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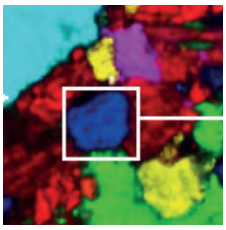
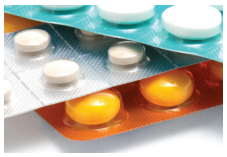
Raman Spectroscopy

Raman Hyperspectral Imaging: An essential tool in the pharmaceutical field





White Paper
Pharmaceutical

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Abstract

Resulting from the combination of Raman spectroscopy and optical microscopy, Raman hyperspectral imaging has proven to be an indispensable tool in the pharmaceutical field. This article will broach a number of Raman hyperspectral imaging applications that were developed in our laboratory, in order to demonstrate the significance of the technique.

Introduction

In the pharmaceutical environment, and especially in the research and development field, the quality of the medicine is a critical step as it is facing challenges with increased demand from the regulatory affairs to improve the quality of a pharmaceutical drug product. In order to ensure its proper effect on the patient health, a product has to be manufactured with the appropriate quality. [1-3]

Today, a lot of techniques are used in pharmaceutical laboratories to ensure the quality of a drug product. Several tests such as dissolution profiles, stability studies or control of active content are required from the pharmaceutical guidelines and authorities to ensure that the analysed product is included within pre-determined specifications. In the QC labs, most of the analytical tools are based on chemical analyses (liquid chromatography, dissolution apparatus…) which generally damage the sample, require solvent and a lot of time or important human resources.

In the last decade, the use of vibrational spectroscopy has grown quickly and has appeared as an alternative analytical tool to usual techniques [4-5]. By allowing fast and non-destructive analysis, without needing sample preparations in most cases, these analytical tools are particularly appreciated by the analysts.

Since its discovery in 1928 by Raman and Krishnan [6], Raman spectroscopy, which is based on the inelastic scattering of light, has attracted increasing attention due to its numerous advantages, such as the lack of need to use organic solvents, the reduced sample preparation step and the ability to perform qualitative as well as quantitative analyses in relatively short data acquisition times [7].

Recently, Raman spectroscopy has been combined with optical microscopy, giving rise to Raman hyperspectral imaging [8]. The use of the latter has expanded in the pharmaceutical and biomedical fields since microspectroscopic techniques present several advantages by combining the acquisition of spatial and spectral information from a sample. Raman hyperspectral imaging allows to characterize the sample in terms of chemical (API and excipients identification and content for example) and physical properties (solid state, particle size, amongst others) by adding information on the spatial distribution. Therefore, data are collected in the form of a three-dimensional hyperspectral datacube (x,y and λ) with x and y as spatial dimensions and λ as spectral dimension.

Hyperspectral data analysis is based on chemometrics and may be divided in three main parts: (i) preprocessing of the raw spectral data, aiming at correcting the perturbations that occurred during the analysis or at limiting the effect of undesired phenomena to ease the access to the relevant information, (ii) processing of preprocessed data based on univariate (peak height, area or ratio) or multivariate data analysis (for instance PCA, ICA, MCR-ALS or PLS) depending on the data complexity and (iii) post-processing of the image. Each chemometric tool has its own specificities and will be used depending on the available and desired results [9].

However, more and more user-friendly software are provided directly by the manufacturer to help the analyst to manage conventional sample analysis.

In order to illustrate the power and the suitability of Raman chemical imaging, several pharmaceutical applications developed in our laboratory will be presented in this article.

Material and methods

For the collection of the hyperspectral datacube, a LabRAM HR Evolution (HORIBA Scientific) equipped with a two-dimensional EMCCD (1600 x 200 pixels sensor) detector was used. A 785 nm laser (XTRA II single-frequency diode laser, Toptica Photonics AG), a 50x Fluotar long working distance objective (NA=0.55) and a 300 gr/mm grating were selected. The data were collected with the LabSpec 6 software (HORIBA Scientific) and analyzed by using MATLAB homemade functions and PLS/MIA toolbox (Eigenvector).

Before analyzing the samples, their surfaces were prepared with a Leica EM Rapid milling system (Leica Microsystems) equipped with a tungsten carbide miller to obtain a plane and smooth surface.

Results and discussion

Identification and spatial distribution

During pharmaceutical development or quality control, analysts must ensure the distribution of active(s) and excipients in the tablet. Indeed, the distribution of compounds is directly linked to the product stability (i.e. modification of crystalline form or degradation), active content (especially required for score tablet) and drug release (agglomerates can modify the profile). The Raman images make possible the characterisation of the pure compound in a pharmaceutical drug product and can provide its spatial distribution on the surface of a sample.

In this example, an effervescent tablet for fever and pain relief was analyzed by Raman imaging. First, a map of 4 x 4 mm^2 with a step size of 20 µm (low lateral resolution) was realized (*Figure 1b*). Once obtained, the datacube underwent pre-processing (baseline correction) and processing (Multivariate Curve Resolution – Alternating Least Squares analysis (MCR-ALS)) to resolve the different component spectra and spatial distribution [10]. After data treatment, the map allowed us to identify each component of the tablet (API and excipients alike) and to obtain information on both particle size and spatial distribution of each compound.

Based on this information, one may be able to detect counterfeit tablets even if the correct API is present in the correct amount but if one excipient is different in nature or amount [11]. It is also possible to explain inconsistencies in dissolution profiles due to inhomogeneity in spatial distribution of API or to select the most homogeneous formulation without destructive analysis [12].

Another very important information that may be obtained is the particle size. In this case, the lateral resolution may be a critical parameter. This resolution can be enhanced by a decrease in the step size. The step size is the distance between two adjacent pixels. The bigger the step size, the lower the spatial (or lateral) resolution. However, at constant analyzed area, the lower the step size is, the higher the pixel (and therefore the spectra) number will be. If the number of pixels' increases, the analysis time also does. The analyst must keep this in mind and design the analysis in function of the desired information. *Figure 1b* to *figure 1d* show three Raman hyperspectral images with the same number of pixels (200 x 200) and consequently the same analysis time. However, as the step size decreases the details increase but the obtained information is completely different. The blue particle highly resolved in *Figure 1d* (very accurate particle size with an effective lateral resolution of ~1 µm) is hardly observable in *Figure 1b*. But the spatial distribution and chemical composition of the tablet can only be observed in *Figure 1b*.
Particle size is not a crucial issue for tablets since this parameter may be obtained accurately on the raw materials. However, hyperspectral imaging is nearly the only technique that can provide this information on semi-solid formulations due to the melt step of the raw materials and recrystallization when the formulation is cooled [13].

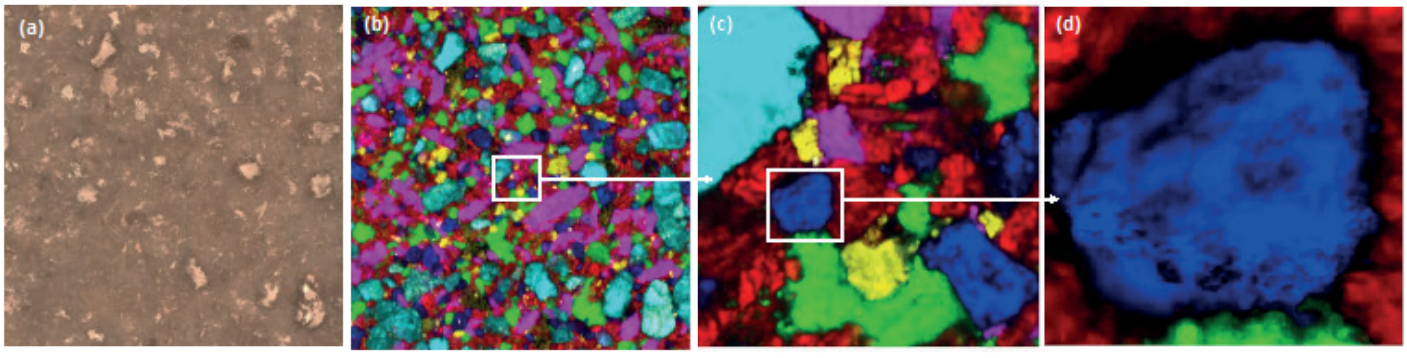

Figure 1: Visible image of a tablet (a), distribution maps indicating the composition and the spatial distribution of six components (sodium bicarbonate, citric acid, acetylsalicylic acid, sorbitol, ascorbic acid and paracetamol) for a map size equal to 4 x 4 mm^2 with a step size of 20 µm (b), 500 x 500 $µm^2$ with a step size of 2.5 µm (c) and 100 x 100 $µm^2$ with a step size of 0.5 µm (d).

Solid state investigation

Solid state information is becoming more and more important in the pharmaceutical industry partly because of patent issues and partly because of the physical properties of the different polymorphs. Indeed, the pharmaceutical industry uses a lot of API coming from a total chemical synthesis, which are often BCS class II APIs [8]. It is therefore interesting to obtain specific polymorphs or the amorphous form of the API to enhance its aqueous solubility and increase its bioavailability. As the solid state is crucial for some formulations, it is crucial to be able to monitor it properly, during drug discovery, pharmaceutical development or stability studies.

Since Raman spectroscopy is one of the European Pharmacopoeia's recommended technique (EP 9.1 general text 5.09) to characterize solid states of raw materials, it is consequently the same for hyperspectral Raman imaging.

In this example, a tablet composed of the three polymorphs of mannitol (produced in house from the commercial β form) was analyzed by Raman hyperspectral imaging. Once obtained, the spectra were baseline corrected and processed by MCR-ALS. This analysis allowed us to resolve both the spectra and distribution maps of each polymorph (*Figure 2*). It is therefore possible to use hyperspectral imaging as a tool to detect solid state transitions on samples placed in stability studies, especially because of its non-destructive character.

Even if it is often possible to characterize solid states based on the usual spectral range (200-1800 cm^{-1}), it is recommended to work in the low-frequency Raman scattering (0-400 cm^{-1}) as this range is the most affected by differences in crystalline lattices [14]. It is now possible to reach very low frequencies (up to 5 cm^{-1}) on conventional Raman microspectrometry systems when using Volume Bragg Gratings (VBG) as laser rejection filters [15].

Surface-enhanced Raman scattering

The main issues limiting the implementation of Raman imaging in the pharmaceutical field are its low sensitivity and the appearance of autofluorescence especially with formulations comprising colored constituents or colored coatings. However, by combining Raman imaging with surface-enhanced Raman scattering (SERS), it is possible to fix these issues and to increase the number of pharmaceutical applications.

In this context, one case which was studied in our laboratory was the quantitative detection of an impurity in a pharmaceutical formulation [16]. Impurities are, most of the time, present in a very low dosage in the pharmaceutical products, consequently preventing their detection using Raman imaging. Surface-enhanced Raman chemical imaging (SER-CI) was therefore used for the quantitative detection of an impurity. A rather simple pharmaceutical formulation based on paracetamol tablets, comprising a small number of excipients, was selected as a model. The aim of this study was to detect quantitatively 4-aminophenol (4-AP), the main impurity of paracetamol, using SER-CI. 4-AP is actively researched due to its hepatotoxicity and nephrotoxicity.

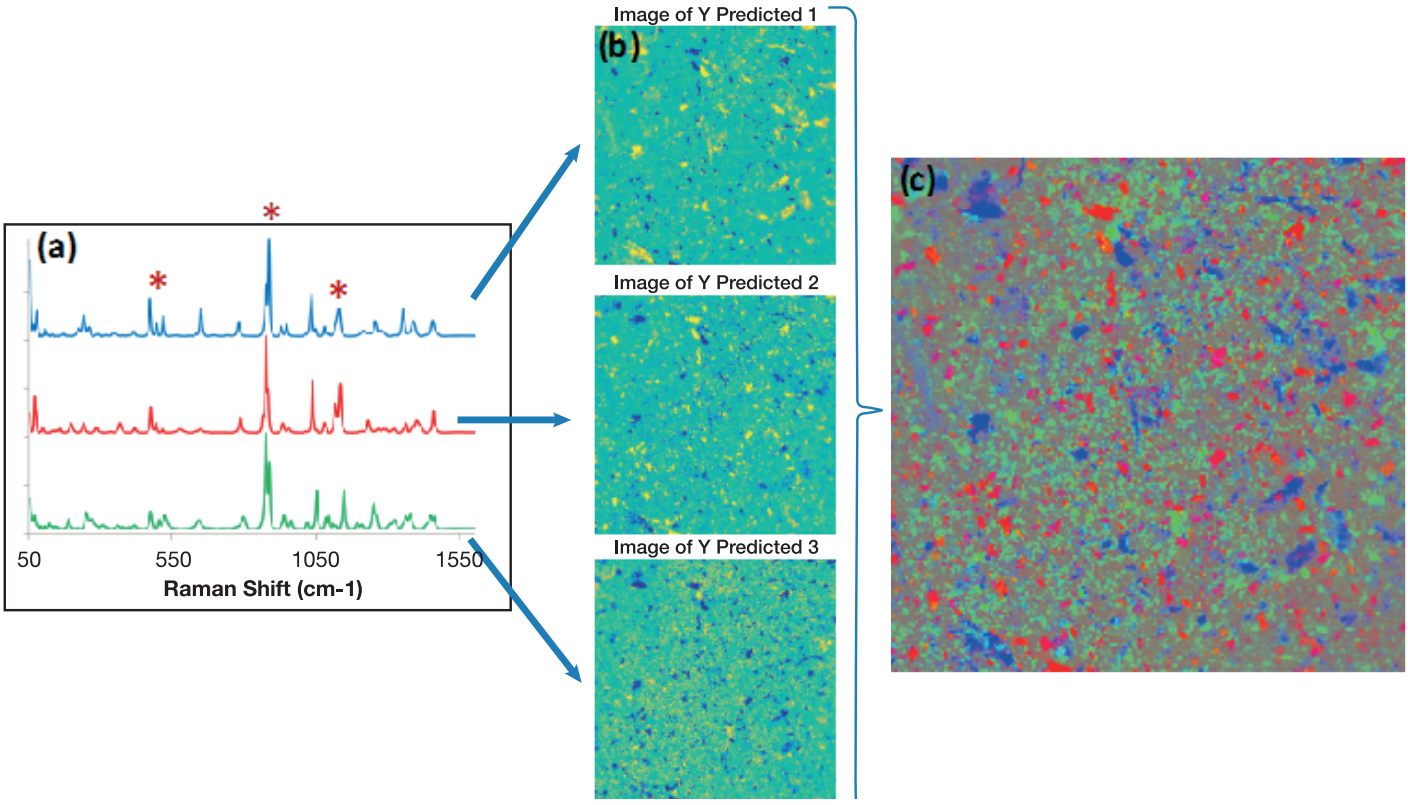

Figure 2: Raman spectrum of α, β, δ mannitol (a), distribution maps indicating the spatial distribution of mannitol forms (b) and α, β, δ distribution map indicating the composition and the spatial distribution of mannitol forms of a tablet (c).

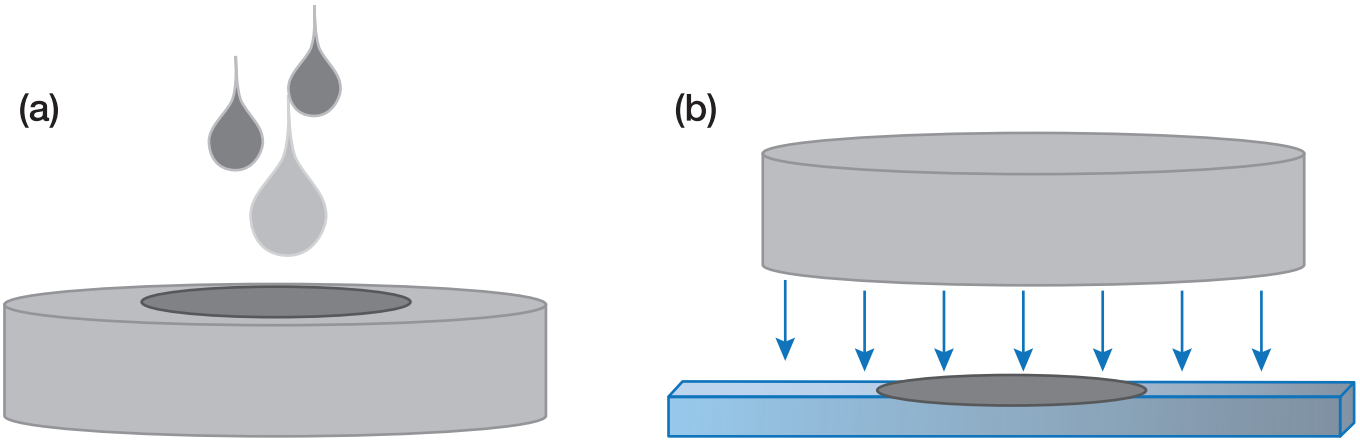

Figure 3: Strategies tested to cover the surface of a tablet with a suspension of nanoparticles: dropping nanoparticles on the tablet (a) or placing of a drop of nanoparticles on a glass slide and putting the tablet on it in order to let the drop be absorbed by the tablet by capillarity (b).

During the development of this method, the SERS substrate synthesis was firstly considered. Silver nanoparticles (AgNps) were synthesized according to the protocol described by Lee and Meisel [17]. After their characterization in terms of size and shape, the surface of these AgNps was modified using 1-butanethiol in order to follow the nanoparticles distribution along the paracetamol tablet surface. Then, different strategies to cover the tablet surface with the colloidal suspension of functionalized AgNps were tested and are described in *Figure 3*. In a first time, the tablet surface was covered by dropping AgNps on the top of these tablets as it is illustrated in *Figure 3a*. In a second time, the tablet surface was covered by dropping the AgNps on a microscope glass slide and by putting the tablets on the drop as it is illustrated in *Figure 3b*. The AgNps were absorbed by capillarity on the top of the tablets. A homogeneity study of the AgNps covering was further performed in order to select the best way to cover the tablet surface. Linescanning acquisitions were done along the tablet surface using Raman spectroscopy and it was observed that the method using the capillarity absorption was more appropriate.

Then, paracetamol tablets comprising different concentrations of 4-AP were prepared. These tablets were covered by AgNps using the capillary method before being analyzed using Raman imaging. The data acquired were processed using two different normalization approaches and the normalization by the band intensity of 1-butanethiol was shown to be the best one, reducing the inter-series variation. Finally, as it is illustrated in *Figure 4*, a SER-CI quantitative method was developed allowing the 4-AP detection from 0.025% to 0.2% in paracetamol tablets.

These researches will pave to way to new developments using Raman imaging applied to low dosage drugs or impurities in the pharmaceutical field.

Conclusion

As a conclusion, Raman microscopy can be considered as a powerful analytical tool in the pharmaceutical environment through the entire drug product life cycle. Indeed, it retains the advantages of Raman spectroscopy, namely compound identification, solid state characterization, but also add those of imaging, such as spatial distribution of compounds, particle size analysis or homogeneity of distribution analysis. Moreover, the application of hyperspectral imaging to SERS allows the analysis of low dosage drugs or impurities, which extends the field of application of the technique.

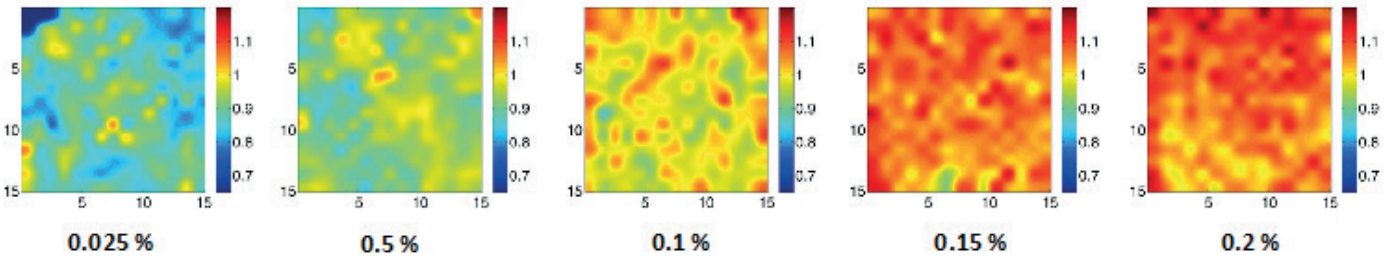

Figure 4: Concentration maps of 4-AP in a paracetamol tablet from 0.025% to 0.2% obtained by SERS chemical imaging [11].

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