NMR applications to fats and oils

The following article on NMR applications, linking molecular structure to physical properties, was written by Thomas M. Eads and William R. Croasmun of Kraft Inc.'s Technology Center in Glenview, Illinois. It was prepared under the guidance of William McShane of Kraft, JAOCS' *Associate Editor for the News for Instrumentation.*

Nuclear magnetic resonance plays an ever-increasing role in the study of properties of fats and oils of animal and vegetable origin. However, the nonspecialist will encounter a bewildering array of NMR experiments in the literature, with little guide to selecting the one he or she needs to answer a particular question about an oil sample. In this article, we will walk through some NMR techniques that answer some of these basic chemical and physical questions (Table 1). Some basic reviews that cover applications of NMR spectroscopy to foods include Schoolery (1), Horman (2) and Weisser and Harz (10).

NMR basics

In the NMR experiment, magnetic nuclei (e.g., carbon-13, hydrogen, phosphorous-31, oxygen-17, nitrogen 15) are aligned in the strong magnetic field established by an electromagnet or superconducting solenoid. Under these conditions, they may absorb energy from a pulsed radio frequency excitation field, causing them to align in a new direction. As the excited nuclei return to their original alignment, they induce a signal in a receiver coil. The NMR signal then can be presented as a plot of intensity as a function of time, as in pulsed NMR, or it may be converted, as in Fourier transform NMR, into a plot of intensity versus frequency, the result being an NMR spectrum.

In an NMR spectrum, the position tion of a resonance line on the frequency axis (its **chemical shift**) depends on the chemical environment of the nucleus, thus revealing local molecular structure. The **shape** of the line contains several kinds of information:

- Splitting of a resonance (called spin-spin or J-coupling) is due to the influence on a nucleus by its magnetic neighbors, transmitted through chemical bonds between them, splitting thus revealing more extended molecular structure. J-coupling is particularly useful in proton (1H) NMR of liquids.
- A broad line appears when the resonance frequency is spread out, and this occurs either when the molecular motion is somewhat slow compared to a liquid (e.g., a viscous fluid), or when the molecule is hopping between environments in which it has different chemical shifts (e.g., chemical exchange).

Line shapes are most important in carbon (^{13}C) NMR of fats, especially semicrystalline samples. However, for most analytical applications, the information contained in the NMR line shape is sacrificed (sharp lines are obtained by liquifying or heating the sample, or by other physical and NMR tricks) in order to get as much **chemical** information as possible from the chemical shifts.

In summary, NMR signals may be characterized by one or more of the following parameters: intensity, chemical shift, J-coupling, line shape and relaxation time. In any specific type of NMR experiment, only a subset of these parameters is measured (Table 2). The remaining parameters are eliminated from consideration by the method of measurement or the nature of the sample itself. The broad classes of NMR experiments may be distinguished by the method of data presentation (raw signal or Fourier transform), the state of the sample (solid, semisolid or liquid), the parameters measured and the experimental technique required.

Pulsed NMR

In pulsed NMR experiments, the initial intensity of an NMR signal from a sample is directly proportional to the number of resonant nuclei within the bounds of the receiver coil. The intensity decays with relaxation times that differ in the different phases (liquid, solid) in a sample. This is the basis for pulsed NMR analysis of solid fat content (AOCS Official Method Cd 16-81), oil estimation and water estimation.

High resolution FT NMR of liquids

The spectrum of a liquid shows many sharp lines because molecular motions have averaged out the physical interactions that otherwise would cause the lines to be broad. As an example, the ^{13}C NMR spectrum of neat margarine oil obtained at a temperature where all fat crystals should be melted is shown in Figure 1. The detail obtainable is evident particularly in the region containing carbon resonances from sites of unsaturation in fatty acid chains (inset to Fig. 1). Such a spectrum is rich in chemical information and can be used for compositional analysis, by comparison with a set of spectra obtained on known coupounds. In addition, the assignment of resonances is becoming simpler due to schemes relating chemical shift to carbon position in saturated chains (4). Identification of the source of an oil by principal component analysis has been accomplished with crude petroleum samples (7). Although source identification seems feasible and useful for edible oils, no such applications have appeared yet.

High resolution FT NMR of solids

Pulsed NMR experiments can distinguish liquid from solid in a heterogenous sample. High resolution solids NMR, however, has the potential to extend and refine the solid fat content determined by pulsed NMR, by preserving the information about solid and liquid ratios and at the same time, because a spectrum is obtained, by providing information about the chemical

TABLE 1

Uses of NMR in Lipid Science

Information wanted	Method to use	Key references
Analysis		
Composition of oils	High resolution NMR	Schoolery (1) Horman (2)
Composition of oilseeds	High resolution NMR	Haw & Maciel (3)
Chemical structure	High resolution NMR	Bengsch (4)
Positional isomerism	High resolution NMR	Ng (5)
Unsaturation, iodine number	High resolution NMR	Schoolery (1) Ng (6)
Identity of source of oil	Principal component analysis of high resolution spectra	Kvalheim et al. (7)
Cis-trans isomerization	High resolution NMR	Schoolery (1) Pfeffer et al. (8)
Solid fat content	Pulsed NMR	Korn (9) Weisser & Harz (10)
Oil content estimation	Pulsed NMR	Horman (2)
Structure		
Crystal polymorphism	High resolution solids	Yannoni (11) Norton et al. (12) Bociek et al. (13)
Crystallization kinetics	High resolution solids	
Phase diagrams of mixtures	FT NMR relaxation Wide-line NMR	Gibon et al. (14)
Crystal structure	High resolution solids	
Molecular motion		
Liquid diffusion Coefficient and emulsion droplet size	Pulsed field gradient NMR	Callaghan et al (15)
Glycerol backbone and chain motion	Wide-line FT NMR	Gibon et al. (14) Davis (16) Griffin (17)
	Pulsed field gradient NMR	Callaghan (18)
	FT NMR relaxation	Bociek et al. (13)

TABLE 2

What is Observed in Various NMR Experiments

Method	Observables
Pulsed NMR	Intensity, relaxation times
High resolution FT NMR—liquids	Intensity, chemical shift, J-coupling
High resolution FT NMR—solids	Intensity, chemical shift
FT NMR relaxation	Relaxation times of individual signals
Wide-line FT NMR	Intensity, line shape

composition of the two phases. This kind of information is required for unambiguous interpretation of x-ray and calorimetric studies of crystallization and melting in semisolid fats.

This potential barely has been tapped. As an example, we examined margarine oil, partially solidified at 7 C (45 F). In a conventional NMR experiment, only resonances from molecules that have liquid-like motions would appear, since resonances from solid regions would be undetectably broad. Upon application of some solids techniques (11), all lines are narrowed, and the intensity represents both liquid and solid. This clearly is observed in Figure 2, in which the integrated intensities of most peaks in the conventional spectrum are 60–70% of the intensities in the spectrum obtained with line-narrowing techniques, in agreement with the estimated 60% liquid content of the sample. The crystalline regions may be observed selectively by other solids techniques, and various polymorphs of triglycerides can be distinguished by their solid state chemical shifts in spectra of the crystalline phase (13). Solids and liquids experiments require different instrumental hardware.

FT NMR relaxation

Excited nuclei return to their original alignment with characteristic times called relaxation times, which reflect molecular motion. Relaxation times for solids and liquids can be measured by relatively simple NMR experiments. For example, NMR relaxation measurements have shown that motions of carbon atoms in a fatty acid chain are slower and more restricted at chain positions closer to the glycerol backbone (18). This is certainly true in liquids, and preliminary evidence suggests it also is true for triglyceride chains in crystalline regions (13). The effects on crystallinity and polymorphism due to unsaturation, *cis-trans* isomerism and mixed chain length may be studied by a combination of spectral and relaxation methods, but very little is published so far.

Fat molecule diffusion rates affect nuclear relaxation, and thus diffusion can be measured by NMR using a method called pulsed field gradient NMR. Such experiments can be used to noninvasively examine the state of the sample. For example, oil molecule diffusion rates have been used to measure size distribution of emulsion droplets by pulsed field gradient NMR (15). Such measurements can be done on the water or the oil in both oil-in-water and water-in-oil emulsions.

Wide-line NMR

Rather than using tricks to produce a high resolution line from a broad one, it sometimes is possible to analyze the shape of a broad line for structural and motional information. For example, the carbon line widths of the conventional spectrum of a semicrystalline oil (Fig. 2, bottom) are certainly larger than those of the melted sample (Fig. 1). This is direct evidence for restricted motion in the liquid domains of the semicrystalline oil. The actual widths can be used to estimate motional rates.

Another example of the application of wide-line NMR is NMR of deuterium (^{2}H)-labeled molecules to study structure and dynamics of phospholipids in bilayers and membranes (16,17). We have not yet seen applications to fats. In principle, deuterium and carbon NMR also could be used to measure bond angles and distances in studies of triglyceride single crystals and their polymorphs, to supplement structural information obtained by x-ray diffraction.

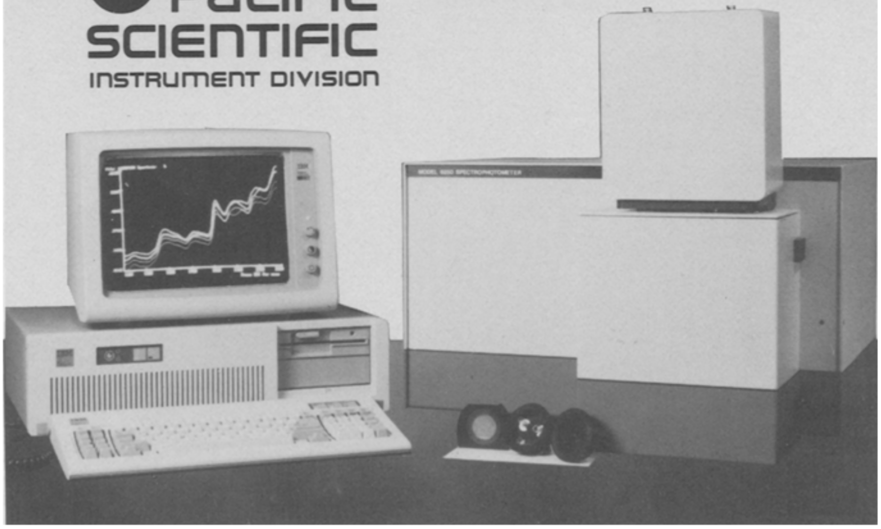
NMR experimental design

The questions to ask in designing NMR experiments always include:

(a) Do we have sufficient sensitivity? Are the concentrations high enough for the compounds we want to observe? Does the nucleus we wish to observe have a large enough inherent sensitivity given those concentrations? In general, in FT NMR, signal-to-noise ratio (S/N) is proportional to the square of the number of scans. Thus, to double S/N, the experiment time must be quadrupled. S/N also is directly proportional to the sample concentration. Thus, the best way to increase S/N is to increase concentration.

(b) Do we have sufficient selectivity? Can we observe the signal of interest in the presence of other signals? A carbon spectrum, for example, would be quite crowded if the sample contained enough carbohydrate and protein to interfere with oil resonances. Selectivity can be improved by isotopic labeling or by a variety of other NMR tricks that allow certain resonances to be observed selectively.

(c) Can the experiment be designed to preserve the native state of the sample? A very important advantage of solids NMR is that whole foods or samples with several phases can be observed without



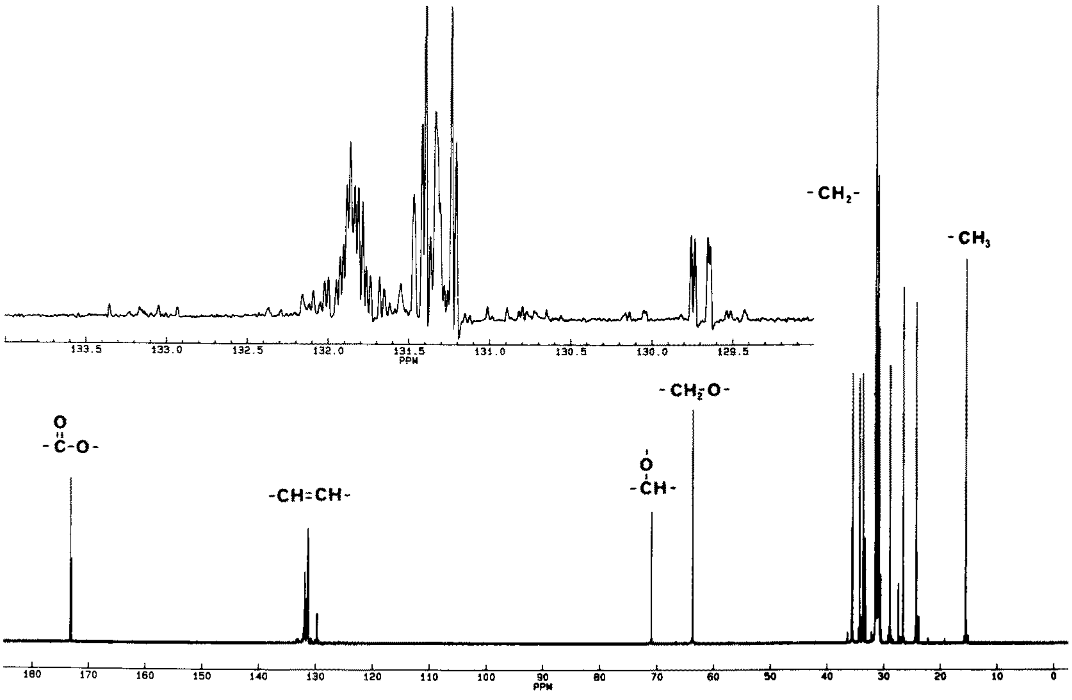


FIG. 1. 100 MHz ^{13}C NMR proton decoupled spectrum of a hydrogenated vegetable oil at 50 C. 1024 scans were obtained on 0.5 ml of the neat oil, using a 90 degree pulse, 5 seconds between pulses, and a WALTZ decoupling scheme. The relative intensity of the carbonyl carbons is attenuated due to rapid pulsing. The chemical shifts are estimated from the chemical shift of the carbonyl carbon of oleic acid in the 1,3 position of palm oil dissolved in deuterochloroform (5). Inset: unsaturated carbon region, plotted using Gaussian resolution enhancement.

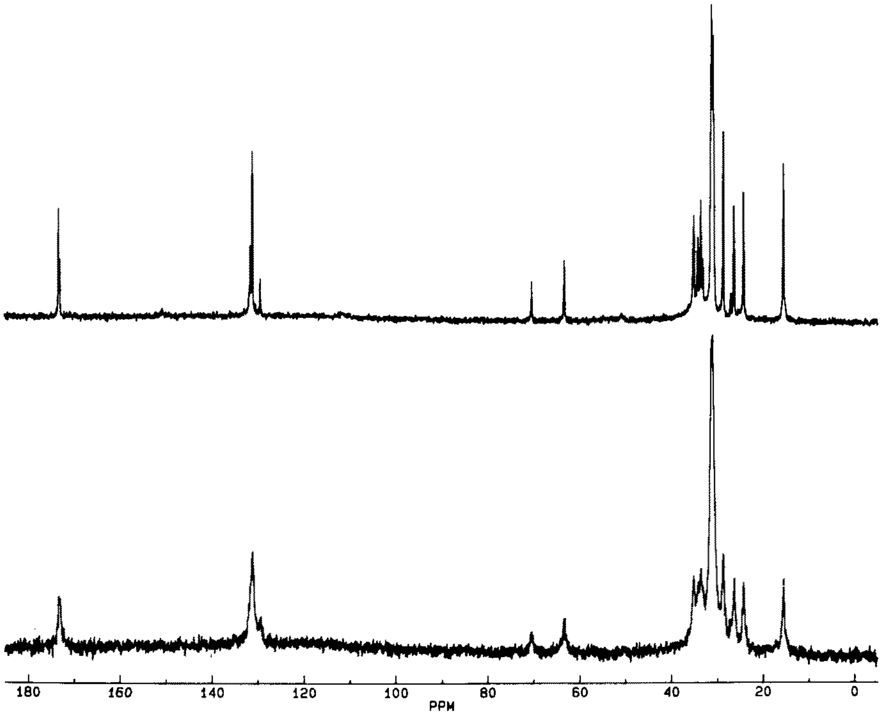


FIG. 2. 100 MHz ^{13}C NMR proton decoupled spectra of a blended margarine oil at 7 C. 128 scans were obtained on 0.5 ml of the neat oil using a 90 degree pulse and 5 seconds between pulses. Chemical shift referencing was accomplished as for Fig. 1. Bottom: low power broad band modulated proton decoupling, power level appropriate for high resolution ^{13}C NMR of liquids, static sample. Top: high power proton decoupling, power level appropriate for high resolution NMR of solids, magic angle sample spinning at 2 kHz.

dissolving, fractionating, heating or otherwise destroying the very structure of interest. In the case of fats, for example, the thermal history is not destroyed by the NMR experiment.

Conclusion

The techniques of pulsed NMR are well-known and widely used, but they do not provide detailed chemical information. High-resolution NMR is being applied to analysis of neat and dissolved liquid oils, and its potential is just being realized in industrial analysis and research. FT NMR relaxation has seen a few applications to molecular motion. With the exception of a few pioneering studies on solid triglycerides (12–14), the potential of high resolution solids NMR and wide-line NMR for chemical analysis and for the study of structure and dynamics in solid and semisolid fats largely is untapped.

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Methodology

Method development update

Collaborative studies

Four collaborative studies have been proposed. They are the following:

- Wax in sunflowerseed oil (nephelometry)
- Improving method Ca 3-46 (insoluble impurities) for the analysis of feedstocks
- Tecator block digestion/rapid steam distillation method for TKN (protein) analysis
- Validation of a sodium ion electrode method for sodium in fat- and oil-containing food products.

Project coordinators and collaborators have been identified for the wax in sunflowerseed oil and insoluble impurities methods; however, there is room for additional collaborators. The extent of interest still must be identified for the Tecator TKN and sodium ion electrode methods before any steps are taken to select project coordinators and collaborators.

Anyone interested in participating in any of these collaborative studies is asked to contact the AOCS technical director as soon as possible.

Technical interest groups

Under the new AOCS Governing Board structure, there is a new committee called the Technical Activities Coordinating Committee. This committee is chaired by John Heilman of Continental Grain. The chairmen of the various AOCS technical programs—Smalley, Uniform Methods Committee (UMC), Examination Board and Technical Interests—are a vital part of this committee.

Ted Matson of Vista Chemical is the chairman of the Technical Interests Committee. The purpose of this committee is to define technical interest areas/topics, for which people can meet on an informal basis (at AOCS national meetings) and hold discussions about various elements of technical importance (new methods, problems, concerns). These discussion groups are not