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⁂Estimation of Solid-Liquid Ratios in Bulk Fats and Emulsions by Pulsed Nuclear Magnetic Resonance

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ABSTRACT

Several pulsed nuclear magnetic resonance (NMR) methods were evaluated to estimate the solid fat content of fats and oil-in-water emulsions. The methods were checked with samples of paraffin oil or triolein containing known quantities of crystalline tristearate. A method based on the signal of solid fat (with use of a correction factor, the "*f*-factor") was rejected in this work for general use. Correct results were obtained with methods that used only the signal of the liquid phase. With emulsions, disturbances could arise due to the surfactant present and to possible solubilization of water in the oil phase, presumably by monoglycerides. Without these disturbances, solid fat content in emulsions could be estimated as in bulk fats, after correction of the liquid phase signal for the contribution of protons from the aqueous phase. The signal from fat crystals inside emulsion droplets differed from that of crystals of the same fat in bulk, which may have been due to difference in crystal size but not to difference in crystal modification. Measurements on natural cream showed that disturbances were also possible in this type of emulsion.

INTRODUCTION

Knowledge of the solid fat content of fats and emulsions is important in the food industry as well as in research. Of the methods available for measuring the solid fat content, pulsed nuclear magnetic resonance (NMR) is the most promising (1,2). The method is based on the difference in spin-spin relaxation time T_2 of hydrogen nuclei in the solid and liquid states, on the assumption that differences in proton content of solid and liquid fats are negligible (as is usually the case for natural fats).

The magnetization decay after a 90° pulse allows estimation of the solid fat content directly at one temperature, but a correction factor (the "*f*-factor") is needed since the signal can only be measured some 10 μsec after the pulse. The solid fat content can also be estimated indirectly by taking the signal of the liquid phase into account at a temperature at which the solid fat is dissolved, analogous to the continuous-wave wide-line NMR method (3).

However, the pulsed NMR method can introduce errors if the *f*-factor is used. The use of a mean *f*-factor is justified when it is estimated for a specific group of fats exposed to the same temperature treatment, e.g., margarine fats (1). Care should be taken, however, not to use this mean *f*-factor for other fats or for fats that are exposed to a different temperature treatment. The *f*-factor depends on adjustment of the equipment and on mobility of protons, hence on fat composition, formation of compound crystals, polymorphism, crystal size and temperature (1,3,4).

An attempt has been made (5) to avoid use of the *f*-factor by extrapolation of the free induction decay after a 90° pulse to time zero. Since the exact shape of a signal after a pulse is unknown, this method should be discouraged. In our opinion, use of this erroneous method caused, at least in part, the large discrepancy with other methods (5).

Estimation of the solid fat content in emulsions is hampered by the contribution of protons of the aqueous phase to the NMR signal. Saturation may offer a possibility of distinguishing the aqueous phase from the oil phase (6). To suppress the water signal significantly, however, fat protons will also be saturated somewhat. Another suggestion was addition of paramagnetic ions, which lower the relaxation time of water so that oil and water protons can be distinguished by their relaxation times (7). Addition of ions to an emulsion, however, could cause its instability. A possibility that remains is to correct the signal of the liquid phase by subtracting the contribution of the aqueous phase. This contribution can be calculated from the fat content of the emulsion when the signal of unemulsified aqueous phase has been measured separately. Trumbetas et al. (8) did so, but they used a very short trigger time (i.e., the time between pulses), namely 100 msec, so that fat signals must also have been partly saturated. Oil signals become saturated when the trigger time is below 800 msec.

The aim of this study was to evaluate several pulsed NMR methods for estimation of the content of solid fat in bulk fats as well as in oil-in-water emulsions containing fat crystals in the dispersed phase. The methods were checked with samples containing oil and tristearate crystals; the content of solid fat in these samples was precisely known since the solubility of tristearate in oil is known.

EXPERIMENTAL PROCEDURES

Materials

Paraffin oil (Ph. Ned. VI, density 860 kg m^{-3}, viscosity 68-81 mPa sec) was obtained from Lamers & Indemans, 's-Hertogenbosch, The Netherlands.

Triolein was supplied by K&K Laboratories Inc., USA (no further specifications available).

Tristearate was kindly supplied by P. de Bruyne and D. Waddington of Unilever Research, Vlaardingen, The Netherlands. We found that 99.3% of the fatty acids in this triglyceride was stearic acid, and the content of mono- and diglycerides was less than 0.5%.

Deuterium oxide (D_2O) was obtained from Biorad Chemicals.

The surfactants used were sodium dodecylsulfate (SDS), obtained from BDH Chemical, England (specially pure), and sodium caseinate (NaCas), prepared from fresh skim milk by repeated precipitation with hydrochloric acid and redispersion in weak NaOH (to pH 7.6); it was subsequently spray-dried.

Natural cream was obtained by centrifugation at ca. 35 C. The cream was pasteurized (13 min at 86 C).

Equipment and Methods

A Bruker Minispec p20 was used with an operating frequency of 20 MHz. The apparatus was equipped with an oscilloscope. A 90° pulse was used with a width of 3 μsec; the dead time of the receiver was 7 μsec. Phase-sensitive detection was used throughout. The signals from 5 pulses were read and averaged.

The magnet temperature was kept constant at 33 C. If total measuring time is below 10 sec, the sample holder need not be thermostated, but in this study it was always done; the magnet temperature was allowed to return to equilibrium before starting measurements. A difference in temperature between the thermostat and the sample holder was possible, so that the temperature was measured inside the sample holder.

The sample tubes were 10 cm long and had internal diameters of 10 mm. When placed in the sample holder, the bottom of a tube was still inside the receiver coil. Tubes were filled in such a way that the samples were well within the receiver coil. The samples were weighed (0.5-1 g).

Direct Method

A 90° pulse was given at various trigger times (i.e., time between pulses) and the NMR signal was measured at 12 μsec and at 90 μsec after the pulse (point s' and L, respectively, in Fig. 1). Because of the very short relaxation time T_2 of solids, the signal has already decayed from s to measuring point s' and a correction factor has to be introduced, so that $s = fs'$. After 90 μsec, the contribution of solid fat protons to the signal is negligible, whereas the signal of the liquid protons is virtually the same as directly after the pulse. The fraction of solid fat is then (1):

$$S_{dir} = \frac{fs'}{fs' + L} \qquad [I]$$

Indirect Method

In the indirect method, the liquid signal L is measured at the desired temperature, at which part of the fat is solid, and also at such a temperature that all fat is melted: signal $M.M > L$, and the increase is due to the originally solid fat protons. A correction has to be applied for the temperature dependence of the oil signal; this is done with a reference oil that is liquid at both temperatures (e.g., olive oil, triolein, paraffin oil), giving signals L_R and M_R. The fraction of solid fat is then calculated as (1):

$$S_{indir} = 1 - \frac{LM_R}{L_R M} \qquad [II]$$

Weight Method

The signal of oil (L) is a measure for the mass of the oil phase. Consequently, the content of solid fat can be estimated at one temperature when the signal per unit mass of oil (specific oil signal) is known. The mass of oil phase in a sample can then be calculated from signal L and since the total mass of the sample is known by weighing, the content of solid fat can be calculated. The specific oil signal can, of course, be determined easily for model systems of paraffin oil or triolein. For natural fats, it could be determined by melting the fat and plotting NMR signals as a function of temperature above the melting point. A linear relationship is then found, provided that saturation is avoided at every temperature. The specific oil signal at a certain temperature can be obtained by extrapolation (see results on natural cream).

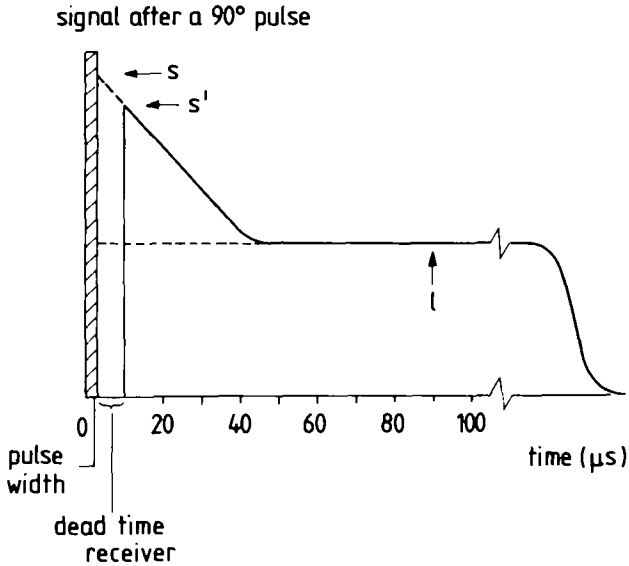

FIG. 1. Schematic representation of signal decay after a 90° pulse for a partially crystalline fat.

X-ray Diffraction

X-ray diagrams were made with a Guinier de Wolff camera (FR 552, ENRAF-NONIUS, Delft, The Netherlands) and Cobalt radiation K_α with wavelength 0.17889 nm. The exposure time was 2 hr and the temperature 22 C.

Preparation of Emulsions

Oil phase and water phase (containing 4 kg m^{-3} SDS or 10 kg m^{-3}/NaCas) were premixed with a Vibromixer, and the mixture was then homogenized with a Condi laboratory homogenizer (Foss-Electric type 127-05) or an Ultra Turrax with a device to exclude air. When the emulsions were to contain tristearate crystals in the oil phase, these were first dissolved in the oil phase by raising the temperature, and homogenization was then carried out at a temperature at which all fat was liquid. After homogenization, emulsions containing dissolved tristearate were cooled deeply to ensure crystallization in all globules. Homogeneous nucleation starts some 20-25 C below the final melting point of the α-modification of tristearate in the mixture (9).

The particle size distribution of the emulsion was estimated by spectroturbidimetry (10).

RESULTS AND DISCUSSION

Content of Solid Fat in Bulk Fats

Experiments were performed with known amounts of pure tristearate in triolein and paraffin oil. The solubility of tristearate at room temperature was negligible, even in the α-modification (11).

Tristearate-in-oil samples were exposed to different temperature treatments. First, tristearate was added to oil at room temperature without any further treatment. Second, tristearate was added to oil, melted, slowly crystallized at 60 C during 24 hr and then slowly cooled to room

TABLE I

The *f*-Factor Calculated for Tristearate in Oil at 22 C

Temperature treatment	*f*-Factor for paraffin oil containing tristearate (% w/w)			*f*-Factor for triolein containing tristearate (% w/w)		
	10.4%	25.4%	39.7%	12.4%	20.9%	33.0%
No treatment	2.18	2.35	2.34	1.85	1.91	1.90
Slowly crystallized	2.30	2.37	2.30	1.82	1.98	1.95
Quickly crystallized	2.02	2.00	1.93	1.47	1.45	1.42

TABLE II

Results of the Weight and Indirect Methods

Temperature treatment	Tristearate in paraffin oil (% w/w)			Tristearate in triolein (% w/w)					
				12.4%		20.9%		33.0%	
	10.4% wm[a]	25.4% wm	39.7% wm	wm	im	wm	im	wm	im
No treatment	10.1	25.5	39.8	12.0	11.4	20.4	21.0	32.4	33.5
Slowly crystallized	10.5	25.0	39.2	12.2	12.2	20.9	20.8	32.6	33.5
Quickly crystallized	10.0	25.3	39.4	12.8	12.8	21.2	21.6	33.7	34.4

[a]wm = weight method; im = indirect method.

temperature. Third, tristearate was added to oil, melted, quickly crystallized at 0 C during some hours and then slowly warmed to room temperature. NMR measurements were made at 22 C. To avoid saturation of tristearate protons, the trigger time was >5 sec.

Since the solubility of tristearate in oil was negligible at room temperature, the *f*-factor was calculated from the known (added) amounts. The results as given in Table I show that the *f*-factor depended strongly on the temperature treatment. X-ray diffraction experiments showed that the diffraction lines were at equal positions for the untreated, slowly and rapidly crystallized samples. They corresponded to spacings of 0.366 - 0.383 - 0.457 - 0.520 - 0.535 nm, which fits the β-modification of tristearate (12). Hence, the difference in *f*-factor for tristearate in oil exposed to a different temperature treatment is not due to a difference in crystal modification. The suggestion is therefore made that the difference in *f*-factor is due to a difference in crystal size, since crystal size depends on the temperature treatment during crystallization. The area between oil and crystals increases with decreasing crystal size. The molecules in the surface of a crystal are, on average, more mobile so that their relaxation time T_2 may increase. Consequently, the average *f*-factor may decrease with decreasing crystal size.

The difference in *f*-factor for tristearate in paraffin oil and triolein (as shown in Table I) was caused by a difference in proton content (paraffin oil contains more protons than triolein or tristearate).

The use of a fixed *f*-factor is thus not always warranted. By no means can tristearate be used to determine an *f*-factor, which is then used for other fats, as has been done (13,14).

The same tristearate samples in triolein or paraffin oil exposed to the same temperature treatments as just described were used to test the weight and indirect methods (the indirect method could not easily be used for tristearate samples in paraffin oil because of the difference in proton content). The trigger time was chosen in such a way that the oil phase was not saturated (>800 msec) (only the liquid signal was used in these two methods). The results are given in Table II.

Excellent agreement was found between the amounts added and the results of these NMR methods. Table II also gives some idea about the reproducibility of the methods, which was usually below 1% solid fat (standard deviation). The advantage is that the weight and indirect methods do not depend on temperature treatment. Admittedly, the weight and indirect methods are more laborious than the

TABLE III

Increase in Signals Measured (%), with Respect to Signals Calculated, of a Sodium Caseinate Emulsion

Fat content (w/w)	Trigger time (sec)						
	1	2	4	6	8	10	20
57.4	1.2	1.4	1.0	1.0	0.7	-0.5	-1.3
33.2	2.4	1.8	1.2	0.1	-0.5	-0.5	-0.9
11.5	1.0	0.7	1.4	-0.2	0.5	0.5	-0.2

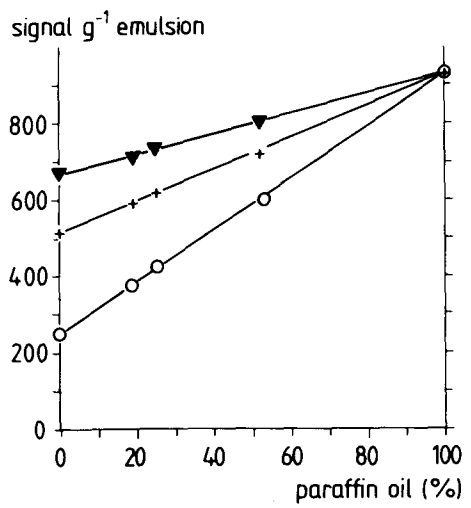

FIG. 2. NMR signals of oil-in-water emulsions stabilized by SDS. Measurements were made at 22 C. Trigger time was varied: 1 sec (○), 3 sec (+) and 6 sec (▼).

TABLE IV

Increase in Signals Measured (%), with Respect to Signals Calculated, of Samples Containing Triolein and Water

Sample	Trigger time (sec)					
	0.6	1	3	6	10	20
Triolein on water after 0 hr	0	0	0	0	0	0
Triolein on water after 2 hr	2.9	2.3	1.2	0.2	–	–
Triolein on water after 24 hr	4.9	4.6	2.0	1.0	–	–
Emulsion of triolein and SDS	3.8	6.2	6.9	3.4	1.8	0.9

TABLE V

Content of Solid Fat (% w/w) in the Oil Phase of SDS-Stabilized Emulsions as Estimated by the Pulse NMR Weight Method

Added amount of tristearate in oil phase (% w/w)	Trigger time (sec)					
	0.6	1	4	6	10	20
10	10.0	10.3	10.8	10.4	10.9	9.4
30	29.7	29.2	29.1	29.6	29.7	30.0
50	49.7	48.7	48.3	48.3	50.4	49.9

direct method, and may result in errors when done by inexperienced operators.

Content of Solid Fat in Emulsions

Water protons disturbed the NMR signal when the sample height was above the receiver coil (loss of signal). Samples containing water should thus be well within the receiver coil and it may also be necessary to readjust the tuning of the probehead. Sodium caseinate also disturbed NMR signals when it stabilized emulsions. Signals measured from sodium-caseinate-stabilized emulsions with liquid oil only (no crystals) were different from the ones calculated from the signals of the separate phases and the fat content. The effect depended on trigger time (Table III). No systematic effect of particle size or fat content was found. When an oil layer was placed upon a layer of sodium caseinate solution, no effect was found. With emulsions, an effect was always found, but the extent of it was not very reproducible. Skim milk powder disturbed wide-line NMR measurements on margarine in a somewhat similar way (7), though the effect was much larger. Trumbetas et al. (15) reported that the presence of sodium caseinate in freeze-dried emulsions increased NMR signals. The dependence on trigger time (Table III) suggests that the effect is caused by a change of the spin-lattice relaxation time T_1 of either the oil phase or the water phase. Relaxation time measurements may provide better understanding. For the moment, the phenomenon cannot be readily explained.

The deviation shown in Table III may not seem large, but it considerably influenced the calculation of the content of solid fat; for instance, a sodium-caseinate-stabilized emulsion containing 30% tristearate in the oil phase was calculated to contain 26% tristearate at a trigger time of 1 sec. An SDS-stabilized emulsion with paraffin oil showed no disturbances; the deviations in signals measured were ca. ±0.5%, independent of trigger time (Fig. 2).

Another complication was found with triolein and water. When a layer of triolein was placed upon an aqueous layer (with or without surfactant) the oil layer became turbid and the NMR signals were higher than the ones calculated from the separate signals of oil and water. Probably, the oil was not very pure and water was solubilized in micelles by monoglycerides. The mobility of solubilized water will be less than in bulk, so that the relaxation time T_1 of solubilized water protons will be shorter, leading to a higher signal than that of bulk water at the same trigger time. The effect should then decrease with increasing trigger time. This was indeed so (Table IV). Included in Table IV are results on an SDS-stabilized emulsion with triolein. An SDS-stabilized emulsion with paraffin oil (which does not contain monoglycerides) showed no such effect; see Figure 2. When a triolein layer was placed upon a D_2O layer, no increase in NMR signal was found, though the triolein layer became turbid. These results all point to the conclusion that the effect was caused by water, solubilized in oil. Here again, relaxation time measurements may provide a better understanding.

Since an SDS emulsion with paraffin oil showed no discrepancies, this emulsion was chosen to test the pulse NMR method for estimation of the content of solid fat in emulsions. The tristearate used contained practically no monoglycerides, so that water probably was not solubilized during emulsification. Emulsions were made with known amounts of tristearate in the oil phase. The fat content of the emulsion was 45% and the volume-surface average particle diameter was 0.8 μm. The solid fat content was estimated by the weight method after correction of the liquid signal for the contribution of protons from the aqueous phase.

The results are shown in Table V. Good agreement was found between the added and measured amounts and the method was independent of trigger time. Since the emulsion droplets were small (0.8 μm), crystals inside the

TABLE VI

Calculated *f*-Factor for Tristearate Crystals in Emulsions

Amount of tristearate in oil phase (% w/w)	Trigger time (sec)			
	6	8	10	20
10	1.69	1.81	1.77	1.70
30	1.79	1.85	1.75	1.74
50	1.76	1.71	1.72	1.79

TABLE VII

Content of Solid Fat (% w/w) in the Oil Phase of Natural Cream at 15 C

	Trigger time (sec)									
	1		3		6		8		10	
Temperature treatment	im[a]	wm	im	wm	im	wm	im	wm	im	wm
8 → 11 C	34.6	30.3	37.3	34.2	37.3	37.7	37.3	37.4	37.3	37.4
8 → 25 → 11 → 21 → 11 C	30.4	27.5	31.6	28.3	32.5	31.9	32.5	33.0	32.5	33.3

[a]im = indirect method; wm = weight method.

droplets also must have been small. It has been suggested that very small crystals contribute to the "liquid signal" because of their mobility (16). From the results of Table V, one would conclude that the effect of small crystals on the liquid signal is negligible.

The *f*-factor that should have been used in the direct method to estimate the true content of tristearate in emulsions was calculated from the known amounts of tristearate (Table VI). It is questionable whether one can speak of an *f*-factor in these systems, where complex relaxation occurs. Nevertheless, comparison of Table VI with Table I shows that the *f*-factor was consistently lower for tristearate crystals in emulsion droplets. This might be due to two effects: smaller crystals or more crystals in the α-modification. X-ray diagrams of tristearate crystals in emulsified paraffin oil droplets showed, again, that all crystals were in the β-modification (spacings at 0.366 - 0.383 - 0.457 - 0.520 - 0.535 nm). An explanation that remains is the effect of crystal size on the relaxation. The crystals in the emulsion droplets were probably very small. They are always smaller, and usually much smaller, than the emulsion droplets (17) and droplet diameter ranged from ∿0.2-1.5 μm, average 0.8 μm. The molecules at the surface of a crystal may relax more slowly because they are, on average, more mobile than those inside the crystal, and the specific surface area increases with decreasing crystal size. Unfortunately, variation of droplet size, hence of crystal size in emulsion droplets, was not possible because these emulsions were very unstable whenever the average droplet size was larger than 1 μm (11).

Finally, measurements were made on natural cream. The signal from the aqueous phase was eliminated by calculating its contribution from the fat content and the signal measured from skim milk. Two methods were used: the weight method and the indirect method. For the weight method, the specific oil signal was needed, but milk fat begins to crystallize at temperatures below 40 C. To solve this problem, attempts were made to extrapolate NMR signals, obtained at temperatures higher than 40 C, to lower temperatures. A linear relationship was found between NMR signals of liquid oil and temperature with triolein, but the linear relationship broke down when the trigger time was such that saturation occurred. Since the sensitivity to saturation (in fact the relaxation time T_1) changes with temperature, care must be taken to avoid saturation at every temperature. In this way the specific (liquid) milk fat signal was obtained at every temperature by extrapolation.

Two temperature treatments were given to the cream. In the first one, the cream was cooled (after pasteurization) to 8 C during 2 hr, then held at 11 C during 16 hr. In the second treatment, cream was (after pasteurization) cooled to 8 C during 2 hr, warmed to 25 C during 8 min, cooled to 11 C during 2 hr, warmed to 21 C during 8 min and cooled to 11 C during 16 hr; such a treatment is expected to give less solid fat if compound crystals are formed (17). The content of solid fat was measured at 15 C. The results are in Table VII.

Agreement between the two methods was good at the longer trigger times. However, the dependence on trigger time showed that also with natural cream disturbances are possible, as with sodium caseinate emulsions or water solubilized in oil. The solubility of water in milk fat is ca. 0.12% at 15 C (17) and, in addition, some water may be solubilized in micelles in the fat. But a complete explanation cannot be given. The composition of cream is too complicated to explain the phenomena simply on the basis of the results in Table VII. Relaxation time measurements may provide a better understanding.

Some of the problems that may arise when NMR measurements are made on bulk fats and emulsions have been indicated. These problems can be solved, even though the nature of disturbances is not fully understood. It should be clear, however, that NMR measurements to estimate content of solid fat must be interpreted with great care.

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