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Raman Spectroscopy of HPLC Fractions from Retina Oil Droplets

5-7-2014

Sample preparation: Jeri Timlin

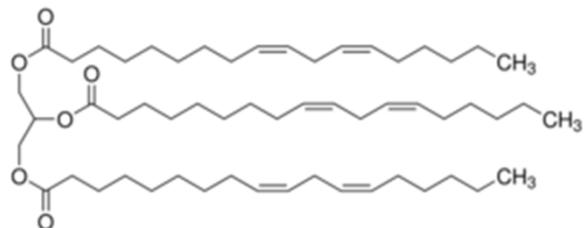
Imaging: Anthony McDonald

Analysis: Jeri Timlin



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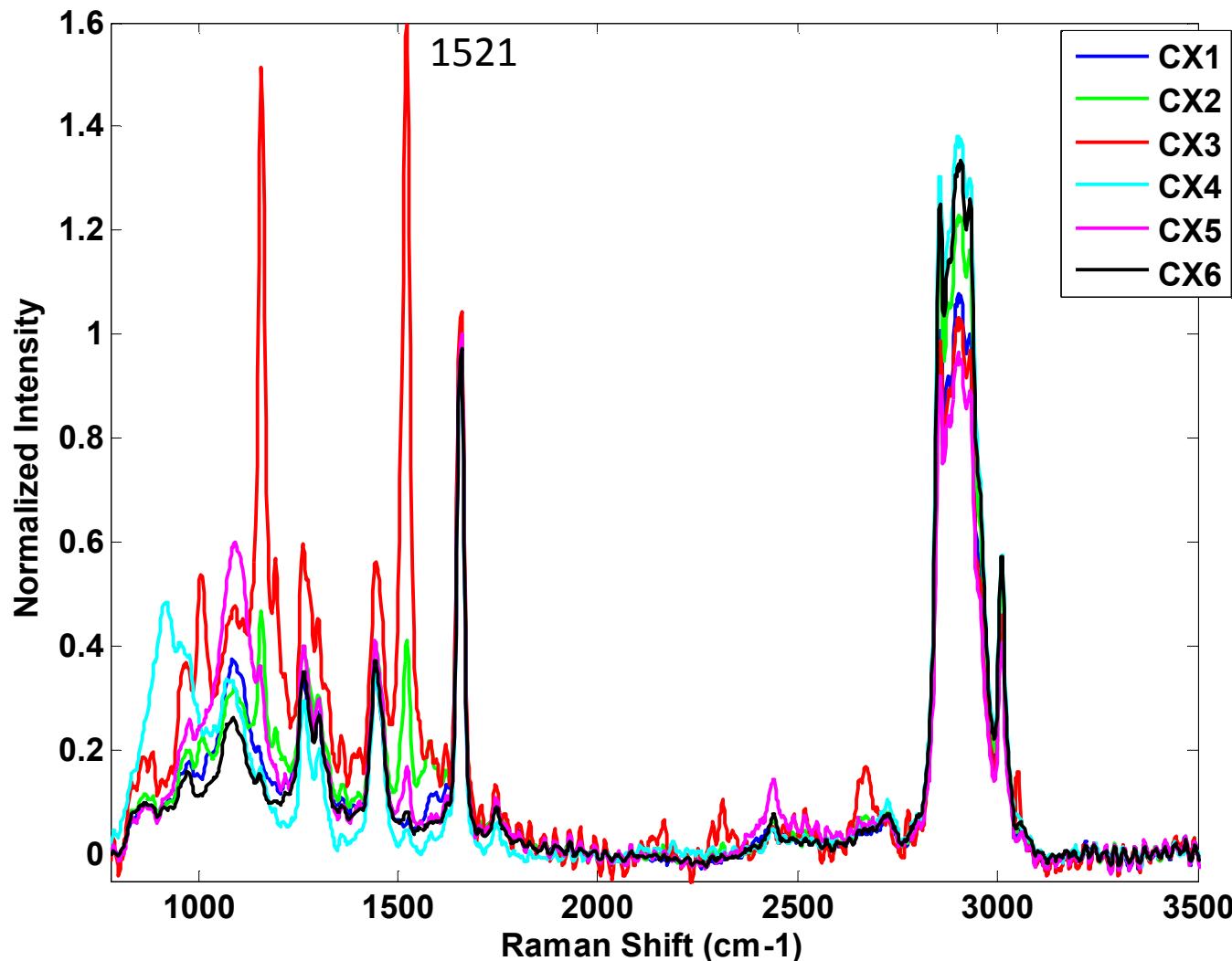
- Carotenoids are resuspended in glyceryl trilinoleate
- A few microliters of lipid mixture was place in well of slide and coverslipped
- Spectra were collected in “microprobe mode”. It was challenging to find the carotenoid inside
- We do see the lipid in the standards
- Heterogeneous spectra depending on location (variable amount of lipid +/- carotenoid)
- Raman peaks in the finger print region from the lipid are:
 - 1741 C=O ester stretch
 - 1660 C=C str (cis?)
 - 1442 CH₂ methylene scissor
 - 3011



Standards

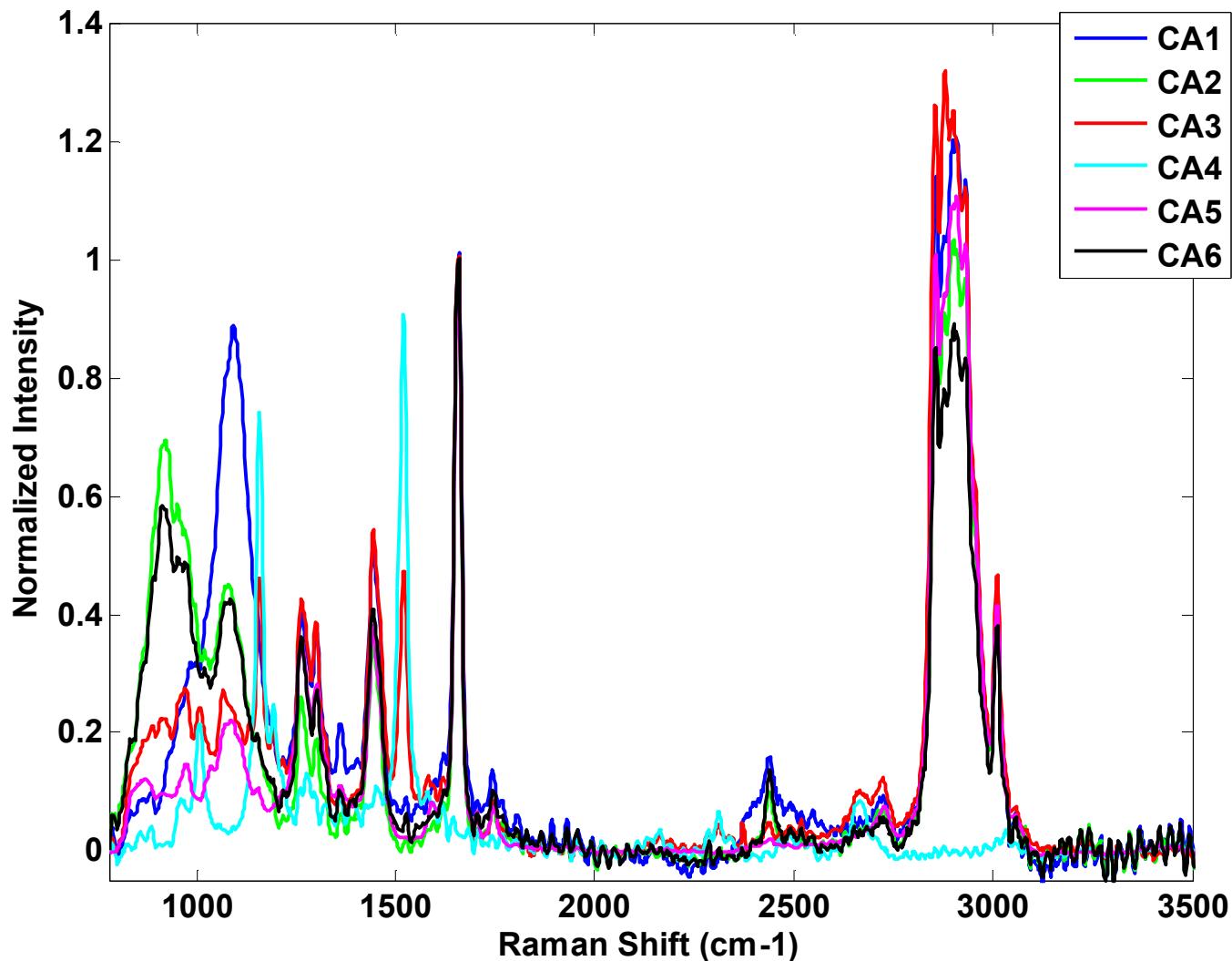
- **H1 - 11,12-dihydrogalloxanthin – no detectable carotenoid bands**
- **CX1 – galloxanthin – no detectable carotenoid bands (1150)**
- CX2 - cis-lutein 1521.6 and 1158 (maybe relative intensity change with cis)
- **CX3 - lutein 1521.6 and 1158**
- **CX4 – zeaxanthin 1516 & 1522 (doublet) & 1156.3**
- CX5 – unknown 1521 & 1154
- CX6 - e,e - carotene* **1518.5 & 1524 (doublet) & 1153.7**
- CA1 - cis-astaxanthin – **no detectable carotenoid bands**
- CA2 - cis-astaxanthin – **no detectable carotenoid bands**
- CA3 - cis-astaxanthin 1520 and 1157 diagnostic of astaxanthin (maybe relative
- **CA4 - astaxanthin** intensity change with cis)
- CA5 - cis-astaxanthin – **no detectable carotenoid bands**
- CA6 - – **no detectable carotenoid bands**

1158 CX Standards



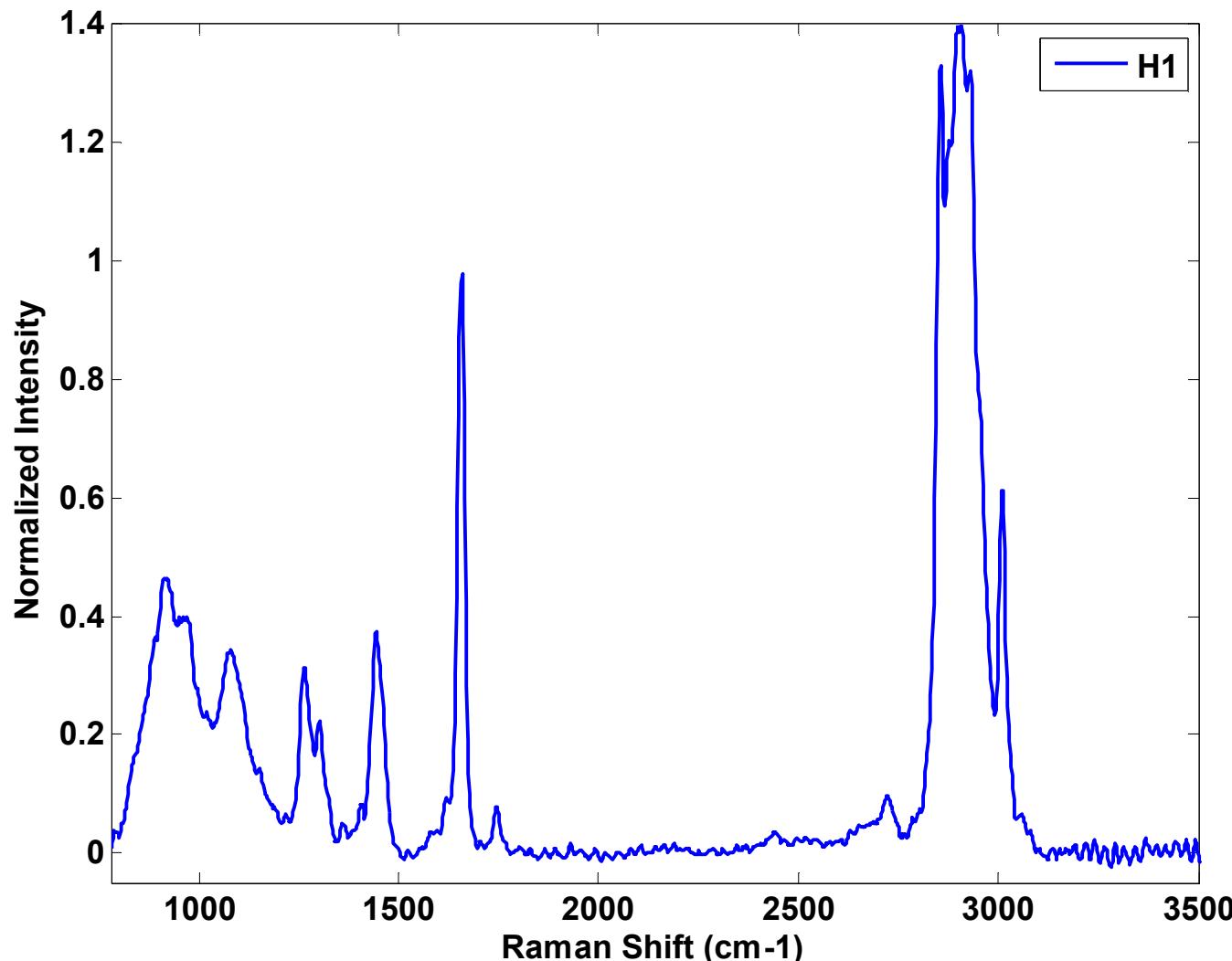
Spectra have been baseline corrected (3rd order polynomial), normalized to 1660 peak, and are displayed after a basic 3 pt S-V smoothing operation.

CA Standards



Spectra have been baseline corrected (3rd order polynomial), normalized to 1660 peak (except for CA4), and are displayed after a basic 3 pt S-V smoothing operation.

H Standard



Spectra have been baseline corrected (3rd order polynomial), normalized to 1660 peak, and are displayed after a basic 3 pt S-V smoothing operation.