# Raman Microscopy:
# An Essential Aid in Characterizing Drug Delivery Products

**Abstract**
Raman microscopy provides information about molecular organization and crystalline order that is being used to characterize drug delivery materials. This white paper by Horiba Jobin Yvon discusses the mapping of creams, timed-release solids and stents; Raman maps provide hyperspectral cubes from which images can be reconstructed that show distribution, dissolution and molecular associations.

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Raman microscopy provides chemical and crystallographic information at the resolution of optical microscopy – at the sub micron scale.  The information is derived from the scattering phenomenon which is mediated by atomic motion in molecules and crystals. The way different atoms move in concert depends on the mass of the atoms, how they are bonded together (geometrical arrangement) and the strength of the bonds (single, double, triple bonds). Thus, there is exquisite differentiation of molecules in the spectra. In addition, as the molecules pack into the orderly arrays of crystals, there are effects of the crystal structure on the atomic motions, as well as interactions between molecules in the crystals. These phenomena enable the differentiation of different crystal forms of the same molecule.

These characteristics of Raman scattering can be quite valuable for the characterization of drug delivery products such as creams and patches, timed-release solids, and stents.

In the case of **creams and timed-release solids,** Raman mapping will show the distribution of the various phases.  An example of a map of an antibiotic cream shown below will illustrate that crystals of the antibiotic active pharmaceutical ingredient (API) are suspended in the delivery cream.  Statistical analysis of the map will provide measures of the sizes, shapes, and distribution of the crystals. In the case of time-released solids, it will be possible to confirm the encapsulation of one phase in another.

**Skin patches** are used for delivery of API transdermally. In general, the API is somehow contained and released to the surface in a calculated fashion. Very often, the API is dissolved in a low crystallinity polymer. The polymer will dissolve small molecule APIs exclusively in its amorphous phase where the network of polymer molecules is open. In addition, the API molecules can diffuse through the polymer network where it is non-crystalline. A depth profile of a commercial nicotine patch will illustrate that a Raman microscope can monitor the distribution of the API as a function of distance from the surface.

## Antibiotic cream
The goal of this study was determine if a Raman map can provide information on the distribution of the API in the hydrophilic base which could subsequently provide information on the mode of action of the drug.

A commercial sample of sulfadiazine cream (manufactured by BASF for Par Pharma) was used for this study. According to the label it contains "silver sulfadiazine (10mg/g) in a hydrophilic base containing cetyl alcohol, isopropyl myristate, polyoxyl 40 stearate, propylene glycol, purified water, stearyl alcohol, NaOH, sorbitan monooleate and white petroleatum with 3% methyl paraben (added as a preservative)." No information on the method or rationale of formulation was available for these measurements. Therefore this was a blind study.

Optical examination of the cream in the microscope indicated the presence of broad needle-like crystals in a hydrophilic matrix. Presumably the crystals are of the API sulfadiazine whose structure is shown below.



Silver sulfadiazine
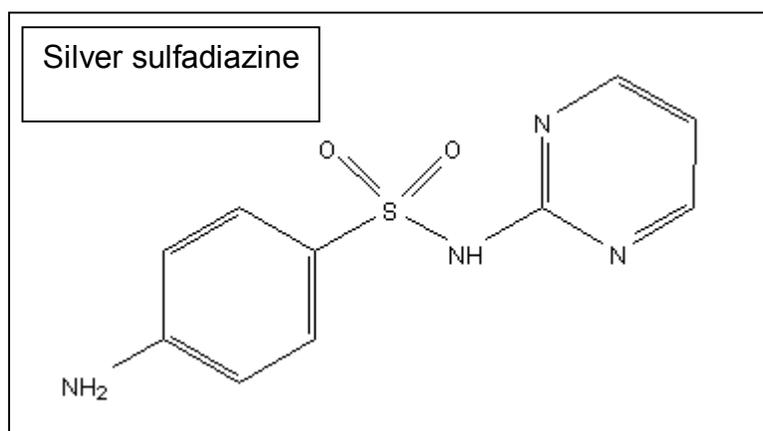
Since this molecule, like many other API's, contains aromatic groups, it was anticipated that its Raman spectrum would be quite intense and easy to recognize, even without reference materials. 2-aminopyrimidine, is a molecule with the ring to the right on this figure and an -$NH_2$ group attached to the carbon between the 2 nitrogens. The strongest features in its spectrum appear near 870 and 1580 $cm^{-1}$ and similar bands are expected in the spectrum of the API. A 6-

carbon membered aromatic ring with 1,4 substitutions usually have a diagnostic band near 1600 cm$^{-1}$. Sulphonamides have strong bands in the mid 1100 and 1300 cm$^{-1}$ regions.

On the other hand, a hydrophilic cream base containing a variety of molecules with linear hydrocarbon chains is expected to have spectral features similar to that of disordered polyethylene – 1060, 1130, 1300 and 1420-1460 cm$^{-1}$.
A sample was prepared for a map. A slide with a well was filled with the cream and covered with a coverslip for stabilization (and to avoid evaporation). The spectra were recorded with a 60 x biological objective corrected for the cover slip.
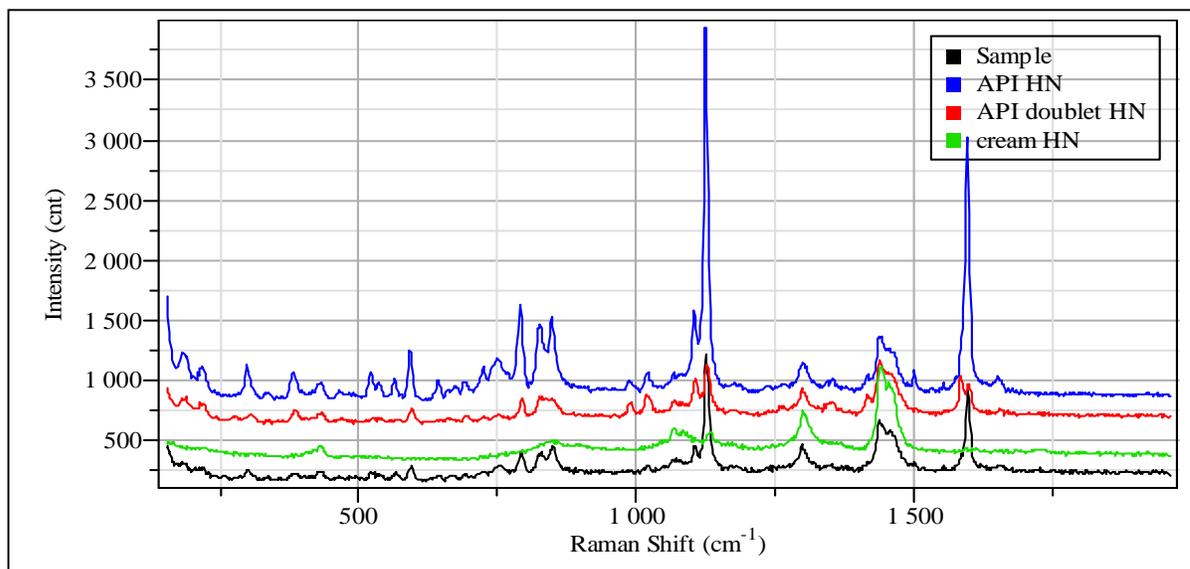


The spectra in this figure were extracted from a Raman map of the cream. The top two spectra have strong sharp bands consistent with our expectations for an aromatic molecule. The third spectrum down is consistent with our expectations for a hydrophilic base. The spectrum at the bottom appears to be a mixture of the API and the cream base.

LabSpec, the software on the instrument, has many capabilities for creating a Raman image from the hyperspectral cube created by the mapping capabilities. The easiest function is to bracket peaks unique to 3 particular species and create an RGB image by integrating the intensity between the cursors, and subtracting the background. For complicated systems such as this one, where each species has many peaks, it is often the case that it is not possible to identify unique spectral features. In this case it is possible to "Model" the image by using selected full spectra of each species. These spectra can be extracted from the map, or can be recorded from reference materials.

The following results were achieved by using these modeling capabilities. In order to provide cleaner results, the entire hyperspectral cube was first subjected to background subtraction. The basis spectra used for the image are displayed again in the following figure in a manner that better shows their differences. The

second API spectrum (shown in red, 2nd from the top) indicates some changes in the spectrum of the API (doubling of the band below 1600 cm⁻¹, for example) as well as contributions from the hydrophilic base (green spectrum, 3rd from top). Since it is not clear whether these differences represent a spectrum taken from a mixture of the cream and a crystal at another orientation, or of an interacting species of the API and cream, this spectrum was defined as one of the basis spectra. The image created by these spectra are shown below the spectra.
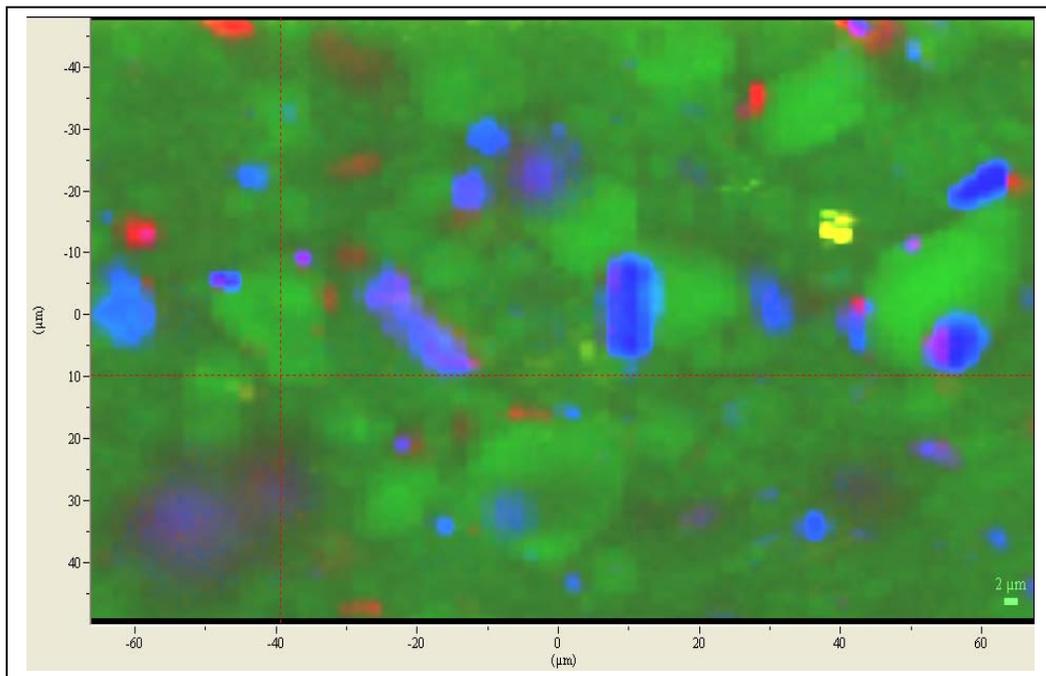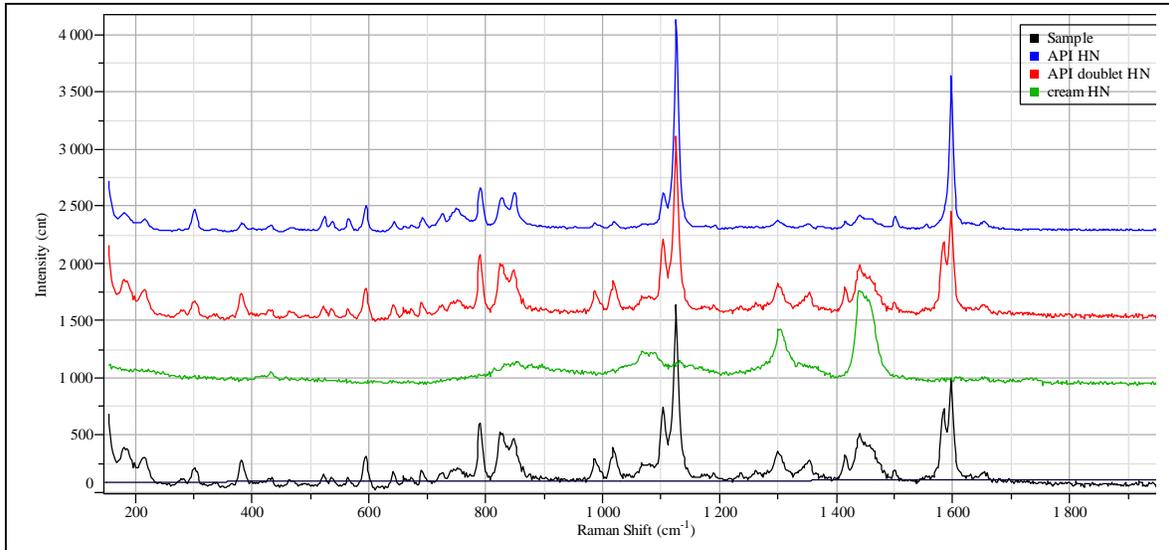
What one can say after inspection of this map is that, as expected, the hydrophilic base forms the matrix for this cream, and the API crystals are dispersed in the cream. The "red species" shows up both as crystal particles and, in some places, overlapping either the "normal" API spectrum or the matrix cream.

After one begins to produce such Raman maps, it becomes clear that more extensive image analysis could provide more information on such samples. Multivariate analysis data treatment of spectroscopic maps enables extraction of spectral information not obvious to casual inspection, and statistical information on the occurrence of multiple species. The hyperspectral cube that was used to create the Raman images in LabSpec was then processed in ISys™ (Spectral Dimensions, Olney, Maryland), a software package that provides a suite of multivariate analysis tools.

The algorithm that was used on this data set was Factor Analysis. In Factor Analysis the data set is multiplied by its transpose, making it a cubic matrix, and then diagonalized. The result of this operation is to produce "factors" which are mathematical constructs of pseudo spectra. These factors can be manipulated until they "look like" spectra. When Factor Analysis was performed on this data set, the following factors were created.
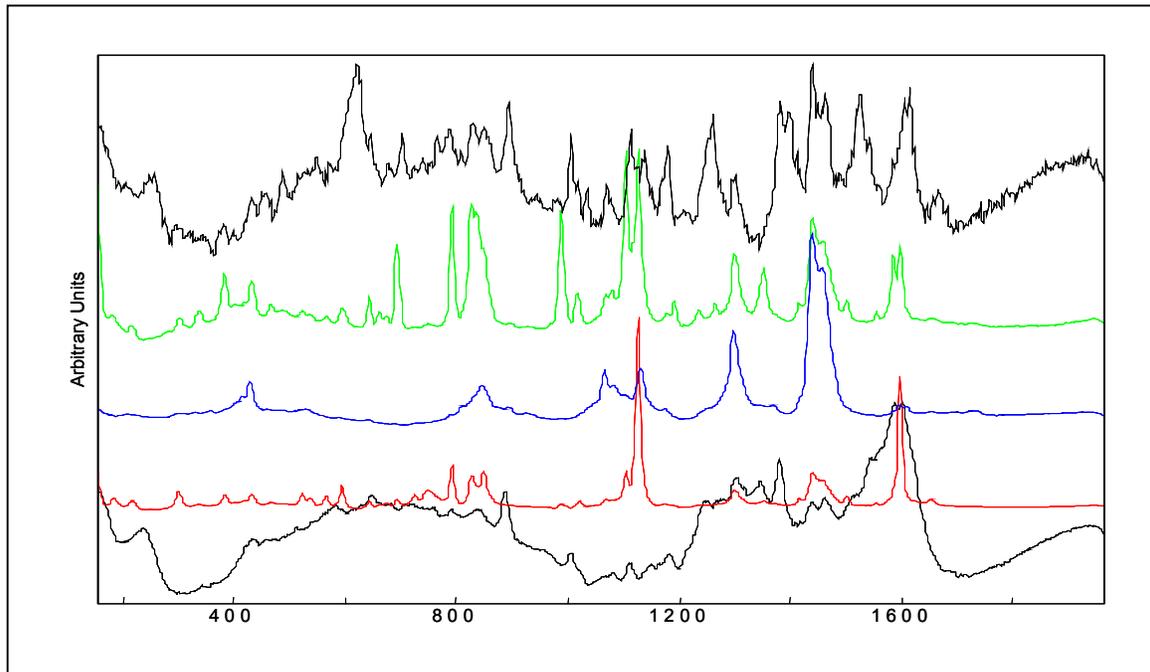


Factors 2, 3, and 4 are quite similar to the basis spectra identified in LabSpec above. The image produced by these factors is quite similar to that created by LabSpec.

133µm
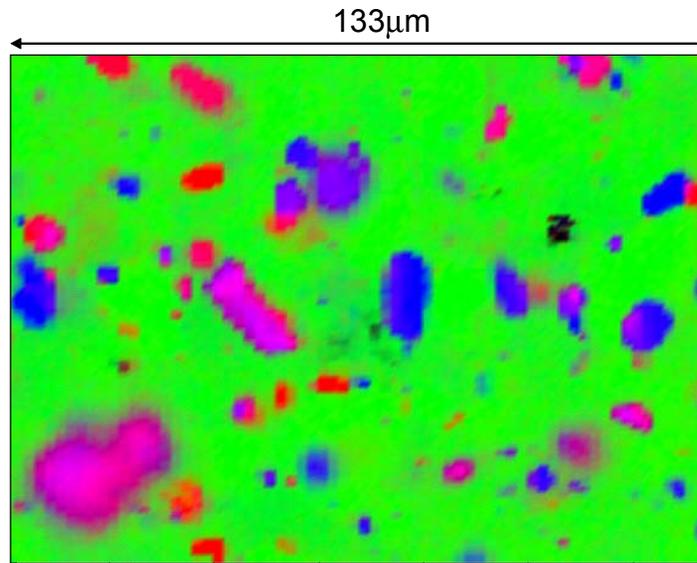


It is interesting to explore the additional variables identified in ISys™'s multivariate techniques. For instance, the images showing graded intensities of the color hues corresponding to variations of the Scores from the Factor Analysis can be converted to binary images where a threshold has been selected to indicate where the species is present or not. For instance, a binary image of this map is shown next.

133 µm
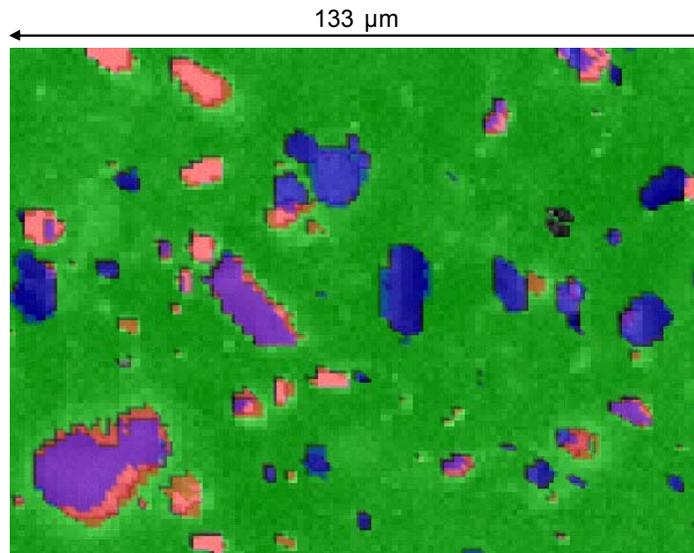


And from this, particle statistics can be derived.

| Red | | Blue | |
|---|---|---|---|
| **Red** | | **Blue** | |
| Number of Particles | 40 | Number of Particles | 49 |
| Percentage Area Covered | 10 | Percentage Area Covered | 9.7 |
| Mean Area | 32.1 | Mean Area | 25.5 |
| Area STD | 54.3 | Area STD | 59.9 |
| Mean Diameter | 5.0 | Mean Diameter | 4.3 |
| Diameter STD | 4.0 | Diameter STD | 3.8 |
| Mean Nearest Neighbor Distance | 14.7 | Mean Nearest Neighbor Distance | 13.4 |
| Center of Mass X | -5.6 | Center of Mass X (pixels) | -21.6 |
| Center of Mass Y | 3.7 | Center of Mass Y (pixels) | 7.8 |

## Nicotine Patch

A depth profile was performed on a nicotine patch (Nicoderm CQ™) as it came out of its commercial packaging.  Spectra of 6 layers were recorded by optical sectioning (scanning confocally) and identified on the next figure.  The top and bottom layers are PET (polyethylene terephthalate).  The second and 4th layers are polyethylene of one form or another; details in the spectra indicate that the 4th layer is more crystalline or of higher density than the 2nd (well defined band at 1420 cm$^{-1}$).   The other two layers were unknown when first examined, but comparison with a reference spectrum of nicotine indicated that these two layers contain nicotine.
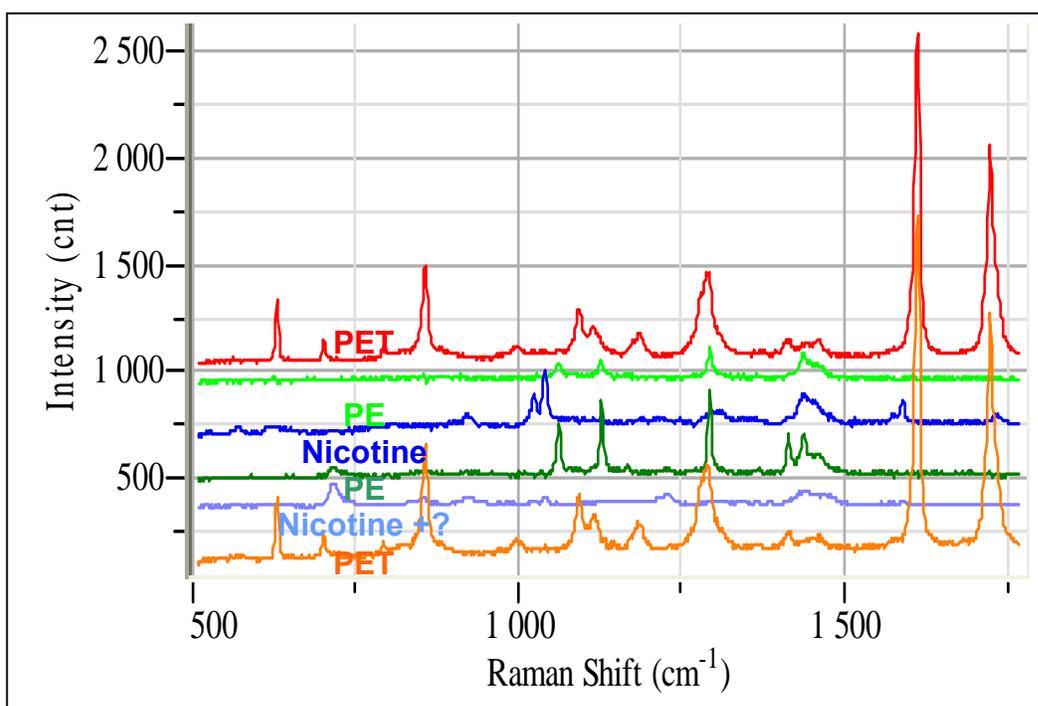
The reference spectrum from the Verlag Chimie collection is shown on the next figure, and the spectrum of the 2nd layer is replotted to better compare it to the reference spectrum (axis now oriented right to left). The one major difference is the presence of a carbonyl band near 1735 cm$^{-1}$ which is not present (and would not be expected) in the spectrum of nicotine. This may indicate the presence of the polymer that is serving as the solvent for the nicotine. Another interesting observation is the presence of a broad band at about 700 cm$^{-1}$ in the 4th layer that is not present in the 2nd layer; this would indicate that the nicotine is mixed/dissolved with different materials in the two layers.
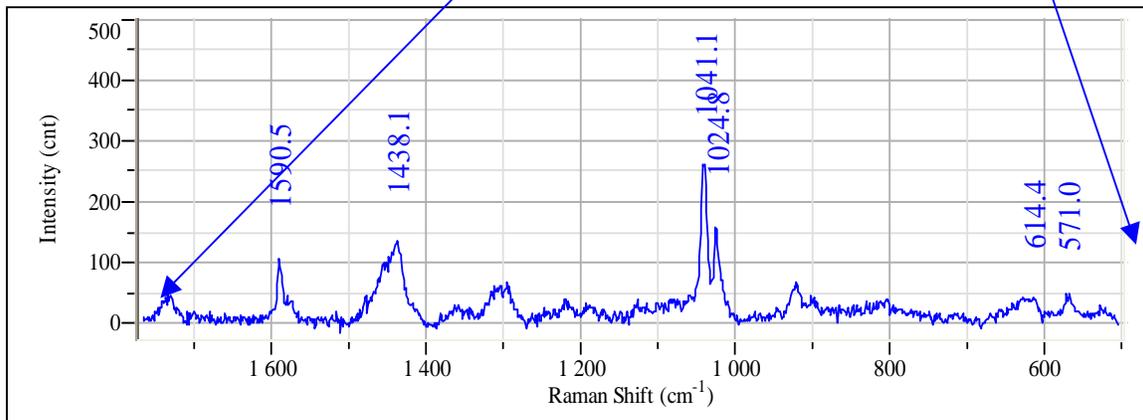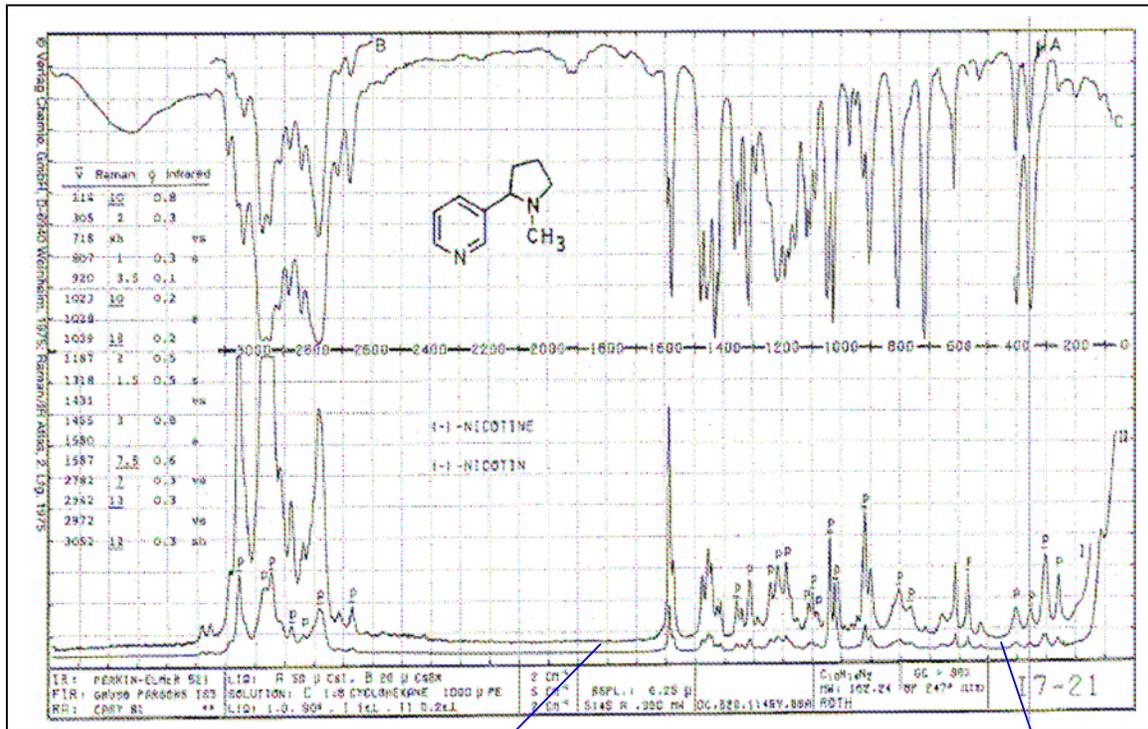


What really is of interest is to profile the components as a function of depth. Depth profiles of 3 components – polyethylene terephthalate, polyethylene, and

nicotine – are shown in the last figure.  The X axis is the depth scanned, and has not been corrected for the index of the material; since the index of most polymers is close to 1.5, the X axis numbers would be increased by that factor.
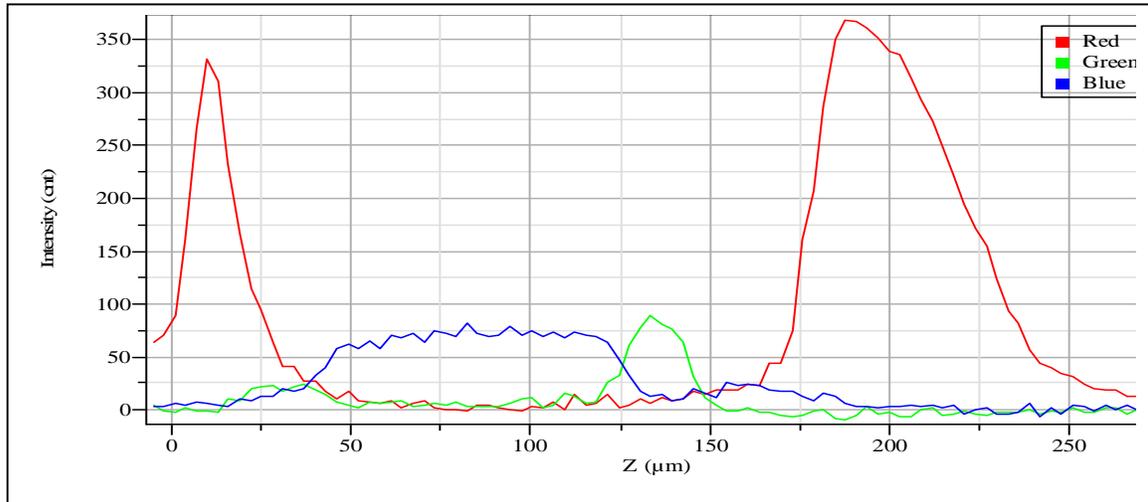


The uncorrected values for the FWHM of the layers is tabulated:

| PET | PE | Nicotine | PE | Nicotine | PET |
|-----|-----|----------|-----|----------|-----|
| 15μm | 24μm | 81μm | 21μm | 45μm | 48μm |

In this case, the profiles were constructed by using two cursors to bracket bands specific to the species of interest.  Even though all the spectra have many bands, it was not impossible to find bands that were fairly unique to a given species.  Examination of the profiles does indicate at least one interesting phenomenon.  The second nicotine band was rather diffuse.  Dynamic inspection of the multifile indicated that there was nicotine diffusing into the PE and PET layers.  Since the confocal conditions were rather tight, it is not believed that this is an optical artifact, but indicates the real molecular diffusion.

**Stents** are cylinders of scaffold mesh that are inserted into occluded coronary arteries to prevent total stenosis and consequential myocardial infarction.  While they have been in use for more than 10 years, it has been found that restenosis is not an uncommon event.  In order to prevent restenosis the stents are now being coated with products designed to prevent restenosis.  Raman microscopy has been used to characterize the distribution of the API in the stent coating.  Unfortunately, because of the IP investment in these products, it is not possible to include an example.  Suffice it to say that depth profiling and molecular mapping is providing invaluable information on the performance of these products.

**Summary**

Raman microscopy spectra of an antibiotic cream and a transdermal nicotine patch have demonstrated the potential to elucidate the molecular dispersion of these pharmaceutical products.  The information generated will be invaluable to the engineering of these and other drug delivery products.

**About Horiba Jobin Yvon**

Horiba Jobin Yvon manufactures a complete range of high-quality spectroscopic instrumentation. We are the world leaders in Raman spectroscopy. Our Raman systems are used throughout the pharmaceutical industry for drug discovery—including high-throughput screening, formulation development and control, failure analysis and process control. We also manufacture the highest sensitivity fluorescence spectrometers available today, including highly advanced lifetime systems such as time-correlated single photon counting systems for macro and micro applications. For elemental analysis, we manufacture both EDXRF microscopes and ICP spectrometers.

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