope, but colors and hues are not reliable chemical and structural markers. X-ray diffraction (XRD) provides both chemical and structural information, but only at an ensemble level: no local information about where and how big the API particles are in a formulation can be obtained. Thermal analysis, such as differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), are very powerful methods for structural investigations of solids (polymorphism, crystallinity, *etc.*), but lack any local information too. Raman spectroscopy on the other hand, which probes the vibrational modes of a sample by detecting the inelastic scattering of a monochromatic laser beam, is capable of both.[2] Molecular vibrations depend on the masses of the atoms and their bonding, resulting in a Raman spectrum that is specific for every molecule. By comparing a measured spectrum with a database, chemical identification is straightforward. Moreover, any modification of the chemical bond structure will affect the vibrational modes too, so Raman spectroscopy can detect crystallinity and polymorphism by tracking subtle variations of the position and width of the peaks.[3] Based on a light scattering process and involving no absorption of light, Raman spectroscopy is also non-destructive and almost sample-preparation-free. But the most appealing feature of Raman spectroscopy is probably its capacity to generate chemical images with micrometer resolution: with an optical microscope and an X-Y motorized stage, a two-dimensional matrix of Raman spectra can be recorded.[4–6] By mapping the intensity of a particular peak, local chemical and structural information of a definite surface area appear (Fig. 1).

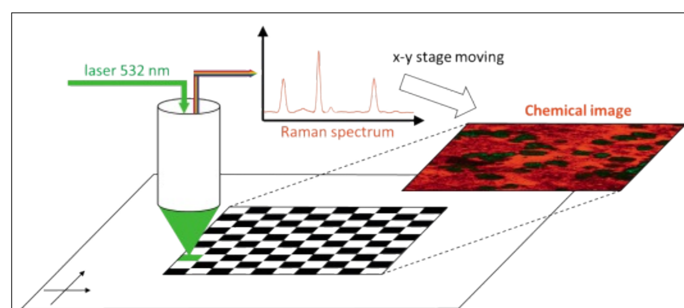

Fig. 1. Principle of Raman chemical imaging.

In this paper, we present a practical example of chemical imaging to visualize and quantify the spatial distribution of an API inside microparticles of excipient. The API is a peptide, and the excipient is a copolymer,[7] polylactic-co-glycolic acid, commonly called PLGA.[8,9] Due to its biodegradability and biocompatibility, PLGA is commercially used for the *in situ* delivery of small molecule drugs inside the human body, as well as for tissue engineering applications.

An example of the samples that we studied is shown in Fig. 2. Microparticles of PLGA[10] containing a peptide are embedded in a microtome resin, then sliced into fine lamella and deposited on a glass substrate. On the low-magnification optical image (Fig. 2a, objective 10×), several particles are readily visible (isolated dark areas), the rest being resin. Most particles exhibit a diameter of less than 100 µm. A zoom-in (objective 50×) to the marked particle is presented in Fig. 2b. No further sample preparation is necessary for Raman investigation. As explained above (Fig. 1), we have recorded a rectangular map (blue rectangle) around the particle by moving the sample in X and Y directions on a motorized stage (the 50× microscope objective is fixed).

The experiments were carried out with a confocal micro-Raman spectrometer (HORIBA LabRAM HR800, combined with an optical microscope Olympus BX41). We use a 531.8 nm laser for excitation, attenuated with a neutral density filter (ND1, 90% attenuation), resulting in laser power of 4 mW on sample with the 50× objective employed for mapping. Because of the concentration of the light onto a very small spot (diameter approx. 2 µm), the attenuation of the laser power with a filter was necessary to avoid thermal degradation of the sample. With 2 µm steps of the motorized stage, and acquisition time of 3 × 0.5 s at each point of the map, the total map acquisition time was about 2 hours, depending on the size of the particle. After recording the Raman maps, we performed a numerical analysis of the particles size distribution with a homemade LabVIEW-based program (National Instruments).

The first step of the study consists in determining which spectral region is specific to the API. For this purpose, we re-

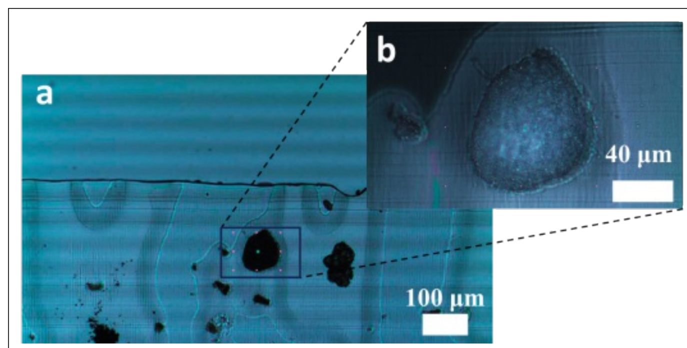

Fig. 2. Optical images of PLGA particles containing API embedded in resin (a: objective 10×, b: objective 50×).

corded the individual Raman spectra of pure PLGA, pure API, and microtome resin (Fig. 3). Very clearly, the peak at 1369 cm^{-1} is specific to the API: no parasitic peak from either PLGA or resin interferes in this spectral region.

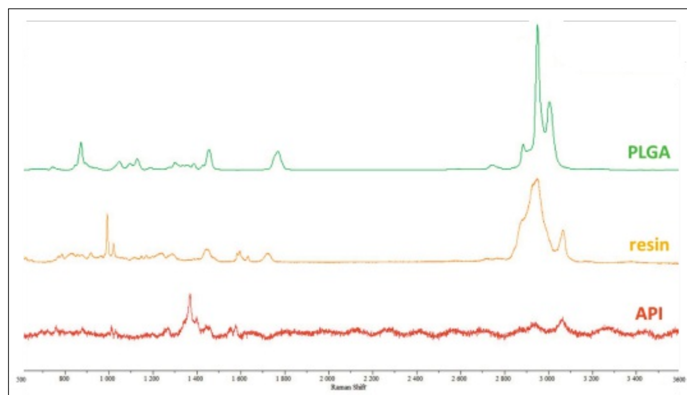

Fig. 3. Individual Raman spectra of the three species.

Therefore, we have chosen to map the intensity of this API-specific Raman peak at 1369 cm^{-1} on the complete microparticle shown in Fig. 2b. The resulting chemical image is presented in Fig. 4. On the left image (a), the intensity of the API Raman peak is represented by the intensity of the red color, the green regions are PLGA-specific peaks, and the black surrounding is the resin. We can observe, as expected in this case, that the particle is composed of PLGA and contains isolated spots of API, well-distributed over the whole particle. Of course, no API is detected outside the particle (microtome resin only).

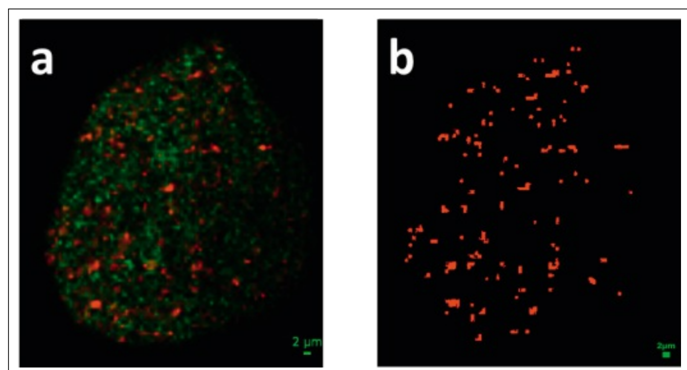

Fig. 4. Raman chemical image (a) and threshold-processed image (b) of a PLGA microparticle containing API (colors: red = API, green = PLGA, black = resin).

In order to gain more quantitative information on the location and aggregation of the API inside the particle, we have numerically treated the Raman map (Fig. 4b). The treatment is based on a red-green-blue color code, with intensities from 0 to 255 (arbitrary units), and we selected only the red areas with an intensity between 100 and 255 (arbitrarily chosen threshold, but same on all maps for comparison purposes), with an additional smoothing filter (erosion 3x3, holes filling). A solid black-and-red image emerges (Fig. 4b), and then a histogram representing the number of occurrences of API islands *vs.* their surfaces (in μm^2) is generated (Fig. 5).

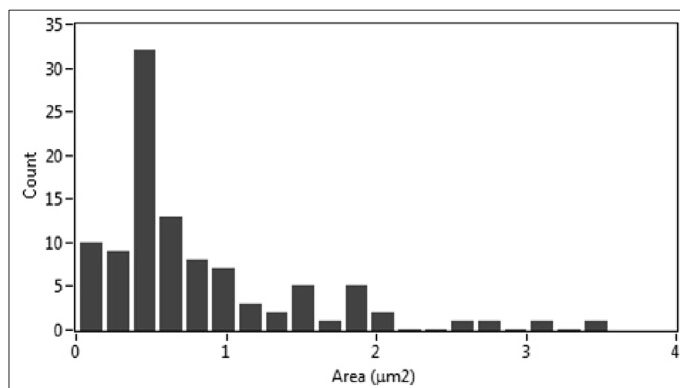

Fig. 5. Histogram of the size distribution of the API islands in the PLGA particle.

This numerical filtering allows us to determine the size distribution of the API islands: in this case, most of the aggregates are below 2 μm^2, with approximately twice as much of 0.5 μm^2 than of any other areas.

In conclusion, we have shown that Raman micro-spectroscopy has the potential to provide very useful information on where is what in a solid sample: by mapping the intensity of an API-specific Raman peak on a region of interest of the sample, we can create a color-coded chemical image with micrometer resolution. A subsequent numerical treatment delivers the quantitative size distribution of the API islands. Since basically every chemical species is Raman active (with varying cross-sections of course) except pure metals, and with almost no sample preparation (except the microtome preparation if one wants to study the inside of a three-dimensional object, like here), this technique can be employed in a large variety of applications: pharma (like here), polymers, semiconductors, and even natural samples (wood for instance).

Received: October 28, 2013

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