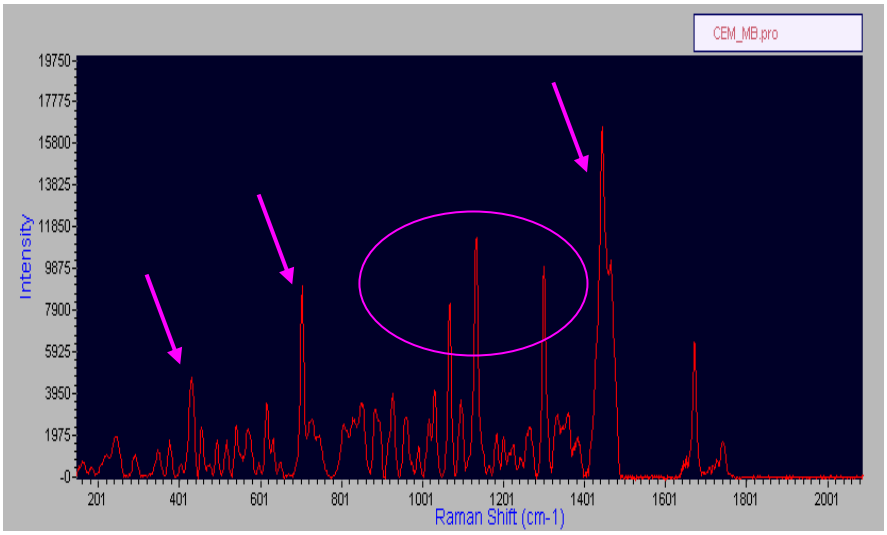
Lambda Solutions, Inc.

Dimension-P1™ Raman System

Now You Have the Power

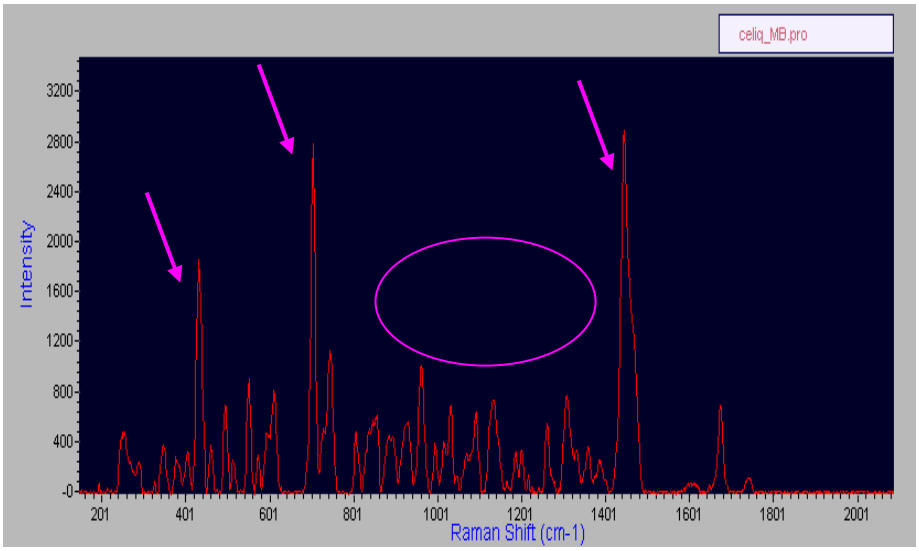
Raman Analysis of Cholesterol Esters: Liquid and Crystal States

Recent interest in cholesterol ester (CE) and the patho-physiology of thrombosis-vulnerable aortic plaque indicates the central role of this lipid in coronary artery disease. Peng et al (Arterioscler Thromb Vasc Biol. 20: 2682-2694, 2000) used NMR to show that significant molecular changes occur across the temperature-dependent crystal to liquid crystal to liquid states of CE. The crystal to liquid crystal transition was at or near body temperature and depended on the acyl chain length. Although Raman has been used to successfully analyze the vulnerable signature of these plaques in vitro, these studies were limited to room temperature. This prompted us to examine whether Raman can detect these transitions of physical states as a means of gaining greater understanding of CE in the disease process. In this preliminary study, we have examined temperature- dependent changes using the LSI Dimension-P1™ Raman System.



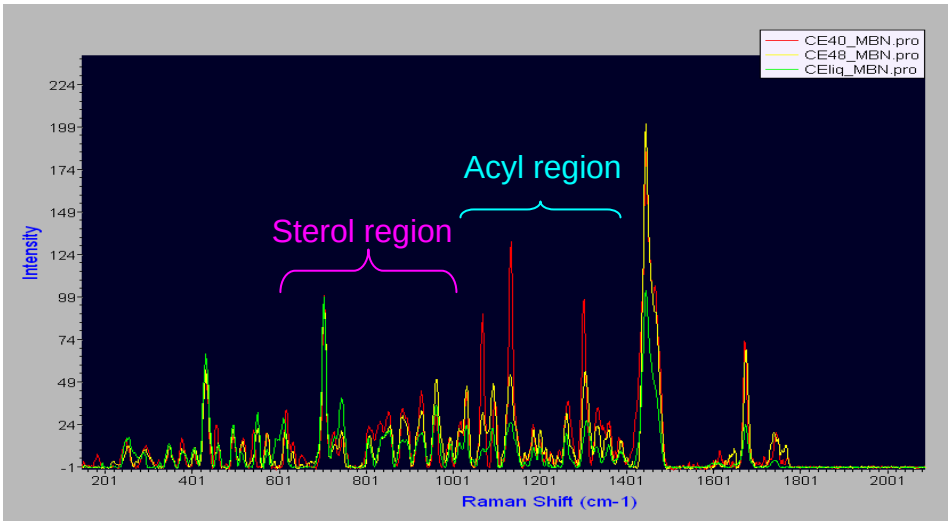
The spectra illustrated here were obtained with the Dimension-P1™ using the LSI External Sampling Module. The sampling module was heated from 25 to 85° C. The spectra were averaged 5 times and background-removed using LSI RamanSoft™. The spectra shown are actual RamanSoft™ screen shots. The Dimension-P1™ was configured for a coverage of 140 -2070 cm^{-1} at 3 cm^{-1} resolution.

The spectrum above was from cholestereyl mysristate powder at room temperature. The spectrum to the right is after it is melted at 85 °C. Several striking changes are evident: the prominent acyl derived peaks from ~1000 to 1300 cm^{-1} are greatly diminished; a decrease in the intensity of the compound peak at 1430 cm^{-1} relative to 710 cm^{-1} and 430 cm^{-1} is observed.

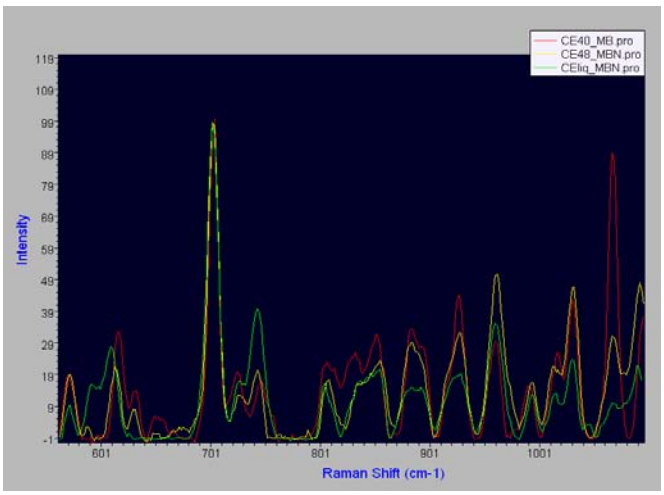


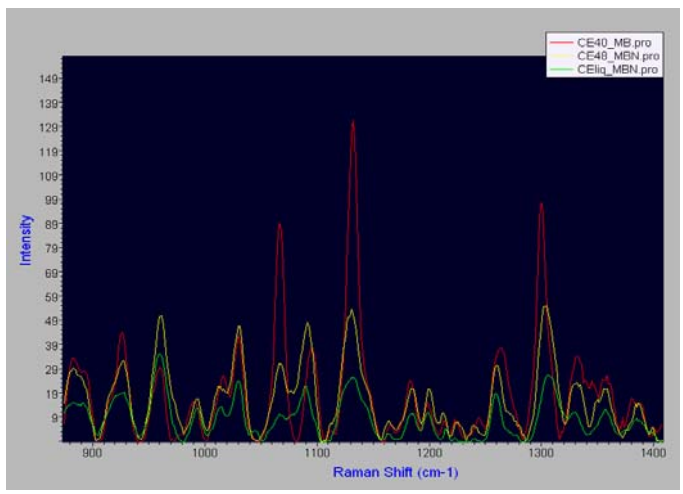
Raman Analysis of Intramolecular Changes

In order to determine the Raman peak transitions associated with liquid to liquid crystal to crystal states more fully, Dimension-P1™ spectra were obtained sequentially as the materials were cooled from 85 °C to 27 °C. The Raman spectrum of cholesteryl-myristate was obtained at 85 (fully liquefied) 60, 48, 40 35 and 27 °C ; characteristic spectra were processed and overlaid in LSI RamanSoft™ and illustrated below. Significant changes occur between 85 °C and 48 °C and again at 40 °C, but no significant changes were observed below 40 °C (data not shown).

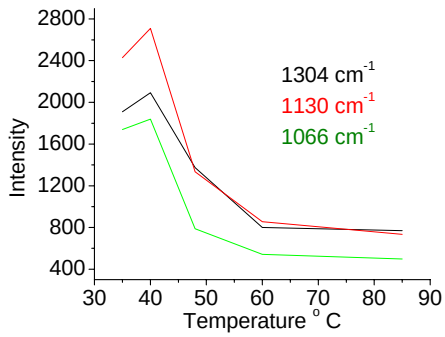


There was little or no change in the major sterol peak at 703 cm^{-1} while some small intensity changes can be detected with the Dimension-P1™ which occur across this region, see expanded view below. As expected, a large change is seen in the acyl region of the spectra. More detailed examination of the intensity of these acyl peaks vs. temperature, see below, suggests that transitions at ~48 °C and ~40°C mark the change of states.





This preliminary study illustrates the power of the Dimension-P1™ Raman System to be an important tool for biomedical research. More detailed studies are underway to specifically isolate these liquid state transitions and to elucidate their dependence on acyl chain length.



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