



My career path in the field of Raman spectroscopy and microscopy

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I have worked in the field of Raman spectroscopy since the 1960's, starting in Professor Eli Burstein's semiconductor group, and then post-doc'ing in Professor Britten Chance's Biophysics Department at UPENN. In 1978 I started with Instruments SA/Jobin Yvon who had recently introduced the Raman microscope designed in Lille, France by Professors Michel Delhaye, Paul Dhamelincourt and Edouard DaSilva. This instrument was conceived as an instrument for providing chemical information with $1\mu\text{m}$ spatial resolution, and with my background in physics I was able to expand its applications into materials science. That is, I recognized that spectra taken with $1\mu\text{m}$ spatial resolution can provide valuable information on crystalline forms of a material as well as their chemical forms which has use in elucidating function. © Anita Publications. All rights reserved.

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1 Introduction

In this article, I will survey what types of measurements I have done over the years, with emphasis on demonstrating a synergy between spectroscopy and materials science. That is, I was always interested in how the technology could reveal more information than simple compound identification. There will be a short section on my work in semiconductor physics as a student, and then in biophysics as a post-doc/research assistant professor. Then I will discuss the work that I did as an applications scientist at JobinYvon/ISA, now known as Horiba. This included establishing the use of silicon as a universal instrument test, and then development of various applications that combined microscopy with spectroscopy. Usually the spectroscopy had been developed to some extent, but had not been applied to microscopic samples. Finally, I will describe two projects that are currently active where I am using Raman spectroscopy to characterize a biopolymer, and to identify extractables and leachables from polymers used as pharmaceutical containers.

2 Semiconductor Physics

During the late 60' and the 70's a good percentage of Raman measurements were done in the field of semiconductor physics. By a judicious selection of laser wavelengths either the bulk semiconductor, or its surface could be measured. The effects of dopants that provided free carriers had effects on the spectra that could then explain things like surface charges and surface fields that were useful in understanding how the developing devices worked. I did not work on silicon or the III-V semiconductors, but on crystalline Se which has an interesting exciton whose energy is in the red part of the spectrum. But exciting with the He-Ne laser (63.817nm) and or the 647.089nm line of a newly acquired Kr-ion laser, I made an interesting set of measurements; the longer wavelength line excited the TO (transverse optical) mode at 148 cm^{-1} , whereas the shorter wavelength line excited the LO (longitudinal optical) mode at 152 cm^{-1} because of its closer proximity to resonance. This work was only published in my thesis.

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3 Biophysics

When I started as a postdoc at UPENN in the biophysics department in the Medical School (Eldridge Reeve Johnson Foundation), Professor Tom Spiro and his student Tom Streckas in Princeton had just published the resonance Raman (RR) spectrum of cytochrome c [1]. I was working at an important center of mitochondrial research where the staff was using absorption spectroscopy to understand the control of energy transduction facilitated by electron transfer between cytochromes at different redox states. There are two types of cytochromes with slightly shifted absorption. I immediately realized that by combining redox control of mitochondrial preparations and the excitation wavelength of the RR spectrum I could spectrally separate contributions from the different cytochromes. Of particular interest was the fact that there are two b-type cytochromes, and the spectra are only resolved in carefully prepared materials. That meant to me that there are spectral interactions between hemes on the mitochondrial membrane related to the geometrical relationship between the hemes. My goal was to relate spectral interactions in the RR spectra to the physical chemical state controlling electron transfer and energy transduction [2,3].

During my time at the Johnson Foundation Prof. Martin Gouterman, chemistry professor at Univ. of Washington in Seattle, would come to Philadelphia to visit family and spend some time talking to us. As a graduate student at the University of Chicago, he had worked out the molecular orbital theory of porphyrins, which of course, include hemes. I had been noticing a broad background on my RR spectra of reduced cytochromes, which I believed, fit the expected fluorescence, if it was not totally quenched. I discussed this with him, and after making a systematic set of measurements on a set of hemeproteins whose absorption bands exhibited varying widths, we published a paper which showed that we were able to calculate the excited state lifetimes, which then could be related to the remaining fluorescence and the RR quantum yields [4]. Martin also recognized that the different widths of the Raman bands of oxidized and reduced cytochromes that I was measuring were related to low-lying electronic states of the ferric iron when the hole moves between different states of its various t_{2g} levels [5]. In pulling up this reference I note that Martin is not a co-author, which from my perspective today seems odd; my guess is that because I was at the beginning of my career and experiencing some difficulty getting established, he did not want to dilute my contribution. Thank you Martin!

In both of these fields of research, I was utilizing the RR spectra of hemes to infer complimentary information. For the biological preparations, I was demonstrating the potential to extract biophysical information. For the spectra of the pure cytochromes, I was showing that the Raman spectra provide indirect information on electronic states.

3 Career re-boot with instruments, S.A. (ISA), now HORIBA Scientific:

By this time it was fairly clear that I would not be able to secure an academic position, so when I was approached to become an applications scientist at ISA I was happy to make the change. Here is what Ruth Bader Ginsburg replied to Sandra Day O'Connor who asked her "Suppose you and I had gone to law school in days when there was no barrier to women in the legal profession. Where would we be now?" RBG answered "We would be retired partners of a large law firm. But because we didn't have that path available to us, we had to find a different one, and both of us ended up on the US Supreme Court" [6]. I cannot compare myself to our first lady Supreme Court judges, but the opportunity that I accepted has enabled me to achieve far more than I would have ever expected.

The Raman microscope was developed in the early to mid 1970's at the Spectrochimie Infra-rouge et Raman C.N.R.S., Universite' de Lille [7,8]. I believe that Professor Delhay and Monsieur DaSilva were motivated to provide microprobe access to chemical information that Castaing's electron microprobe [9] could not. But because my education was in Physics where the properties of the condensed phase are as important as chemical composition, I recognized immediately that determining the crystallographic (or

amorphous) phase of a material and/or its orientation could be as important as identifying the chemical species. As soon as I started attending conferences and writing trade articles, I made this point. My identification of tetragonal zirconia in a 2 μ m defect in a fiber optic from AT&T Bell Labs brought this point home exquisitely [10]. In the early 1980's AT&T was making fiber optics by pulling cylindrical boules of SiO₂ in a furnace. The furnace had two sources of ZrO₂, one used as the susceptor to heat the boule, and the second as the furnace insulator. EDX (Energy Dispersive X-ray) analysis told the Bell Labs Staff that an inclusion causing a break in a fiber was composed of ZrO₂. Unfortunately, that did not provide Bell Labs the identity of the inclusion because they knew that there were two sources of ZrO₂. It turned out that Raman analysis was able to distinguish them because one source was monoclinic and the other was tetragonal. But in addition, ZrO₂ tends to have lots of rare earth (RE) impurities and those impurities were different in the two materials. So by comparing the Raman signature as well as the RE luminescence, we were able to identify the source of the impurity. The answer was that the particle came from the "grog" which is what they called the insulation, that was in the monoclinic phase.

This work was published in 1984. But beginning in 1978 when I started I had to start learning about all kinds of materials about which I knew nothing. So my publication list shows articles on semiconductors, ceramics, glasses, oxide/corrosion films, polymers, especially fibers, and pharmaceuticals. When we published work demonstrating microscopic strain in silicon, we were the first to do that at the microscopic scale [11]. During that time period I also worked with David Clarke, then at Rockwell International, but now at Harvard University, measuring the transformation zone in ZrO₂ that had been stabilized in the tetragonal form, but reverted to the monoclinic form after the application of stress with a micro indent [12]. And I worked with Herman Noether, a polymer physicist retired from Celanese, quantifying orientation and crystallinity in fibers of polyethylene terephthalate (PET) as a result of the spinning process [13]. I was fortunate to be able to explore these and other possibilities because I was at the right place at the right time.

4 Silicon, the perfect sample for instrument tests

Before I proceed to describe work that I am doing today, I can describe what I did to standardize the equipment. Anyone who has made Raman and Infrared (IR) measurements understands that in contrast to IR spectroscopy, the abscissa axis in Raman is arbitrary because a Raman measurement is a single beam technique. We measure the number of counts in the scattered band, and can normalize that to the laser power, but there is a plethora of other phenomena that affect the signal...slit width, dispersion, grating reflectivity, transmission of optics (lenses and mirrors), laser focusing and Raman collection optics, and the quantum efficiency of detectors. This presents several phenomena that needed to be addressed. As an instrument manufacturer, we needed to know that each instrument of a particular type produces the same signal within some limits. We also needed a way to compare signals from one instrument type to another. Coming from a solid-state physics background, the solution was obvious. We could use a small chip of silicon as a test sample. The material is clearly available in this age of computer technology that is based on silicon. A sample with its surface perpendicular to the crystallographic Z axis (001) can be easily identified because silicon cleaves long (110) axes, producing right angle corners. When polished the sampling optics are reproducible and the Raman signals are reproducible (within ~10%) when all conditions are defined. These conditions include sample orientation, laser wavelength and power, microscope objective, grating groove density, etc. When back scattering off such a (001) surface, the scattering is polarized if the laser polarization is parallel to a cleavage axis (110) but crossed if the laser is polarized along a crystallographic axis (100). Because some gratings, especially the high groove density gratings, have vastly different reflectivities for the two polarizations, we can check the polarization properties of the instrument with this sample, determined largely by the grating reflectivities of the two polarizations. In addition, silicon can be used for any excitation wavelength. So we immediately started using this sample to test instrument performance, and it was subsequently adopted by the manufacturing group in France, and by all other manufacturers.

5 Today

It has been a pleasure to watch how each of these publications have opened new areas of research [10-13]. Among them, Ingrid deWolf has done an enormous amount of work showing that the mapped strain in silicon in the vicinity of device structures aids in the engineering of these structures [14]. Several groups took up the work on polymeric fibers and the orientation and crystallinity parameters that have been derived provide information on the physical properties of the fibers. Because this field is so rich, I will cite several articles [15-19]. The field of Raman spectra in ceramics has seen many publications, but I have not seen many where Raman has been used to follow solid state changes in chemistry and polymorphism. Many years ago, I had looked at a SiC protective coating for the space shuttle. It was formed by reacting silicon powder with the carbon fiber surface of the shuttle in order to protect it during re-entry when the shuttle would experience high temperatures and oxidative corrosion of the carbon surface. We observed multiple phases of SiC, B₄C, and Si with varying levels of B doping (I was told that the boron was added for its refractory properties). It was clear that the Raman maps could provide excellent information on these changes [20]. One worker that I am aware of who has pursued this is Philippe Colomban [21]. And a group in Bonn has specifically looked at changes that occur during sintering of ceramics at high temperatures [22]. Their analysis which uses Raman images taken as a function of temperature (T) is useful in the study of ancient ceramics, metamorphic rock formation in nature, and the production of industrial, hi tech ceramics. Specifically deriving information from spectral maps, they consider the thermodynamic favorability vs. time and temperature of reaction processes, measuring shrinkage, densification, and grain growth, noting that grain size and porosity are important because of their effect on strength and toughness that are better for small grains. Here is what they say: “These first results already highlighted the power of Raman spectroscopy as an analytical tool that can deliver in situ information about the growth and breakdown of distinct phases, including the detection of metastable or intermediate phases, without the necessity to quench the sample to room temperature (RT) [22].

6 My current interests

Unlike an academic researcher, my successful projects have been a result of what “walked through the door”. I have always suspected that this diminished the importance of my work. On the other hand, this guaranteed that the topic would be of current relevance. And the reality is that I have done more than routine measurements. I am currently working with Isao Noda on his PHA biodegradable polymers that are being commercialized, and on extractables and leachables which is a topic of relevance for the pharmaceutical industry.

6a PHBHx – Poly (hydroxybutyrate-co-hydroxyhexanoate)

This polymer developed by Isao Noda while at Procter & Gamble was engineered as a biodegradable polymer. In fact, it is both manufactured by a fermentation process and is biodegraded at its end of life [23]. It is a semi-crystalline, random co-polymer. The hydroxyhexanoate content lowers the crystallinity, and by lowering the melting temperature significantly below the temperature of degradation, the material is easier to process. [Figure 1](#) shows the structure of PHBHx.

The presence of the hexanoate replaces the methyl side group of PHB with a propyl side group and has the effect of interrupting the crystallinity. Both the percentage of hexanoate (Hx) and the molecular weight of the molecule, together with the degree of crystallinity determine the grade of the copolymer, and thus its chemical and physical properties, especially ductility and toughness; that is, if the crystallinity is too high the polymer will be brittle and of limited use. In addition, the length of the side chain can also be selected, and it will affect the flexibility of the polymer as well. The work that we will show here has all been done on PHA with propyl sidegroups (PHBHx).

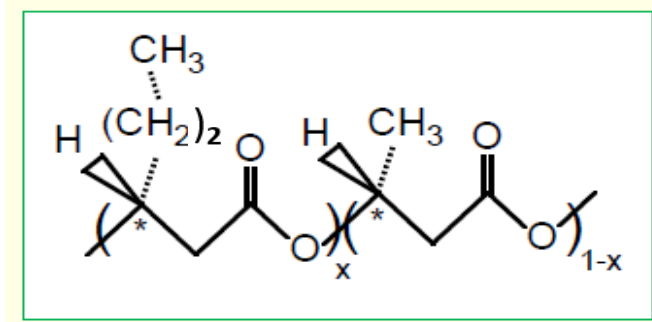


Fig 1. Molecular composition of PHBHx

PHBHx exhibits some unusual behavior with which Raman measurements can assist. In 2004 IR studies documented the presence of a methyl group CH stretching band at fairly high frequency which was attributed to an interaction between a methyl proton and a carbonyl oxygen ($\text{C-H}\cdots\text{O}=\text{C}<$) possibly between the two helices in the unit cell [24]. In a publication in 2018 there is a detailed analysis of the growth of single crystals from dilute solutions using XRD (x-ray diffraction) and TEM (transmission electron microscopy) which reinforced this conjecture [25]. Together these techniques showed that the crystals exhibited needle-like habit with rapid growth along the a axis, and the c axis (molecular axis) oriented normal to the plane of the needles. By inspecting the atomic coordinates from the XRD it was possible to argue that the rapid growth is enabled by “cooperative $\text{-CH}\cdots\text{O}=\text{C}<$ bonding” in the direction of the a axis. In addition, because XRD parameters for PHBHx are the same as those for 100% PHB, it is argued that the hydroxyhexanoate is not incorporated into the crystals. The lamellar thickness for single crystals varies between 3.6nm and 5.2nm, depending on the growth conditions and this corresponds to about 6 to 12 monomer units.

How can Raman spectroscopy help with this structural analysis? Standard spectra can be acquired between about 100 and 4000 cm^{-1} . However, with an ultra low frequency (ULF) accessory, spectra can now be acquired to less than 5 cm^{-1} from the laser line. That enables monitoring low frequency characteristics as well as the molecular vibrations detailed in the 2004 reference. Any spectral features that appear in the ULF region can be assigned to several origins. There can be accordion-like motion where the frequency is proportional to the inverse of the dimension of the lamellar thickness (longitudinal acoustic modes, LAM's). There can be librational motion where the chains rotate around their own axes. And there can be molecular phonons where the molecules in the unit cell can beat against each other; this last phenomenon is only possible where there are at least 2 molecules in the unit cell, which is the case for PHBHx.

There are many ways to perform crystallization experiments. A sample can be melted and then the spectrum can be monitored as it is cooled. Or it can be slowly heated, and the loss of crystallinity can be monitored. We opted instead for an isocrystallization measurement. In this case the sample is melted, quenched in liquid nitrogen, and then followed spectrally as a function of time at room temperature. This method is particularly advantageous when one is measuring the low frequency region because the temperature-dependent Boson factor which modifies the spectral intensities in the low frequency region, will not be changing during the measurement because the temperature is not changing. The early and late spectra are displayed in Figs 2 and 3. Figure 2 shows the Stokes and anti-Stokes spectra in the low frequency region; by recording both sides of the laser line we demonstrate that the observed spectral features are not artifact. Figure 3 shows the spectra between 10 and 3250 cm^{-1} broken into three parts in order to better reveal the details of the spectra.

The classic method for analyzing such spectra is to band-fit the spectral regions where changes are observed. Anyone who has band-fit spectra is aware that such fitting does not produce unique fitted

parameters. Professor Noda has developed an alternative mathematical method for such analysis that is called 2D-COS (Two Dimensional Correlation Analysis) [26,27]. In 2017 a publication describing the application of 2D-COS to the study of PHBHx was published [28]. Here I will show results taken over a much longer period of time, and include the complete spectrum out to 3250 cm^{-1} .

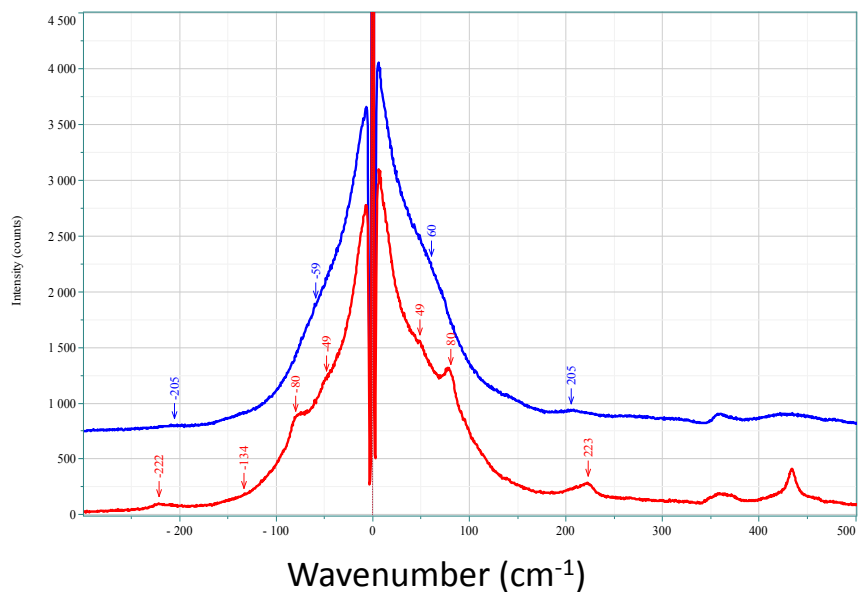
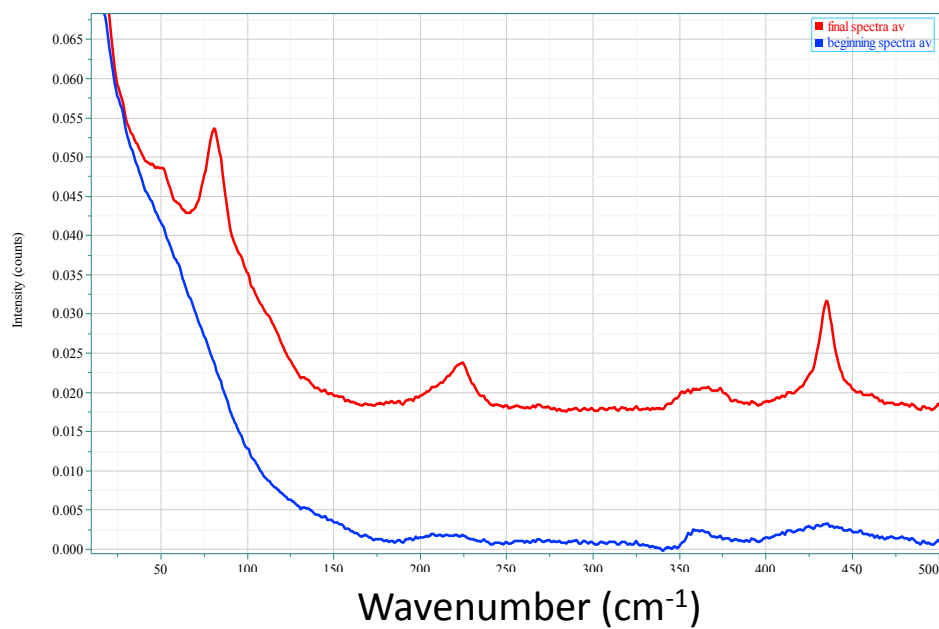


Fig 2. Stokes/Anti-Stokes spectra of PHA (13.8% Hx) at the beginning (top) and end (bottom) of an isothermal crystallization.



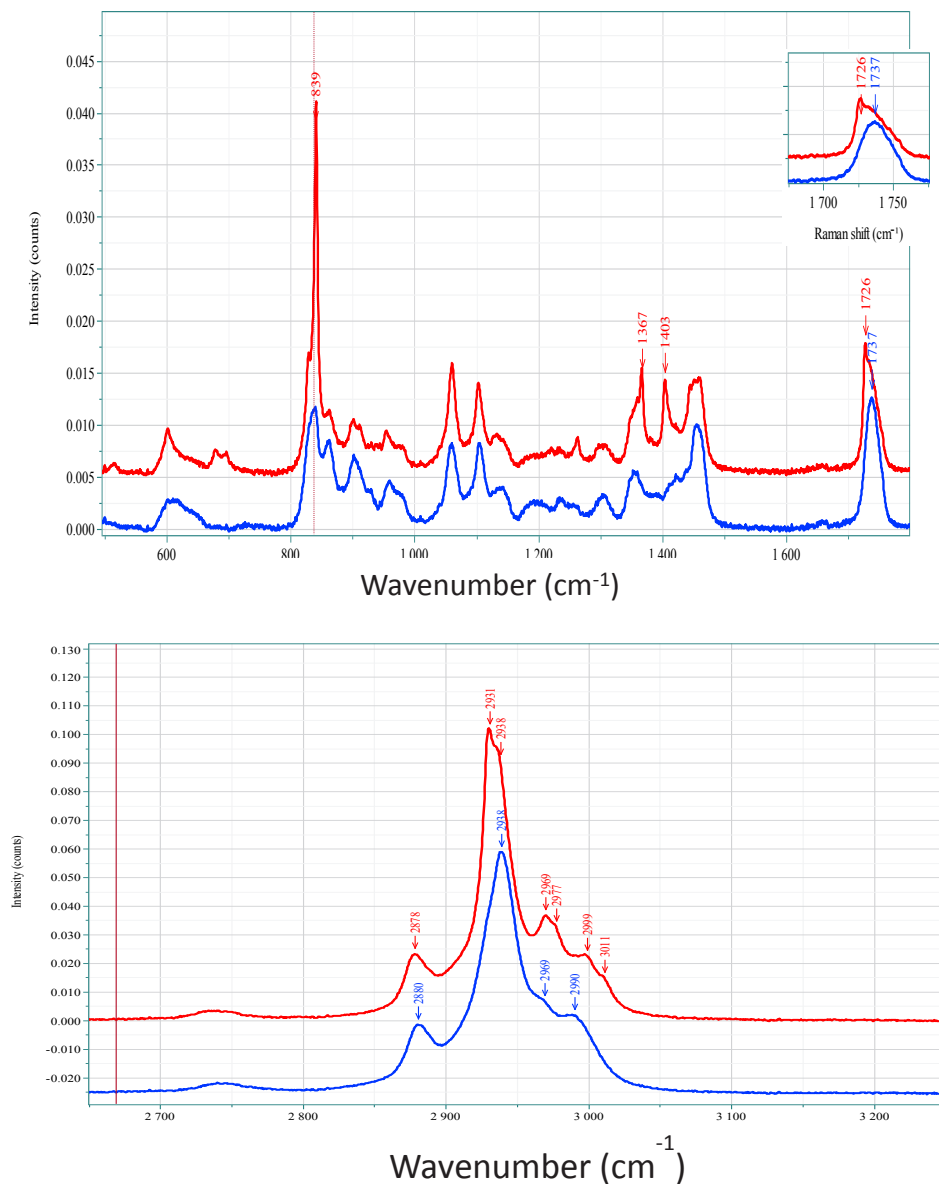


Fig 3. Raman spectral pattern at the beginning (bottom) and the end (top) of an isothermal crystallization study of 13.8% hexanoate in PHBHx. The spectra are broken into three regional displays in order to better reveal the details of the spectra. From 10-500 cm^{-1} , 500-1800 cm^{-1} , and 2650-3250 cm^{-1} .

In examining the behavior of the spectra, I used the standard LabSpec™ software to plot the behavior of three of the spectral features that grow with time. Refer to Fig 4. The abscissa in the plot is the intensity between 2 cursors that bracket the spectral feature of interest, with the background subtracted. The three traces were normalized so that their maximum intensities overlap. It is clear that the growth of the three peaks is not exactly parallel, which means that 2D-COS should provide good information on what is changing in what order.

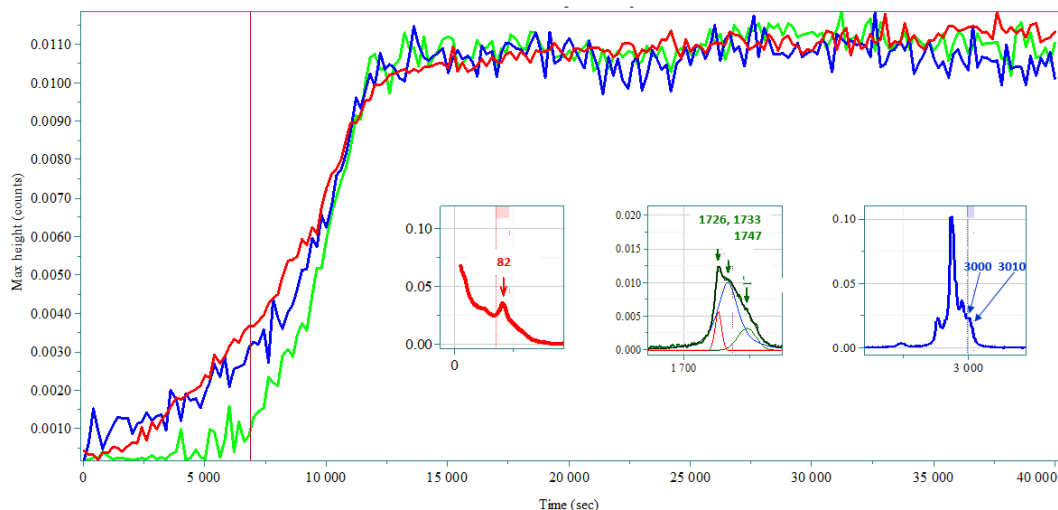


Fig 4. Intensity variation of bands associated with the crystalline phase of PHBHx (13.8%) as a function of crystallization from the melt at room temperature. The band fit of carbonyl band near 1730 cm^{-1} is displayed but the time dependent plot followed the crystallization peak at 1725 cm^{-1} .

A full explanation of 2D-COS in this article is not possible; therefore for those interested, references [26-28] are recommended. However, I can attempt to summarize what 2D-COS is doing. For a series of spectra that are changing in a systematic way, the synchronous and asynchronous correlation for each spectral pair is calculated. The results of the synchronous calculation is plotted on a two-dimensional plane, and where there is intensity, spectral features are changing “synchronously”; the spectral pair can either both be increasing, both be decreasing, or the two bands are changing in opposite directions. The results of the asynchronous calculation is also plotted on a two-dimensional plane but here the results become more interesting. By comparing the signs of the changes in the synchronous and asynchronous plots for a given spectral pair it is possible to say which spectral band is changing first. I am going to focus here on the changes in the spectrum of PHBHx (13.8%) as it is crystallizing at room temperature from a melt quench over a 12 hour period, focusing on the spectral features that are known to be sensitive to the crystalline form that will be of focus here. The spectral features of interest are shown in Figs 2 and 3. There is the ultra low frequency band at about 80 cm^{-1} that grows with time, the carbonyl band that shifts and sharpens, a marker band in the CH region for the crystalline form (2930 cm^{-1}) and two unusual bands in the CH region (above 3000 cm^{-1}) that are discussed in references [24 and 25]. The interest here is to use the 2D-COS plots to determine the sequence of changes from the amorphous form to the crystalline form. Figures 5-8 show the 2D-COS plots for the CH region, for the carbonyl region compared to the CH region, the ULF region compared to the CH region, and then the carbonyl region compared to the ULF region. The Figure legends will indicate the order in which selected bands are changing.

By comparing the sequences of changes in the figures, it is possible to reconstruct the global order of all these changes which comes out to be $3000, 1723/1725, 2930, 80, 1730/1715, \text{ and } 3010\text{ cm}^{-1}$. From the publications cited earlier (24 and 25), it is now known that the bands at 3000 and 3010 are unusually high because of a cooperative interaction between the carbonyl group and one of the methyl CH's along the a axis. We assume that the low frequency mode at 80 cm^{-1} can be attributed to the beating of the two chains in the unit cell against each other in the crystalline form, although the other two possibilities have not yet been eliminated. What is interesting is that this analysis is telling us that the first thing to happen is for the methyl to starting interacting with the carbonyl, so the carbonyl changes next, then the crystal marker at 2930 cm^{-1}

grows, then the 80 cm^{-1} low frequency marker appears, then more changes in the carbonyl region and finally the 3010 cm^{-1} band that is an indicator for the methyl-carbonyl interaction. The question is why the 3000 and 3010 bands do not appear together. The conjecture is that there is only one such band at the beginning, and it is only after the 80 cm^{-1} marker appears for the two chains beating against each other that the CH band can split.

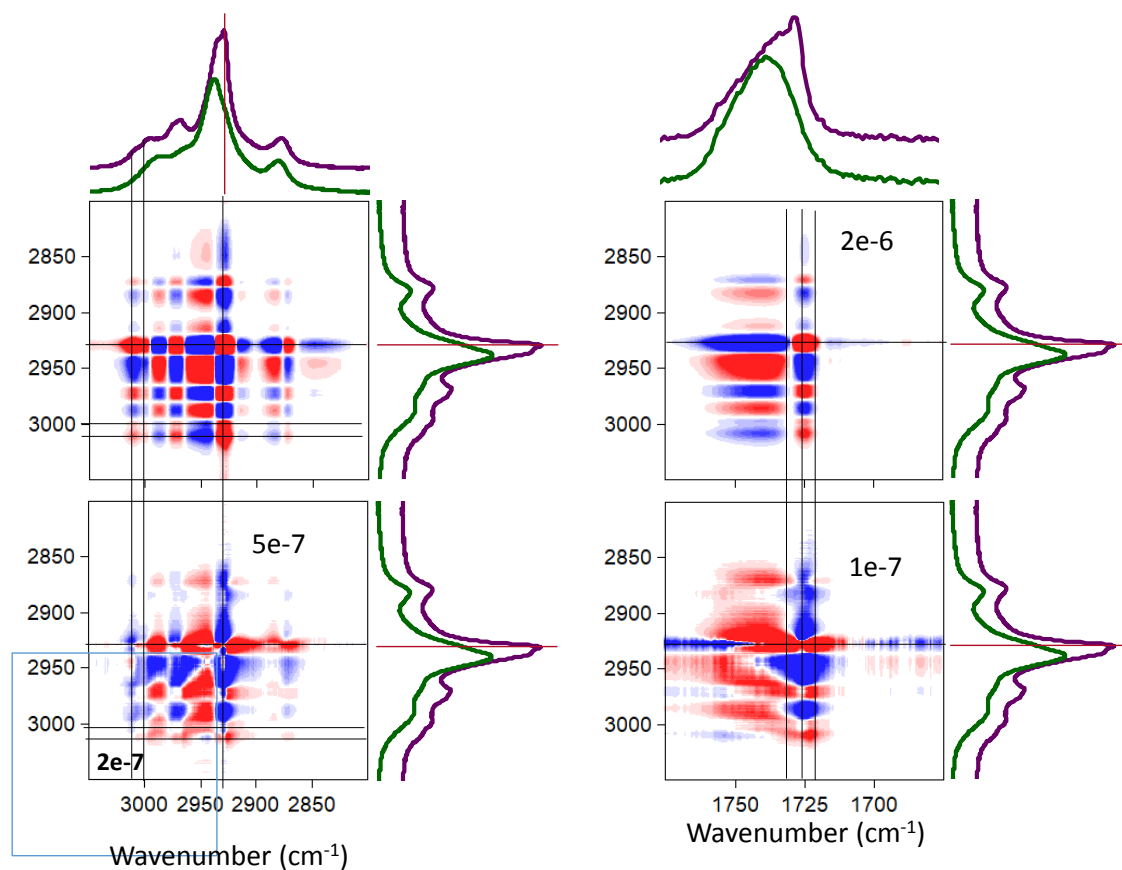


Fig 5. Changes in the CH stretching region: The 2D-COS predicts the changes occurring in the order of the bands at 3000 , 2930 , and 3010 cm^{-1} . **Fig 6.** Changes in the CH stretching region compared to the $>\text{C}=\text{O}$ region: The 2D-COS predicts the changes occurring in the order of $1723/1725$, 2930 , 1730 and 1715 cm^{-1} .

6b. Extractables and Leachables

Several years ago, I began receiving multiple emails announcing conferences on Extractables and Leachables (E & L's). I thought this would be an ideal target for Raman microanalysis. But demonstrating it required samples collected from a relevant source. I did get some samples, was told that they were from implants, but at the last-minute permission to reveal the source was removed. (The source being the organization that had collected the samples, not the product from which they came of which I was never informed.) I did work up the results and published them in one of my columns [29]. While interest in the pharma community was non-existent, I did receive a favorable response from the spectroscopic community. The most interesting response came from Mark Witkowski. Mark and a colleague at the U.S. FDA's Forensic Chemistry Center (FCC) were working on an unrelated project and encountered what appeared to be extractable material originating from centrifuge tubes. After reading my column he proposed sending

me a collection of samples to help identify what was going on with the centrifuge tubes. I now have spectra collected from residues of all the samples. There are ten tubes from each of three different vendors, with ten protocols for solution exposure. I am currently in the process of interpreting the spectra and hope to finally come up with some coherent information on the composition of the additives to the polymers. It is interesting that all tubes are made from polypropylene, but the stuff coming out is different for the three vendors. Polymeric additives include such things as plasticizers, stabilizers, lubricants, antioxidants, colorants, etc. and are selected to engineer the physical and chemical properties for the purposes for which the polymers will be used.

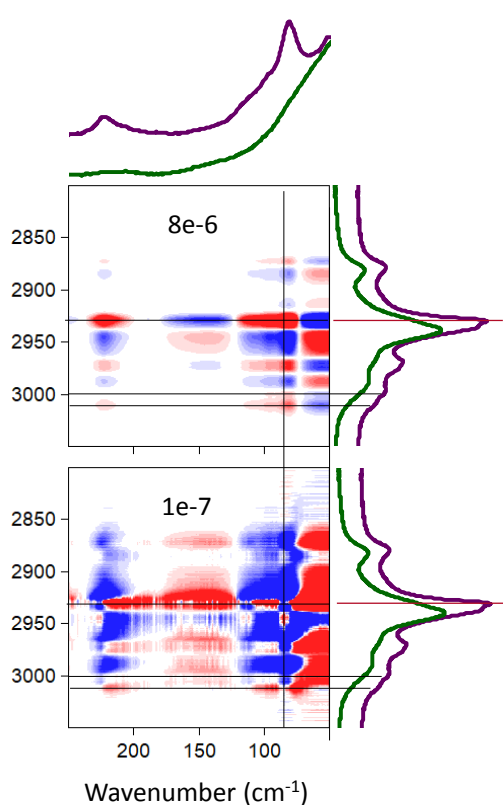


Fig 7. Changes in the CH stretching region compared to the low frequency region: The 2D-COS predicts the changes occur in the order of 3000/2930, 80, 3010 cm^{-1} .

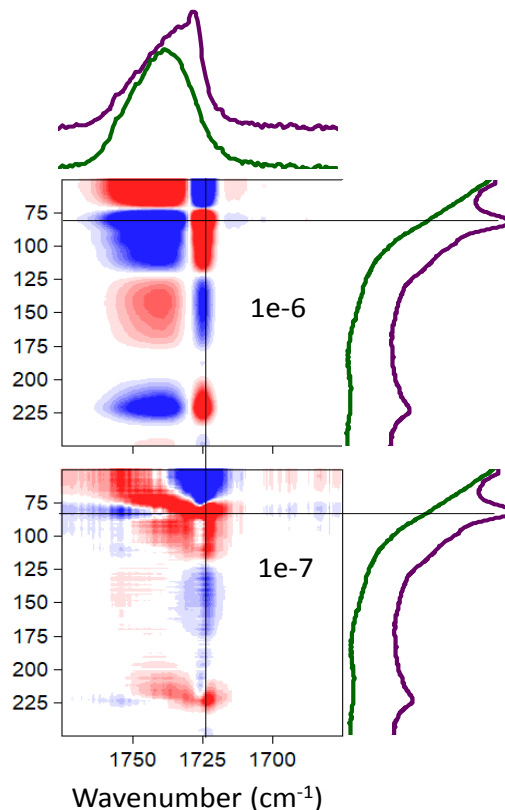


Fig 8. Changes in the $>\text{C}=\text{O}$ region compared to the low frequency region: The 2D-COS predicts the changes occurring in the order of 1725, 80, 1715/1730 cm^{-1} .

The concern regarding extractable and leachable compounds from pharmaceutical or other commercial containers is the impact that these compounds may have on the drug product or device if/when a patient or consumer uses or ingests the product. The E&L compounds may be toxic or may interact with the drug product (or excipient) producing a toxic or otherwise undesirable product. For this reason, all medical devices and products coming into patient contact have to be pre-qualified. Routine methods have been developed over the years, usually using GC-MS (gas chromatography-mass spectroscopy) or LC-MS (liquid chromatography-mass spectroscopy). In my random attempts to keep up with literature in the fields where I work, I have identified two recent articles on the analysis of E&L's [30,31]. Not having ever worked with MS and knowing how widespread this technology is in the pharma industry, I had assumed that identification of

organic unknowns is routine and straightforward. Reading one of these articles [31] one is surprised at how much skill is required in measurement interpretation. The fact that the titles of many sections of this article start with the word “Challenges” is already a clue that the technique is not straightforward. Section 4. Entitled “Challenges associated with the identification of extractables and leachables” has a sentence “*The primary challenge in identifying extractables and leachables is the quality of a mass spectrum which is often affected by interferences from co-eluted compounds and other factors.*”

Ultimately in this work, I want to answer my own question: why bother doing Raman microscopy if these contaminants are being identified by GC-MS or LC-MS. I am beginning to take heart that all the effort I am putting into my Raman study is worthwhile. What are the challenges that Raman analysis faces? First one has to record a spectrum. The difficulties introduced by fluorescent impurities that obscure the spectrum have largely been overcome in the last 20 years with microscopic sampling and a variety of laser wavelengths that can be chosen to avoid the fluorescence. (The laser wavelength most often cited is 785nm because the photon energy is too low to excite the fluorescence. I have found that a red laser, 633 or 638, or 660nm can also often avoid the fluorescence but still provide the full spectrum out to 4000cm^{-1} shift, which the 785nm laser cannot do because of the dropping sensitivity of the detector in this wavelength range). Second is the interpretation of the spectrum, and here is the biggest challenge. Vibrational spectroscopy is not being widely taught anymore. In my opinion, analytical chemistry departments are doing their students a disservice because vibrational spectroscopic interpretation can be a major asset in landing industrial employment. Of course, spectral data bases and algorithms for searching are a “solution”, well partly. Reading the cited MS literature [30] and [31] one can see that easy identification is not a given even for MS. The Raman databases [32-35] are probably an order of magnitude smaller than those of MS and whether Raman will get a good “hit” will depend on whether your unknown compound is in the data base. If the spectral unknown is not in the Raman data base, it will not be identified. Having said all of this, let’s look at some of the Raman results.

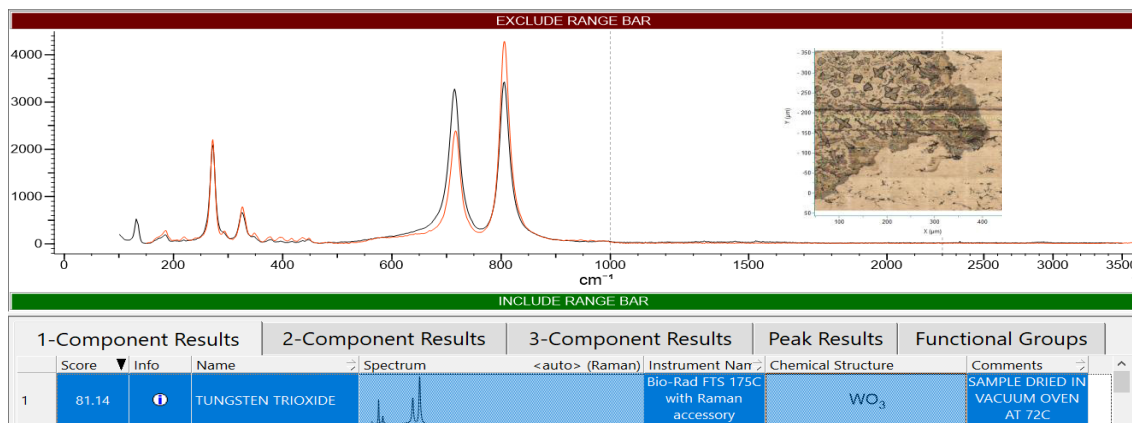


Fig 9. Spectrum of unknown deposit identified as WO_3 by KnowItAll™. Inset shows the field of view from which the spectrum was recorded.

I received 30 polypropylene vials, ten from each of three vendors, each one filled with one of ten solvents. From each vial, I deposited a microdroplet on a stainless steel slide – stainless steel so that I could avoid the signal of glass. Figure 9 shows our spectrum in black overlaid with the match from KnowItAll™. The spectra are identical except that the relative intensities are different which happens when the sample is crystalline, with crystals larger than the beam spot and oriented arbitrarily. This was an unusual match for these samples – tungsten oxide (WO_3) that possibly came from a residual of catalyst used to manufacture the polymer, or another unknown source.

Figure 10 shows the spectrum of a deposit compared to that of the polypropylene container. Clearly the bands overlay but the bands of the deposit were considerably broader compared to the spectrum of the vial. We believe that this is a sample either of amorphous polypropylene or to a polymer oligomer that was released into the solvent. KnowItAll™ listed amorphous polypropylene as a possible hit, but not at the top of their listings. This is the reasons that all spectroscopists will warn new users not to rely on the hit quality index (HQI) without human judgement for identification.

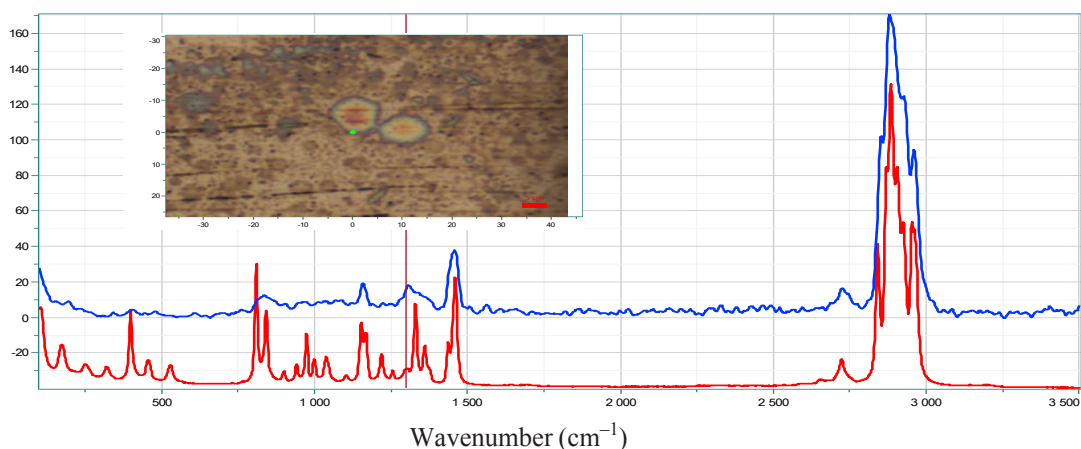


Fig 10. Raman microscope Spectrum of Deposit (top) and the spectrum of the Polypropylene Container (bottom). The micrograph shows the region from which the spectrum was measured; clearly it was a film less than 1 μm thick.

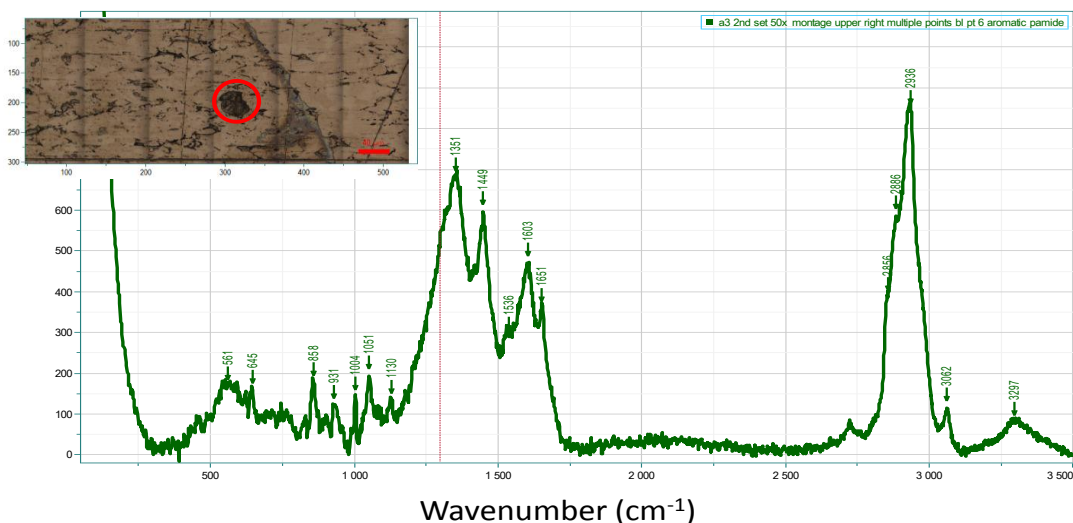


Fig 11. Spectrum of an organic material derived from the spot shown in the figure. The broad bands near 1360 and 1600 cm^{-1} are carbon bands.

The next example shown in Fig 11 is of a spectrum with a clear background of carbonaceous material. Actually many spectra of organic unknowns that come from contamination or some kind of degradation often have the carbon signature superimposed on the spectrum of the unknown(s). The analyst must be familiar with the carbon signature, or many errors can occur; unfortunately this is not so straightforward because the carbon Raman spectrum is quite variable. The carbons are related to graphite which has a strong reasonably

sharp band at 1583 cm^{-1} . When it is disordered a second band appears somewhere in the mid 1300 cm^{-1} region; I am purposely imprecise about its position because the center of the band depends on the excitation wavelength. This band is called the D band which over the years has meant disorder or diamond-like. In fact, it is a band that is not allowed within the strict rules of solid state physics, but appears because of the double resonance with electronic transitions when the crystals are small or there is a defect, a phenomenon which is unique in graphene/graphite. This has all been explained in great detail by Reich and Thomsen [36]. The correspondence between the non-zero phonon wavevector and the two electron transitions (incident and scattered photon) leads to the conclusion/observation that the phonon frequency will depend on the excitation wavelength, which is, in fact, observed. In fact, because the spectrum is so variable (peak positions, peak widths, relative intensities, wavelength dependence) I have found that the most practical advice sometimes is to manually subtract that spectrum in the instrument software and then search on the residual.

Figure 12 shows the spectra of three carbon fibers provided by Herman Noether, retired from Celanese, and is shown to demonstrate how much variability there can be. The fiber made from pitch is the closest to graphite which has no band in the $1330\text{--}1370\text{ cm}^{-1}$ region. The two fibers made from polyacrylonitrile (PAN) were made by different processes that accounts for the different spectra reflecting different microstructure. In addition, the top two spectra show an intense overtone at 2665 cm^{-1} , almost exactly twice the frequency of the D band and is explained in reference 36. Because the spectral behavior of carbons is so complex and interesting, I am suggesting that you get some familiarity with these spectra [37] even if you don't understand all the theoretical subtleties.

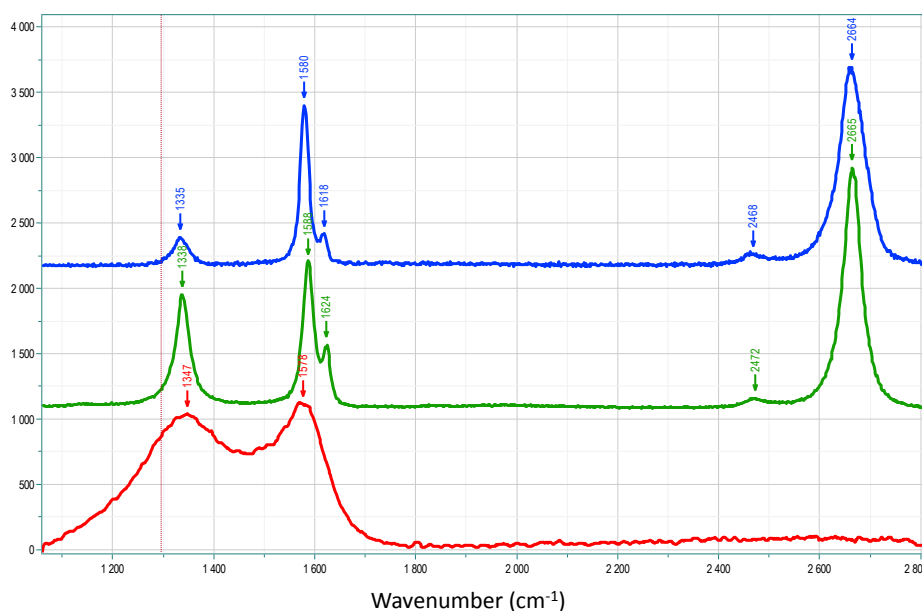


Fig 12. Raman spectra of carbon fibers made from pitch (top), and polyacrylonitrile (PAN) (middle and bottom).

After having made the case for being careful when dealing with spectra with a carbon background, there are some cases in which the library searching programs can determine that a spectrum represents a mixture of an organic with a carbon. The next figure illustrates when this does work well. Figure 13 shows the results in of a search in KnowItAll™ of the spectrum shown in Fig 11. Note that the grey regions were deselected from the search so that those regions where there is no information is eliminated from the search and cannot them contribute noise to the result.

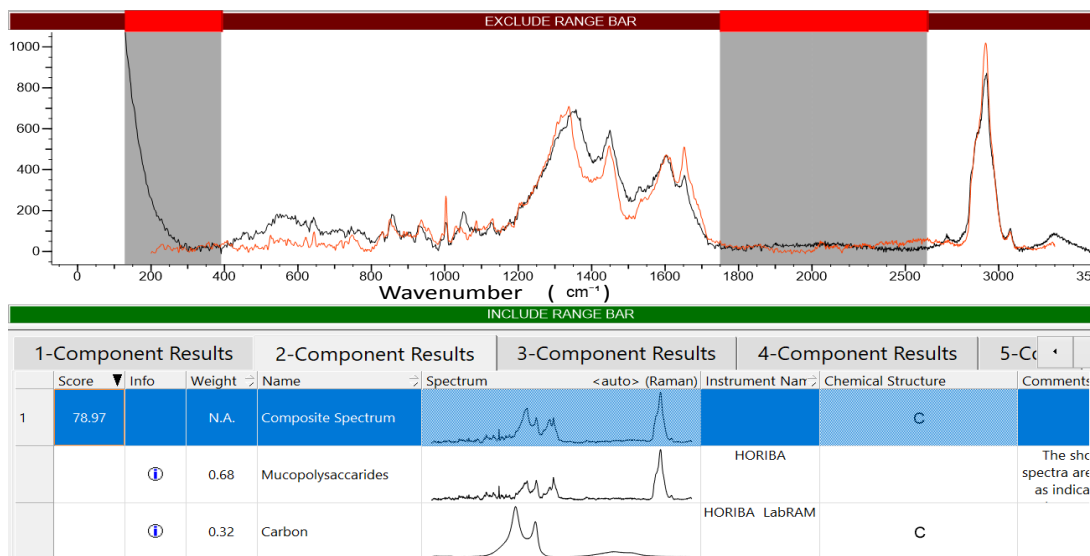


Fig 13. Result of a search in KnowItAll™ showing a mixture of a carbon spectrum and that of a mucopolysaccharide.

In my spectral searching of these E&L samples, I have found a hit for mucopolysaccharides often. As yet I do not know if this is legitimate or if there is an industrial material with a similar structure.

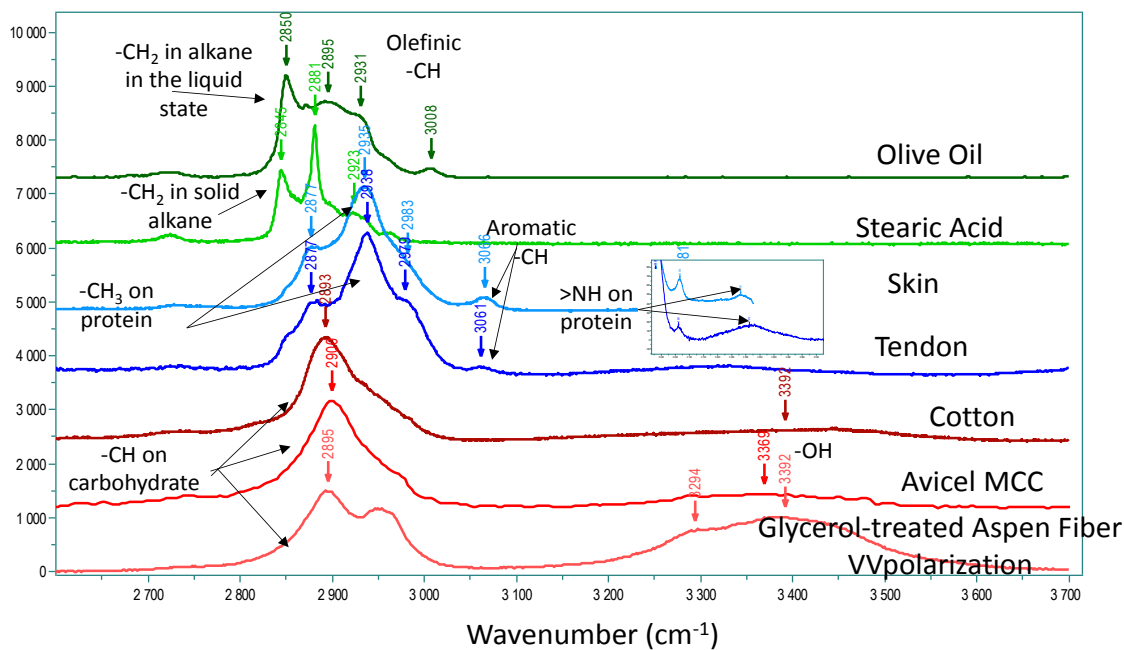


Fig 14. Raman spectra in the CH/OH region of some common biological polymers. The origin of the bands in terms of what is vibrating is indicated in the figure.

But what happens when the search does not easily produce something reasonable. In the course of searching many spectra, I have noticed, surprisingly, that the CH region can be used for classification.

Figure 14 shows the CH region for a number of organic materials. Olive oil is a triglyceride of mostly oleic acid which has one double bond; the CH band at 3008 cm^{-1} is an analytical band for olefinic CH. Stearic acid is solid and is representative of the class of saturated free fatty acids (FFA's) which are solid at room temperature. Any CH band below 3000 cm^{-1} is normally attributed to a saturated hydrocarbon; while the CH bands in this region overlap, the overall structure of the spectrum is quite different from that of liquid alkanes and can be used to differentiate liquid or amorphous from solid hydrocarbons. The next two spectra represent two proteins – skin and tendon. Note how different is the envelope of the CH bands below 3000 cm^{-1} from that of the lipid. This spectrum, peaked at $\sim 2930\text{ cm}^{-1}$ with shoulders on either side, is typical for proteins. It is also possible to identify the aromatic CH near 3060 cm^{-1} and the $>\text{NH}$ from the protein amide group at about 3350 cm^{-1} . The bottom three spectra were recorded from three fairly pure forms of cellulose – cotton, Avicel, and wood fiber. The Avicel and cotton spectra are almost identical with maybe a slight shift in the broad, poorly defined OH band of the cellulose. The wood spectrum was acquired under well-defined polarization conditions and shows a clear splitting between ~ 2900 and 2960 cm^{-1} as well as a significant OH band.

In my column [29] I have also discussed the region of the spectrum below 1800 cm^{-1} , so I encourage anyone who is interested to take a look at this article that is available on line. I have found these classifications useful, even the ones in the CH region which originally surprised me. I actually should not have been surprised because these differences are used extensively in CARS (Coherent Anti-Stokes Raman Spectroscopy) [38]. In addition, I have seen the relative intensities in the CH region of linear chains to vary with chain length. The bands near 2840 and 2880 cm^{-1} are assigned respectively, to the symmetric and asymmetric stretches of the $>\text{CH}_2$ methylene group whereas the symmetric and asymmetric $-\text{CH}_3$ methyl stretches occur close to 2885 and 2930 cm^{-1} . Because there is only one methyl group on a fatty acid, or two on an unsubstituted alkane chain, the intensity of the upper methyl (asymmetric) band relative to the rest will diminish as the chain lengthens.

7 Summary

After moving from Physics to Biophysics I set up a program to measure physical interactions between cytochromes on functioning biological membranes. Then with the move to Jobin Yvon/ISA (which has become HORIBA Scientific) I started exploring the potential of the Raman microscope. It has truly been a privilege to be the person to explore what was then a new technology. In this article, I have tried to survey what I have been doing over the past 40-some odd years. My background made me uniquely suited for this opportunity. I had already crossed over from semiconductor physics to biophysics where I had learned some chemistry. And I had acquired the ability to identify the relevant question that needed an answer and which Raman spectroscopy could provide. After determining that silicon can be used as a reliable instrument test, I started exploring applications in fields that included polymer physics, ceramics, corrosion mechanisms, and pharmaceuticals. And as the French designers understood, contaminant identification would always have an important role to play. Always looking for new ways to exploit the technology, today I am working on characterizing the Nodax™ biopolymer using 2D-COS analysis. I have also applied multivariate analysis (MVA) techniques for mapping and for studying chemical/physical changes (not included in this article). As the applications of the technology have expanded, the number of instruments sold has increased many-fold, and this has enabled the development of new capabilities that are integrated into commercial products making the instruments easy to use so that even more problems can be solved. Since the global consensus is now that We, the world's population, has to solve problems of global warming and energy efficiency and poverty, it is clear that analytical instruments will have an increasing role to play. And I am humbled to have been able to be part of the Raman revolution in making this possible with Raman spectroscopy.

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She has received numerous awards, the latest ones being the Abbe Award from the New York Microscopy Society in November, 2019 and the Gold Metal Award from the Society of Applied Spectroscopy in 2021. Since 2000, Dr. Adar has been writing a column for Spectroscopy whose goals are to point out where Raman spectroscopy and microscopy are having an impact on evolving technologies, and to guide new users into the field. Dr. Adar continues to work with new and experienced Raman users developing applications, and pushing instrumentation developments to accommodate new applications enabled by evolving technologies.