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Raman hyperspectral imaging: a single tool to characterise pharmaceutical products

Raman hyperspectral imaging is an increasingly used tool in the pharmaceutical field because it allows for the investigation of many characteristics on a solid sample. This paper delves into Raman spectroscopy and imaging, including spectral and spatial information, and presents some applications of Raman hyperspectral imaging in the pharmaceutical field.

Introduction

Hyperspectral imaging – or spectral imaging – was first used in the material, food and biological domains. But it is increasingly being employed by pharmaceutical professionals, since it combines the dual advantages of spectroscopic techniques and imaging. Hyperspectral imaging allows the user to acquire the spectrum at each pixel of a defined image. It is thus possible to visualise properties invisible to the naked eye, as the spatial distribution of the constituents of a pharmaceutical solid form, the polymorphic form and so on. The available information is thus a function of the spectroscopic technique used. Each technique has its advantages, disadvantages and specificities and must be selected according to the information sought [1-2].

Chemical imaging

After a hyperspectral imaging analysis, the data obtained are presented as a three-dimensional matrix, the so-called hyperspectral data cube. This matrix contains two spatial dimensions (x and y) and a spectral dimension (λ). The treatment of this cube is divided into three parts: pre-processing, processing and image processing. Firstly, the three-dimensional data cube must be unfolded in a two-dimensional matrix concatenating the spatial dimensions. The pre-processing step reduces the artifacts and makes it possible to work under better conditions. Different treatments are possible: spikes and dead pixel correction, de-noising treatment, baseline correction and normalisation[3-5]. A corrected data cube is thus obtained.

After pre-processing, the data are processed using either univariate data analysis if a specific peak is not interfered with by any adjacent peak or multivariate data analysis (MVA). The latter consists of resolution, regression or classification techniques that can be applied depending on the sought information. The resolution techniques divide the pre-processed data in a matrix of concentration and a matrix of spectra. Some techniques do not need prior knowledge and are called explora- tory techniques among which Principal Component Analysis (PCA) is the most often used. PCA indicates the variables which most impact the variability of the data, reducing the number of dimensions of the problem. Therefore, it may be used to estimate the number of compounds present in the studied sample.

Raman hyperspectral imaging provides an accurate tool for the qualitative and quantitative analysis of a pharmaceutical solid form

Finally, in order to obtain more chemically meaningful and reliable information, Multivariate Curve Resolution – Alternating Least Squares (MCR-ALS) may be applied. This technique is able to approximate the qualitative and quantitative composition of an unknown sample without a priori information. If quantitative information is desired, one may use the Classical Least Squares (CLS) algorithm. This performs 'least squares regression' using the pure spectra of the compounds. It is thus very sensitive to any variation which impacts spectra. If more complex or noisier data are used, it is wise to use a Partial Least Squares (PLS) algorithm. It establishes a linear model with a calibration set. Therefore, it requires analysing calibration samples with a reference technique (e.g. HPLC). This time-consuming approach provides more robust and precise results for well-established formulations. If the objective is to extract qualitative information and to classify data in groups (e.g. to determine the presence of counterfeits) classification techniques such as the K-means algorithm are used[2,4-7].

Raman hyperspectral imaging: applications

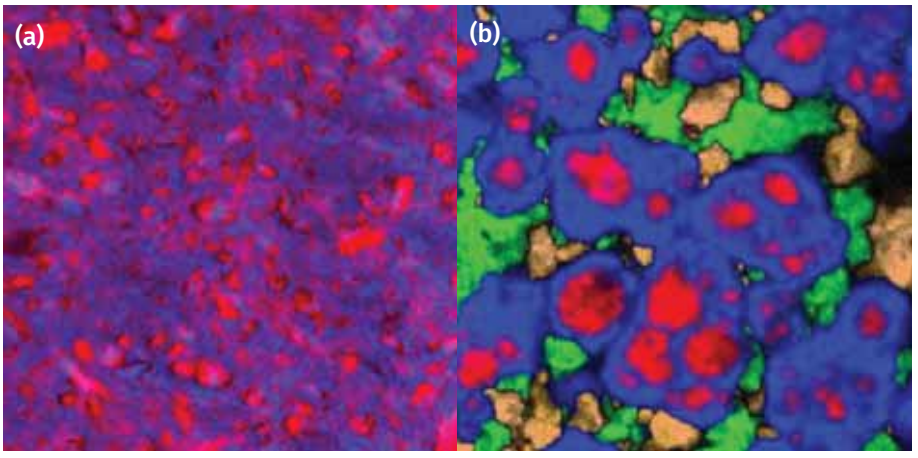
Raman hyperspectral imaging is an interesting tool that can be used to analyse a pharmaceutical sample. Some of its uses and advantages are presented in the following paragraphs.

1. *Identification of the composition of a sample*

An important thing to consider when analysing pharmaceutical drugs is the determination of the composition of a sample even if the components are unknown. Indeed, it is important to evaluate whether

the qualitative composition of a sample is correct, to track the apparition of the impurities during a stability study or even to study the interactions of the compounds.

Distribution maps illustrating the composition of tablets are presented in Figures 1a and b. After acquiring the data cube, pre-processing and processing algorithms were applied in order to extract spectra of the different components to identify the composition. It then becomes possible to observe the spatial distribution of the identified compounds within the sample as it is shown in Figures 1a and b.



Figures 1a and b: Distribution maps indicating the composition and the spatial distribution of two pharmaceutical tablets with the same pharmaco-technical properties and API content

Moreover, sometimes it is interesting to identify different polymorphic forms of a compound. It is possible to determine them and evaluate their spatial distributions by acquiring the Raman spectra of each solid-state.

2. *Size determination*

It can be interesting to investigate the size of various parts of a sample or the thickness of the coating of a tablet. Raman chemical imaging of a coated pellet is presented in Figure 2. The thickness of the coating was measured and in this case was equal to 50µm. Determining and monitoring this parameter during a production process is important. Indeed, the coating of a pharmaceutical solid form has a role to play on the API release and on the API activity.

Moreover, using a confocal Raman microspectrometer, it is possible to acquire a three-dimensional image of a pellet, and to acquire spectra on the z dimension and so analyse different characteristics inside a sample according to the confocality parameter. The determination of the composition and the spatial distribution of the pellet are presented in Figure 3a and the volume/three-dimensional image of the pellet is presented in Figure 3b.

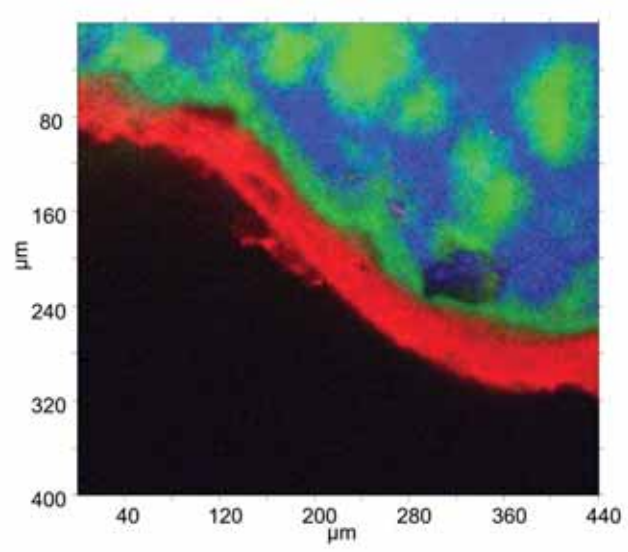


Figure 2: Distribution map revealing the coating thickness of a pharmaceutical pellet

3. *Homogeneity*

Because of the combination of spectral and spatial information, Raman chemical imaging is able to determine the spatial distribution of a specific compound in a sample. Indeed, two samples may have the same API content, the same results for dissolution tests and so on, but have a different spatial homogeneity. As shown in Figures 1a and b, it is possible to note the differences at a spatial level of two different samples which have the same pharmaco-technical properties and API content. The obtained chemical images highlight differences in formulation architecture and manufacturing.

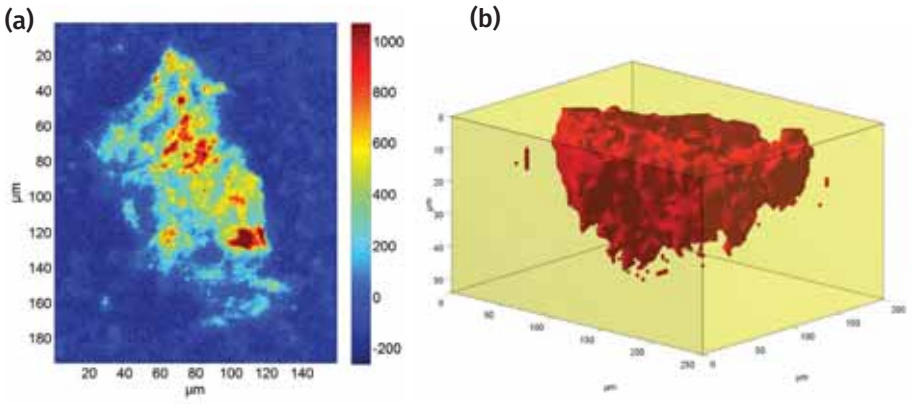


Figure 3: Distribution maps indicating (a) the composition and the spatial distribution of a pharmaceutical pellet and (b) a three-dimensional image of a pharmaceutical pellet

4. *Quantitative approach*

In addition to qualitative information (identification, solid-state and so on) it is possible to obtain quantitative information with Raman hyperspectral imaging. Calibration samples are prepared and the model is developed based on the Raman spectra. This model is compared with a reference technique (e.g. HPLC). The advantages of developing a quantitative model with Raman hyperspectral imaging is that it is developed

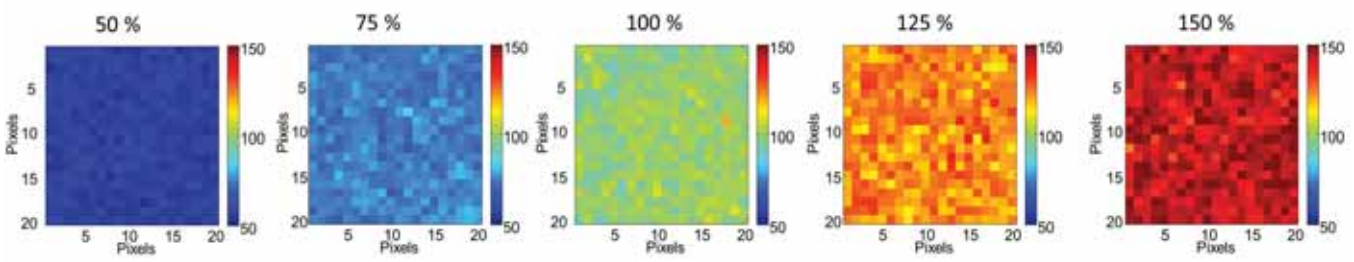


Figure 4: Distribution maps at different API concentrations

at a pixel level (Figure 4) and not at a sample level without the destruction of the sample.

5. *Other applications*

It is also possible to identify counterfeit drugs. The main advantage of Raman hyperspectral imaging in this case is to detect the presence of excipients that are absent from the original formulation. The application of classification techniques on drugs allows one to separate the counterfeit drugs from the original ones.

Raman hyperspectral imaging can also be used to gain an understanding of processes; for example, it is able to detect process variability, dysfunctions and so on, controlling the final product quality. Indeed, using Raman hyperspectral imaging for this purpose ensures product quality during the entire manufacturing process. It is thus a versatile and nondestructive tool for qualitative and quantitative analysis of solid pharmaceutical formulations[2,4,7-8].

Because of the combination of spectral and spatial information, Raman chemical imaging is able to determine the spatial distribution of a specific compound in a sample

Conclusion

Raman hyperspectral imaging provides an accurate tool for the qualitative and quantitative analysis of a pharmaceutical solid form. Indeed, it combines the advantages of Raman spectroscopy and imaging, bringing together spectral and spatial information. The technique is able to identify the composition of a sample, to determine the solid-state of the different compounds and to calculate the size of some parts in the sample. It is also possible to identify counterfeits or to increase understanding of a manufacturing process. One of the most important characteristics of Raman chemical imaging is the ability to investigate spatial distribution. Indeed, it determines the homogeneity of the sample at a spatial level indicat- ing the repartition of the components on the sample. Hyperspectral Raman imaging is thus an accurate and complete tool to analyse pharmaceutical samples.

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