

Introduction

Raman spectroscopy is increasingly being recognized as an important analytical tool, particularly in pharmaceutical, biomedical and biological applications, as a result of its high chemical “fingerprint” information content.¹⁻³ The advantages of Raman relative to IR spectroscopic methods include the relative insensitivity of Raman to water as well as its compatibility with optical microscopy and CCD camera detection methods.⁴ However, since Raman signal are relatively weak, Raman spectral features may be obscured by even trace quantities of fluorescent impurities.

This application guide demonstrates a very simple method for reducing fluorescent impurities to produce high quality Raman spectra of analytes such as proteins.⁵ The effectiveness of this method derives from the fact that components in the solution tend to precipitate in different regions on the substrate. The entire process of deposition and Raman data collection requires only a few minutes.

Raman Instrumentation

This application requires a Micro-Raman Instrument with a CCD detector equipped with a high magnification objective (e.g. 100X).

Materials and Reagents

- Tienta SpectRIM™ Slide
- Myoglobin (MW 17,200)
- Phosphorylase B (MW = 97,200)
- Water (18.2 MΩ cm⁻¹)
- Micropipette capable of delivering 1 – 10 uL of liquid

Preparation of Protein Solutions

Prepare a convenient volume of the proteins by dissolving 1.03 mg/mL of Myoglobin (60 uM) and 0.32mg/mL Phosphorylase B (3.3 uM) in water

Note: Care should be taken to avoid fluorescent contaminants from water, glassware, and other laboratory equipment.

Procedure

For a reference spectrum deposit a small amount of powder on the metal surface (substrate) of the Tienta SpectRIM™ Slide. Take a Raman spectrum.

On a separate SpectRIM™ Slide, deposit a 4-uL volume of the 60 uM Myoglobin solution and the 3.3 uM Phosphorylase B solution. Deposit the drops at least ½ inch or 1 cm apart. Allow the sample to dry approximately 20 minutes. A clear ring should form (see figure 1). No spectral changes should be observed in the Raman spectra after drying periods as long as several weeks.

Position the slide with the deposited sample on the microscope stage. Focus on an area of the outer ring and collect the Raman Spectrum. Take spectra from inside the ring for comparison. No protein should be found inside the ring.

Note: A low illumination power should be used to avoid burning the sample.

Results and Discussion

Figure 1 shows the white light images of myoglobin on the substrate after solvent evaporation. The deposition volume is 4 uL, and the concentration of the deposited solution is 60 uM.

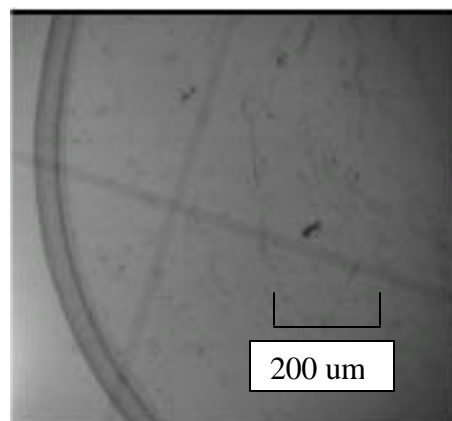


Figure 1. White light optical image of a portion of the myoglobin ring.

Figure 2 shows the Raman spectra obtained from a compressed pellet of myoglobin powder and a dried solution of myoglobin deposited on the Tienta substrate.

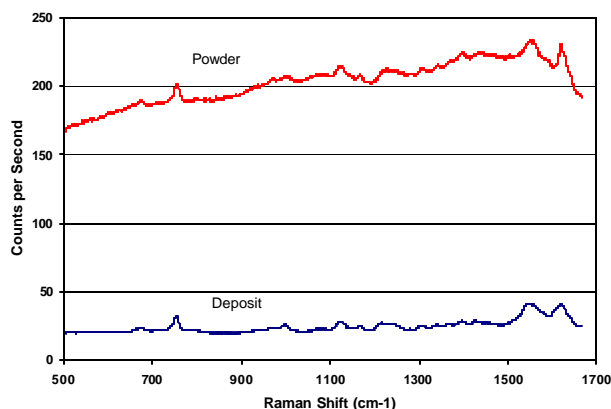


Figure 2. Raman Microscope spectra of myoglobin powder and deposit of 60 μ M solution on the Tienta SpectRIM™ Slide. A laser power of 0.25mW, at the sample and an integration time of 200 s were used to collect both spectra.

Figure 3 shows the Raman spectra obtained from phosphorylase B powder and a dried solution of phosphorylase B deposited on the Tienta SpectRIM™ Slide.

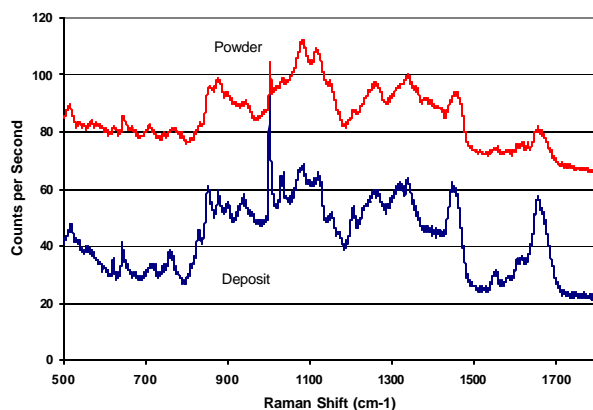


Figure 3. Both spectra were recorded on a JY LabRAM with a 100x objective, 1800 gr/mm

grating, 632.8 nm laser, 8 mw @ sample with an integration time of 100sec.

The spectra of as-received protein powders, often sit on a high fluorescence background. In this application guide the fluorescence of two such protein powders was reduced by simply dissolving the protein in water and then depositing the solution on the Tienta SpectRIM™ Slide prior to taking the Raman spectra.

Results may vary depending on instrumentation, illumination wavelength integration time and the thickness and purity of the sample.

References

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This Application Guide was produced jointly by Tienta Sciences, Inc., Jobin Yvon, Inc. and Dr. Dor Ben-Amotz of Purdue University.