The Use of a High Resolution NMR Spectrometer Controlled by a Dedicated Computer for Quantitative Analytical Chemistry[1]

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Abstract

Operational complexity of high resolution NMR spectrometers has severely limited their use in quantitative analysis in spite of the many inherent advantages of the NMR technique. In order to demonstrate the feasibility of routine analyses in a rapid and accurate fashion with no special demands on the skill of the operator, the authors have programmed a small general purpose computer to control an NMR spectrometer, maintain adjustment of critical operating parameters, acquire data, numerically integrate appropriate spectral regions, calculate results and errors, and print out a teletype record of results. The unsaturation in natural edible oils, expressed as the iodine number and a polyunsaturation factor, has been chosen to illustrate in a specific case the application of this general approach to the analysis of multicomponent mixtures. It is shown in this paper that iodine numbers obtained by NMR agree with those obtained by the classical titration method within two or three iodine numbers over a wide range of values. The effect of varying NMR instrumental parameter settings over a wide range has also been investigated and reported. In view of the results obtained in the present work, it appears that computers aided quantitative analysis using sophisticated analytical instrumentation such as NMR spectrometers, and should now be given serious consideration by many high volume, multicomponent analyses in the food, drug and chemical industries.

Introduction

Although it has been widely accepted as a structural tool, high resolution NMR spectroscopy has not come into routine use for quantitative analysis. While the reasons for this are complex, one factor frequently appears to be determinative, namely, that obtaining high quality quantitative analytical data from a high resolution NMR spectrometer has placed excessive demands upon the skill of the operator. Consequently, other analytical means are often used that are simpler, although more time consuming and lacking in the structural information that is obtainable from the NMR method. The ability of a small dedicated computer to control a high resolution NMR spectrometer, to acquire the data, and to calculate and print out the analytical results in a convenient form has led to a reevaluation of the usefulness of this instrument for quantitative analysis. If the spectrometer is controlled by a computer, the operational complexity of obtaining high quality data is greatly diminished or eliminated altogether, and the computation of results is carried out automatically with high precision. Two recent review articles by Hopkins (1) and Lustig and Moniz (2) have discussed a wide variety of applications where NMR is used in quantitative analysis.

The analysis of the NMR spectrum for edible oils (fatty acid triglycerides) has been reported by Hopkins and Bernstein (3). Johnson and Shoolery (4) determined the average molecular weight and degree of unsaturation in edible oils and related these values to the Wijs iodine value. The present work evaluates the computerization of the Johnson-Shoolery method using a high resolution NMR spectrometer controlled by a small dedicated computer.

Experimental Procedures

Instrumentation

The NMR spectrometers used in this work were the Varian A-60D and T-60 NMR spectrometers, both of which operate at 60 MHz in a field of 14,096

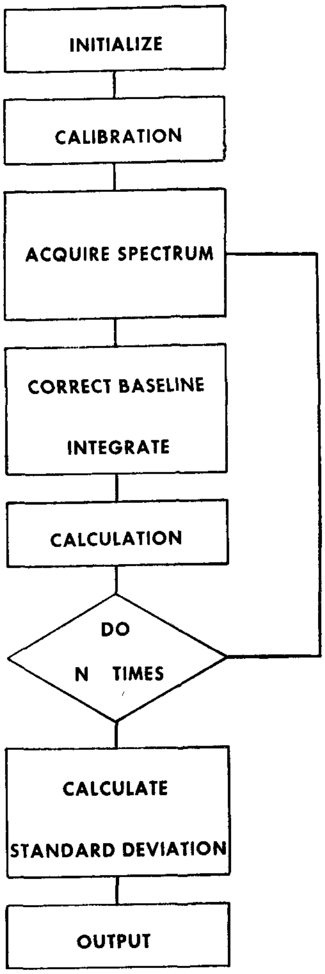

FIG. 1. The operation flow chart of the computer program for the analysis of edible oils by NMR.

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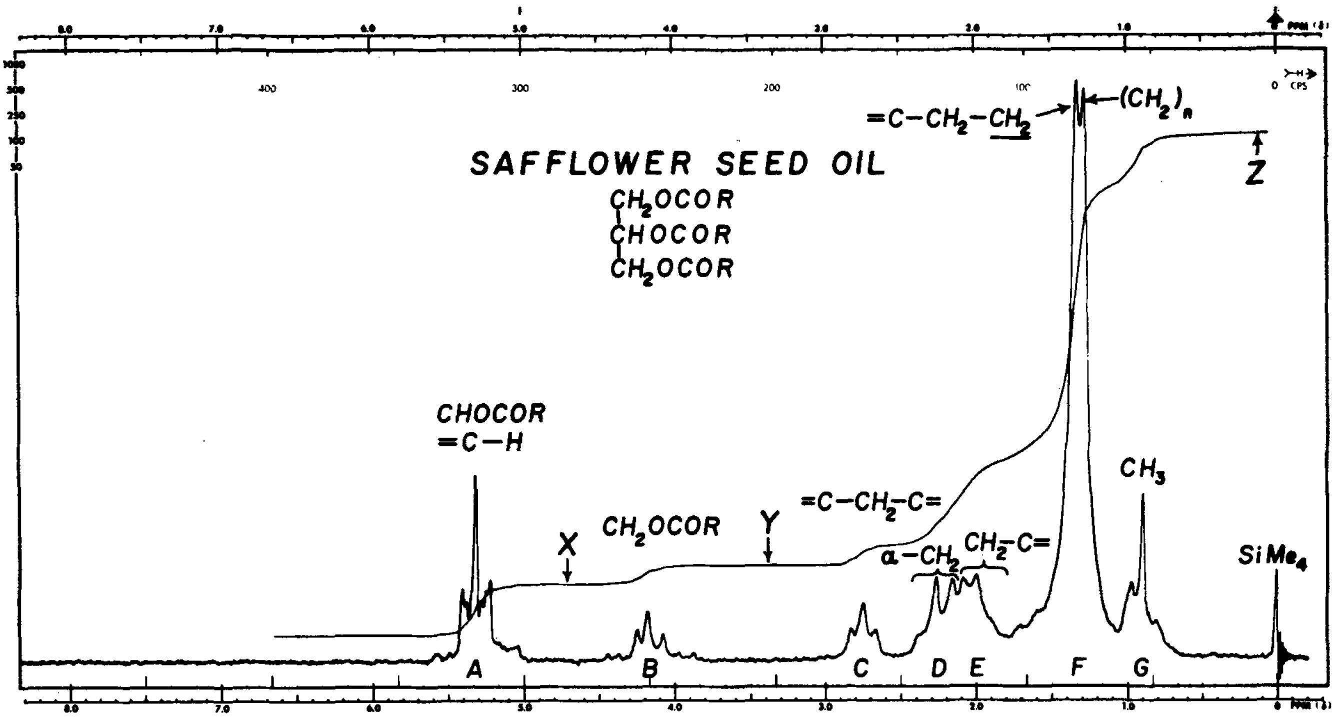

FIG. 2. 60 MHz spectrum of safflower seed oil (from Johnson and Shoolery). Note that in this work, $SiMe_4$ was not used in the samples as an internal reference.

oersteds. The spectrometers were controlled by a Varian Spectro-System 100 consisting of a Varian computer-spectrometer interface system (SIS), and a Varian Data Machines 620i computer.

Procedure

Samples of commercial grade edible oils were diluted to approximately 50% (vol) with Matheson Coleman and Bell spectro grade CCl_4. The solutions were introduced into 5 mm (o.d.) NMR tubes (Varian Part No. 905370–00), which were then inserted in the NMR spectrometer and the sample spinning rate adjusted to approximately 40 rps. Analyses were then carried out by the automated system using the following procedure. The operator depresses the "Carriage Return" key on the ASR 33 teletype (primary input/output device for the 620i computer) and the system responds by typing "Sample No." The operator now may type in any combination of letter or numbers to identify the sample. Sample identification is concluded by typing a "Period," which gives the computer control of the system and starts the analysis. At the conclusion of the analysis, the average molecular weight, iodine number, average number of vinyl groups and a polyunsaturation factor with their respective standard deviations are printed out on the teletype. The sample is withdrawn from the spectrometer, a new sample is inserted, and the routine repeated.

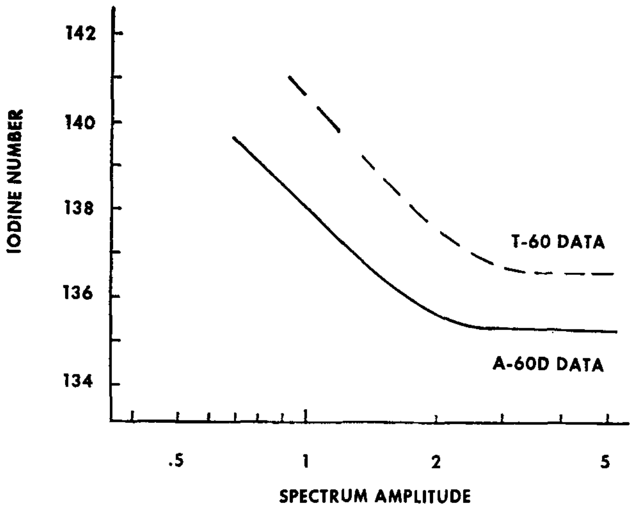

FIG. 3. The effect of spectrum amplitude on iodine number sunflower seed oil: INO = 134.6–136.0 by titration.

Computer Program

The logical flow chart for the generalized quantitative analysis routine is shown in Figure 1.

After the operator has depressed the "Period" key of the teletype, the computer takes control of the spectrometer, deactivates the teletype to external control and sets the instrumental parameters for scanning the spectrum. The detector zero, spectrum amplitude, spectrum phase control and RF irradiating power are still controlled by the operator. The routine initializes all temporary memory locations and parameters and calibrates the spectrometer sweep circuit and the SIS sweep output digital-to-analog converter (DAC).

In edible oils, the absorption due to the methylene groups of the fatty acids has a chemical shift of 1.3 to 1.4 ppm (75 hertz at 60 MHz) downfield from the position where the internal reference compound, tetramethylsilane, would be found if it were dissolved in the sample. The computer scans through the region where this absorption is expected, finds the highest peak and sets it to 75 Hz. Any subsequent scan by the computer is referenced to this calibration and the appropriate offset to the sweep DAC's is calculated. Next, the computer acquires the spectrum for the sample by scanning the spectrum from 7.17 ppm to −0.67 ppm (430 Hz to −20 Hz) in 18 sec, digitizes and stores the spectrum in 1024 words in memory. Figure 2 shows a spectrum of a typical edible oil.

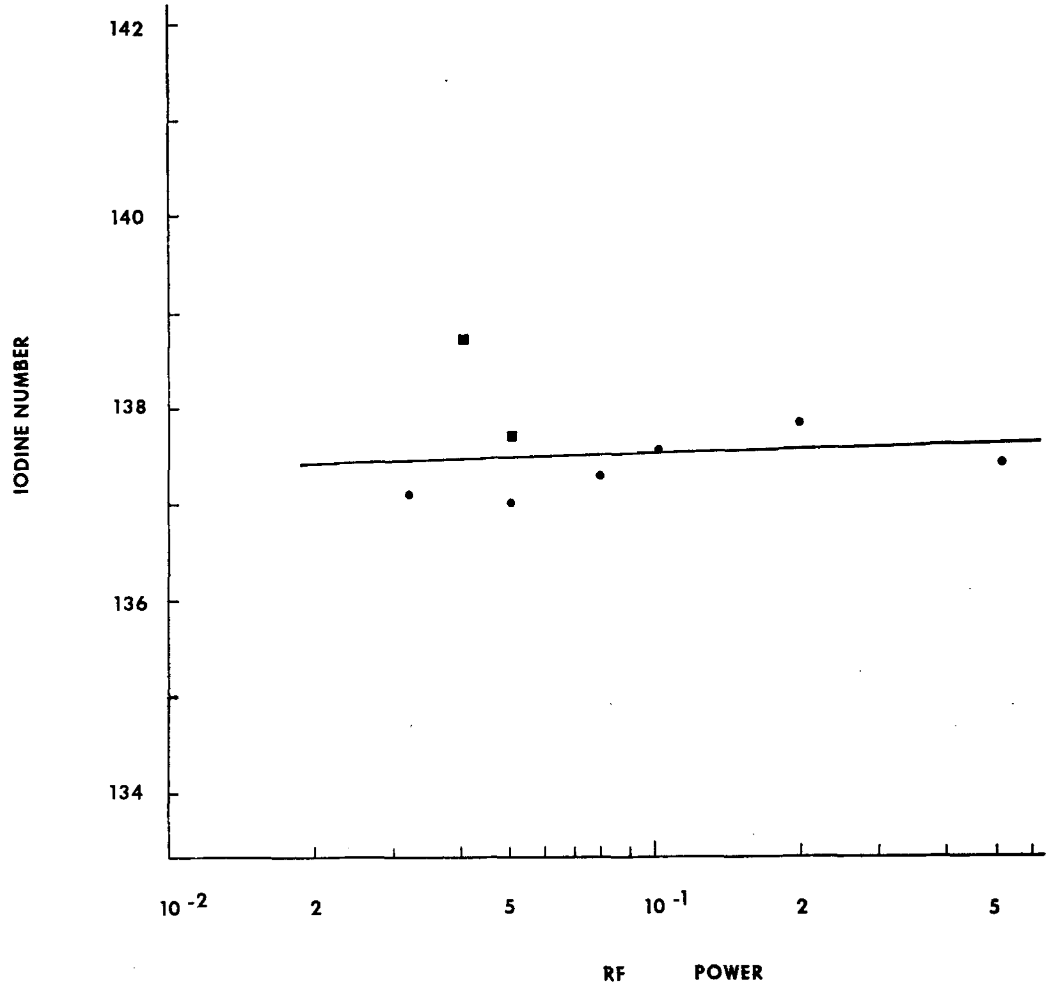

FIG. 4. The effect of rf power on iodine number sunflower seed oil: INO = 135.6–136.0 by titration, solid squares T-60 data, solid circles A-60D.

After the spectrum is stored in memory the computer calculates the average value of a word using the first 128 words of the spectrum. This is a region of the spectrum where there are no signals. The baseline is adjusted by subtracting this value from every word in the spectrum. The spectrum is then digitally integrated and the integrals are stored for the following five regions: A, 7.17 to 4.37 ppm; B, 4.37 to 3.50 ppm; C, 3.50 to 2.55 ppm; DE, 2.55 to 1.80 ppm; and FG, 1.80 to 0.33 ppm.

Calculation

It follows from Johnson and Shoolery (4) that given the five integrals (Fig. 2, A, B, C, DE and FG); the area per proton, X, is B/4 which is simply the absorption due to the glyceride methylene protons divided by four. The number of olefinic protons, V, is

$$\frac{4\,(A)}{(B)}-1 \qquad [1]$$

the number of protons on a methylene group adjacent to two vinyl groups, P, is

$$\frac{4\,(C)}{B} \qquad [2]$$

and the total number of protons, T, is

$$\frac{[(A)+(B)+(C)+(DE)+(FG)]4}{(B)} \qquad [3]$$

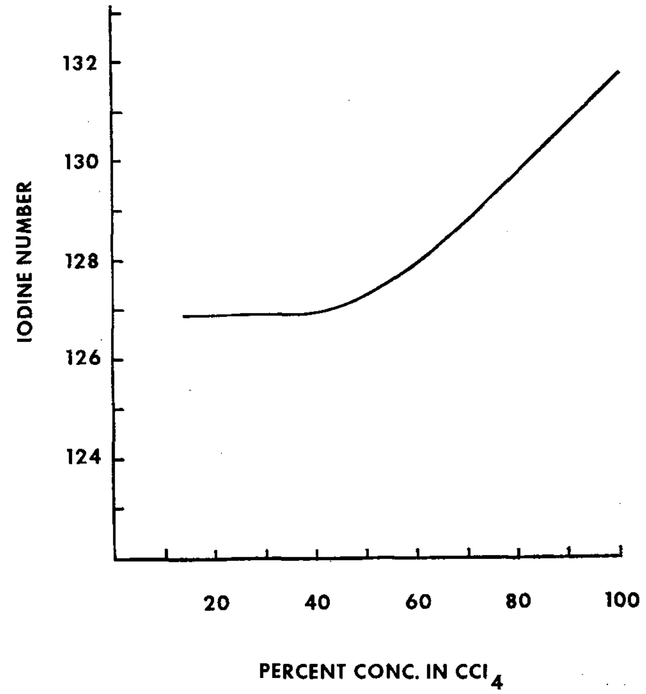

FIG. 5. The effect of concentration on iodine number corn oil: INO = 127.5–128.2 by titration.

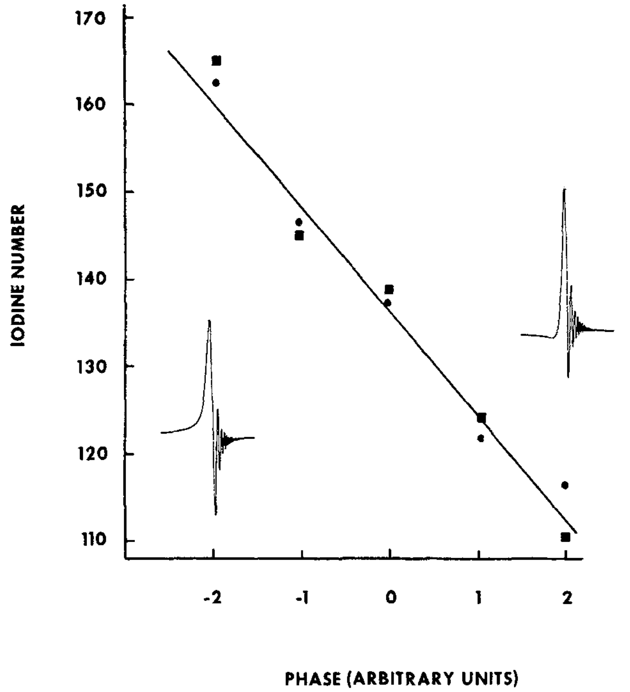

FIG. 6. The effect of phase on iodine number sunflower seed oil: INO = 135.6–136.0 by titration.

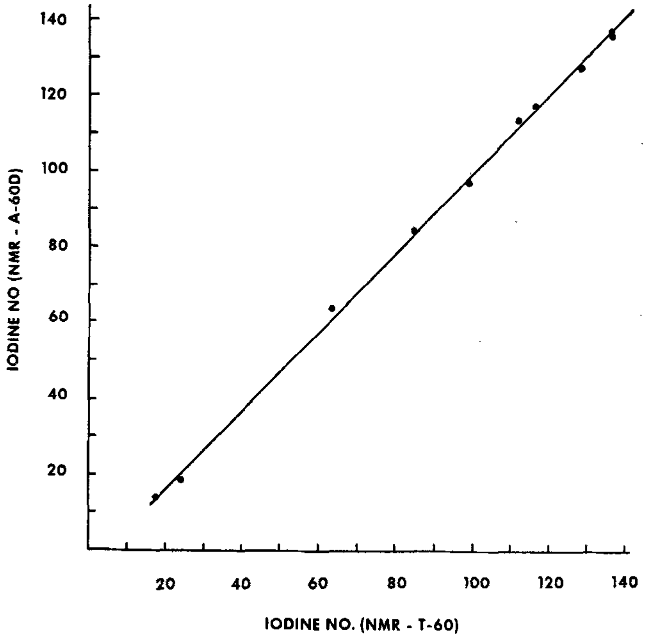

FIG. 7. Determination of iodine number by NMR.

Using these relationships and making corrections for the natural abundance of C_{13} (4) the following values are calculated and stored in the calculations portion of the computer program:

$$\text{Avg. Mol. Wt.} = 120.0 + 7.013\,T + 6.006\,V \qquad [4]$$

$$\text{Iodine Value} = \frac{(12792)(V)}{\text{Avg. Mol. Wt.}} \qquad [5]$$

$$\text{Avg. No. of vinyl groups} = \tfrac{1}{2}V, \text{ and polyunsaturation} = 2P/V \qquad [7]$$

Where

$$V = \left[\frac{(A)-(B)(5.5\times10^{-3})}{X}\right] - 1 \qquad [8]$$

$$P = \frac{(C)-[(B)+(DE)](5.5\times10^{-3})}{X} \qquad [9]$$

$$T = \frac{(A)+(B)+(C)+(DE)+(FG)}{1.011\,X} \qquad [10]$$

and

$$X = \frac{(B)-[(A)+(C)](5.5\times10^{-3})}{4} \qquad [11]$$

The program now acquires another spectrum, calculates another set of values, and stores them as partial sums and partial sums of the squares. In principle, this can be done any number of times but in practice, the number of repetitions is five. Finally, the averages and standard deviations are calculated and are typed by the teletype.

The five-run analysis takes less than 5 min. The computer program requires less than 2000 words of memory and thus fits conveniently in the basic Varian Spectro-System 100 with 4096 words of memory.

Discussion

Iodine values have been calculated for a series of edible oils using data from both 60 MHz NMR spectrometers. Since the complete analysis, i.e., acquisition of data to calculation is computerized, it has been possible to investigate quickly and in some depth how the instrumental parameters for an NMR spectrometer affect the accuracy of the results.

Figure 3 shows the effect of the spectrum amplitude control setting for both the A-60D and T-60 on the iodine number of sunflower seed oil.

It is seen from this figure that as the spectrum amplitude becomes smaller (the degree of amplification of the RF receiver signal from the detector) there is a small increase in the calculated value of the iodine number. Experimentally, there is a wide region where the differences in the iodine value data from the A-60D and T-60 spectrometers fall within the standard deviation of the measurements and the values themselves fall within one unit of the titrated value for the iodine number. Consequently, the setting of this control is not a critical factor in the analysis.

The effect of RF (irradiating) power on the iodine number (Fig. 4) shows little, if any, variation over a large range. The effect of concentration on iodine number (Fig. 5), shows a rather large "flat" region, approximately 20–50%, where the iodine value is not affected by concentration. Above 50% concentration, ADC overloading on the large methylene peaks gives a low value for the total absorption and consequently a low molecular weight. This leads to a high value for the iodine number.

The instrumental parameter that has the greatest effect on the iodine value is the rf phase angle. An NMR signal is a mixture of an absorption mode signal and the dispersion mode signal. In quantitative work, the spectrometer should be adjusted so that the dispersion mode signal is zero. Figure 6 shows how the iodine number for sunflower seed oil varies over a range of phase settings. The NMR curves shown on this figure are recorded for $CHCl_3$ with the instrumental phase control displaced from the optimum setting by −2 and +2 arbitrary phase units (1 arbitrary phase unit represents a phase angle of approximately 10°). It is clear that, with a change of one iodine number for each degree of phase misadjustment, this can be a serious source of error. The proper phase can be obtained experimentally quite successfully, however, by adjusting the phase

TABLE I
Summary of Results

Natural oil (refined and bleached)	Iodine No. (titration)		Iodine No.[a]		Avg. No. groups vinyl[b]	
	Plant A	Plant B	A-60D	T-60	A-60D	T-60
Coconut	8.6	8.8	12.1	17.8	0.3	0.4
Palm kernel	17.0	16.6	18.1	23.0	0.5	0.5
Soybean	61.6	61.7	64.7	63.8	2.3	1.9
Soybean	81.8	81.9	83.9	83.7	2.9	2.6
Peanut	96.0	96.0	97.9	98.9	3.4	3.1
Cotton seed	110.4	110.2	112.7	112.3	3.8	3.4
Soybean	118.9	117.2	117.0	117.3	4.0	3.8
Corn	127.5	128.2	128.4	128.8	4.4	4.2
Soybean	133.0	133.1	137.3	138.9	4.9	4.5
Sunflower	135.6	136.0	137.1	137.9	4.7	4.4

[a] Standard deviation, 1.4 iodine number.
[b] Standard deviation, 0.1 to 0.2.

for a known standard sample until the teletype prints the correct iodine number. This is the procedure which was used to set the phase in all experiments for this work. After the phase is set, no further adjustment of this control is necessary over an 8–10 hr time period.

Figure 7 and Table I shows the correlation of iodine numbers with the A-60D and T-60 NMR spectrometers as well as the correlation of the NMR results to the values determined by titration (AOCS official method Cd 1–25). The Figure indicates an excellent agreement between the two spectrometers. At low iodine numbers the T-60 NMR spectrometer is slightly biased toward higher iodine numbers than those obtained from either the A-60D or by titration. The table compares titration iodine values from two different laboratories with values obtained by NMR. The slightly higher iodine number values by NMR are consistent with the comparison of titration values to iodine values obtained by Beadle et al. (5) using vapor phase chromatography. Further it is reported (Conway, private communication) that Wijs iodine numbers can be affected by the choice of reaction times or the ratio of iodide to chloride ion concentrations or both.

Precision and Errors

A sample of pure trilinolein (Applied Science Laboratories, Inc., Lot No. 0006) was prepared and analyzed by this method. Fifty individual analyses were made and the following results (and standard deviations) were obtained. The instrument used was the A-60 with rf power level set at 0.05 milligauss, spectrum amplitude setting 2.5, and filter bandwidth at 4 Hz: average molecular weight, 875.4 ± 30.9 (Theor. 879); iodine number, 174.2 ± 1.8 (Theor. 173.3); average number of vinyl groups, 6.01 ± 0.24 (Theor. 6.00); and polyunsaturation, 0.99 ± .02 (Theor. 1.00).

Several possible sources of errors have been considered during the progress of this work. The most important is the phase setting, for which the degree of error and how it is minimized has previously been discussed. Magnetic inhomogeneity can introduce another significant error in that the presence of linear field gradients results in spinning sidebands which appear as small signals on either side of a peak at a distance equal to the frequency of sample rotation. Their amplitude may approach several per cent of the main peak and if they fall into a different integral region of the spectrum than the parent peak, they lead to errors in the calculated results. For example, with 2% spinning sidebands, the average NMR molecular weight for trilinolein was measured as 870 (Theor. 879) and the iodine number at 171.0 (Theor. 173.8). As the spinning sidebands increase, both the molecular weight and iodine value decrease still further. It is normally not difficult to maintain instrument adjustment so that spinning sidebands do not exceed 2%. An alternative approach would be to determine the errors in the iodine numbers for primary standards as a function of spinning sideband amplitude and program the computer to correct for this effect.

ACKNOWLEDGMENTS

The Varian Data Systems group assisted. T. F. Conway, Moffett Technical Center, Corn Products Company International, furnished the samples of commercial edible oils and the Wijs Iodine values.

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[Received June 16, 1969]