Raman Analysis of Unsaturated Fatty Acid Composition

Introduction

This study is a continuation of the study "Raman Analysis of Fatty Acid Unsaturation" as reported on Spectroscopy – The Application Notebook, February, 2005, p.20. However, in this study we concentrated on those Raman peaks that reflected specific fatty acid species and not solely the average number of double bonds. While the Raman peaks at 1655 cm^{-1} and between 1200 - 1350 cm^{-1} are useful in quantification (see Figure 1 of "Raman Analysis of Fatty Acid Unsaturation", Spectroscopy – The Application Notebook, February, 2005, p.20), they do not permit one to determine the relative fatty acid composition. For example, it does not allow one to distinguish between C-18 fatty acids with 2 double bonds (e.g., linoleic acid) from a 1:1 mixture of fatty acids with 1 double bond (e.g., oleic acid) and 3 double bonds (e.g., linolenic acid) (see Figure 1 below).

While the peaks used to quantify the number of double bonds are uninformative in distinguishing fatty acid composition, a series of smaller interdependent Raman peaks carries the constituent information. By taking advantage of the uniquely high signal-to-noise ratio and resolution of the LSI Dimension Raman systems, we illustrate how the Raman spectra can be used to carry out constituent analysis.

Results

Figure 1 shows an overlay of the Raman spectra of linoleic acid (yellow) and a 1:1 mixture (red) of oleic acid and linolenic acid. As one would expect, the Raman peaks between 1200-1700 cm^{-1} are completely super-imposed. However, in the 800-1100 cm^{-1} region of the spectrum significant differences exist.

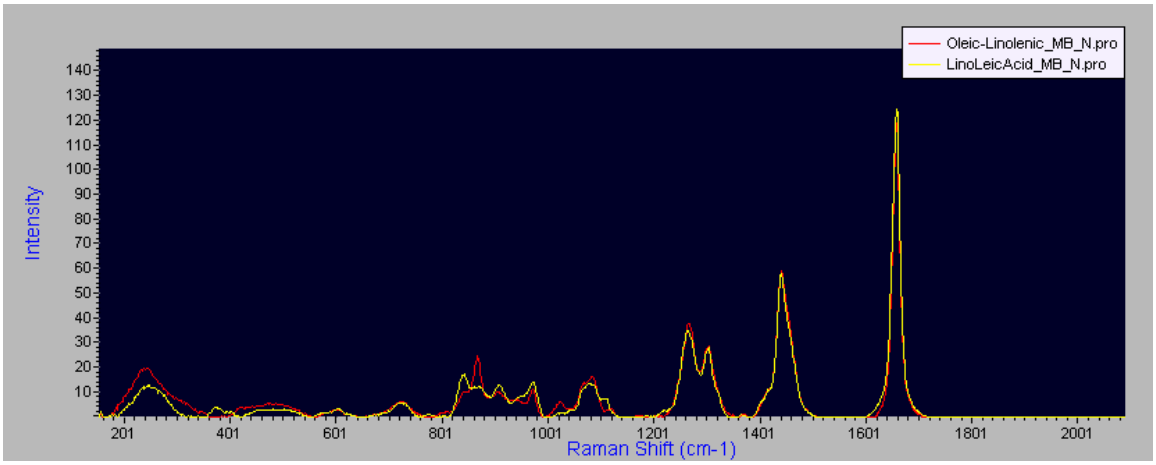

Figure 1. Overlay of normalized spectra of linoleic acid (yellow) and a 1:1 mixture of oleic and linolenic acids (red). Spectra were overlaid and normalized using LSI RamanSoft. This figure and subsequent figures are direct screen shots from RamanSoft.

In order to fully explore the information carried in the differences in the lower wavenumber portion of the spectra, a more careful comparison of different fatty acids and fatty acid mixtures is needed. Figure 2 shows the graded changes with different compositions of these fatty acids.

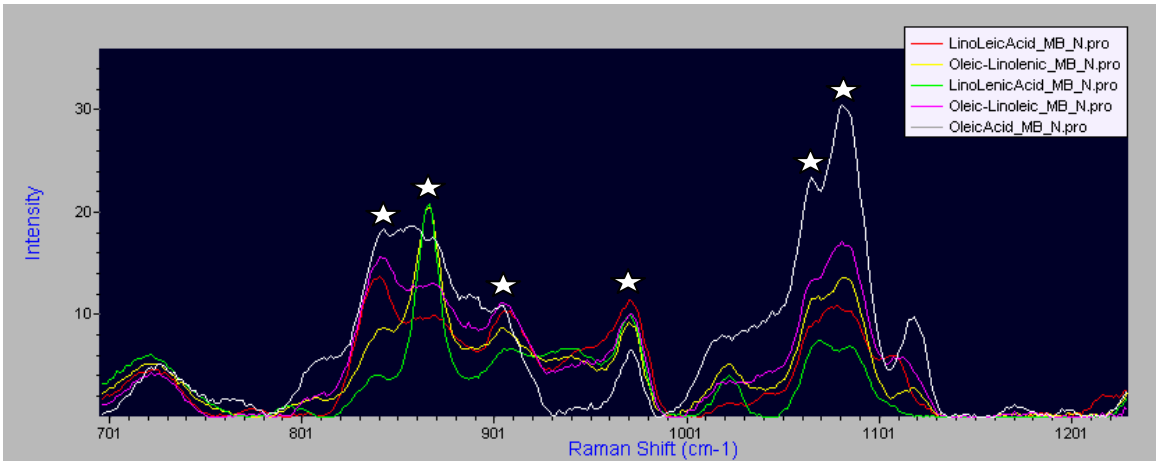

Figure 2. An expanded view of normalized, overlaid spectra from a series of pure fatty acids oleic (grey), linoleic (red), linolenic (green), and 1:1 mixtures of oleic and linoleic (purple), and oleic and linolenic (yellow) acids. The Raman peaks noted in the text are marked with white stars.

In the region between 1000 - 1100 cm^{-1}, the relative peak heights at around 1065 cm^{-1} and 1085 cm^{-1} reflect the number of double bonds in each of the component fatty acids. In oleic acid (grey spectrum), the 1085 cm^{-1} peak is dominant; in linoleic acid (red spectrum), these two peaks are roughly equal leading to a relatively featureless broad peak; in linolenic acid (green spectrum), which has 3 double bonds, the 1065 cm^{-1} peak is the higher peak. In the case of the 1:1 mixtures intermediate patterns are observed. However, these Raman peaks are coupled and together they reflect larger scale structural elements and not just the number of double bonds. In this case therefore, the Raman spectra of the 1:1 mixture of oleic and linolenic acid (average 2 double bonds, yellow spectrum) is clearly different from that of the pure linoleic acid (red spectrum) with 2 double bonds.

A similar and even more pronounced relationship exists in the relative peak heights in the region between 800 - 980 cm^{-1} in these graded fatty acid composition. The four peaks in this region exhibit a complex pattern which could be used as the fingerprint region that corresponds to these different molecular species. All these observations are made clearer when the oleic-linolenic acid mixture is compared directly to linoleic acid, as shown in Figure 3. The family of 4 peaks in the 800 - 980 cm^{-1} region clearly distinguish the oleic:linolenic mixture (red spectrum) from the pure linoleic acid (yellow spectrum).

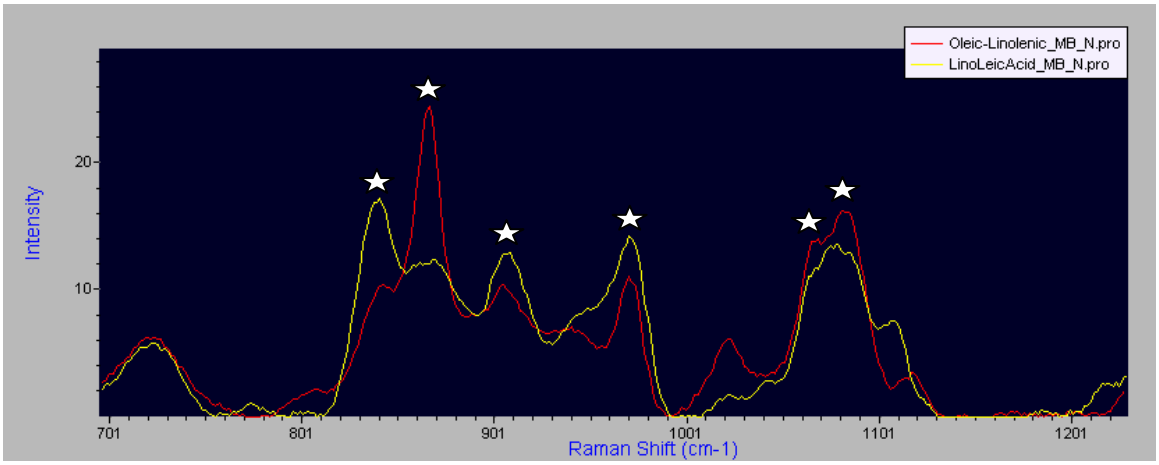

Figure 3. An expanded view of the overlaid and normalized Raman spectra of linoleic acid (yellow) and a 1:1 mixture of oleic and linolenic acids (red). Raman peaks noted in the text are marked with white stars.

Conclusions

The fact that two regions of the Raman spectrum, each with multiple informative peaks, can be used to identify component fatty acids, indicates that the Dimension-P Raman systems can rapidly quantify double bonds and at the same time characterize the fatty acid constituents. In conjunction with LSI RamanSoft, the Dimension-P Raman systems provide a powerful tool for fatty acid analysis in biomedical, pharmaceutical, and nutritional and health sciences.

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