Application Note AN-RS-055

Low-frequency Raman spectroscopy

Raman spectroscopy is an analytical tool that enables the measurement of molecular structure and the identification of materials based on the rotational and vibrational modes of a molecule. Most laboratory Raman systems cover the fingerprint spectral region ranging from 200–3400 cm^{-1}. However, access to the lower-frequency areas provides additional information for applications in protein characterization [1], polymorph detection and identification [2], along with material phase and structure determination.

The lab-grade fiber optic sampling probe for laboratory spectrometers from Metrohm can access lower-frequency modes down to 65 cm^{-1}, providing a cost-effective solution for wider range measurements. This Application Note describes the use of low-frequency Raman spectroscopy for studying amino acids, polymorph detection, and phase change monitoring.

The low-frequency region augments the information content from the fingerprint region of the Raman spectrum and broadens possible applications. Detection of hydrogen bonds and other structural features, such as polymorphism, can be differentiated using this region of the spectrum. The result is increased specificity for very similar materials.

EXPERIMENT

Metrohm's i-Raman Plus 785S laboratory Raman spectrometer with a 785 nm, 300 mW (maximum power output) laser and a sensitive, thermoelectrically cooled back-thinned CCD (charge-coupled detector) is used to collect Raman spectra.
The i-Raman Plus is equipped with a unique fiber optic probe that supports data collection over a full spectral range of 65–3500 cm^{-1} with a spectral resolution of 4.5 cm^{-1}.
Raman spectra are collected at room temperature using SpecSuite software with integration ranging from 100 ms to 10 s.

AMINO ACIDS

Raman spectroscopy is used to study the structure and conformation of amino acids – the building blocks of proteins. The low-frequency portion of the Raman spectrum is a necessary source of information for a comprehensive study of amino acids. This is obvious in the 65–3200 cm^{-1} region of the Raman spectrum of L-asparagine in **Figure 1**. Specifically, the vibrational information in a Raman spectrum can help interpret molecular interactions and biological processes [**3**].
Figure 1 shows both the fingerprint region (blue) as well as the low-frequency Raman region (red) for L-asparagine; note the three dominant bands below 200 cm^{-1}.

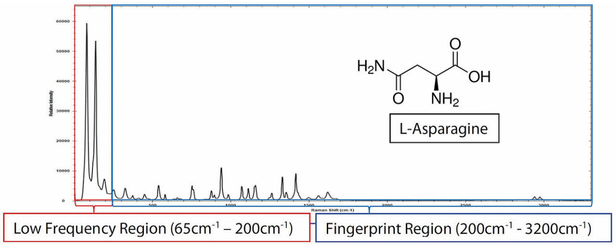

Figure 1. Metrohm's i-Raman Plus with standard lab-grade probe is used to collect the low-frequency spectra of L-asparagine with a total integration time of 1.2 s.

POLYMORPH DETECTION

Determining the structural form of active pharmaceutical ingredients (APIs) is of great concern for the pharmaceutical industry. This is especially true during drug development, manufacturing, and final product quality control.

APIs exhibit polymorphism – identical chemical composition but different solid-state structures. Polymorphs may affect bioavailability and therapeutic index, so the efficacy of a drug can be compromised if the wrong form is used [**2**]. Pseudo-polymorphs include solvents suspended in a lattice structure.

Figure 2 provides an example of the pseudo-polymorph D-glucose, demonstrating the ability of the i-Raman Plus probe to detect differences between monohydrate and anhydrous forms at frequencies below 200 cm^{-1}.

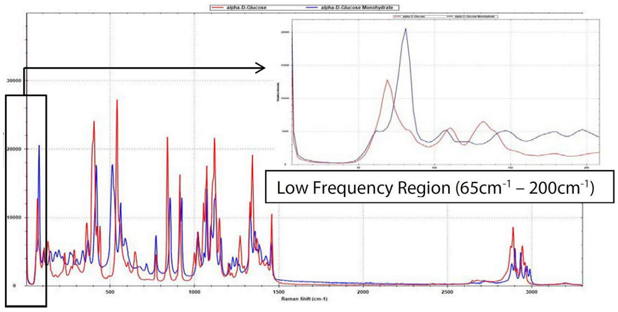

Figure 2. Raman spectra of α-D-glucose (red) and α-D-glucose monohydrate (blue), taken with 10 s integration time. Note the significant difference between the two pseudo-polymorphs within the low-frequency range (see inlay).

MONITORING PHASE CHANGE

A change from one polymorph to another as a result of melting followed by recrystallization can be observed in the low-frequency region. Exceptional specificity is required to monitor such phase changes, and the probe of the i-Raman Plus is well-suited, as demonstrated for sulfur (**Figure 3**).

Solid -sulfur is deposited onto an aluminum tray and heated with a hot plate while Raman spectra are collected with an i-Raman Plus and fiber probe, using a laser power of 300 mW and 0.1 s integration time to measure both the solid and liquid phase.

After the sample is heated above its melting point at 115.2 °C, the low-frequency peak at 83.6 cm^{-1} broadened and shifted, indicating the change from the - to the -form. Note that there are no observable changes within the fingerprint region (**Figure 3**).

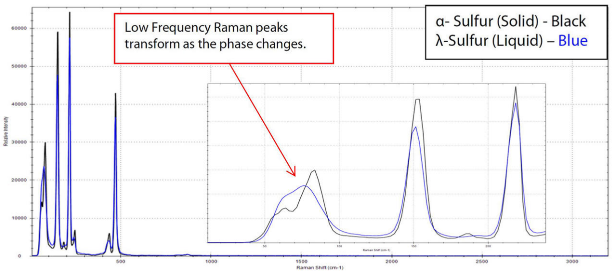

Figure 3. Raman spectra of sulfur transitioning from the α-crystalline form to the λ-liquid form, taken with 0.1 s integration time. Note significant broadening in the peaks located in the low-frequency region (see inlay).

CONCLUSION

The i-Raman Plus 785S Raman spectrometer is a valuable tool for applications requiring low-frequency Raman detection down to 65 cm^{-1}. The ability to characterize polymorphs and solvated forms supports manufacturing and formulation processes in pharmaceutical and biological industries.

Along with protein, polymorph, and phase characterization, low-frequency Raman spectroscopy can also be used to study semiconductor lattices [**4**], carbon nanotubes [**5**], solar cells, and an assortment of minerals, pigments and gemstones.

REFERENCES

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CONFIGURATION

i-Raman Plus 785S

i-Raman Plus 785S785 nm65~3350 cm1TECCD

SERS

XYZSpecSuite785S

i-Raman Plus 785S

i-Raman Plus:

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- i-Raman PlusXYZ
- i-Raman PlusSpecSuite

(785 nm)

1.5i-Raman Plus 785S 785HR