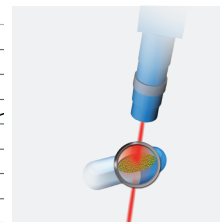
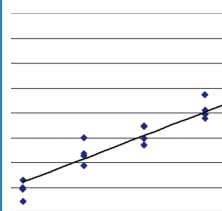


Transmission Raman Spectroscopy: review of applications



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Abstract

The use of the transmission raman mode instead of the traditional backscattering one brings additional flexibility for the analysis of material, especially when averaged information of bulky samples is required.

Key words

Transmission, Bulk samples, Pharmaceuticals, Quantitative analysis.

Introduction

The measurement of samples in the transmission configuration offers some possibilities that cannot be addressed by the conventional backscattering coupling with a microscope. Although not new, transmission Raman Spectroscopy (TRS) has known recently a rising interest, due to the possible combination of high power NIR lasers and dedicated accessories that permit the analysis of samples with this geometry.

TRS requires diffusing materials, such as tablets, and was initially developed for pharmaceutical applications. It works as well for capsules and other diffusing and translucent materials - not only in pharmaceutical field - requiring whole sample analysis. Examples of applications can be found in various domains, such as biomaterials, (tissues, food) or polymers. It can be used successfully to measure samples through the packaging.

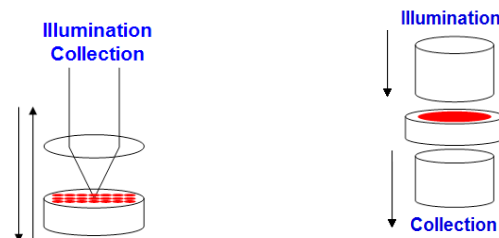


Fig. 1 Geometry of the transmission measurement (right) as opposed to the backscattering mode (left).

Transmission Raman Spectroscopy

Raman spectroscopy has undergone tremendous hardware improvements over the past decades. It is now very common to interface a microscope to a Raman spectrometer to analyze objects down to the sub-micron level. However in some cases, the information provided by the coupling with the microscope is too restrictive as it focuses on a very small volume of a sample, close to the surface, and may give incomplete information if the sample is rather heterogeneous.

Transmission Raman spectroscopy provides an interesting alternative when bulk measurements of heterogeneous samples are required. By collecting the signal at the opposite of the laser illumination, the Raman spectrum obtained from the transmitted light will represent the sample in its totality and provides an answer to the sub-sampling issue (Fig. 1.)

To the contrary, backscattered measurements, especially when coupled to a microscope, might give partial information. For example, pharmaceutical tablets are often coated and backscattering measurements will account mostly for the coating. Fig. 2 shows the transmission and backscattered Raman spectra of an anti-inflammatory Advil tablet: the blue spectrum shows the overwhelming presence of TiO_2 – anatase form attested by the peak at 145 cm^{-1} – and sugar, which are the main constituents of the coating. On the other hand, the transmission spectrum is more complex: besides the Raman peaks of anatase – coming from the coating - the peaks of the other compounds located in the core, such as the API (ibuprofen) are observed.

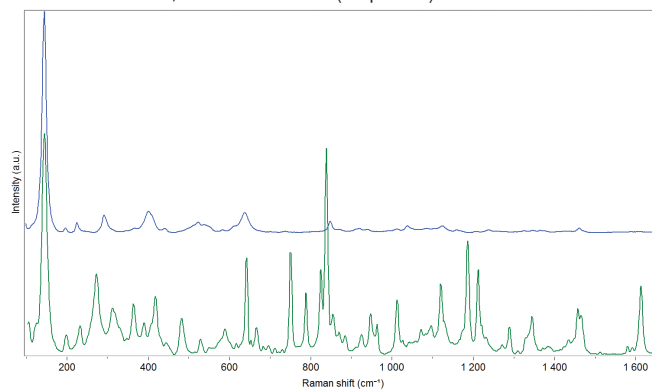


Figure 2. Backscattered (blue) and transmission (green) Raman spectra of an anti-inflammatory (Advil) tablet

Transmission Raman Spectroscopy (TRS) for pharmaceutical applications

Quantitative evaluation and calibration method

The monitoring of the active pharmaceutical ingredient (API) within the formulation is of prime importance to ensure that the formulation dosage is correct. Since transmission Raman spectra of tablets or capsules represent well the entire sample and are not sensitive to local variations, quantitative determinations of the different constituents are possible.

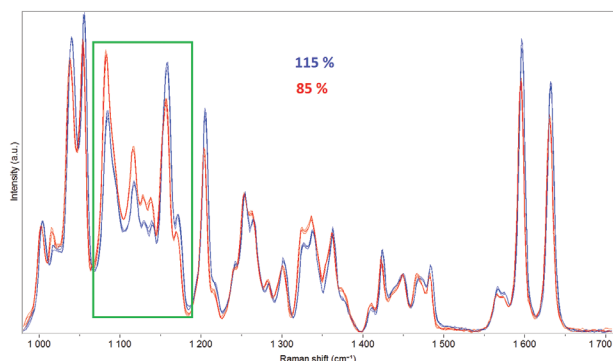


Fig. 3: transmission spectra of tablets with two different amounts of API (85 and 115 % of the target value).

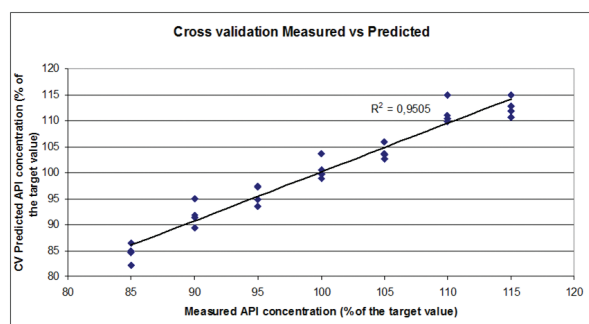


Fig. 4: Cross validation results obtained after the PLS calibration of a set of tablets formulated with various amounts of API around the target value.

By measuring in transmission a set of calibrated reference samples, and applying appropriate chemometrics algorithms (such as PLS – partial least squares), it becomes possible to build a calibration method which can be applied on unknown test samples to obtain quantification data. Fig. 3 illustrates the spectral differences observed in transmission spectra, with amounts of API varying between 85 % and 115 % around the target value (equivalent to 21,2 and 28,7 % of the total weight). Fig. 4 shows the cross validation results associated with the calibration of the set of tablets, illustrating the capabilities of the method to give acceptable quantitative results.

Polymorphism

Many active ingredients have several polymorphic forms, with one of them only being the desired form to be formulated in the drug. It is thus imperative to control within the mixture which form is present. Being non-invasive, and very chemically selective to discriminate polymorphs, Raman spectroscopy is well adapted to determine within tablets or capsules which form(s) are included.

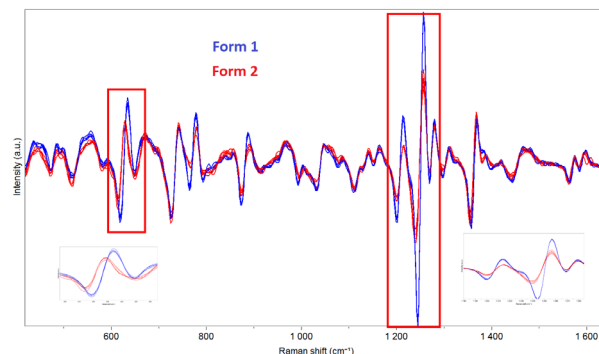


Fig. 5: Derivative spectra of capsules containing Form 1 and Form 2 of the Active ingredient.

Clear differences can be observed, showing the ability of discrimination of the polymorphs in formulations. It is even possible to quantify the proportion of each form to detect the presence of the undesired form down to low levels (estimated at 2% of form 2 in form 1 in this example).

Analysis of fat composition in food without sample preparation

The fatty acid composition of foods dictates a diversity of aspects regarding food quality. For example, the consumption of trans fats increases the risk of health problems and is therefore submitted to regulations in many countries.

Variation of composition in fatty acids was established between the different fat layers (outer and inner) of adipose tissues. In that respect, being able to get an averaged spectrum of a bulk sample is necessary, if global information is required.

Transmission Raman spectroscopy provides such averaged information. Adipose tissues of lamb, veal and pork chops were measured: samples of various sizes and thicknesses were analyzed without any preparation by transmission Raman spectroscopy.

Raman spectra give multiple indications about the sample composition. For example, trans fatty acids are readily observable from the spectra: the peak at 1668 cm⁻¹ is directly linked to the trans configuration of the C=C double bond of fatty acids.

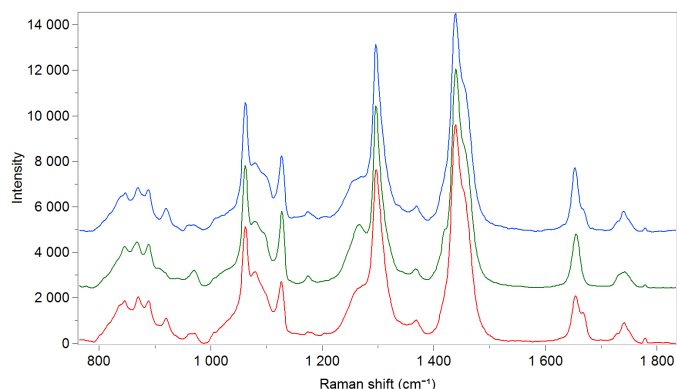


Figure 6: Transmission Raman spectra of adipose tissues from different species (lamb, pork, veal) using the transmission accessory operating at 785 nm.

In a similar way, Raman spectra could be used to derive quantitative information about the fatty acid profiles. Classification of species according to the Raman signature of their adipose tissues is also possible, as shown by the score plot in Fig. 7.

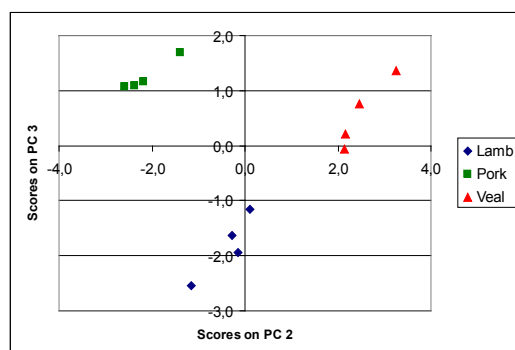


Figure 7: Principal component analysis score plot of samples of lamb, pork, and veal adipose tissues.

New Applications for Transmission Raman spectroscopy

Polymers

Although particularly well adapted to pharmaceutical applications, transmission Raman might be successfully adapted to various sample types.

Polymer samples are also good candidates for this technique. Fig.8 shows the transmission Raman spectra of polymer materials including a 2 layers polyethylene – polypropylene sample, exhibiting the spectral features of both material.

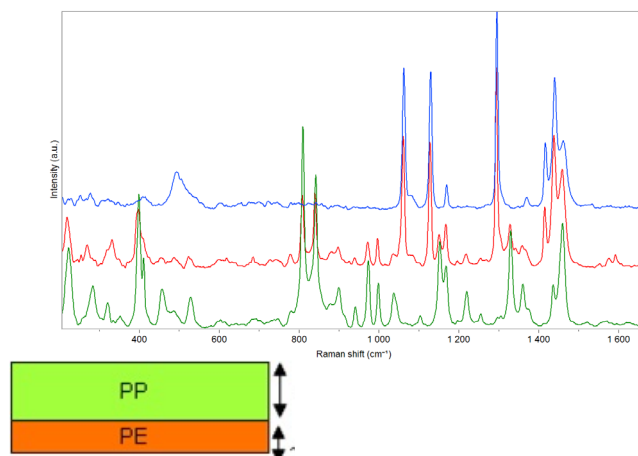


Fig.8: Transmission spectra of polymer materials: polyethylene, polypropylene, and a bi-layer sample made of a 1.5 mm polypropylene layer and a 0.75 mm polyethylene one.

Such technique can then be envisaged for the quality-control of polymer samples: this can include a quantification step of the different layers and / or components.

Measurement through packages

As demonstrated in the previous examples, transmission is the chosen geometry of analysis when bulk measurements are required. It might also be considered when the content of a packaged material has to be analyzed without removing it from the package. Fig.9 shows the spectrum of a shower gel measured through the PET package. In this example, the contribution of the PET is minimal, making this measurement technique suitable for the quality-control of the gel.

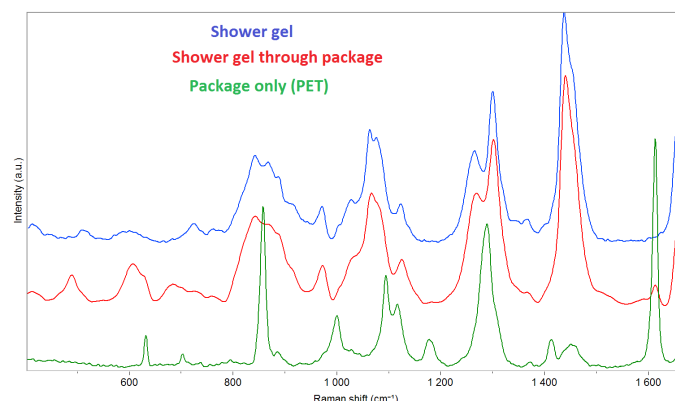


Fig.9: Transmission spectra of a shower gel: only the gel, the gel through the package and the PET package.

Stability of products in their original packaging over time might be evaluated using this technique, without having to transfer a sub sample into any vial – with the risk of transferring a non representative fraction of the products or add contamination.

Summary

The transmission design has demonstrated to be the technique of choice whenever Raman spectral information of a bulk material is required. It has already proven its utility for pharmaceutical applications, as tablets or even powder mixtures are good candidates for this measurement mode. However, transmission Raman might be applied successfully to other sample types, such as polymers, bio-tissues or any translucent material, and can be envisaged for evaluating the content of product inside a package. In addition, as TRS provides a global spectral information of the measured sample, it will be a technique of choice when quantitative evaluation of mixtures is needed.

Further Reading

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Transmission Raman devices for pharmaceutical applications are available in locations other than the United States of America, Europe, and India.

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