The feasibility of cryo in-SEM Raman microspectroscopy.

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The combination of noninvasive compositional analysis by Raman microspectrometry with high-resolution imaging in the scanning electron microscope greatly expands the analytical capabilities of the electron microscope [1, 2, 3, 4]. However, the chemical preparation of scanning electron microscope (SEM) specimens, although adequate for low-resolution imaging of superficial detail, is not the true representation of the chemistry and composition of the sample, as extraction and aggregation artefacts as a result of dehydrating and cross-linking agents are abundant. The original chemical composition and ultrastructure is only preserved using cryo preparation methods [5, 6, 7, 8]. Therefore, a complete cryo transfer flange was designed and built to ad cryogenic control of specimens to the configuration of the EMRAM instrument, a combined Raman spectrometer and XL-30 ESEM instrument. The Raman spectra of two model specimen, polystyrene beads and 2.3M sucrose were studied at ambient and cryogenic temperatures as well as during a heating ramp.

For cryo In-SEM Raman measurements, a 2.3 M sucrose specimen was prepared by placing a small drop on a Leica specimen stub and was immersed in cold Nitrogen gas for vitrification. The glass state of the sample was inspected under the optical system of the cryo ultramicrotome (Leica Ultracut UCT + EM FCS, (Leica Microsystems GmbH, Austria) used for cryo planing the sample block face. After cryo transfer into the SEM, the collected frost at the sample surface was extensively sublimed at -85° for 20 minutes to ensure that all frost was removed. Raman spectra were collected at -150m °C using the EMRAM MCS-A1 and a focused laser spot at 10 seconds integration time. A small volume of polystyrene beads (diameter 10 µm), for cryogenic experiments, was dried on a sample stub and cooled during transfer inside the cold stage of the microscope. Raman spectra were collected at -150m °C using the EMRAM MCS-A1 and a focused laser spot at 10 seconds integration time. The spectra were baseline subtracted using Wire spectral software (Renishaw plc, United Kingdom).

Figure 1a shows a single polystyrene bead dispersed over a TEM finder grid and imaged under low temperature conditions (-150 °C). Figure 1d shows a vitrified 2.3 M sucrose solution covering a relocation grid kept at -150 °C. The spectra depicted in figure 1b & c for polystyrene and figures 1 e & f for sucrose show the resulting Raman signals collected both at ambient conditions and cooled to -150 °C. In the fingerprint region the characteristic spectrum of polystyrene was collected both at ambient and under cryogenic conditions. In the 700 – 400 cm^{-1} region a series of well resolved bands can be observed at cryogenic conditions (1c, arrows). The bands are due to wagging modes, rocking modes and out of plane vibrations, which appear as broader and weaker features at elevated (room) temperature, due to dynamic broadening effects as a result of the large amount of transient interactions.

Comparing the fingerprint regions of polystyrene and sucrose, both measured at ambient and at cryogenic conditions, only small spectral differences were observed for the main peaks of both molecules (figure 1). A pronounced sharpening of the bands however occurred in the 800–400 cm−1 region, a result of the reduction of intermolecular interactions (figure 1 c & f). This enhanced visibility of the lower frequency modes may offer interesting potential for more detailed interpretation of Raman spectra. In general, cryo preservation results in an undisturbed chemical and structural

preservation which, when combined with the aforementioned higher specificity of the Raman signal, results in a more accurate correlation of microstructure and molecular composition.

References

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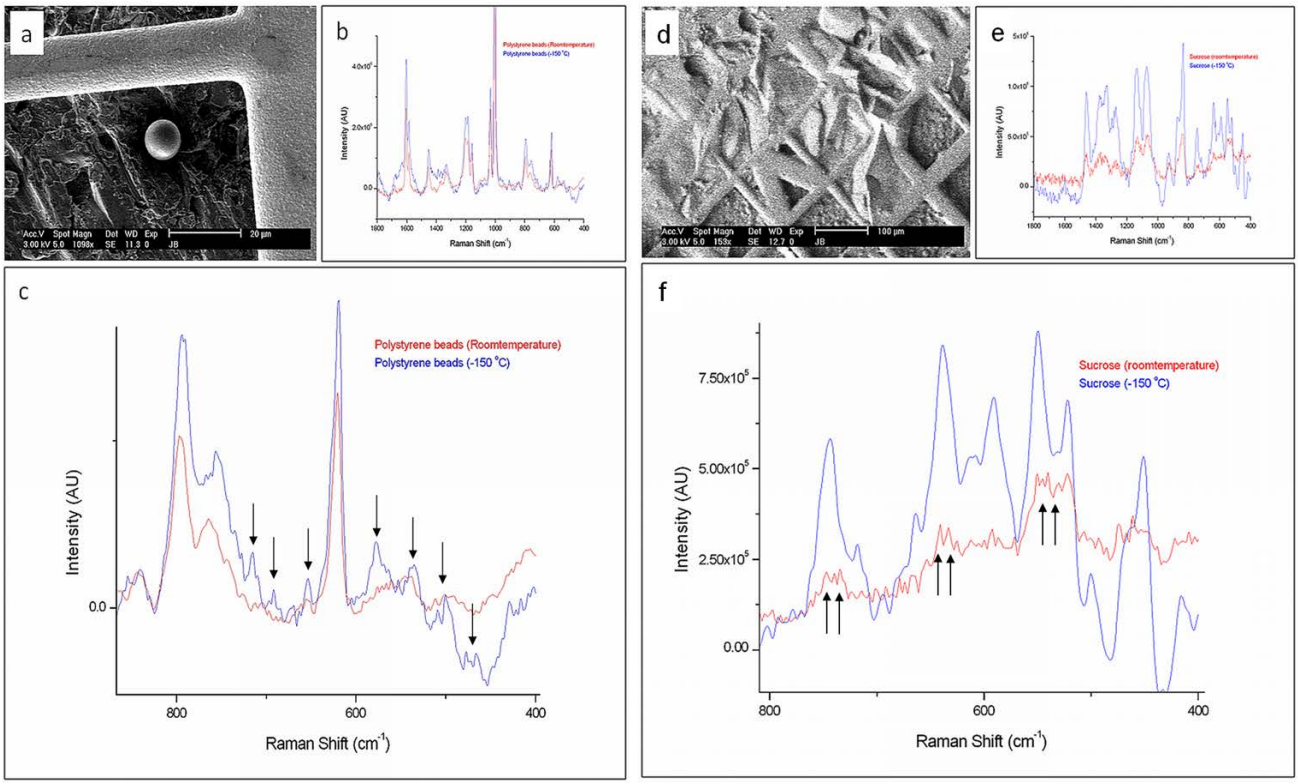

Figure 1. *In-SEM Raman analysis of polystyrene beads (a,b,c) and of 2.3M sucrose (d, e, f) using a 685 nm laser in the EMRAM In-SEM Raman instrument. The unprocessed Raman spectra collected at ambient (b/c & e/f, red curves) and cryogenic (−150◦C, blue curves) conditions. Under low-temperature conditions, the peaks in the 800–400 cm−1 regions sharpen (c & f, arrows) as a result of the reduction of dynamic interaction.*