Determination of Oil in Aqueous Emulsions by Wide-Line NMR[1]

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ABSTRACT

The radio frequency (r-f) saturation method was shown valid for determination of four edible oils in aqueous emulsions but not in commercial margarine. The interference in case of margarine was shown to be due to nonfat solids. The signal from water at low RF level was eliminated by converting to water of crystallization. Of three anhydrous salts studied for this purpose, only magnesium perchlorate was satisfactory. The method could be made quantitative for oil in margarine only by addition of carbon tetrachloride to the margarine before addition of magnesium perchlorate.

INTRODUCTION

The margarine industry has great need for a rapid and accurate method for measurement of oil content of its products to allow control of oil content at minimum standards; wide line NMR should be a suitable method. Several techniques have been mentioned as possibilities for measurement of oil in presence of water using this instrument (1-3): drying the sample to 4% moisture, r-f saturation, paramagnetic additive and freezing.

The effect of the radio frequency (r-f) level on determination of oil in aqueous emulsions and application of information obtained to the development of a quantitative determination of oil in margarine were studied.

MATERIALS AND METHODS

NMR Spectrometer

The wide line NMR Spectrometer used for this work was a Varian Model PA-7 Process Analyzer equipped with an electronic integrator Model V4221. Sample weight was dialed into the weight setting control so the NMR integral signal readout obtained was on a unit weight basis, arbitrary NMR Units per gram sample. A 2.5 ml or a 40 ml probe was used with this instrument. The temperature of the sample was controlled by a Variable Temperature Accessory Model V-4540. Only the small probe could be used with this. The instrument parameters used were as follows: time constant, sec, 0.5; sweep time, min, 0.5; sweep amplitude, gauss, 2.0; sensitivity, variable; modula-

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tion amplitude, gauss, 0.5; r-f attenuation, variable; integrator threshold, mu, 0.1; integrator signal multiplier, 1; integrator readout multiplier, variable.

Materials

Corn, soybean, cottonseed and safflower oils were used for oil-water mixtures and emulsions. All were winterized. Span 80 was used as an emulsifying agent for water in oil (w/o) emulsion and Tween 60 was used for oil in water (o/w) emulsion. The mixtures were emulsified in a bottle with an ultrasonic vibration device. Samples of each emulsion were transferred to test tubes for NMR readings.

The commercial margarine was supplied by the quality control laboratory of a manufacturer. Each sample was the remainder of a coded unit that had been analyzed by that laboratory. The oil contents, reported here (4) were also provided by that laboratory. (Kraft Foods Co., Champaign, Ill.)

Procedures

For NMR readings, the sample was added to a tared 2.5 or 40 ml sample tube. Only the work done with pure water, corn oil and oil-water emulsions was done with the 40 ml tube at room temperature. All other work was with the 2.5 ml tube at 40 C. Sample weight was determined to the fourth decimal place and dialed into the integrator. The mv/g signal at the desired r-f level was recorded.

When inorganic salts were used in the system, about one gram of the salt was added to the weighed sample, 0.2 to 0.5 g in case of the 2.5 ml sample tube, and mixed by shaking for 10 min. When CCl_4 was used, 1 ml was added to the weighed sample, 0.2 to 0.3 g, and the sample was dispersed by slowly shaking the tube. When NMR readings were taken at elevated temperature, the weighed samples were held in a 40 C water bath for 30 min before analysis and the probe was controlled at 40 C.

RESULTS AND DISCUSSION

The first approach taken was the r-f saturation method. It is known that as the r-f level is increased from 36 db to 4 db, the NMR signal will be reduced in direct relation to the reduction in mobility of the hydrogen nuclei (1). This phenomenon is demonstrated by our data in Figure 1. The corn oil signal is always higher than the water signal because it has more hydrogen nuclei per gram of sample.

Of great interest in Figure 1 is the finding that the signal for water almost disappeared at the highest attenuation, 4 db, while the signal for corn oil at 4 db was still one third the maximum value obtained at 28 db. Thus, in case of an

TABLE I

NMR Signal for Water at High RF Level in Presence of Skim Milk Powder (SMP) and Sodium Chloride

Sample	NMR units at RF 4 db		
	Per gram sample	Expected due to water per gram sample	Increase in signal due to solids per gram sample
100% Distilled water	1.0	1.0	---
100% SMP	0.0	---	---
9% SMP + 91% water	2.35	0.91	1.44
10% NaCl + 8% SMP + 82% water	6.95	0.82	6.04

11
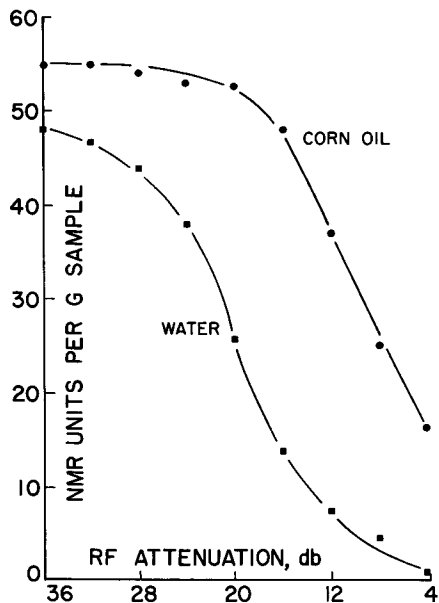
FIG. 1. Effect of r-f level on NMR signal from corn oil and water.

FIG. 2. NMR signal at high r-f level (4 db) from edible oils in oil-water emulsions.

FIG. 3. NMR signal at high r-f level (4 db) and 40 C from oil in margarine samples taken from same production line at different times.
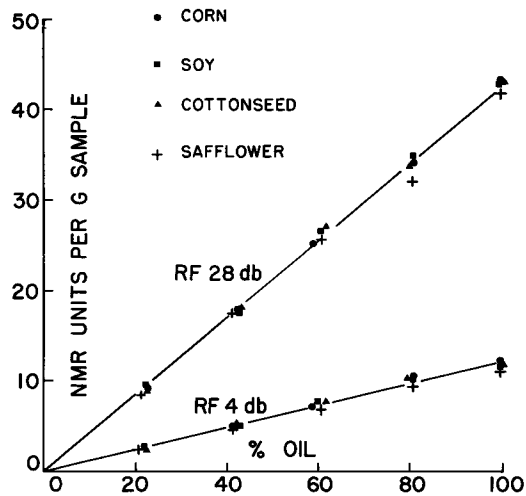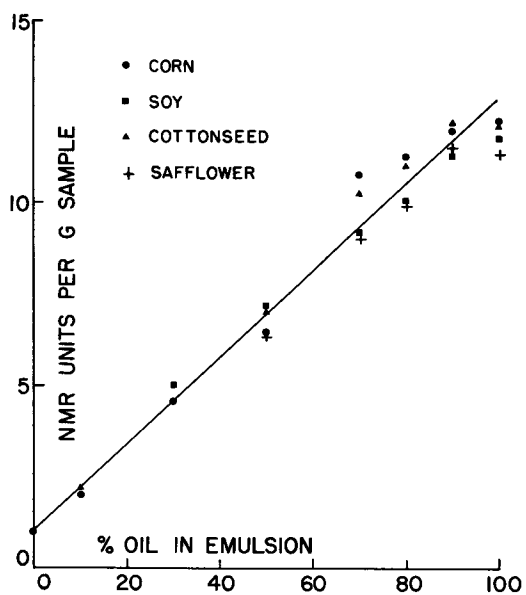

FIG. 4. NMR signal at two r-f levels (low, 28 db and high, 4 db) from edible oils in presence of inert material.
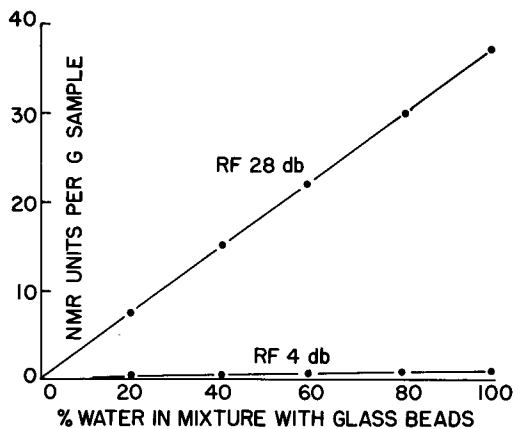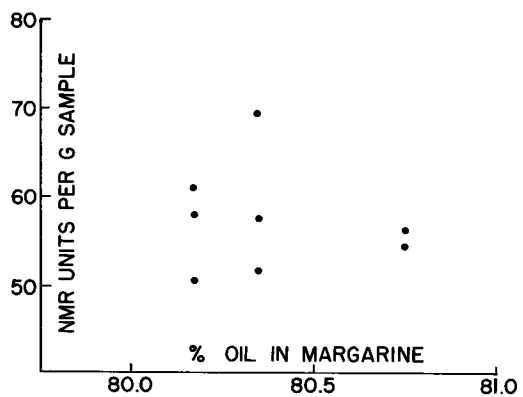

FIG. 5. NMR signal at two r-f levels (low, 28 db and high, 4 db) from water in presence of inert material.
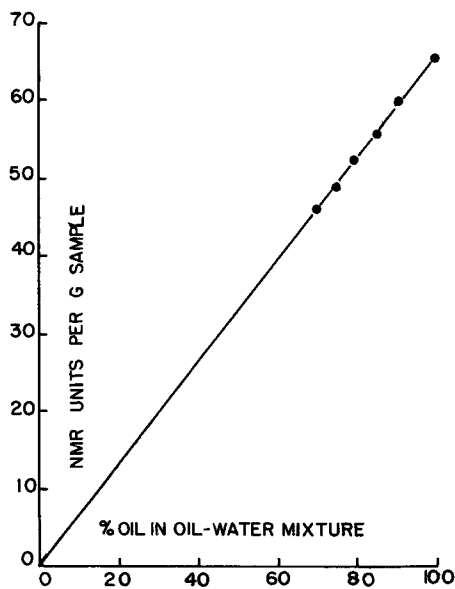

FIG. 6. NMR signal at low r-f level (28 db) at 40 C from soy oil in oil-water mixtures that have been taken up in carbon tetrachloride and anhydrous magnesium perchlorate added.
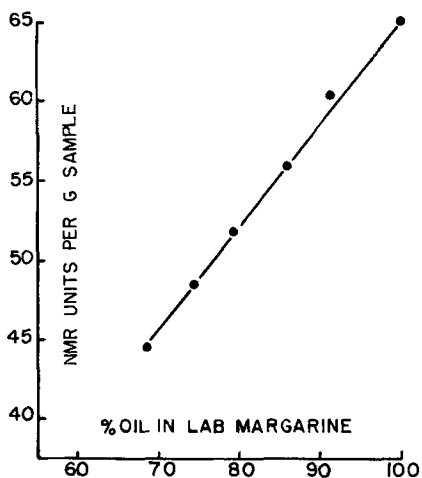
FIG. 7. NMR signal at low r-f level (28 db) at 40 C from the soy oil in laboratory margarine taken up in carbon tetrachloride and anhydrous magnesium perchlorate added.
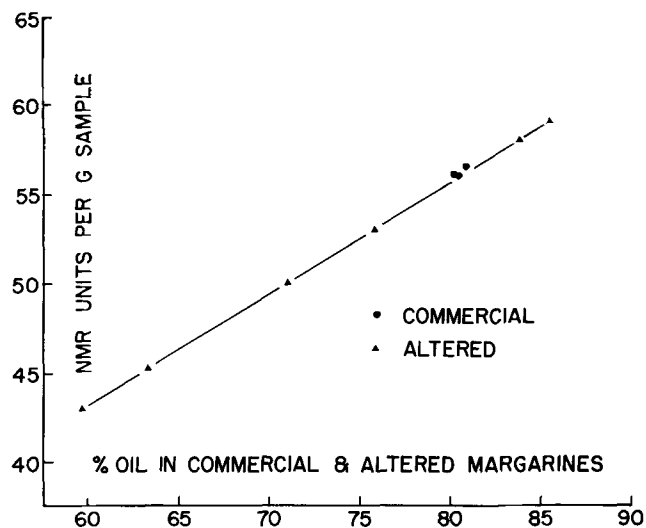

FIG. 8. NMR signal at low r-f level (28 db) at 40 C from the oil in margarine samples obtained by diluting a commercial margarine with varying amounts of water or oil.

emulsion, a reading at 4 db should measure oil only and another reading at 28 db will indicate a weighted mean of oil plus water.

This technique was applied to determination of four different oils in oil-water emulsions. The data and their regression line are shown in Figure 2. Theslope was 11.55, intercept 1.14 and the coefficient of determination was 0.985, indicating excellent agreement to the linear relationship. Deviations from the line are probably due to variation in molecular weight and degree of unsaturation. For better accuracy a calibration line should be obtained for each oil used.

When this r-f attenuation technique was applied to commercial margarine samples taken from the same production line at different times of the day, the data in Figure 3 were obtained. A calculated regression line showed a slope of -76.8, an intercept of 76.0 and a coefficient of determination of 0.0162, showing a very poor, negative correlation. Therefore, this technique cannot be used for margarine, and the r-f attenuation technique was investigated using model systems.

Since margarine contains milk solids and salt in addition to oil and water, the former two components were investigated for interference at r-f 4 db by means of model systems. As shown in Table I, addition of skim milk powder to water in the same proportion as that found in margarine, gave about 150% increase in signal above that expected. When both salt and milk powder were added to water in the proper proportions, the increase in signal was over 600%. Thus, if the signal contributed per unit weight of the interferring substances and their weight present were known, it might be possible to apply corrections to margarine readings. However, the method would no longer be rapid.

It was desired to determine whether the interference was peculiar to margarine additives or any solids would give similar results. Therefore, four oils were mixed with varying

amounts of glass beads, representing an inert solid, and readings taken at r-f 4 db and r-f 28 db. As shown in Figure 4, the NMR signals for all four oils fell on two straight lines, one for each r-f level. The next step was to do the same with water. Figure 5 shows the results obtained, a straight line relationship, were as expected. Therefore, it was concluded that the interference was due to margarine solids.

The next approach was to return to the low r-f level (28 db) and try to eliminate the NMR signal from the water by converting it into crystallization water (4). When anhydrous Na_2SO_4 was added to margarine components the signal was increased by about 5% over that of the control so this salt was discarded as a possibility. Addition of anhydrous $CaSO_4$ in a similar experiment showed a reduction in signal for all the samples. This indicated the crystallization of some water. However, the reduction in signal was not proportional to the total amount of water present, so this salt was discarded.

Experiments made with the addition of anhydrous Mg $(ClO_4)_2$ to margarine components show that a marked reduction in signal was obtained (Table II). This indicated that Mg $(ClO_4)_2$ can eliminate the signal due to water. However, the reduction in signal was proportionately greater than the amount of water present in the system. Since this system is semisolid, not only all the water but some of the oil was held in the matrix, thus reducing the mobility of some of the H atoms in the oil and giving a reading below that expected for the oil present.

To increase the contact between Mg $(ClO_4)_2$ and water in the sample and to keep the H in the oil samples mobile, margarine components were taken up in CCl_4 (5) before addition of Mg $(ClO_4)_2$. Also, the samples were adjusted to 40 C by placing them in a hot water bath for 30 min; the probe was kept at this temperature during measurement to

TABLE II

NMR Signal at Low RF Level From Margarine Components as Affected by Presence of Anhydrous Magnesium Perchlorate

Sample	NMR units at RF 28 db per gram sample	
	Without MG $(ClO_4)_2$	With MG $(ClO_4)_2$
83.5% Oil + 16.5% water, layered	39.8	27.0
83.5% Oil + 16.5% water, emulsion	38.2	27.15
82% Oil + 16.4% water + 1.6% SMP, emulsion	38.7	29.20
80% Oil + 16.4% water + 1.6% SMP + 2% NaCl, emulsion	37.6	30.45

TABLE III

NMR Signal at Low RF Level From Water
and From Soybean Oil as Affected by
Presence of Carbon Tetrachloride and
Anhydrous Magnesium Perchlorate

Reagents	NMR units at RF 28 db per gram sample		
	Alone	In water	In oil
None	---	63.2	68.1
CCL$_4$	0.0	118.0	122.0
Mg(ClO$_4$)$_2$	0.0	0.0	68.0
CCl$_4$ plus Mg(ClO$_4$)$_2$	---	0.0	122.0

assure that all the margarine oil was in the liquid state. NMR signals obtained from water and oil in the presence of CCl$_4$ and Mg (ClO$_4$)$_2$ singly and together, are given in Table III. Addition of CCl$_4$ to either H$_2$O or oil almost doubled the NMR signal because of its effect on relaxation time. Addition of Mg (ClO$_4$)$_2$ completely eliminated the water signal either with or without CCl$_4$. Furthermore, addition of Mg (ClO$_4$)$_2$ to either oil alone or oil dissolved in CCl$_4$ had no effect on the oil signal. This technique was further investigated with oil-water mixtures containing 70% to 100% oil. Figure 6 shows the signal obtained at r-f 28 db and 40 C when the mixture was taken up in CCl$_4$ and anhydrous Mg (ClO$_4$)$_2$ added. The relationship between per cent oil and NMR units was a straight line passing through the origin. This showed that oil can be quantitatively determined in the presence of water by this technique.

Margarine samples containing 68% to 91% oil were prepared in the laboratory and determined for oil. The results, plotted in Figure 7, showed a straight line relationship between oil content and NMR signal.

Four commercial margarine samples were taken and one was altered by addition of either water or soy oil so that oil varied from 59% to 86%. Figure 8 shows the NMR signal plotted against the per cent oil in the commercial and altered margarines. The straight line relationship obtained indicates the possibility of using this technique for accurate determination of oil in margarine.

However, due to the weak field of the instrument, 1715 gauss, and the small weight of margarine that could be taken as a sample, 0.3 g, the accuracy in this case was only about ± 0.5% oil. Present accuracy in the margarine

industry is about the same. New instruments available today have more powerful magnets and should give accuracies of ± 0.1% oil. Then, continuous passage of a substream through the instrument with readings taken at frequent intervals and fed back to automatic controls may allow continuous margarine production with very close oil tolerances.

The method developed in this study should be applicable to other emulsion products such as mayonnaise and salad dressing as well as to margarine.

PROCEDURE FOR OIL IN MARGARINE

A recommended procedure for oil in margarine is based on instrumentation available to us. A larger sample size and stronger magnet should be used for greater accuracy.

For a 2.5 ml sample tube tare, add 0.2 to 0.3 g margarine to the bottom of the tube, and reweigh to 0.1 mg. Transfer of the sample must be made without coating the tube above the 2.5 ml mark where it will contribute to weight but not proportionately to NMR signal. Add 1 ml CCl$_4$ and slowly shake so that all the sample remains below the 2.5 ml mark. Slowly add 0.8 to 1.2 g powdered anhydrous Mg (ClO$_4$)$_2$. Bring sample temperature to 40 C and place sample in probe controlled at 40 C. Take NMR reading at a low r-f level such as 28 db. The weight per cent oil in the margarine is read from a calibration curve obtained under the same sample conditions, especially temperature instrument parameters and r-f level. For highest accuracy a new calibration curve should be obtained whenever there is a change in kind or proportion of oil used as raw material.

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