Determination of Water Droplet Size in Margarines and Low-Calorie Spreads by Nuclear Magnetic Resonance Self-Diffusion

B. Balinov[a,1], **O. Söderman**[a,*] and **T. Wärnheim**[b,2]
[a]Division of Physical Chemistry 1, S-221 00 Lund, Sweden and [b]Karlshamns Oils & Fats AB, S-374 82 Karlshamn, Sweden

The nuclear magnetic resonance self-diffusion technique can be used to determine the size of emulsion droplets. In this communication, the method is discussed and applied to some margarines and low-calorie spreads, ranging in fat content from 40 to 80%. The data show that the droplet size distribution is fairly polydisperse, at least for the solid margarines studied. A liquid margarine, containing 80% fat and studied by optical microscopy, showed that a log-normal size distribution function is a reasonable description of its size polydispersity. Therefore, this distribution function was used in the present work, and the parameters of the log-normal function were determined by a least-square fit directly on the experimental raw data for all the systems studied. The uncertainties in the obtained parameters are judged by means of a Monte Carlo technique. The uncertainty is around ±15% (or better). Finally, we discuss the possibility of the presence of nonspherical droplets and how the presence of such droplets would actually affect the measurements.

KEY WORDS: Emulsion droplet size, low-calorie spreads, margarine, NMR self-diffusion, restricted diffusion, w/o emulsion.

The structure and droplet size distribution of the aqueous-phase is important for many properties of margarine. Ordinary margarine, a water-in-oil (w/o) emulsion containing 80 wt% fat as continuous phase, is stabilized by a fat crystal network (1,2). Strictly speaking, margarine or butter are not emulsions because the continuous phase is plastic rather than liquid, but the term "emulsions" will be used for these systems (3). Systems containing 80% fat or more will be referred to as margarines and those containing less than 80% fat will be called low-calorie spreads. A closed water droplet structure imparts good storage properties with respect to microbial deterioration, while the mouthfeel is, to a large extent, affected by the melting properties of the fat phase. However, with low-fat spreads containing 40, 25% or even lower amounts of fat, the emulsion structure may depend on the composition and processing conditions, which will give widely varying properties. In general, a closed w/o structure with small droplet radii (1–5 μm) is preferred with respect to keeping properties because the stability, with regard to microbial deterioration, is maximized. In addition, it minimizes the drying out of the surface. On the other hand, for low-fat spreads, the dense emulsion structure may deteriorate the sensory properties with respect to destabilization of the emulsion and flavor release in the mouth. A compromise is a system that contains both small and large droplets (>10 μm). For spreads containing 25% or less fat, sometimes a bicontinuous structure is found (2,4,5).

It follows that it is important to be able to determine the droplet size of a particular emulsion. Where particle size determinations of oil-in-water emulsions are routine experiments, where a number of different techniques may be used (6), w/o emulsions are generally more difficult to characterize. Several methods presuppose a dilution of the emulsions with the continuous phase; this tends to be difficult for margarine because coalescence is rapid when the crystal network is broken down during dilution. Optical and electron microscopy are sometimes used, although sample preparation and treatment tend to be time-consuming and may introduce artefacts.

The nuclear magnetic resonance (NMR) self-diffusion method can be used without perturbing the system (7). Therefore, it is somewhat surprising that few reports on this method, as applied to low-calorie spreads in particular or emulsions in general, can be found in the literature (8–13). The aim of this paper is to describe the method, to discuss its limitations and finally, to present some results from some common margarines and low-calorie spreads. Also included, as an independent test of the NMR method, are some results of particle size determinations of a liquid margarine of 80 wt% fat content, by means of microscopy.

EXPERIMENTAL PROCEDURES

Samples. Samples used in this study were all obtained from Karlshamns Oils and Fats (Karlshamn, Sweden) and used as obtained. Suitable amounts (a few 100 mg) were transferred to 5-mm NMR tubes. For the analysis of the NMR data, it is necessary to know the bulk diffusion coefficient of the water component. This quantity was obtained in one of two ways. In the first procedure, the margarine or low-calorie spreads were phase-separated by centrifugation at 25°C. The water phase was collected, and the water self-diffusion coefficient of this sample was determined as outlined below. In the other procedure, the water component was obtained directly from Karlhamns Oils and Fats and used for NMR experiments.

Microscopy. Microscopy was performed on a Nikon 104 microscope (Nikon Corp., Tokyo, Japan) by phase-contrast measurements at 400× magnification. Image analysis was performed on the digitized video image with Labeye (Innnovative Vision AB, Linköping, Sweden) Software. The derived size distribution was obtained from 700–1000 counted objects. Because the analysis could not be performed on images from intact samples, they were diluted with deodorized soybean oil. Care was taken to minimize the effect of the dilution on emulsion structure. Thus, only a sample of 80 wt% fat liquid margarine could be diluted without visible disturbances and in a reproducible manner, leading to a reliable droplet size distribution.

NMR experiments. The NMR self-diffusion experiments were performed on a spectrometer of in-house design, equipped with a Varian (Palo Alto, CA) 2.3 T electromagnet. The gradient drivers were of in-house construction, following a design suggested by Stilbs (14). The NMR probe is equipped with quadrupole coils (15), which are capable of delivering up to 0.9 T/m field gradients. Most experiments were performed with a gradient strength of 0.3 T/m. The experiments were performed with

---

[1] On leave from The Institute of Physical Chemistry, Bulgarian Academy of Sciences.

[*] To whom correspondence should be addressed at Division of Physical Chemistry, Box 124, S-221 00 Lund, Sweden.

[2] Present affiliation: KABI Pharmacia Hospital Care AB, S-11287 Stockholm, Sweden.

the ordinary Carr-Purcell sequence. The time between the 90° and 180° pulses was typically 100 ms, and the length of the gradient pulses was varied between 3 and 60 ms. The temperature for all measurements was 17°C. The evaluation of the data and the different least-square analyses were performed on Macintosh computers with the MATLAB package (MathWorks Inc., South Natick, MA).

The NMR method for determining emulsion droplet size. This section briefly describes the NMR self-diffusion method, in general, and its application to the determination of emulsion droplet sizes in particular. A more complete account of the NMR method can be found elsewhere (14,16). The NMR experiment, by which self-diffusion coefficients are determined, is based on a spin-echo experiment, but in addition to the rf pulses used in "ordinary" NMR, one also subjects the sample to two field gradient pulses. The pulse sequence used is depicted in Figure 1. The experiment measures the mean translational motion of the molecules under a time, which is equal to the time between the application of the pulsed field gradients (Δ in Fig. 1). In a typical experiment, Δ is of the order of a few 100 ms, which means that the distance travelled by the spins during this time is around 10 μm for typical low-molecular weight liquids. For molecules that perform Gaussian (free) diffusion on this time scale, the echo attenuation for a sample subjected to the pulse sequence in Figure 1 is given by (7,14,16):

$$ln\left[\frac{E(\delta,\Delta,g,\tau)}{E_0}\right] = -\frac{2\tau}{T_2} - \gamma^2 g^2 D\delta^2\left(\Delta - \frac{\delta}{3}\right) \qquad [1]$$

In Equation 1, E_0 is the echo intensity in the absence of any field gradient, τ is the time between the 90 and 180° rf pulses, T_2 is the transverse relaxation time, γ is the gyromagnetic ratio, g the amplitude of the field gradient, δ the duration of the field gradient pulses and D is the self-diffusion coefficient.

For the case where the molecules experience barriers to their diffusion and the distance between the barriers is of the same order of magnitude as the distance traveled during Δ, the outcome of the experiment is affected. In such a situation, the echo attenuation will depend on the geometry of the barriers. To the best of our knowledge, the exact relation for the echo attenuation for an arbitrary geometry has not been derived (7,17). Instead, one has to resort to different levels of approximation. For molecules confined to a spherical cavity (as would be the case for the dispersed phase in an emulsion), Murday and Cotts (18) have derived an equation for the echo attenuation, which is based on a simplifying assumption regarding the phase acquired by the molecules as they perform random motions:

$$ln[E(R)] = -\frac{2\gamma^2 g^2}{D}\sum_{m=1}^{\infty}\frac{\alpha_m^{-4}}{\alpha_m^2 R^2 - 2}$$

$$\times\Big(2\delta - \{2 + \exp[-\alpha_m^2 D(\Delta - \delta)] - 2\exp(-\alpha_m^2 D\delta) - 2\exp(-\alpha_m^2 D\Delta) + \exp[-\alpha_m^2 D(\Delta + \delta)]\}/\alpha_m^2 D\Big) \qquad [2]$$

where α_m is the m^th root of the Bessel function equation:

$$\frac{1}{(\alpha R)} J_{3/2}(\alpha R) = J_{5/2}(\alpha R) \qquad [3]$$

or equivalently:

$$(\alpha R)J'_{3/2}(\alpha R) - \frac{1}{2}J_{3/2}(\alpha R) = 0 \qquad [4]$$

R is the droplet radius and D is now the bulk diffusion coefficient of the dispersed phase. The use of the bulk value of D for the dispersed phase is justified by the fact that only two or three layers of molecules adjacent to the emulsion droplet interface have altered properties as compared to the bulk. Thus, the fraction of molecules within the droplets that have their diffusion coefficient altered is small for typical emulsion droplet radii. The assumption underlying Equation 2 has recently been tested by computer simulations, and it was found that the deviation of Equation 2 in describing diffusion within spheres was never worse than 5% (17).

Depicted in Figure 2 are the predictions of Equation 2 for typical parameters and different radii. It shows that the echo attenuation depends on the droplet size, and thus, the radius of an emulsion droplet can be determined by recording the echo attenuation from the dispersed molecules in an emulsion. For an emulsion in which the

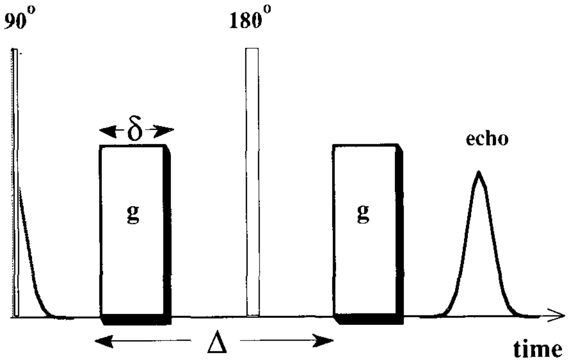

FIG. 1. The pulse sequence used in the pulsed gradient spin echo self-diffusion experiment.

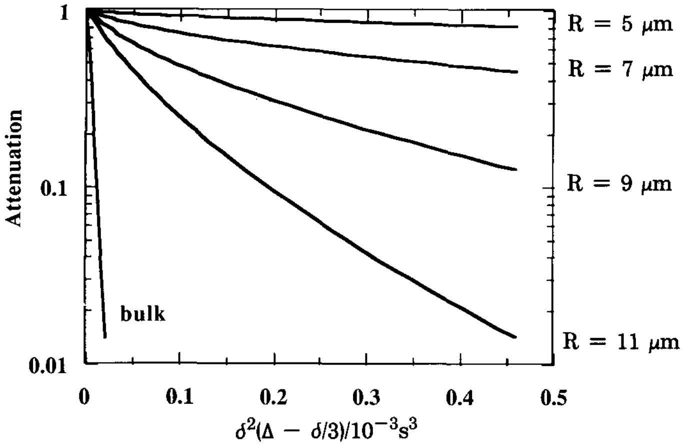

FIG. 2. The natural logarithm of the echo attenuation E (according to Eq. 2) as a function of $\delta^2(\Delta - \delta/3)$ for different radii of emulsion droplets with $\Delta = 0.100$ s, $\gamma g = 10^7$ rad m^{-1}s^{-1} and $D = 2\ 10^{-9}$ m^{2}s^{-1}.

droplets are polydisperse with respect to their size, the situation is slightly more complicated. If the exchange of molecules between droplets is slow on the NMR timescale (which is essentially equal to the value of Δ), then droplets of each size will obey Equation 2, and the measured NMR echo intensity is a sum of the echo contributions from the various droplets weighed by the volume fraction of each droplet size (the NMR signal is proportional to the number of spins). Thus, we may write:

$$E_{poly} = \frac{\int_0^\infty R^3 P(R) E(R) dR}{\int_0^\infty R^3 P(R) dR} \qquad [5]$$

In Equation 5, E is given by Equation 2 and $P(R)$ is the size distribution function. From the NMR experiment, it is not possible to determine the functional form of $P(R)$. However, given a functional form of the distribution function, we may compute the parameters of that particular distribution function. There are several different types of distribution functions used for describing the polydispersity of emulsion droplets. A wide variety of emulsions can be described with a log-normal distribution function (6) (see also Fig. 6, later in text). Moreover, this particular function is simple to use, as it is determined by only two parameters. The log-normal distribution function is given by:

$$P(R) = \frac{1}{2R\sigma\sqrt{2\pi}} \exp\left[-\frac{(\ln 2R - \ln d_0)^2}{2\sigma^2} \right] \qquad [6]$$

In Equation 6, d_0 is the diameter median, and σ is a measure of the width of the size distribution. We have used this distribution function in evaluating the droplet sizes in the present work. One parameter that can be derived from the distribution function in Equation 6 is the volume-based geometric mean diameter d_{33} [defined as d_0 $\exp(3\sigma^2)$], and, hence, we will also report the value of this parameter.

The main advantages of the NMR approach as compared to more conventional means of determining droplet sizes include: (i) it is independent of the physical status of the sample and can thus be used on optically dense systems or highly viscous system; (ii) it is nondestructive, which is important when one wants to follow the properties (such as the stability of an emulsion) of a system over a long time; (iii) fairly small sample volumes are needed (in the order of 100 mg in a typical case); and (iv) the method is rapid—the experiments performed in this work took about 15 min each.

RESULTS AND DISCUSSION

Determination of parameters. The equations used in describing the data are rather complicated (*cf.* Eqs. 2 and 5, and one might wonder how accurate the parameters are determined from a given set of experimental raw data. To investigate this, we have performed Monte Carlo analyses along the lines suggested elsewhere (19,20). First a least-square analysis by means of a Nelder-Meade simplex algorithm is performed on the experimental raw data. This procedure yields the values for the parameters of the distribution function. Subsequently, normally distributed random noise (generated from the actual uncertainties in the experimental data points as determined from repeated measurements) was added to the experimental raw data. The generated data set was then subjected again to a least-square analysis, yielding once more the values of the parameters of the distribution function. This process was repeated 100 times. A typical result for a low-calorie spread of 60% fat is presented in the histograms in Figures 3, 4 and 5 for R_0 ($=d_0/2$), σ and d_{33}, respectively. The uncertainties in the determined parameters may then be determined from the histograms, and the uncertainties quoted in this work have all been determined in this way. The reported values correspond to a 67% level of confidence. Again, it should be stressed that we cannot distinguish between different forms of the size distribution function.

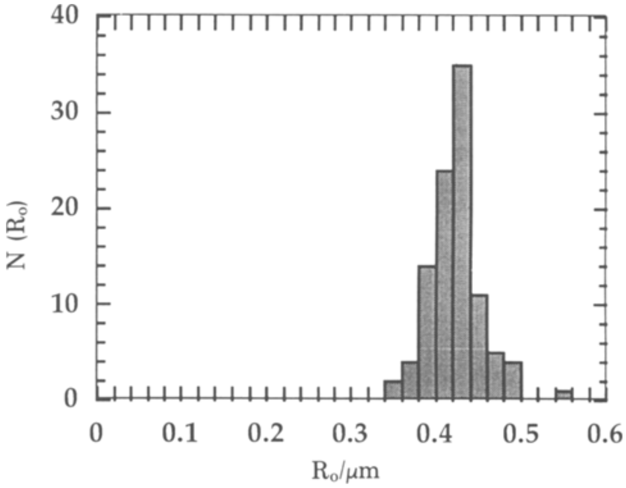

FIG. 3. $N(R_0)$ for a low-calorie spread containing 60% fat. The resulting radius of the droplet is $R_0 = 0.42 + 0.024$ μm.

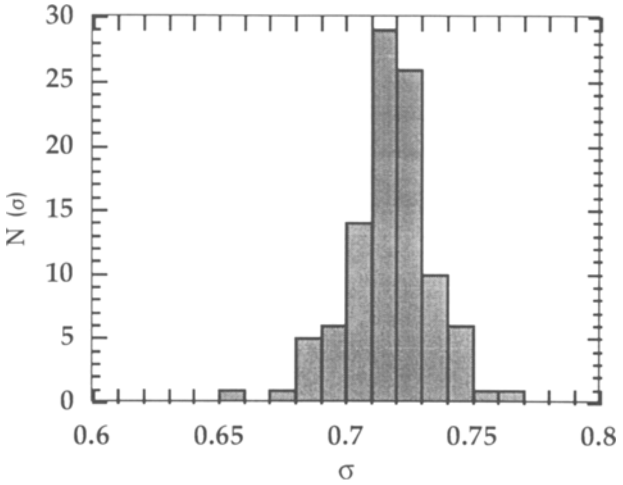

FIG. 4. N (σ) for a low-calorie spread containing 60% fat. The resulting width of the distribution is $\sigma = 0.72 \pm 0.012$.

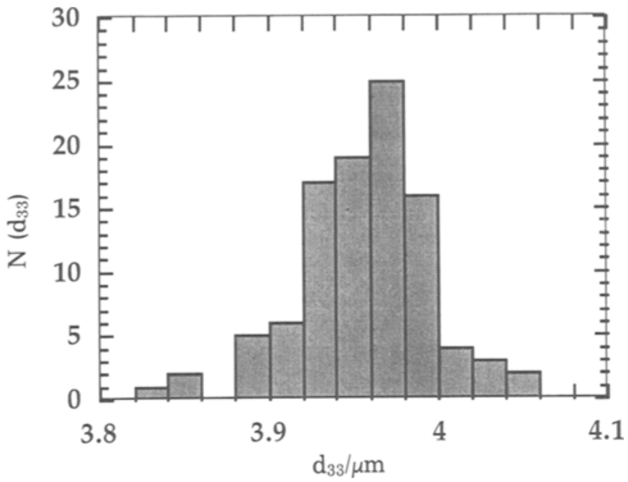

FIG. 5. N (d_{33}) for a low-calorie spread containing 60% fat. The resulting value of d_{33} is 4.0 ± 0.031 μm.

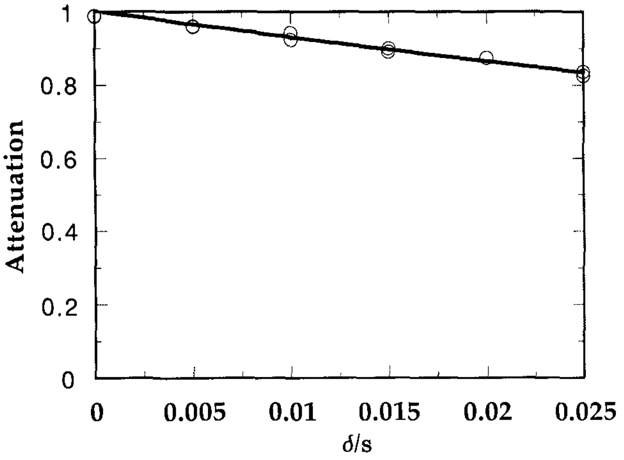

FIG. 7. Echo intensity vs. δ for a liquid margarine containing 80% fat. The solid line is a fit of Equation 2 (monodisperse case with d_0 = 2.7 μm) to the data. Polydisperse model (Eqs. 5 and 6 give d_0 = 3.0 μm and σ = 0.056.

Bulk diffusion of the water components. As can be inferred from Equation 2, it is necessary to know the bulk diffusion coefficient of the water component in the margarines and low-calorie spreads. These diffusion coefficients were obtained by measurements directly on the water component, which was obtained either through a phase separation or directly from the manufacturer. The water component contains a number of additives, and, thus, one cannot directly use the diffusion coefficient of pure bulk water. The reduction in the values of the water self-diffusion coefficients for the margarines and low-calorie spreads was generally around 10%.

A liquid margarine. The results of the droplet size determination by means of optical microscopy, as described previously, are shown in Figure 6. The original sample, containing 80% fat, was diluted with oil. Around 800 drops were counted, and the size distribution given in Figure 6 was derived from this procedure. Also given is the result of fitting a log-normal distribution function to the data. As can be seen, this functional form for P(R) gives a reasonable representation of the data. The derived parameters are: d_0 = 2.5 μm and σ = 0.063. The same brand of liquid margarine, but not the same sample, was then investigated by the NMR method. The raw data are presented in Figure 7, together with a fit of Equations 2, 5 and 6 to the data, yielding: d_0 = 3 μm and σ = 0.0056. Taking into account the uncertainties in the determined parameters, as discussed above, and the fact that two different batches of the liquid margarine were used, the differing results of the two methods are not significant. Both methods indicate that the size distribution of the droplets in the liquid margarine is fairly narrow; in fact, the data in Figure 7 can be represented equally well by means of monodisperse droplets (the solid line in Fig. 7 is the prediction of the monodisperse case).

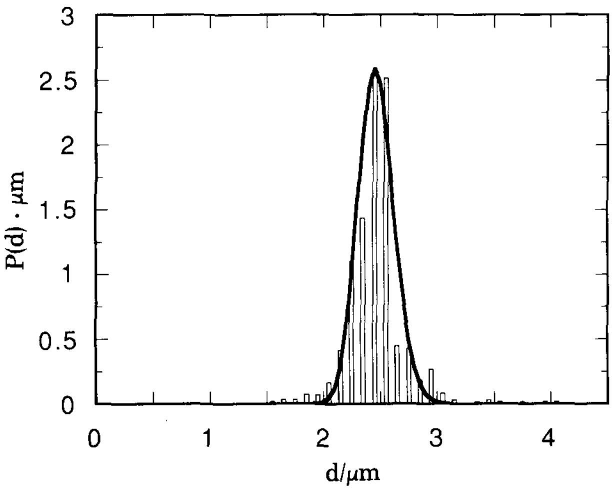

FIG. 6. Size distribution of water droplets for a liquid margarine containing 80% fat as determined by optical microscopy. The solid line is the best fit for a log-normal size distribution (Eq. 6) with d_0 = 2.5 μm and σ = 0.063.

Results for margarines and low-calorie spreads. Presented in Table 1 are the results for one margarine and two different low-calorie spreads, with fat contents ranging from 40 to 80%. Also included are the results from the liquid margarine previously discussed. A typical data set for a low-calorie spread containing 60% fat, and the

TABLE 1

The Results of the Nuclear Magnetic Resonance Size Distribution Determinations for Four Different Margarines and Low-Calorie Spreads of Indicated Fat Content

Fat content (%)	d_0 ± error (μm)	σ_0 ± error	d_{33} ± error (μm)
40%	0.82 ± 0.08	0.82 ± 0.02	6.15 ± 0.07
60%	0.84 ± 0.05	0.72 ± 0.013	3.96 ± 0.03
80%	0.98 ± 0.54	0.61 ± 0.12	3.05 ± 0.26
80%[a]	2.96 ± 0.46	0.0056 ± 0.28	2.97 ± 0.17

[a] A liquid margarine.

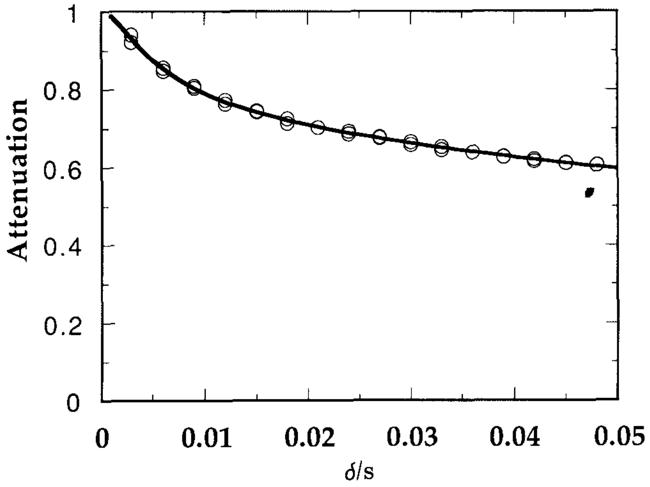

FIG. 8. Echo intensity vs. δ for a low-calorie spread containing 60% fat. The solid line is a fit of Equations 2, 5 and 6 (polydisperse case) to the data.

results of fitting Equations 2, 5 and 6 to the data are presented in Figure 8 (the same data set underlies the results presented in Figs. 3–5). Judging from the Monte Carlo procedure, the accuracy of the calculated parameters can be estimated to be around ± 15%. The uncertainty in the determination of σ for the liquid margarine is rather large. This is due to the fact that this sample is almost monodisperse (in fact, a least-square fit to the monodisperse case is equally good and yields a value of $d_0 = 2.7$ μm). Note also that the uncertainty in d_{33} is lower than in d_0. This indicates that there is a certain amount of covariance in the values of the parameters d_0 and σ, as d_{33} is determined by means of the relation $d_0 \exp(3\sigma^2)$. It is clear that the average droplet size is around 1 μm in all the samples studied (except for the 80% liquid-fat sample). The polydispersity in droplet size is rather large, and it appears to increase as the fat content is decreased. A typical distribution function, as derived from the parameters in Table 1, can be found in Figure 9.

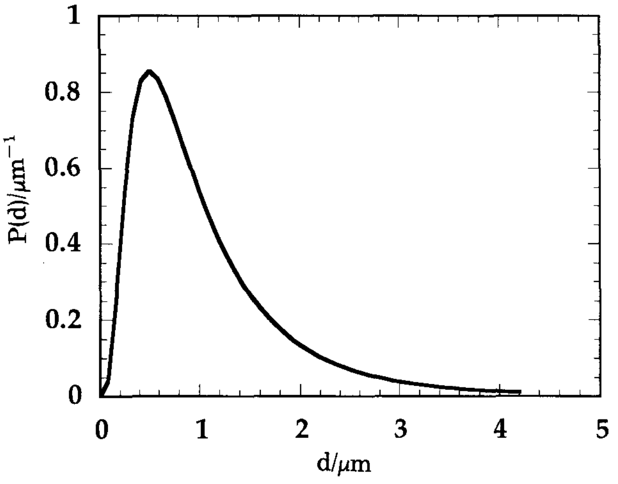

FIG. 9. Log-normal size distribution for a low-calorie spread containing 60% fat as obtained from the data in Figure 8.

The presence of nonspherical droplets. While it is certainly true that the spherical shape is the most likely one found in liquid emulsions, this is not necessarily true for the margarines, and, in particular, the low-calorie spreads, where the continuous phase is plastic rather than liquid. The analysis described here hinges on the fact that the droplets are indeed spherical in shape. If this were not the case, the degree of deviations from the predictions of Equations 2 and 5 would depend on how far the actual shape is removed from the spherical shape. It seems reasonable to assume that if the deviation is quite small, as for instance, would be the case for spheroids of moderate axial ratios, then Equations 2 and 5 would give a fairly accurate representation of the echo decay (albeit with values of d_0 and σ that now depend on the size/shape of the confinement). An added complication is that the NMR experiment measures the diffusion in one dimension. Thus, the orientation of the nonspherical confinement becomes important. Consider, as an example, the case of a prolate-shaped confinement. The prolate, oriented with its major axis perpendicular to the field gradient, will be perceived as smaller (recall that the NMR experiment measures the molecular displacements along the direction of the field gradient) than the one oriented with its major axis parallel to the magnetic field if the result is analyzed in terms of droplets of spherical shape. Because there is no reason why a specific orientation would be preferred over another, one would, if analyzing the data in terms of droplets spherical in shape, reach the conclusion that the droplets were polydisperse in size. It seems unlikely that, with this method, one would be able to differentiate between nonspherical droplets and spherical droplets polydisperse in size. It would appear that the presence of nonspherical aggregate shapes (if these do not deviate too much from the spherical shape) would lead to an artificial broadening of the size distribution function if the data are analyzed in terms of polydisperse spheres.

Presented above is an NMR method to determine size distributions of emulsion droplets, which has some rather definite advantages compared to more conventional sizing methods. We have applied it to the case of low-calorie spreads, which are systems where most other sizing techniques fail. The accuracy of the determined parameters of a particular size distribution function has been assessed by means of a Monte Carlo technique, and it is found that the parameters can be determined with an accuracy of about ±15% (or better). The method may be applied to a number of different emulsion systems, for instance, water in crude oil emulsions, highly concentrated emulsions and emulsions in the pharmaceutical industry.

ACKNOWLEDGMENTS

This project has been financially supported by the Swedish National Board for industrial and Technical Development (NUTEK) and by Karlshamns Research Foundation.

REFERENCES

1. Heertje, N., M. Leunis, W. van Zeijl and E. Berends, *Food Microstruct. 6*:1 (1987).
2. Kroog, N., T. Riisom and K. Larsson, in *Encyclopedia of Emulsion Technology*, edited by P. Becher, Dekker, New York, 1985, p. 347.
3. Larsson, K., in *Emulsions—A Fundamental and Practical Approach*, edited by J. Sjöblom, Kluwer, Dordrecht, 1992, pp. 41–51.

4. Hermansson, A.H., and E. Jordansson, in *Gums and Stabilizers for the Food Industry*, vol. 6, edited by G.O. Phillips, D.J. Wedlock and D.A. Williams, IRL Press, Oxford, 1992.
5. Buchheim, W., and M.S. Schneider, *Streichfette 50*:1610 (1990).
6. Orr, C., in *Encyclopedia of Emulsion Technology*, edited by A. Becher, Marcel Dekker, Inc., New York, 1988, pp. 137–169.
7. Callaghan, P.T., *Principles of Nuclear Magnetic Resonance Microscopy*, Clarendon Press, Oxford, 1991.
8. Callaghan, P.T., K.W. Jolley, and R. Humphrey, *J. Colloid and Interface Sci. 93*:521 (1983).
9. Packer, K., and C. Rees, *Ibid. 40*:206 (1971).
10. van den Enden, J.C., D. Waddington, H. van Aalst, C.G. van Kralingen and K.J. Packer, *Ibid. 140*:105 (1990).
11. Lönnqvist, I., A. Khan and O. Söderman, *Ibid. 144*:401 (1991).
12. Söderman, O., I. Lönnqvist and B. Balinov, in *Emulsions—A Fundamental and Practical Approach*, edited by J. Sjöblom, Kluwer, Dordrecht, 1992, pp. 239–258.
13. Li, X., J. Cox, and R. Flumerrfelt, *AICHE J. 38*:1671 (1992).
14. Stilbs, P., *Progr. Nucl. Magn. Reson. Spectrosc., 19*:1 (1987).
15. Ödberg, G., and L. Ödberg, *J. Magn. Reson. 16*:342 (1974).
16. Callaghan, P., *Aust. J. Phys. 37*:359 (1984).
17. Balinov, B., B. Jönsson, P. Linse and O. Söderman, *J. Magn. Reson. A. 104*:17 (1992).
18. Murday, J.S., and R.M. Cotts, *J. Chem. Phys. 48*:4938 (1968).
19. Stilbs, P., and M.E. Mosley, *J. Magn. Reson. 31*:155 (1978).
20. Alper, J.S., and R.I. Gelp, *J. Phys. Chem. 94*:4747 (1990).

[Received June 7, 1993; accepted January 14, 1994]