NMR and IR Spectroscopy for the Structural Characterization of Edible Fats and Oils

An Instrumental Analysis Laboratory

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Edible fats and oils are triglycerides that are solids or liquids at room temperature, respectively *(1, 2)*. They have been at the center of many heated nutritional debates such as the popular "margarine versus butter" controversy *(3)*. Recently, the FDA has required that the quantity of trans fat be cited on food labels *(4, 5)*. A survey of the *JCE* index reveals over 20 articles on the analysis of fats and oils but only one using NMR (*6,* a lipid model) and only two using IR *(7, 8)*. Most analyses used GC or HPLC *(9–11)*, electrochemical *(12)*, or wet chemical methods *(13)*. NMR and IR require no sample treatment and can easily provide equivalent information in a single analysis.

This article describes an upper-level instrumental laboratory for undergraduates (typically third-year) that explores the complementary nature of IR and NMR spectroscopy for analysis of several edible fats and oils that are structurally similar but differ in physical properties and health implications. Five different fats and oils are analyzed for average chain length, degree of unsaturation, and trans fat content. Careful interpretation of peak areas in ^{1}H NMR spectra provides the extent of unsaturation and the average chain length. IR spectroscopy can easily identify the presence of trans fat. Instruction on acquisition, processing, and interpretation of spectra goes beyond what is typically taught in an organic undergraduate laboratory. This includes specifics on acquisition and digital filtering, examples of complex coupling, and introduction to 2D NMR experiments. After analysis, students must match each oil or fat to a given fatty acid distribution *(1)*. Students can then reconcile the structural composition to the physical properties and health implications. Students work independently, are guided by literature, and get good results on a challenging problem.

Experiment

This lab may extend over three laboratory periods (3 hours each) and requires careful planning by the students. Students are provided with three oils (olive, coconut, and canola) and two fats (lard and Crisco) for analysis. Several fatty acids (Table 1) are also available to run as spectral references, if elected. Because of time constraints and the many spectra that need to be run (^{1}H NMR, ^{13}C NMR, and IR spectra of the five fats and oils plus reference spectra), the students are allowed to work in groups and share spectra. Each person should get hands-on experience with both instruments. Spectral interpretation and report are the responsibility of each individual. Students are directed to specific literature references for NMR *(14)* and IR *(15)* spectral assignments relevant to fats and oils. Excellent review articles on high-resolution NMR (proton and carbon) are noted and may be of interest to the instructor *(16, 17)*.

A suggested schedule for the laboratory periods is given here but can be modified according to the needs, available time, and number of students. If time is short or the class is large, for example, a schedule can be made so that each student comes in on his or her own time during the week to acquire spectra or the instructor can provide some spectra. During the first laboratory period, background information is reviewed, training on the instrumentation is given, and students organize and begin to acquire some spectra. By the second laboratory period, students are expected to have made the basic band assignments in the infrared *(15)* and to have drawn some preliminary conclusions regarding the characterization of the different fats and oils. During the second laboratory period, students should aim to finish obtaining the IR and ^{1}H NMR spectra. Training and acquisition of ^{13}C NMR spectra can begin. By the third laboratory period, students are expected to have made the basic band assignments in the ^{1}H NMR *(14)*. During the third laboratory period, the ^{1}H NMR spectral assignments, coupling patterns, and interpretation of peak areas are reviewed. Any outstanding laboratory work can also be completed.

Materials

Five different fats and oils were chosen to represent a variety in terms of source (animal vs plant), chain length, and degree of hydrogenation or processing. The following fats and oils were purchased from local grocery stores: Crisco (hydrogenated vegetable fat), lard (animal fat), canola oil, olive oil, and coconut oil.

Table 1. Characteristics of the Common Fatty Acids in Edible Fats and Oils

Name	MeltingPoint/°C	Class	Structure
Lauric acid	47	saturated C_{12}	CH_3–$(CH_2)_{10}$–CO_2H
Myristic acid	58	saturated C_{14}	CH_3–$(CH_2)_{12}$–CO_2H
Palmitic acid	63	saturated C_{16}	CH_3–$(CH_2)_{14}$–CO_2H
Stearic acid	71	saturated C_{18}	CH_3–$(CH_2)_{16}$–CO_2H
Oleic acid	16	monounsaturated C_{18}	CH_3–$(CH_2)_7$–CH=CH–$(CH_2)_7$–CO_2H
Linoleic acid	–5	polyunsaturated C_{18}	CH_3–$(CH_2)_4$–CH=CH–CH_2–CH=CH–$(CH_2)_7$–CO_2H

NOTE: The literature data are from ref *1*.

Deuterated chloroform with 1% TMS (Aldrich Chemical, Inc.) was used as the NMR solvent. Fatty acids of 95% purity or better were purchased as standards for reference and are listed in Table 1. Dried and desiccated KBr powder (International Crystal Labs) was used to prepare pellets for some of the solid fatty acid standards.

Equipment

Infrared spectra were obtained on a Midac M Series IR Spectrometer with Grams/AI (version 7.01) software from ThermoGalactic. The spectra of all fats and oils were obtained with a horizontal ATR (attenuated total reflectance) accessory (Spectra-Tech Inc.) using a 70° Zn–Se boat. Students were given an industry style SOP (standard operating procedure, see the online material) for operation of the IR spectrometer. Students were instructed on proper use of the accessory that includes maximizing the through-put and adjusting the gain so that the full range of the ADC is utilized. A background spectrum using the accessory and empty Zn–Se plate was acquired. The wavelength range is reduced to 4000–650 cm^{-1} because of the absorption cutoff of the crystal. Typically, 16 scans with 4 cm^{-1} resolution were averaged together. The solid fatty acid samples (used for spectral references) were prepared as KBr pellets.

The NMR spectra were obtained with a Bruker AC200 instrument with NTNMR (2.3.7) software from Tecmag, Inc. Samples were dissolved in deuterated chloroform at about 1 wt % for proton and 10 wt % for carbon spectra in a 5 mm NMR tube. Instruction on operation of the instrument and a handout summarizing important acquisition and processing parameters was given (see the online materials). In short, a 90° pulse width and long enough relaxation delay (5 × longest T_1) to ensure quantitative spectra are obtained in the proton acquisition. The line broadening parameter (decay constant) for the exponential multiply (digital filtering) was chosen to match the natural line width so that the both signal-to-noise and resolution are optimized. Spectra were carefully phased (0 and 1st order) so that the baseline is flat on both sides of the peak and integrated with enough range to include the tail of the Lorentzian peak shape. In the acquisition of carbon spectra, a 45° PW and relaxation delay equal to approximately 1 T_1 are used for the most efficient pulsing. The carbon spectra were not presumed to be quantitative because of the insufficient relaxation delay and variable NOEs. The line broadening parameter (decay constant) for an exponential multiply (digital filtering) was chosen to be greater than the natural line width to enhance signal-to-noise.

Hazards

Deuterated chloroform causes irritation to skin, eyes, and respiratory tract and may cause cancer. Avoid contact and use in a well-ventilated area or hood. The edible fats and oils are essentially non-hazardous. The fatty acid standards are irritants. Avoid contact and use in a well-ventilated hood.

Results and Discussion

Most naturally occurring fats and oils are "mixed" triglycerides in which the three fatty acid side chains are not identical (Figure 1). The most common fatty acids found in edible fats and oils and their structure and melting point are given in Table 1. Proton NMR cannot resolve the different fatty acids on the glycerol backbone, but can easily provide an average chain length and average degree of unsaturation for the fatty acid components. These two characteristics as well as the geometric isomerism (cis or trans) of the unsaturation are critical in defining the physical properties of the fat or oil as well as the health implications.

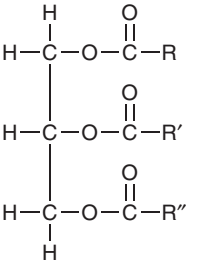

Figure 1. Structure of triglyceride: R, R′, R′′ are fatty side chains.

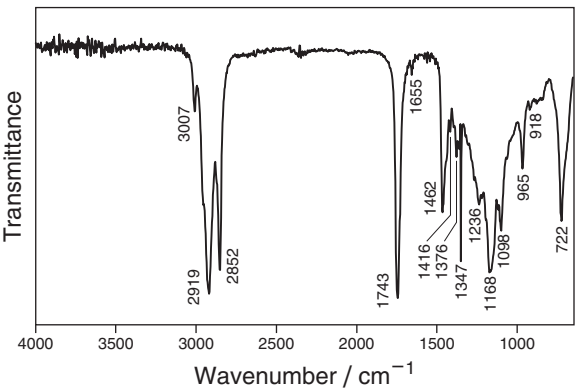

Figure 2. IR spectrum for Crisco using the HATR sampling accessory.

Table 2. Major Band Assignments for the IR Spectra of Edible Fats and Oils

Frequency/cm^{-1}	Assignment	Intensity
3007	=C–H (cis)	m
3025	=C–H (trans)	vw
2919	–C–H (CH_2)	vst
2852	–C–H (CH_2)	vst
1743	–C=O (ester)	vst
1462	–C–H (CH_2, CH_3)	m
1168	–C–O–C of ester (asym)	st
1098	–C–O–C of ester (sym)	m
965	–CH=CH– (trans)	w
914	–CH=CH– (cis)	vw
722	$(CH_2)_n$– (rock)	m

NOTE: vw = very weak, w = weak, m = medium, st = strong, vst = very strong.

Infrared Spectroscopy

Guillen and Cabo *(15)* give an excellent review on infrared spectroscopy of edible fats and oils. The IR band assignments were based upon their work and other correlation tables, particularly the extensive work of Nakanishi *(18)*. The IR spectrum for Crisco is shown in Figure 2 and a summary of band assignments is given in Table 2.

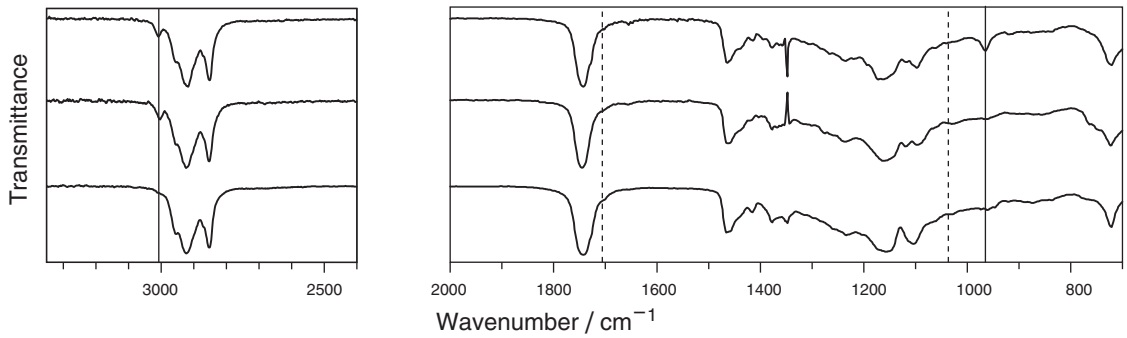

Figure 3. Stacked plot of Crisco (top), olive oil (middle), and coconut oil (bottom). The solid lines mark bands at 3007 cm^{-1} and at 965 cm^{-1} that show the relative degree of unsaturation and the relative quantity of trans fat component, respectively. The dashed lines indicate where bands for free fatty acid (1704 cm^{-1}) or free glycerol (1043 cm^{-1}) would be, if present.

Important differences can be seen immediately with a stacked plot of IR spectra for Crisco, olive oil, and coconut oil (Figure 3). The large trans component in the spectrum of the hydrogenated fat (Crisco) at 965 cm^{-1} is clearly evident and is lacking or much smaller in the other unprocessed fats and oils. The lack of unsaturation at 3007 cm^{-1} for coconut oil (saturated) is also evident. The band for unsaturation was the largest for canola oil, which is consistent with the proton NMR peak integration. This semi-quantitative comparison of the fats and oils by IR is valid since the HATR provides an identical path length (on the order of microns) for all the fats and oils that were run "neat" on the ATR crystal. Also, note that a free fatty acid carbonyl band at 1704 cm^{-1} is absent (or a very small shoulder) as is the strong alcohol CO band at 1043 cm^{-1} that would indicate free glycerol. The broad OH band at ~3300 cm^{-1} is also noticeably missing.

^{1}H NMR Spectroscopy

Peak Assignments

The fist step in interpretation of the proton NMR spectra is peak assignments. Correlation tables, reference libraries, on-line resources *(19)*, and literature references *(14)* are made available for the student. A 2D COSY spectrum was obtained (see the online material) and can be reviewed with students to demonstrate the utility in clarifying and confirming assignments. The 200 MHz proton spectrum of olive oil with the peak assignments is given in Figure 4.

The protons on the glycerol backbone represent an AA′BB′X spin system as labeled on Figure 5. The protons of an individual methylene group on the glycerol backbone are not chemically equivalent (they are diastereotopic) and hence the AB designation. The sets of protons (AA′ and BB′) from both methylene groups are chemically equivalent but not magnetically (they may couple differently to each nuclei in the other set) and hence the AA′ and BB′ designation. This complex system, however, can be treated as a A_2B_2X system. The methylene (A and B) protons have different chemical shifts (between 4.0–4.5 ppm) and exhibit identical geminal coupling (J_{AB} = 12 Hz) to each other in addition to slightly different vicinal coupling (J_{AX} and J_{BX} ~5–7 Hz) to the methine proton (X) on the backbone (Figure 5).

Peaks that are *not* present should also be noted. There is no evidence for free glycerol (at 3.5–3.8 ppm) in the proton spectra. (This is consistent with IR and ^{13}C NMR.) The student should also look for free fatty acid. This absence is more evident in the IR (1704 cm^{-1}) and ^{13}C NMR (181 ppm) for the acid carbonyl. The student can run spectra for these compounds or refer to reference libraries or online resources *(19)* of NMR spectra to confirm their presence or absence.

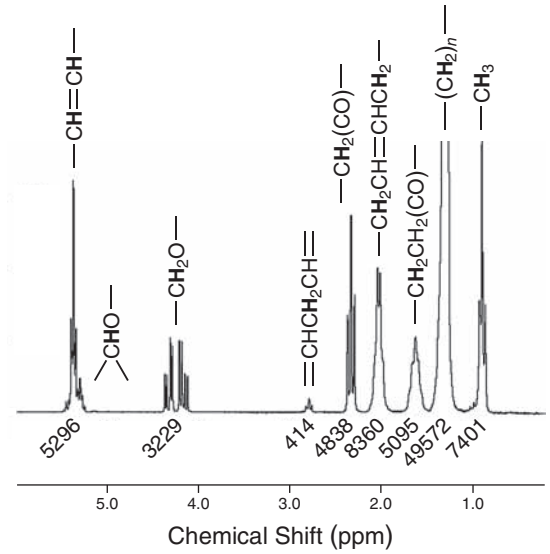

Figure 4. The 200 MHz proton spectrum of olive oil with peak assignments. The integration values are given below the peaks.

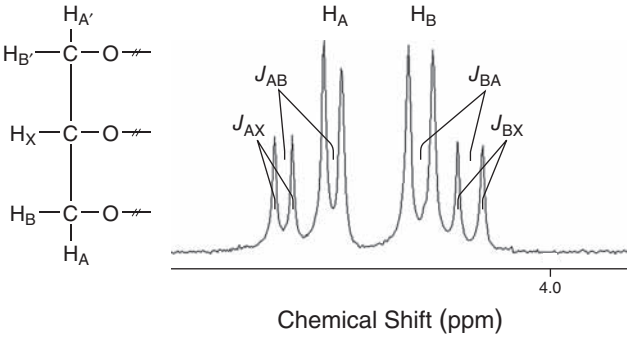

Figure 5. The A_2B_2X coupling observed for the methylene protons on the glycerol backbone.

Peak Areas

One of the biggest advantages of proton NMR over other techniques is the fact that peak areas are directly proportional to the molar ratio of components. In other words, if data are acquired quantitatively, all protons present have a response factor of one. No standards are necessary, unless one wants to quantitate the absolute percent of the compound(s) dissolved in the NMR solvent. Interpretation of peak areas is checked and reviewed in the second meeting. Students are encouraged to attempt analysis on their own with guidance from ref *14*. The basic procedure and example results are demonstrated for the spectrum of olive oil with the integrals supplied in Figure 4.

To calculate the mole ratio of components (saturated protons, unsaturated protons, fatty chain end or methyl group, and glycerol backbone), the following general procedure is followed. The integrals are divided by the number of like substituents per molecule (i.e., one glycerol backbone or three fatty chains), then divided by the number of protons in the substituent (i.e., three protons per methyl group), and finally divided by a constant (value of one proton) to normalize the integrals. Table 3 summarizes the process and shows the final normalized integrals from which average chain length and average degree of unsaturation are derived.

The shifts for the methine proton on the glycerol backbone (5.3 ppm) and the unsaturated protons (5.4 ppm) are not fully resolved and are integrated together. Since there are four methylene protons in the glycerol backbone (4.3 ppm, 3229) and only one methine, the methine proton integral is one fourth of the methylene integral. The total area at 5.3 and 5.4 ppm (5296) minus the area due to the methine proton (3229/4 = 807) is therefore attributed to the protons in the double bond (5296 – 807 = 4489).

The adjusted integrals for the chain-end methyl (0.9 ppm, 822), the methylene beta to the carbonyl (1.6 ppm, 849), the methylene alpha to the carbonyl (2.3 ppm, 806), and the methylene in the glycerol backbone (4.3 ppm, 807) should all agree since they all represent one proton integral in the triglyceride. Students will quickly learn by this comparison how important is careful phasing and choice of the start and end of the integral. NMR line shapes are Lorentzian and not Gaussian (such as a chromatographic peak). For this reason, integrals should not start and stop too close to the peak center since most of the area, for Lorentzian peaks, lies in the tail. The average proton integral for this spectrum of olive oil was [(822 + 806 + 849 + 807)/4= 821 ± 32, 95% confidence]. This value was used to derive the normalized integral in the last column. The reproducibility (precision) of the integration can be judged by these four values. Students should expect no bigger variation than 5% relative error [(32/821) × 100% = 4%]. A similar variation in integrals from sample to sample can be expected. Even if this increases to a generous 10% relative level, the assignment of fats and oils should not be effected given the many determining factors such as chain length, degree of unsaturation, presence of trans component (processed oil), or the specific presence of polyunsaturates (linoleic or others).

Table 3. Proton NMR Integrals and Normalization for Determining Chain Length

Assignment	Position (ppm)	Integral	Adjusted Integral	Norm Integral
$-CH_3$	0.9	7401	÷ 3 ÷ 3 = 822	1.00
$-(CH_2)_n-$	1.3	49572	÷ 3 ÷ 2 = 8262	10.06
$-C\mathbf{H}_2CH_2(CO)-$	1.6	5095	÷ 3 ÷ 2 = 849	1.03
$-C\mathbf{H}_2CH{=}CHC\mathbf{H}_2-$	2.0	8360	÷ 3 ÷ 4 = 697	0.85
$-CH_2(CO)-$	2.3	4838	÷ 3 ÷ 2 = 806	0.98
$=CHC\mathbf{H}_2CH=$	2.8	414	÷ 3 ÷ 2 = 69	0.084
$-CH_2O-$	4.3	3229	÷ 1 ÷ 4 = 807	0.98
–CHO–	5.3	807	÷ 1 ÷ 1 = 807	0.98
–CH=CH–	5.4	4489	÷ 3 ÷ 2 = 748	0.91

The total degree of unsaturation was given by the normalized integral at 5.4 ppm or the mole fraction of 0.91 or 91%. This is confirmed by the proton integrals alpha to the unsaturation (mono *or* poly) or 0.85 (2.0 ppm) + 0.08 (2.8 ppm) = 0.93 or 93%. This gives an average total degree of unsaturation of 92%. The methylene group at 2.8 ppm sandwiched between two double bonds (linoleic or other polyunsaturate) represents a mole fraction of 0.08 or 8%. This can be used as an additional identifier (5% in olive oil) since Table 4 was given to students, without the last 2 columns and the fat and oil assignments in the first column. The average chain length can be calculated by using a linear sum of the carbons in the chain: 1.00 + 10.1 + 1.03 + 2(0.85) + 0.98 + 0.08 + 2(0.91) = 16.7 + 1.00 (unprotonated carbonyl) = 17.7. A factor of two was used if there are two carbons represented by the proton integral. This was in good agreement with the average composition of olive oil *(1, 2)*. The distribution in Table 4 gives 16(0.07) + 18(0.02) + 18(0.85) + 18(0.05) = 17.7. Many students construct a spreadsheet to repeat these calculations on their various spectra.

Table 4. Literature and Student Data (in parentheses) for All Fats and Oils Analyzed

Fat or Oil	Lauric Acid C_{12} (%)	Myristic Acid C_{14} (%)	Palmitic Acid C_{16} (%)	Stearic Acid C_{18} (%)	Oleic Acid C_{18}, C=C (%)	Linoleic Acid C_{18}, 2C=C (%)	Av Chain Length	Av Degree of Unsaturation
Olive	—	—	7	2	85	5	17.7 (17.7)	0.95 (0.92)
Coconut	60	18	11	2	8	—	13.3 (12.3)	0.08 (0.05)
Canola	—	1	10	3	50	34	17.4 (18.3)	1.18 (1.32)
Lard	—	6	27	14	50	6	17.2 (17.4)	0.56 (0.73)
Crisco	—	—	8	3	56	26	16.6 (17.4)	1.08 (1.03)

NOTE: The literature data are from ref *1*. The unshaded portion of the table was given to the students.

A comparison of the student derived and literature based values for the composition of olive oil is given in Table 5. There is very good agreement. Accuracy is largely affected by the natural variation in the composition of these oils and fats. Based on their integration results and analysis, students are asked to identify every fat or oil by the known approximate fatty distribution given in Table 4. The values in the last two columns are calculated from the distribution provided by literature and by students (in parentheses) using the proton integrations.

Typical carbon spectra are shown for olive oil and coconut oil (Figure 6). The carbons on the glycerol backbone are between 60–70 ppm. The ester carbonyl is evident at ~173 ppm. Saturated carbons appear at 14 ppm (methyl group) and between 20–40 ppm (methylenes). Unsaturation is apparent at 130 ppm in the spectrum of olive oil only. The absence of the unsaturated peaks for coconut oil is consistent with the proton NMR and IR spectra.

Table 5. Literature and Student Data for Olive Oil

Parameter	Student	Literature
Average chain length	17.7	17.7
Average total unsaturation (%)	92	95
Polyunsaturation (%)	8	5

NOTE: The literature data are from ref *1*.

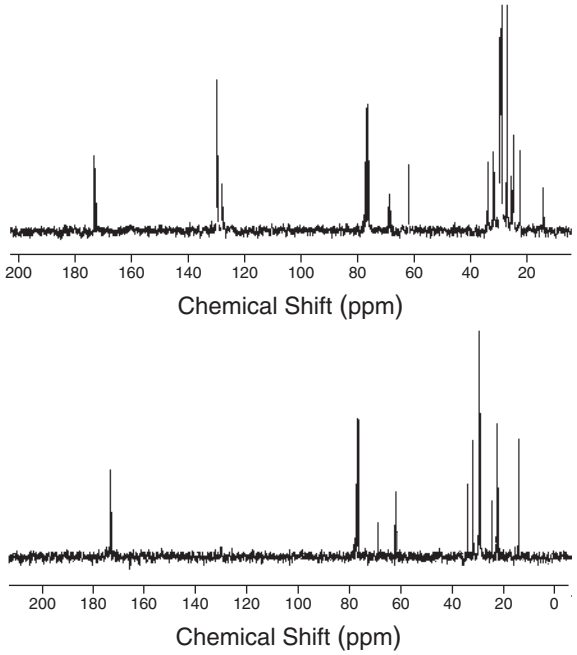

Figure 6. ^{13}C NMR spectra for olive (top) and coconut oil (bottom).

Summary

This lab provides a student with an in-depth analysis of similar compounds using IR and proton, carbon, and two dimensional NMR spectroscopy. The student is given relative independence in the collection and interpretation of spectra. Guidance is given on the complex coupling observed in the proton NMR as well as the analysis of peak areas to yield average chain length and degree of unsaturation. Special consideration is given to the proper acquisition and processing of spectra to reinforce concepts in the lecture of an advanced course in instrumentation. The student will get good results on a challenging and interesting analysis of relevant compounds. The two methods are shown to be complementary to each other.

Acknowledgment

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Abstract and keywords

Full text (PDF) with links to cited URLs and *JCE* articles

Supplement

A student handout with instructions and background material for the experiment; notes for students (and instructor) regarding the acquisition and processing of NMR spectra; the IR SOP; and a two-dimensional proton COSY spectrum with notes that explains the peak assignments by connectivity