Raman Analysis of Fatty Acid Unsaturation

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Part 1. *from* Application Note: in *Spectroscopy*

Rapid analysis by Raman spectroscopy of fatty acid (FA) unsaturation can be employed for quality & process monitoring and biomedical applications.

The unsaturation of FAs and hydrocarbons is pivotal in nutritional, medical, and chemical applications, which makes the analysis of double bond (DB) content critical for quality assurance and monitoring of synthetic and production processes. Evidence that normal tissue and invasive carcinoma cells often differ in membrane fluidity underscores the diagnostic and medical value of FA analysis. When using multiple spectral markers to analyze either simple or complex systems, Raman spectroscopy can provide the speed and specificity needed.

Experimental Methods

C-18 FA standards (stearic acid, oleic, linoleic, and linolenic acids) in vials, two brands of safflower oils (SOs) in glass and plastic bottles, and fish oil FA in gelatin capsules were analyzed using LSI Dimension-P1™ HR Raman System with Vector Raman Probe™ and External Sampling Module (ESM) at 80°C and room temperature. DB values of 1.5 and 2.5 were obtained using 1:1 mixtures of oleic:linoleic and linoleic:linolenic, respectively. Linearity of DB content with different peak intensities was examined at 80°C so as to have stearic acid in a liquid state to obtain zero DB peak values. Raman peak intensities were examined at 1265 and 1655 cm^{-1} for CH ethylene and C=C alkyl stretching, respectively, and the ratio of 1265 and 1301 cm^{-1} (CH_2-alkanes) was also examined. Room temperature studies also included SO and fish oil samples, each analyzed *in situ*. RamanSoft™ was used for data acquisition, background removal, and normalization to laser power and to the peak intensity at 1438 cm^{-1} (CH3, CH2-alkanes).

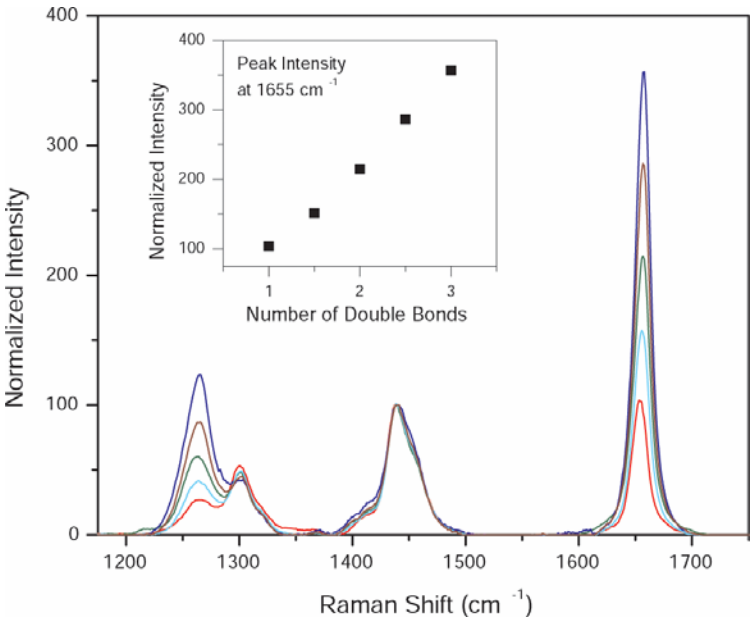

Figure 1. Raman spectra of oleic (red), linoleic (green), linolenic (blue) acids and 1:1 mixtures of oleic:linoleic (cyan) and linoleic:linolenic (maroon). Inset: Quantification of -C=C- content at 1655 cm^{-1}.

Results

A linear relationship of peak intensity with DB content is illustrated for the peak at 1655 cm^{-1} (Figure 1). The peak at 1265 cm^{-1} and the ratio of peaks at 1265 and 1301 cm^{-1} also provided a strong relationship with DB content. However, since the alkane peak at 1301 cm^{-1} decreases slightly with increasing DB content, the relationship of this ratio with DB is non-linear. The three quantitative methods were tested with the two brands of SO and fish oil samples. The expected DB contents were 1.07 and 1.7 for the SOs and 1.75 for fish oil; the average DB content ($\pm$ 6%) for SOs was found to be 1.01 & 1.67 and for fish oil, 1.86.

Conclusions

In situ analysis with an external sampling system and multiple Raman spectral markers permits quantification of FA unsaturation. These quantitative models are the basis for algorithms for use in a range of applications by analytical chemists and food scientists. Moreover, the data indicate that differences in Raman spectra in the 1265 and 1301 cm^{-1} regions, associated with tissue lesions, e.g., invasive breast carcinoma[1], reflect, in part, the population of FAs, and that this may be a useful tool for neoplastic tissue classification.

Reference

1. C.J. Frank and R.L. McCreery, Anal. Chem. **67**, 777-783 (1995)

Part 2. Determination of Unsaturated Fatty acid Composition by Raman Spectroscopy

Introduction:

In the first part of this study we showed how the Raman signal of fatty acids can be acquired and processed for the rapid analysis of double bond content of fatty acids and oils. Such an evaluation is important for grading the nutraceutical content of food products and food supplements as well as the degree of unsaturation of tissue samples for use in *in vitro* and *in vivo* diagnostics. This study is a continuation of the Raman analysis reported above, however, in this portion we concentrate on the Raman peaks that reflect 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 double bond quantification, see Figure 1 above, they do not permit one to determine if the relative fatty acid composition. The reason is they are only measures of specific vibrational (stretch and rotation) of the double bond and are not a reflection of larger structural elements. As the Raman signal more reflects these larger elements the signal obviously becomes more diagnostic of the constituent structure. In this study we identify a constellation of Raman peaks that together reflect constituent structure and that change in concert to yield this information. Being able to distinguish between C-18 fatty acids with 2 double bonds (linoleic acid) from a 1:1 mixture of a fatty acid with 1 double bond (oleic acid) and one with 3 double bonds (linoleic see Figure 2 below) is an example of such an application.

While the peaks used to quantify the double bonds are uninformative for constituent analysis, a series of smaller interdependent Raman peaks carry this information. By taking advantage of the uniquely high signal to noise and resolution of the LSI Dimension Raman systems we illustrate how the Raman data can be used to carry out constituent analysis.

Results:

Figure 2, shown below is an overlay of the Raman spectra of linoleic acid and an oleic acid:linolenic mixture (1:1). As one would expect the Raman peaks between 1200-1700 cm^{-1} are super-imposable making it impossible to use this region of the spectrum to distinguish linoleic acid and the oleic acid:linolenic mixture (1:1). However in the 800-1100 cm^{-1} region of the spectrum (purple oval) significant differences exist between linoleic and the oleic:linolenic mixture. One can appreciate this portion of the spectrum with a broader comparison of different fatty acids and fatty acid mixtures,Figure 3. which show the graded changes with these different compositions.

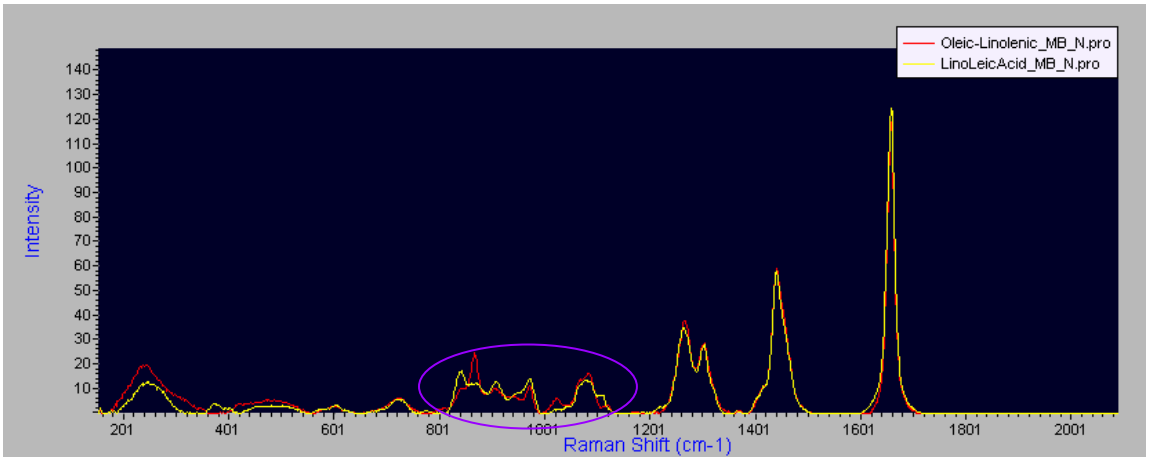

Figure 2. Overlay of normalized spectra of linoleic acid (yellow) and a 1:1 mixture of oleic and linolenic acids (red). Spectra were overlaid and normalized using LSI RamanSoft. This figure and subsequent figures in this section are direct screen shots from LSI RamanSoft.

In the region from 1000-1100 cm^{-1}, we can see that the relative peak heights at approximately 1065 and 1085 cm^{-1} reflect the number of double bonds in each of the component fatty acids. Specifically, in oleic with one double bond 1085 peak is dominant; in linoleic, two double bonds, these peaks are roughly equal leading to a relatively featureless broad peak; linolenic, with 3 double bonds, now reverses the pattern seen with oleic and the 1065 peak is the higher peak. In the case of the 1:1 mixtures, intermediate patterns are observed because these Raman peaks are coupled and together reflect larger scale structural elements and not just the number of double bonds. In this case therefore, the Raman signal of the 1:1 combination of linolenic and oleic (average-2 double bonds, purple spectrum) is clearly different from pure linoleic with 2 double bonds, red spectrum.

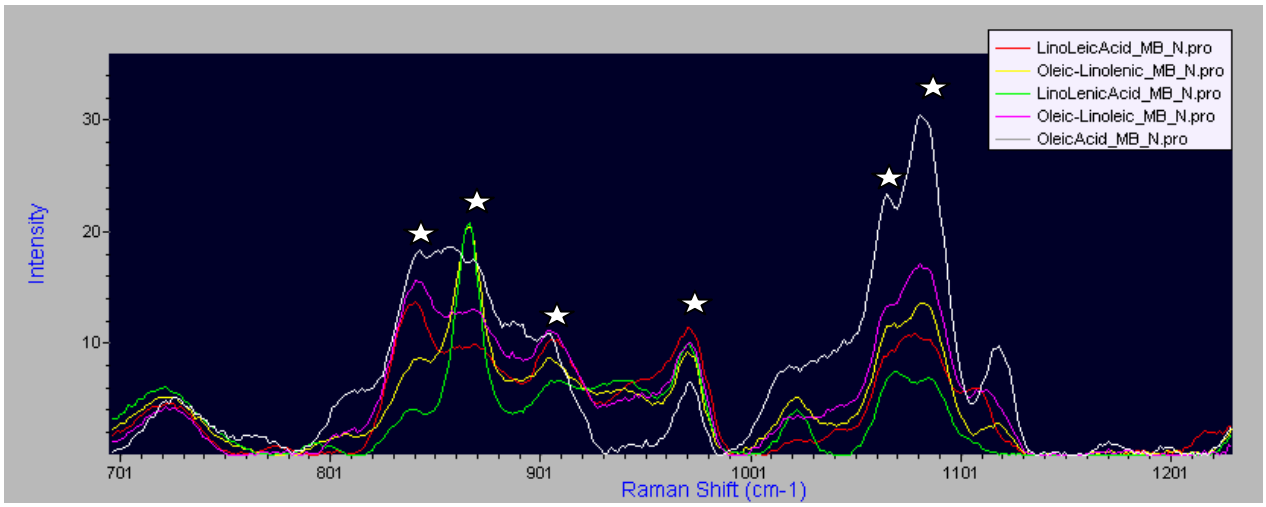

Figure 3. An expanded view of normalized, overlaid spectra from a set of pure and 1:1 mixtures of fatty acids. The spectra for each fatty acid or fatty acid mixture is described in the legend in the top right hand corner. The Raman peaks noted in the text are marked with white stars.

A similar and even more pronounced relationship exists in the relative peak heights in the Raman region between 800-980 cm^{-1} in these graded fatty acid composition. The four peaks in this region exhibit a complex pattern, yielding a unique fingerprint of the corresponding molecular species. It is clear that these patterns reflect, in each peak, a combination of the component spectra and not merely the average number of double bonds as is seen in 1200-1400 cm^{-1} region. This is made clearer when the oleic acid-linolenic 1:1 mixture and linoleic are compared directly, Figure 4. The family of 4 peaks from 800-980 cm^{-1}, clearly distinguish the oleic:linolenic mixture from pure linoleic acid.

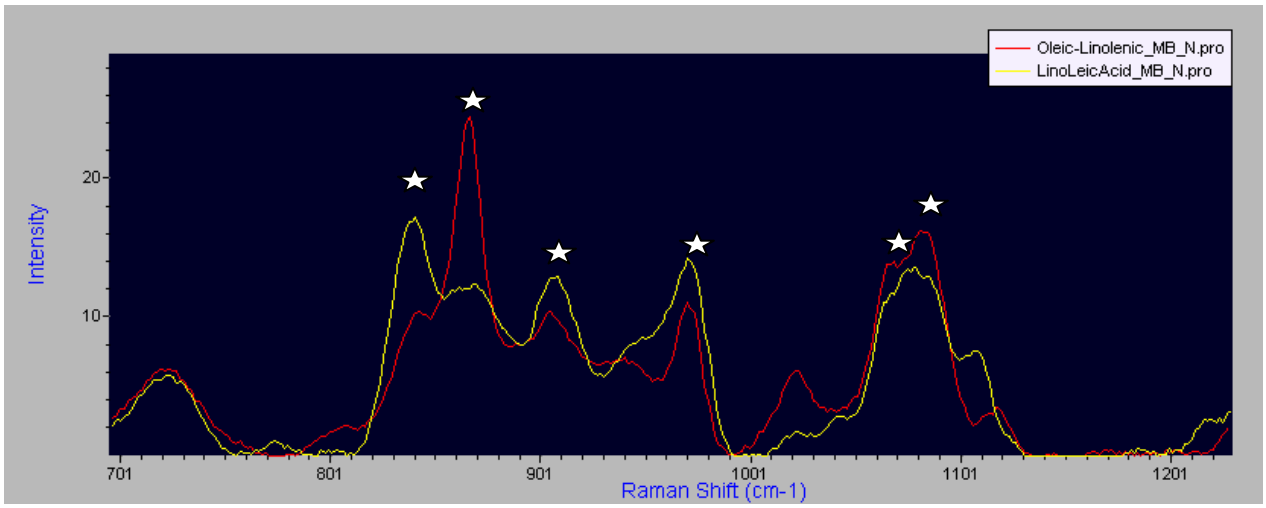

Figure 4. An expanded view of the overlaid normalized Raman spectra of linoleic acid (yellow) and of a 1:1 mixture of oleic and linolenic acids (red). Raman peaks noted in the text are marked with white stars.

Conclusions:

These studies have taken advantage of the high light throughput and sensitivity of the LSI Dimension-P Raman Systems to yield high speed analysis of these important biological species. Two regions of the Raman spectrum, each with multiple informative peaks can be used to identify in seconds component fatty acids and at the same time quantify the number of double bonds. This approach can be utilized for analysis of fatty acids and oils in food products and in biological systems. In conjunction with LSI RamanSoft, the sensitivity and low noise spectra of The Dimension-P Raman Systems provides a powerful tool for fatty acid analysis in biomedical, pharmaceutical and nutritional and health sciences.