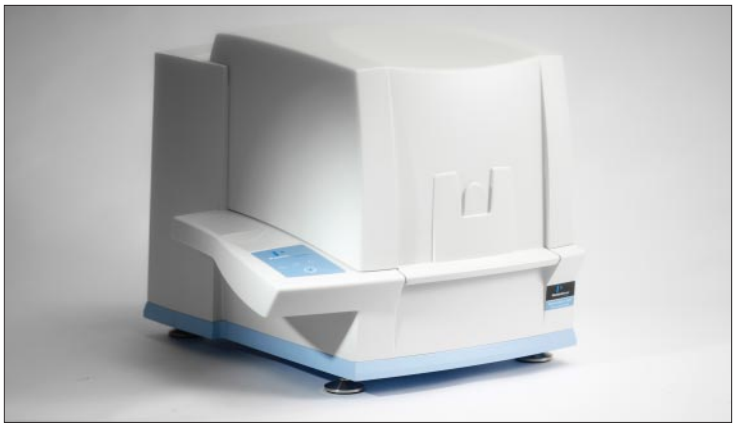
APPLICATION NOTE

RAMAN SPECTROSCOPY

Advantages of Raman Spectroscopy when Analyzing Materials Through Glass or Polymer Containers and in Aqueous Solution



Abstract

This application note discusses the significance of strong and weak Raman scattering materials in routine analysis. It highlights the advantages that Raman has over classical mid-FT-IR spectroscopy in the analysis of samples in glass or polymer containers or in aqueous solution.

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The significance of strong and weak Raman scatterers in Raman analysis

In a modern FT-IR spectrometer or double beam UV/Visible spectrometer, the absorbance (or transmission) spectrum of a sample is determined by measuring the absorbance spectrum through the sample relative to a background or open-beam absorbance. The position of the peaks in the spectrum is defined by the molecular structure of the sample and its chemical environment. The peak heights (or areas) of the spectrum are defined by Beer's Law which states that the peak height (or area) is proportional to the absorptivity (at the peak position) of the molecule, its concentration and the thickness (pathlength) of the sample.

In IR spectroscopy, molecules such as water or acetone are considered to be strong IR absorbers. They contain functional groups such as hydroxyl or carbonyl which have high absorptivity values and therefore strong absorption bands. Other molecules have weaker IR spectra while materials such as sodium chloride or potassium bromide will show no significant absorption over large frequency ranges of the spectrum. These are therefore used extensively as window materials.

When a sample of fixed thickness and concentration is analyzed on the same instrument at several different times, the peak heights will remain the same (within the instrument specifications). If different numbers of scans are accumulated and averaged, the peak heights (signal) will remain the same. However, the inherent, random baseline noise of the measurement will decrease as the number of scans increases. Therefore, increasing the number of scans is the basis for improving the signal: noise ratio of a spectrum.

Assuming that certain parameters such as resolution, apodisation, interpolation, etc. can be normalized between instruments, the peak height for such a sample should be the same when run on different spectrometers. This means that when measuring a sample with IR or UV/Vis, the peak height is a function of the sample and independent of the measurement parameters.

In dispersive Raman spectroscopy however, the situation is different. The ordinate axis normally has arbitrary rather than absorption or %T units. This is because the exact numerical value of the ordinate is simply a measure of the number of scattered photon counts captured by the detector, at any particular frequency in the spectrum, within a specified time interval. If a sample is scanned for five times longer, then the ordinate values (the peak heights) will be five times greater. Equally, if the incident laser power striking the sample is varied, the intensity of the Raman spectrum will vary accordingly. Therefore, the peak height in a Raman spectrum is not simply a function of the sample thickness, its concentration and its Raman scattering characteristics but is also dependent on the analysis conditions (laser power, laser wavelength, scan times, orientation of sample, etc).

In some cases, the instrument software will normalize the ordinate values to some nominal time limit (such as 1 second) but this is totally arbitrary. There are still, however, molecules that can be considered strong or weak Raman scatterers and so it is extremely important to note the scan conditions when comparing the Raman spectra from different samples.

As an example, it is accepted that glass has a relatively weak Raman spectrum. However, the spectrum of glass can be made to appear strong by doing multiple scans (Figure 1).

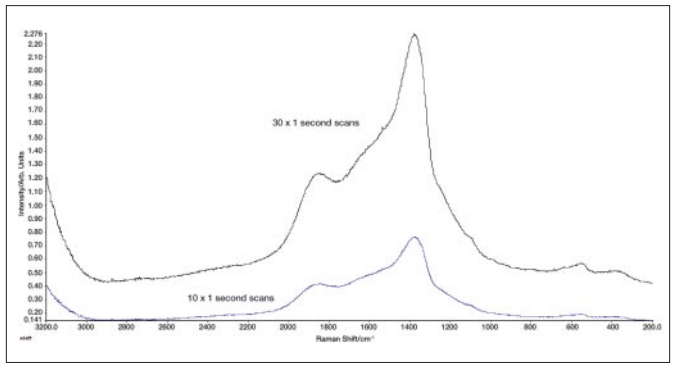

Figure 1. Spectrum of glass after 10 and 30 second accumulations.

Similarly, water has a weak Raman spectrum but if it is scanned for a long time it will show significantly higher ordinate values (Figure 2).

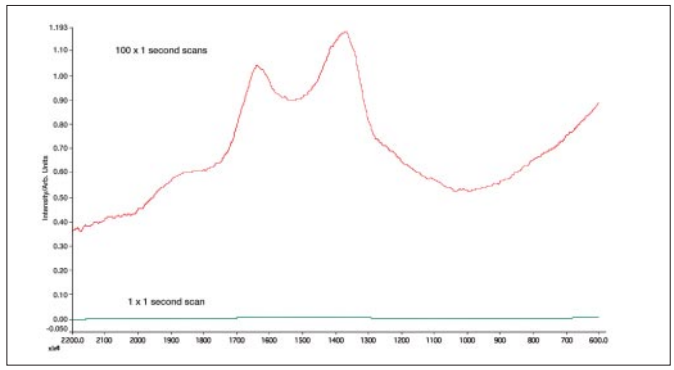

Figure 2. Spectrum of water after 1 and 100 second accumulations.

Cyclohexane on the other hand is classified as being a medium Raman scatterer.

The correct relative scattering powers of these two molecules is highlighted by superimposing a single 1 second spectrum from each sample (Figure 3). The cyclohexane spectrum is clearly stronger.

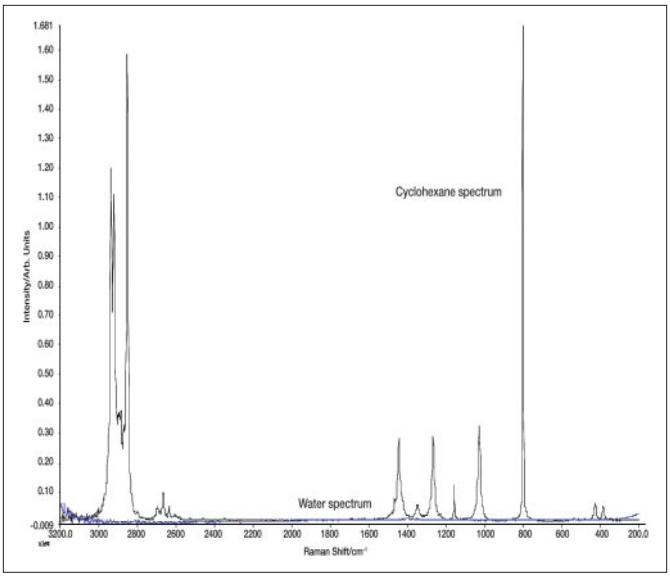

Figure 3. Comparison of spectra of water and cyclohexane after 1 second accumulation.

In Figure 4, the water spectrum is similar in intensity to that of cyclohexane but this is simply a consequence of being the accumulation from a 100 second analysis whereas the cyclohexane spectrum is from a single 1 second scan.

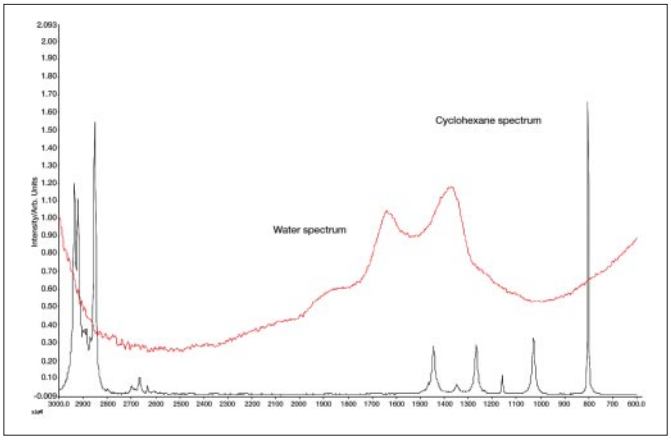

Figure 4. Cyclohexane spectrum after 1 second accumulation and water spectrum after 100 second accumulation.

Therefore, it is vital when drawing conclusions about the relative strengths of Raman signals to note the scan conditions and to ensure that scan times are comparable. As illustrated in Figure 3, when the spectrum of a weak Raman scatterer is compared to that of a strong Raman scatterer under the same collection conditions, then the resultant combined spectrum will be dominated by that of the stronger Raman scatterer.

Qualitative Raman analysis through glass and polymer packaging.

A situation where this is helpful is the measurement of a material contained in a glass bottle or vial. While the number of scans will clearly be the same for both materials, the resultant spectrum is essentially that of the stronger Raman scatterer. Different qualities and colors of glass will have different spectra but because they are all relatively weak, samples can be analyzed through even very highly colored bottles.

Figure 5 shows the spectrum of nicotine liquid (a very powerful toxin by contact or inhalation) taken through a very dark brown sealed glass vial. The result is the spectrum of pure nicotine with no significant contribution from the glass vial.

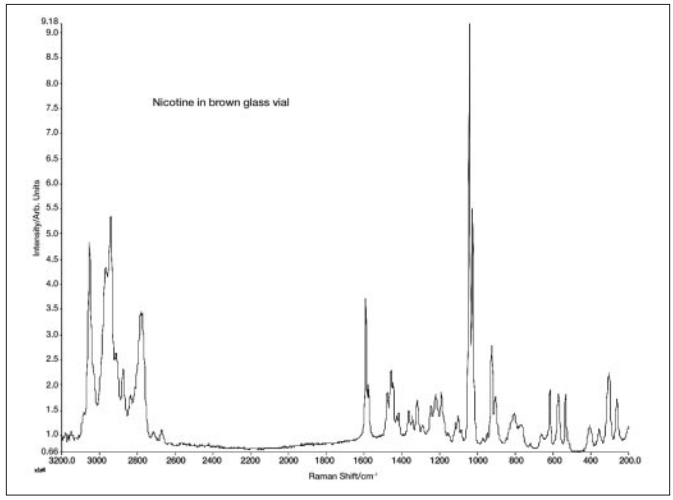

Figure 5. Nicotine in brown glass vial.

In common with most polymers, polyethylene has a Raman spectrum (Figure 6), but when it is in the form of a thin film or coating, a focused laser beam is normally required to obtain a strong Raman signal.

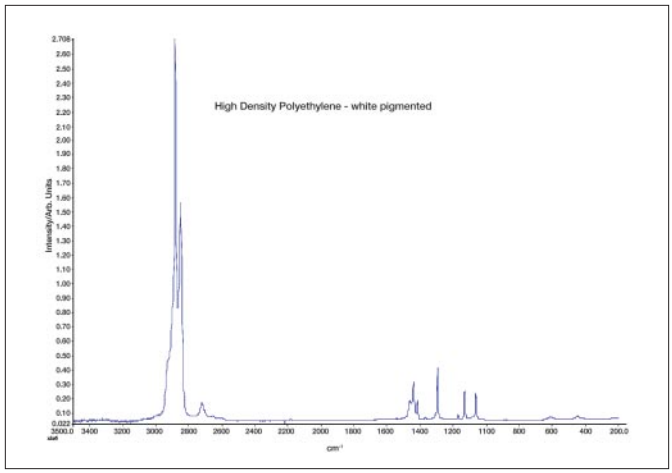

Figure 6. High density polyethylene – white pigmented.

When the incident laser beam is relatively weakly focused, the laser beam tends to transmit through the polymer, generating only a minimum amount of Raman scattering. For instruments such as the RamanStation™ 400 which have a relatively weakly focused laser beam, bulk materials can be measured and analyzed easily through polymer containers with little or no contribution from the container. Figure 7 compares the spectrum from paracetamol taken through a polyethylene bag with the spectrum from pure paracetamol powder. Only the small contribution at 2840-2890 cm^{-1} from the polyethylene bag is evident in the top spectrum. If required, the contribution from the polymer container can be removed by automatic spectral subtraction but this is rarely required for positive material identification.

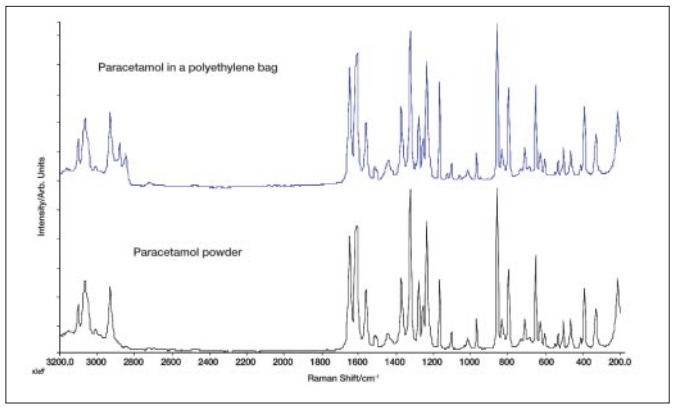

Figure 7. Paracetamol in polyethylene bag.

Raman analyses can also be made through thicker polymer containers such as solvent bottles. Figure 9 shows the Raman spectrum of ethanol taken through a 1.5 mm thick plastic solvent bottle. In this case, there is a significant contribution (1296 cm^{-1}) from the polymer but this is easily subtracted to leave the pure spectrum of the ethanol.

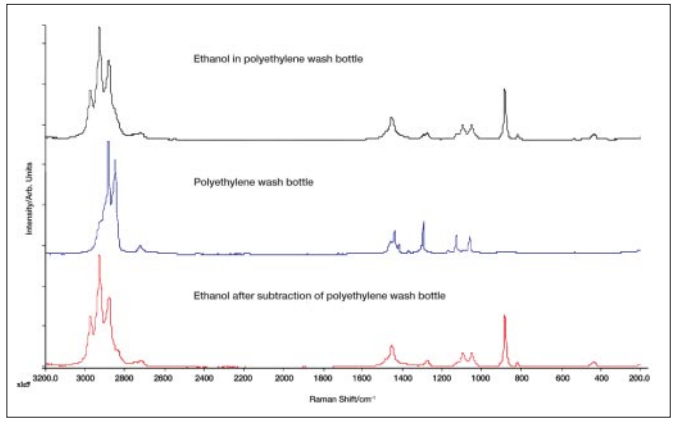

Figure 8. Analysis of ethanol in thick plastic solvent bottle.

Raman spectroscopy is therefore an extremely good and easy technique for the analysis of materials in either glass or polymer containers. These analyses are particularly important where:

1) speed and convenience of analysis is important such as in a QC/QA laboratory
2) samples are toxic or have an unpleasant smell
3) samples may be unstable to air or moisture
4) it is important to maintain the safety of the analyst such as when working with active pharmaceutical materials
5) the properties and toxicity of the material is unknown such as at the scene of an accident
6) confiscated forensic materials of unknown composition or history are being analyzed
7) the integrity of the material is paramount such as in many forensic analyses

Quantitative Raman analysis in solution

In UV/Visible and IR spectroscopy, the spectral contribution from the instrument is monitored and removed by using a reference beam (in a double-beam instrument) or by subtracting a background spectrum (in an FT instrument).

Since Raman spectroscopy is essentially a single-beam technique, any contribution or variation due to the instrument must be considered when doing quantitative analyses. Potential sources of instrumental and sampling variations are laser and detector stability and de-polarization effects. For this reason, it is vital when doing quantitative analyses to have as stable an instrument

as possible and to ensure consistency in measurement parameters (such as laser power, laser focus on sample, scan sequence, spectral resolution, sample orientation) and in any post-run processing (such as baseline correction, noise reduction). This is similar to any quantitative analysis where any sources of unwanted variation are kept to a minimum.

It is also more straightforward to do quantitative analyses on liquids and solutions than on solids since they are more homogeneous and without the problems associated with variable particle sizes and shapes.

For these reasons it is more common to measure relative concentrations of the components in a mixture with band-ratio methods than with absolute quantitative analyses. It is also recommended that a standard reference mixture is measured as part of each analysis regime simply to monitor the stability of the system.

Provided the above considerations are adhered to, Raman can be a very useful quantitative analysis technique particularly for aqueous samples or samples in glass or polymer containers which can be difficult to analyse by IR. This is because relatively strong Raman scatterers can be measured in the presence of weaker Raman scatterers. An example of this is the measurement of ethanol in water in a glass container (Figure 9).

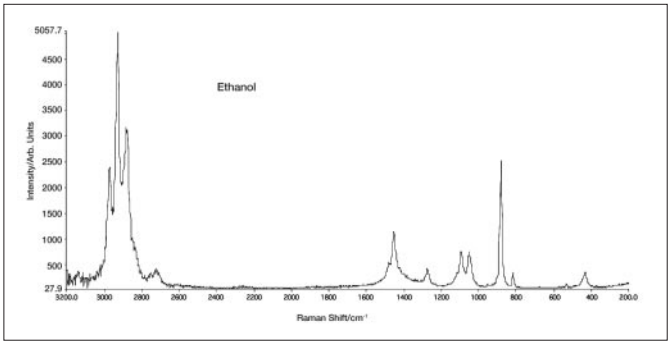

Figure 9. Spectrum of ethanol showing strong, sharp band at 880 cm-1.

The peak at ~880 cm-1 is an ideal band to be used in a quantitative analysis. Figure 10 shows the variation in the height of this peak with varying concentration of ethanol. There is some variation in baseline position but this is easily compensated for by measuring the peak height relative to an adjacent baseline position. These peak height values are given in Table 1 and illustrate a good degree of linearity.

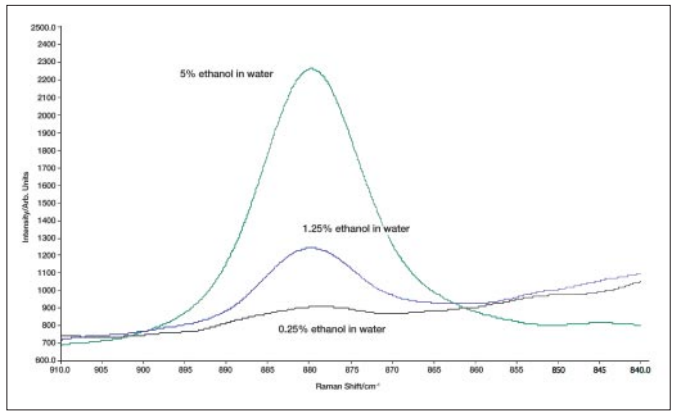

Figure 10. Raman spectra of different ethanol concentrations.

Table 1. Peak Heights of Ethanol at Various Concentrations in Water.

Concentration of Ethanol in water	Peak Height (880 cm-1 relative to baseline 905-850 cm-1)
0.25%	65.6
1.25%	350.1
5.00%	1423.6

The Raman spectra of several alcoholic beverages were measured under the same scan conditions (Figure 11) and the peak heights for the ethanol measured (Table 2). Once again, these peak heights show a good correlation with known alcoholic content for these drinks. A more exact quantitative analysis for these drinks would require separate calibration for each drink type to take into account variation in their composition. The absolute peak height values in Table 2 do not match with corresponding values in Table 1 because the scan conditions were different for the two sets of data.

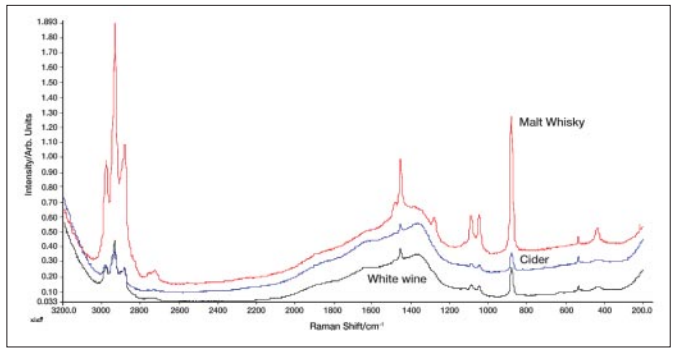

Figure 11. Raman spectra of whisky, wine and cider.

Table 2. Peak Heights of Ethanol in a Variety of Alcoholic Drinks.

Alcoholic Drinks	Peak Height
Scotch whisky (40%)	87208
White wine (9%)	18087
Cider (6%)	11731

Conclusions

The intensity of Raman spectra not only depends on the molecular and physical properties of the material but also on the analysis conditions. Molecules can be classified as strong or weak Raman scatterers depending on their molecular structure. Experimental parameters such as laser power and scan times will also influence the strength of the resultant spectrum. This means that the analyst must take into account both the molecular structure and the scanning conditions when comparing Raman spectra from different samples.

Thin samples such as polymer films or coatings are best measured using a Raman microscope system where the laser power can be concentrated onto these thin samples to minimize unwanted contribution from the surrounding materials.

The fact that glass, polymer films and water have relatively weak Raman spectra when measured using the RamanStation means that this instrument is ideal for the analysis of bulk materials in glass or polymer containers, or in aqueous solution.

Provided adequate experimental procedures are observed, Raman spectroscopy can be used very effectively for quantitative analyses in situations where mid-IR is not appropriate.

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