Evaluation of the Precision of Drop-Size Determination in Oil/Water Emulsions by Low-Resolution NMR Spectroscopy

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The accuracy of the recently reported low-resolution NMR method (Goudappel, G. J. W.; et al. *J. Colloid Interface Sci.* **2001**, *239*, 535) for the determination of drop-size distribution in oil-in-water emulsions is evaluated by comparing the NMR results with precise data from video-enhanced optical microscopy. A series of 27 soybean-oil-in-water emulsions, differing in their mean drop size, polydispersity, oil volume fraction, and emulsifier, is studied. Soybean oil is selected as a typical component of food emulsions. The experimental error of our optical procedure for drop-size determination is estimated to be around 0.3 μm, which allows us to use the microscopy data as a reference for the mean drop-size and distribution width of the studied emulsions, with known experimental error. The main acquisition parameters in the NMR experiment are varied to find their optimal values and to check how the experimental conditions affect the NMR results. Comparison of the results obtained by the two methods shows that the low-resolution NMR method underestimates the mean drop size, d_{33}, by ≈20%. For most of the samples, NMR measures relatively precisely the distribution width (±0.1 to 0.2 dimensionless units), but for ∼20% of the samples, larger systematic deviation was registered (underestimate by 0.3−0.4 units). No correlation is found between the emulsion properties and the relative difference between the microscopy and NMR data. Possible reasons for the observed discrepancy between NMR and optical microscopy are discussed, and some advantages and limitations of the low-resolution NMR method are considered.

1. Introduction

Drop-size distribution is an important emulsion characteristic, which affects various emulsion properties, such as stability to coalescence and sedimentation, rheological behavior, texture, color, and rate of release of volatile components (fragrance and flavor).[1−4] A large number of methods, such as laser light diffraction and scattering, electric sensing, acoustic spectroscopy, dielectric spectroscopy, centrifugal sedimentation, and optical and electron microscopy, are used in research and application laboratories for drop-size determination in emulsions.[1,4−17] The choice of an appropriate method for a particular application depends on numerous factors, such as the method accuracy, reproducibility, and sensitivity; instrument cost; time and cost of the individual analyses; and requirements for special operator skills.

Optical microscopy is arguably the most precise among the existing general methods for drop-size determination.[5,6] The major advantage of optical microscopy is that it is a direct method, with straightforward calibration and well-understood limitations, caused mainly by the wave nature of light and by optical aberrations. Optical microscopy is often combined with image analysis techniques, which enhance the image quality and improve the precision of drop-size determination.[5,6] On the other hand, conventional optical microscopy requires diluted samples (typically around 1 vol % of the dispersed drops),

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it is time-consuming, and an experienced operator is needed to obtain accurate results. The relatively new method of confocal scanning laser microscopy (CSLM) has the advantage that it can be applied to nondiluted emulsions with high drop volume fraction, but the instruments are rather expensive and staining with fluorescent dyes is usually required.[7] For these reasons, optical microscopy is used for drop-size determination mainly in scientific studies in which very accurate results are needed.

Techniques based on light diffraction and scattering are probably the most widespread in research, development, and application laboratories,[4,15−17] because inexpensive commercial instruments provide reproducible results, often without a need for special operator skills. Most of these methods require relatively dilute emulsion samples with nonflocculated droplets, which might be a problem for many emulsions of practical interest. Some laser diffraction methods can be applied to more concentrated samples, if the emulsion is confined in a narrow optical cuvette so as to become transparent to the light beam. This method often raises concerns about possible drop−drop coalescence, induced by the hydrodynamic stress during emulsion flow in narrow gaps.[18] Another serious limitation of all light scattering techniques is that the instruments do not differentiate between the emulsion drops and other particles which may be present and are not easily separated during sample preparation. Typical examples in food emulsions are starch particles and protein aggregates,[3] which can seriously affect drop-size distribution analysis by light scattering techniques.

During the last 15 years, several NMR techniques for drop-size distribution analysis have attracted the attention of researchers, due to some important advantages of the NMR method.[7,19−30] It can be applied to concentrated opaque emulsions of the W/O and O/W types, without pretreatment of the sample (except for loading in the NMR tube). In most cases, the drop-size results are not affected by the drop aggregation or by the presence of other particles in the sample. Along with the drop-size distribution, the NMR techniques can provide information about the sample composition (oil fraction and solid fat content) and the spatial distribution of the components in the sample (NMR imaging).[19] The NMR measurements are usually fast and do not require excessive sample volumes; typically, 0.5−5 mL is sufficient. For these reasons, NMR techniques are among the most interesting and promising methods for studying emulsions of practical importance, for example, in the food and oil industries.[7,22,23,29]

In the pulsed field gradient spin−echo (PGSE) NMR technique, the translation diffusion of the molecules is probed by measuring the amplitude of the so-called "spin−echo" signal[19] (a more detailed explanation of the principle of the PGSE technique is given in section 2.4 below). If the diffusing molecules encounter some boundary during the PGSE experiment (e.g., drop surface, for molecules confined in emulsion drops), the attenuation of the spin−echo signal is smaller compared to the attenuation in the case of free molecule diffusion.[31,32] The echo attenuation strongly depends on drop size, which allows one to determine the drop-size distribution in emulsions from PGSE measurements. This method was successfully tested[20−22] with high-resolution NMR spectrometers, which use magnetic fields of high strength (corresponding to a resonance frequency for protons of 60 MHz and above) and provide separate signals in the frequency domain for the protons in the oil and water phases. The PGSE method was found to be rather accurate for drop-size determination in both O/W and W/O emulsions.[21,22] However, high-resolution NMR spectrometers are expensive, and mainly for this reason, the technique has been used in a limited number of research laboratories.

About 15 years ago, the PGSE technique was adapted[29] for measuring the drop-size distribution in W/O emulsions using low-cost, benchtop NMR instruments, operating at low magnetic fields (corresponding to ≈20 MHz resonance frequency for protons). Currently, the low-resolution NMR instruments are widely used in food research, development, and industrial laboratories due to their low cost, easy operation, and fast measurement of several important characteristics of food emulsions, such as drop-size distribution in W/O emulsions (margarine and low-calorie spreads), oil volume fraction, and solid fat content. These instruments are unable to resolve proton signals originating from the oil and water phases; one common signal is obtained for oil and water in the frequency domain. For this reason, an appropriate NMR pulse sequence was designed to filter out the signal from the continuous oil phase and to measure the echo signal from the water molecules confined in the emulsion drops.[29]

Recently, Goudappel et al.[24] proposed a pulse sequence based on the PGSE method which allows one to measure the drop-size distribution in O/W emulsions with a low-resolution NMR spectrometer. Preliminary tests showed that the method is applicable to vegetable-oil-in-water emulsions, which are typical for a range of food products (e.g., mayonnaise and salad dressing).[24] In a later study, Duynhoven et al.[7] compared results obtained by the method of Goudappel et al.[24] with results obtained by several other experimental methods (conventional optical microscopy, confocal optical microscopy, laser light scattering, and electric sensing) for several oil-in-water food emulsions. The authors showed by statistical analysis that the NMR method has superior *reproducibility* and *repeatability* in comparison with the other methods that measure the mean drop size. With respect to the width of the drop-size distribution curve, the NMR method showed similar reproducibility to the other methods studied.[7]

The *precision* (accuracy) of this reported NMR method[24] has not been studied in such detail. Although a good agreement was reported[7,24] for the values of the mean

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drop size, d_{32}, determined by NMR and other methods, there are several important points that are not thoroughly clarified. First, the NMR results were shown to depend on the particular values of some experimental acquisition parameters (see, e.g., Table 3 in ref 7). Therefore, a careful analysis of the role of the experimental conditions is needed before making a final conclusion about the accuracy of the NMR method. Such an analysis has not been presented so far, though the specific requirements for the low-resolution NMR instrument and the optimal ranges of the acquisition parameters were discussed in refs 7, 24, and 25. Second, the various experimental methods for drop-size analysis provide different types of mean diameter. For example, NMR provides the volume-averaged geometric-mean diameter, d_{33}, whereas the light scattering techniques provide d_{43} or the so-called "z-average diameter", d_z (which is averaged over the intensity of the light scattered by the particles). Since the recalculation of one mean diameter from another could be related to a significant error, a reliable and conclusive estimate of the precision of the method of Goudappel et al.[24] can be made only by using a reference method, which provides precise results with known experimental accuracy. Such a type of comparison has not yet been reported to the best of our knowledge.

This paper is aimed at a careful evaluation of the precision of the NMR method of Goudappel et al.[24] For this purpose, a series of model soybean-oil-in-water emulsions, differing in their mean drop size, polydispersity (including different shapes of the actual drop-size distribution curve), oil volume fraction, and emulsifier, was prepared by several emulsification methods. Five commercial mayonnaise samples (two with starch and three without starch in the aqueous phase) were also studied. Drop-size distribution histograms of all emulsions were determined by video-enhanced optical microscopy. The accuracy of this optical procedure was carefully verified, which allowed us to apply the microscopy data as a reference, with known experimental error, for the mean drop size and for the size distribution width of the studied emulsions. In parallel experiments, the drop-size characteristics of the same emulsions were determined by the NMR method. The main acquisition parameters in the NMR experiment were varied to check how the conditions during signal acquisition affect the final results. In this way, we were able to evaluate the precision of the NMR method for a range of O/W emulsions with different properties.

2. Materials and Methods

2.1. Materials. Soybean oil (SBO) of commercial grade and three different types of emulsifiers—whey protein concentrate (WPC, trade name AMP 8000, Proliant), whole liquid egg yolk (EY), and sodium dodecyl sulfate (SDS, Merck)—were used for emulsion preparation.

Aqueous solutions of the emulsifiers were prepared with deionized water, purified by a Milli-Q system (Millipore). Along with the emulsifier, some of the solutions contained the neutral electrolyte NaCl (Merck, analytical grade, heated for 5 h at 450 °C) and the antibacterial agent NaN_3 (Riedel-de Haën).

2.2. Emulsions. The main characteristics of the studied emulsions are summarized in Table 1. For each sample, we list the oil volume fraction, Φ, emulsification procedure, type of emulsifier, volume-weighted geometric-mean drop diameter, d_{33}, and respective width of the log-normal distribution, σ. The values of d_{33} and σ are determined by fitting the actual drop-size distributions, as measured by optical microscopy, with a log-normal distribution curve (see section 2.3 below for the microscopy measuring procedure and Figure 1 for typical size distribution histograms)

Table 1. List of Emulsions Studied, Ordered by Emulsification Method, Emulsifier, and Mean Drop Size[a]

				drop size from optical microscopy	
no.	Φ, %	emulsification procedure	emulsifier	d_{33}, μm	σ
1	80	commercial mayonnaise	EY	4.50	1.40
2	80	commercial mayonnaise	EY	5.46	1.50
3	80	commercial mayonnaise	EY	5.48	1.43
4	80	commercial mayonnaise	EY, starch	6.10	1.57
5	80	commercial mayonnaise	EY, starch	10.7	1.55
6	73	NGH	EY	7.70	1.55
7	78	NGH	EY	7.75	1.56
8	70	NGH	EY	8.81	1.83
9	78	NGH	EY	9.20	1.46
10	78	NGH	EY	9.70	1.46
11	78	NGH	EY	10.4	1.41
12	70	NGH	WPC	3.93	1.59
13	80	NGH	WPC	3.96	1.46
14	90	NGH	WPC	3.94	1.64
15	28	NGH	WPC	4.12	1.40
16	70	NGH	WPC	8.80	1.65
17	70	NGH	WPC	8.80	1.67
18	70	NGH	WPC	11.7	1.73
19	70	NGH	WPC	12.0	1.75
20	70	NGH	WPC	12.0	1.74
21	60	NGH	WPC	12.4	1.69
22	43	NGH	SDS	6.81	1.53
23	72	NGH	SDS	7.19	1.63
24	92	NGH	SDS	7.91	1.53
25	65	rotor-stator homogenizer	SDS	16.1	1.89
26	60	membrane emulsification	SDS	9.04	1.29
27	60	membrane emulsification	SDS	12.0	1.30

[a] Φ is the oil volume fraction. d_{33} is the volume-weighted geometrical-mean drop diameter, and σ is the width of the log-normal distribution curve, both determined by fit to the microscopy data. The abbreviations are EY (egg yolk), WPC (whey protein concentrate), SDS (sodium dodecyl sulfate), and NGH (narrow-gap homogenizer).

$$P_V(2a) = \frac{1}{2a\sqrt{2\pi}\ln\sigma}\exp\left[-\frac{(\ln 2a - \ln d_{33})^2}{2(\ln\sigma)^2}\right] \quad (1)$$

where a denotes the drop radius. The use of a log-normal distribution to describe the microscopy data was dictated by the fact that this function is implemented in the "oil_droplets" software package of the Bruker minispec mq instrument. Since the NMR signal from a given drop is proportional to the drop volume, the volume-averaged mean drop size should be considered.[24,25] The values of d_{33} and σ obtained by the two methods, NMR and optical microscopy, were compared. (Note that the definition of σ in eq 1 is different from the definition used in refs 7 and 24—our σ is equal to the exponent of the σ used in refs 7 and 24.)

Samples 1−5 were commercial mayonnaise emulsions stabilized by egg yolk. Samples 4 and 5 contained starch in the aqueous phase of the mayonnaise for improved texture. The drop-size distribution in these commercial samples was relatively well represented by a log-normal distribution curve (see Figure 1A, for example). In these emulsions, d_{33} varied between 4.5 and 10.7 μm, whereas σ varied between 1.40 and 1.57 (see Table 1).

Three different emulsification procedures were used to prepare the remaining samples listed in Table 1:

Emulsion numbers 6−24 were prepared by using a custom-made narrow-gap homogenizer.[14] The emulsification in this homogenizer is accomplished by passing the oil/water mixture through a narrow slit (width of 75, 195, or 395 μm) at a moderate pressure (between 2 and 5 bar, depending on the slit). By varying the emulsification conditions and the emulsifier, we prepared a series of emulsions with the mean drop size varied between 3.93 and 12.4 μm and the drop-size distribution width, σ, varied between 1.40 and 1.83 (Table 1). The oil volume fraction in these samples varied between 28 and 92 vol %. Most of these samples

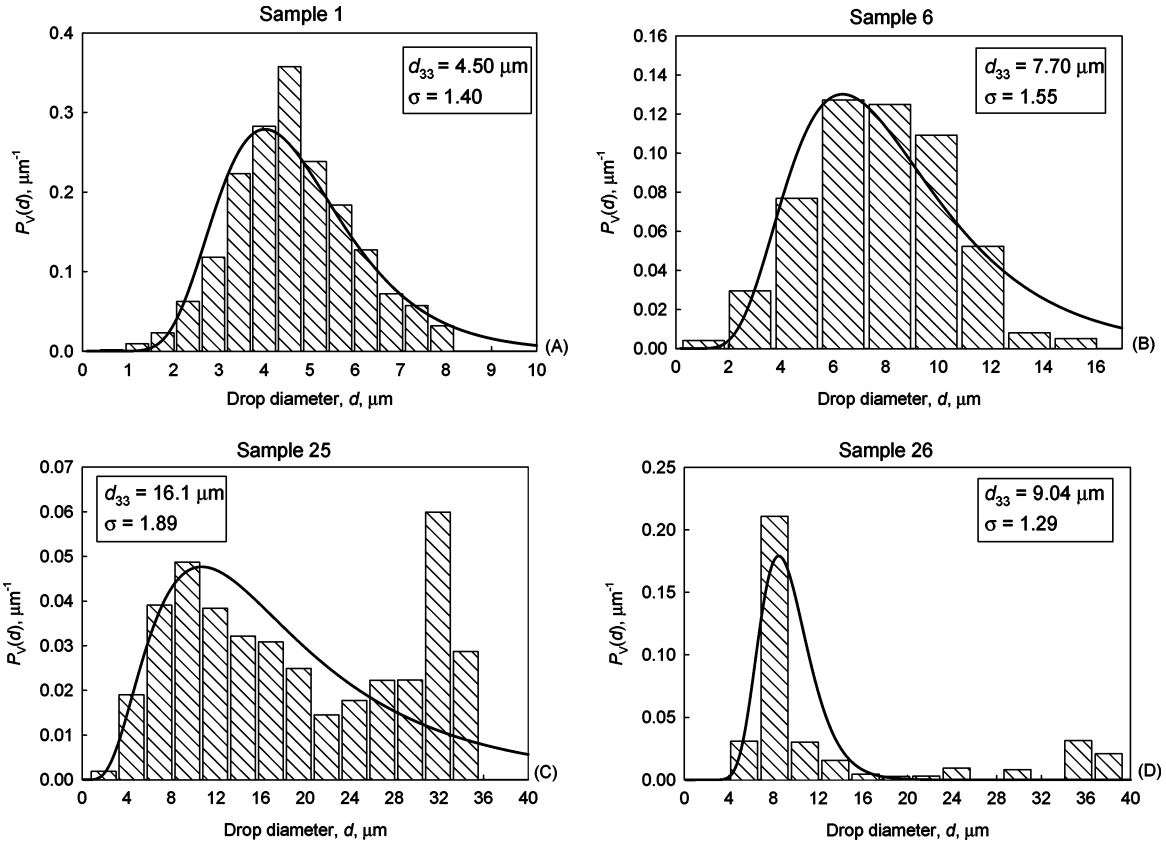

Figure 1. Volume-weighted size distribution histograms of (A) sample 1, a commercial mayonnaise; (B) sample 6, prepared by a narrow-gap homogenizer; (C) sample 25, prepared by a rotor-stator homogenizer; and (D) sample 26, prepared by membrane emulsification. The smooth curves represent a fit by a log-normal distribution curve (eq 1).

were relatively well represented by a log-normal distribution curve (see Figure 1B, for example).

Emulsion 25 was prepared by an Ultra Turrax rotor-stator homogenizer (Janke & Kunkel GmbH & Co, IKA-Labortechnik). This method gave a bimodal drop-size distribution (Figure 1C), which was very different from the model log-normal distribution used to fit the experimental data in the NMR method. The numerical fit of the volume-weighted drop-size distribution data with a log-normal distribution curve gave $d_{33} = 16.1$ μm and a relatively wide distribution of $\sigma = 1.89$.

Samples 26 and 27 were prepared by membrane emulsification.[33−35] In this method, oil was extruded across a porous glass membrane to form oily drops in the continuous aqueous phase. A laboratory-type membrane emulsification module Microkit from Shirasu Porous Glass Technology[33,35] (Miyazaki, Japan) was used. The drop-size distribution in these emulsions was also not well represented by a log-normal curve—a significant fraction of large drops was observed in the "tail" of the histogram (see Figure 1D). The fit of the volume-weighted histograms of these two emulsions, as determined by optical microscopy with log-normal distribution curves, gave $d_{33} \approx 9$ and 12 μm, respectively, and a relatively narrow distribution width of $\sigma \approx 1.30$. This small value of σ shows that the biggest droplets, observed in the histogram, are omitted by the fitted log-normal distribution curve.

2.3. Optical Microscopy and Image Analysis. The emulsions prepared for the NMR experiments were too concentrated to be directly studied by optical microscopy. For this reason, the specimens for optical microscopy were prepared by gentle mixing of ~10 μL of the original emulsions with 1 mL of 20 mM SDS solution. SDS facilitated the deflocculation of the drops and prevented their coalescence. The specimens were transferred for optical examination into microcapillaries of rectangular cross section (depth 0.1 mm, width 1 mm, length 40 mm). For all samples, at least two separate specimens were prepared and the diameters of 5000 drops were measured.

Additionally, for several of the studied samples, specimens for optical observation were prepared by diluting the emulsion with the original aqueous phase. No difference in the measured drop-size distributions was found between these two different dilution procedures.

The optical observations were made in transmitted light with an Axioplan microscope (Zeiss, Germany). An objective Epiplan ×50, with a working distance of 7.0 mm and a numerical aperture of $A = 0.5$ was used. During observation of a given region in the capillary, the focal plane of the microscope was gradually changed in depth of the emulsion to consecutively bring all drops into focus. The images were recorded with a charge-coupled device (CCD) camera (Sony SSC-C370P, 752 × 582 pixels) connected to a video recorder (Samsung SV-4000). The diameters of the recorded oil drops were measured one by one by an operator, using custom-made image analysis software working with a Targa+ graphic board (Truevision, U.S.A.). These data were numerically processed to obtain the volume-weighted drop-size histogram and fitted by a log-normal distribution curve to determine d_{33} and σ. Note that this procedure for image acquisition and analysis excludes the possibility of missing some of the drops, because the entire depth of the capillary containing the emulsion is scanned while changing the focus. As explained in the Appendix, the experimental error in this optical procedure for drop-size measurement is estimated to be around 0.3 μm.

2.4. NMR Spectroscopy. All measurements were performed with a low-resolution NMR spectrometer minispec mq20 (Bruker Optics GmbH, Rheinstetten, Germany), operating at a 20 MHz proton frequency. The instrument was equipped with a variable temperature gradient probehead (mq-PA208) and pulse gradient unit (mq-PGU4), generating a gradient strength of up to 4 T/m. The gradient strength was calibrated with a 0.5 mM aqueous solution of $CuSO_4$. The temperature was stabilized at 23 °C during

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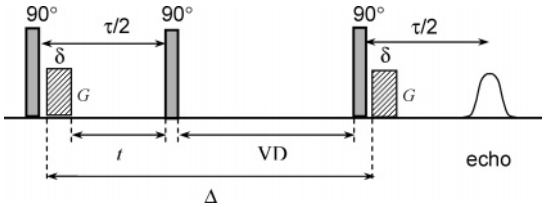

Figure 2. Schematic presentation of the stimulated echo pulse sequence, which consists of three 90° hard pulses and two gradient pulses of duration δ and gradient strength G. τ is the transverse evolution time (called also "echo time"); $\Delta = (\mathrm{VD} + \delta + t + 2\times\text{pulse width})$ is the time between the starting points of the two gradient pulses (diffusion time); t is the gradient settling delay, applied to avoid the influence of eddy-currents on the echo amplitude; and VD (variable delay) controls the time interval, during which the magnetization is longitudinal.

experiments by a thermostat (18205, Bioblock Scientific) and checked with a thermocouple. Standard NMR tubes with a diameter of 10 mm were used.

The applied PGSE technique, as illustrated in Figure 2, is based on the so-called "stimulated echo pulse sequence",[36] which consists of three 90° hard pulses and a pair of gradient pulses of duration δ and strength G. The sample magnetization is measured in the period $\tau/2$ after the last 90° pulse (so-called "echo amplitude"). In this method, the ratio of the amplitudes of the spin−echoes in the presence and absence of gradient pulses is determined. This ratio provides information about the mean-square displacement of the molecules (as a result of translation diffusion) during the diffusion time, Δ.

Briefly, the principle of the method is as follows:[31,32,36] In the absence of gradient pulses ($G = 0$), the spin−echo develops in a homogeneous magnetic field, and the sample magnetization does not depend on changes in the proton positions during the experiment. When $G > 0$, the first gradient pulse creates an inhomogeneous magnetic field, which leads to a partial loss of coherence in the phases of the proton spins, depending on the proton position at the moment of the gradient pulse. In the absence of diffusion, the second gradient pulse would exactly invert the phase shifts of all spins (spin refocusing) and the echo would be the same as in the case of $G = 0$. However, since the molecules in liquids diffuse and change their positions during the diffusion time, Δ, the spin refocusing is incomplete, because the local magnetic field experienced by a given nucleus during the second gradient pulse is different from the field during the first gradient pulse. Larger displacement of the molecule during Δ (i.e., faster diffusion) leads to worse refocusing and to a smaller amplitude of the echo signal.

The echo amplitude in the case of unrestricted diffusion (e.g., Fickian diffusion of molecules in isotropic bulk liquid) is described by[31,36]

$$\ln[R] = \ln\left[\frac{E}{E_0}\right] = -\gamma^2 D G^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right) \tag{2}$$

where E is the echo amplitude in the presence of gradient pulses and E_0 is the respective amplitude in the absence of gradient pulses (i.e., at $G = 0$). R is the spin−echo attenuation ratio, D is the self-diffusion coefficient of the molecules, and $\gamma = 2.67 \times 10^8$ rad/(T·s) is the gyromagnetic ratio for protons.

For restricted diffusion of molecules confined in spherical droplets of uniform radius, a, Murday and Cotts[37] derived the following equation for the attenuation of the echo signal:

$$\ln[R] = -2\gamma^2 G^2 \sum_{m=1}^{\infty} \frac{1}{\alpha_m^2(\alpha_m^2 a^2 - 2)}\left[\frac{2\delta}{\alpha_m^2 D} - \frac{\Psi}{(\alpha_m^2 D)^2}\right] \tag{3}$$

where

$$\Psi = 2 + \exp[-\alpha_m^2 D(\Delta - \delta)] - 2\exp(-\alpha_m^2 D\delta) - 2\exp(-\alpha_m^2 D\Delta) + \exp[-\alpha_m^2 D(\Delta + \delta)]$$

and α_m is the mth positive root of the equation:

$$\frac{1}{\alpha a} J_{3/2}(\alpha a) = J_{5/2}(\alpha a) \tag{4}$$

J_k in eq 4 is the Bessel function of the first kind, order k.

Equation 3 predicts smaller attenuation of the NMR signal (i.e., larger R), for molecules confined in drops, as compared to free molecular diffusion in bulk liquid (eq 2). For very large spheres, eq 3 reduces to eq 2 (the effect of drop boundaries becomes negligible), whereas for very small spheres $R \to 1$ (negligible displacement of the molecules). Therefore, eq 3 is useful in a certain range of drop radii, which depends on the diffusion coefficient, D, of the molecules in the drops and on the values of the control parameters G, Δ, and δ, during the NMR data acquisition process (see section 3.3.3 below for estimates and further discussion).

In the case of a distribution of drop sizes, the measured echo attenuation ratio, R_{obs}, is an average quantity over the volumes of the drops in the sample and can be expressed by the following equation:[38]

$$R_{\mathrm{obs}} = \frac{\int_0^\infty P_{\mathrm{V}}(a)\, R(\Delta, G, \delta, a)\, \mathrm{d}a}{\int_0^\infty P_{\mathrm{V}}(a)\, \mathrm{d}a} \tag{5}$$

where $R(\Delta, G, \delta, a)$ is presented by eq 3 and $P_{\mathrm{V}}(a)$ is the volume-weighted drop-size distribution function. In the following consideration, we represent the drop-size distribution in the studied emulsions by a log-normal function (eq 1) because this is the function implemented in the software package for the NMR instrument.

Low-resolution NMR has the disadvantage that all protons in the sample resonate at approximately the same frequency, and one cannot receive separate signals from the oil and water phases. In the method developed by Goudappel et al.,[24] the water signal is suppressed by means of a filter on the basis of the different diffusivities of the water and oil molecules.[24,39] For triglyceride oils such as the soybean and sunflower oils used in many food emulsions, the self-diffusion coefficient of the oil, $D_{\mathrm{O}} \approx 10^{-11}$ m^2/s, is 2 orders of magnitude smaller than that of free water, $D_{\mathrm{W}} = 2.3 \times 10^{-9}$ m^2/s, at 25 °C. In accordance with eq 2, the rapid diffusion of the water molecules leads to much faster attenuation of the water signal in the presence of gradient pulses, as compared to the oil signal. By making all measurements at a sufficiently long duration of the gradient pulse, δ, larger than a certain minimal value, δ_{min}, one detects the echo created only by the oil protons (the contribution of the water signal is negligible).[24] The value of δ_{min} can be estimated from eq 2, by imposing the requirement that the spin−echo attenuation ratio for water, R_{W}, should be a very small quantity (e.g., $R_{\mathrm{W}} < 10^{-3}$) and using the fact that $\delta \ll \Delta$ in these experiments. Thus, one obtains[24] the following estimate for δ_{min}:

$$\delta_{\mathrm{min}} = \sqrt{\frac{-\ln R_{\mathrm{W}}}{\gamma^2 G^2 D_{\mathrm{W}} \Delta}} \tag{6}$$

Figure 3A illustrates the efficiency of this procedure by showing the simulated amplitude of the echo signal, R, as a function of δ for free water, free oil, and oil confined in spherical drops of radius a (see eqs 2 and 3). It is seen that the water signal is efficiently suppressed at $\delta_{\mathrm{min}} \geq 0.5$ ms, whereas the oil signal is only slightly reduced and can be used to determine the oil drop size.

For data acquisition and analysis, we used the "oil-droplets.app" software package, developed by Bruker and

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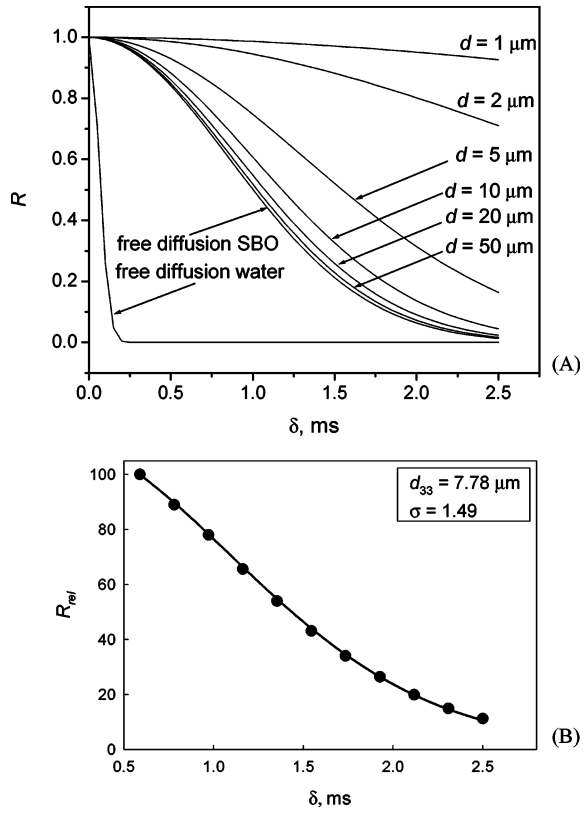

Figure 3. (A) Simulation of the echo amplitude ratio, R, as a function of δ for unrestricted diffusion of water and oil as well as for oil in spheres of different radii. The parameters used for this simulation are $D_W = 2.3 \times 10^{-9}$ m^2/s, $D_O = 1.15 \times 10^{-11}$ m^2/s, $\Delta = 210$ ms, and $G = 2$ T/m. (B) Experimental points, $R_{rel}(\delta)$, for sample 5 and their fit by eqs 1 and 3−5.

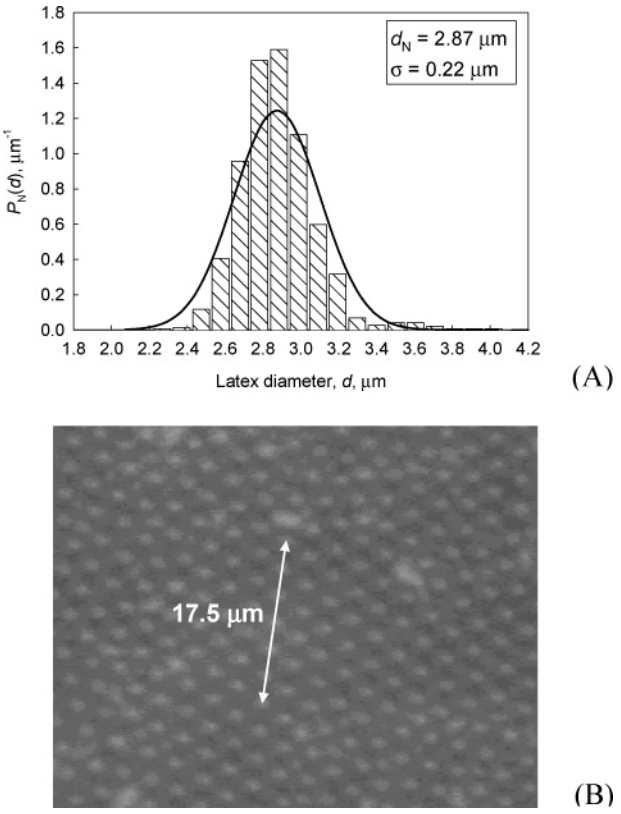

Figure 4. (A) Histogram by number and the respective normal distribution curve for the latex particles, as obtained by the single-particle analysis. (B) Two-dimensional ordered array of latex particles. From the chain of particles, indicated by an arrow, one can determine a mean particle diameter of 17.5/6 = 2.9 μm.

Unilever. During the measurement, the instrument determines the echo attenuation for the oil protons, $E(\delta)$, for a set of several δ values, which are equally distributed between two limiting values, δ_{min} and δ_{max}. The software automatically calculates δ_{min} in accordance with eq 6 after the operator specifies the values of Δ and G.[24] The maximal value, δ_{max}, is set in the range between 2.0 and 2.5 ms to ensure as large as possible variation of the spin−echo attenuation, $E(\delta)$. Larger values of δ_{max} could not be used in our experiments because this approach requires $\delta \ll \Delta \sim 200$ ms. Furthermore, larger values of δ_{max} give very low signal-to-noise ratios. Following data acquisition, the ratio $R_{rel}(\delta) = E(\delta)/E(\delta_{min})$ is calculated and the values of d_{33} and σ are determined by numerical fitting of R_{rel} with eqs 3−5, as shown in Figure 3B.

The source code of the oil-droplets.app package is closed by the producer (Bruker Optics, Germany), so that technical details of the algorithm used for data acquisition and interpretation are not available. Thus, several of the emulsions were studied also by our own software programs for data acquisition (written in Bruker's ExpSpel editor) and for data analysis (written in Fortran 77). Since the results for d_{33} and σ obtained with our programs were very similar to those obtained with the commercial package oil-droplets.app, and the conclusions were the same, we present and discuss below only the data obtained by the commercial package.

3. Results and Discussion

3.1. Accuracy of the Optical Procedure for the Determination of Drop-Size Distribution. Since the results from the microscopy determination of drop-size distribution are used as a reference for comparison with the NMR results, we first estimated the possible experimental errors in the optical procedure and performed a careful check of its accuracy. The estimates, described in the Appendix, show that the experimental error of the microscopy procedure is ± 0.3 μm and is determined mainly by the resolution limit of the microscope.

As a direct check of the accuracy of the optical procedure, we measured the number-averaged diameter, d_N, of monodisperse latex particles (Interfacial Dynamic Corporation, U.S.A.) in two different ways. First, the size distribution of latex particles was determined by optical microscopy, following exactly the procedure for single drop-size measurement, as described in section 2.3. The diameters of 1000 individual particles were measured and $d_N = 2.87 \pm 0.22$ μm was determined. The obtained size distribution histogram of these particles is presented in Figure 4A.

In parallel, the mean diameter, d_N, was precisely measured with latex particles, assembled in well-ordered, two-dimensional (2D) domains (which could not be obtained with oil droplets because the latter were too polydisperse). To obtain such ordered domains, a drop of the latex suspension was spread as a thin layer on a hydrophilic glass plate.[40] As water evaporated from the suspension layer, ordered 2D particle domains appeared under the action of convective hydrodynamic force and lateral capillary force, when the thickness of the aqueous layer became smaller than the particles' diameter.[40,41] By measuring the length of several chains, each comprising five to seven particles (center-to-center distance, as shown in Figure 4B), we determined $d_N = 2.92 \pm 0.05$ μm. The latter procedure of mean drop-size determination is very accurate because an averaging over all particles in the

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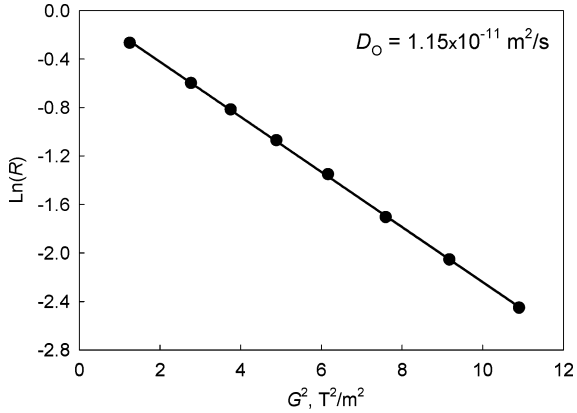

Figure 5. Echo amplitude ratio, ln R, as a function of G^2 for soybean oil at 23 °C. The points are experimental data; the line is a linear fit, in accordance with eq 2. The following acquisition parameters are used: $\Delta = 120$ ms and $\delta = 1.5$ ms.

chain is made and because the measured distance (15−20 μm) is much larger than the optical resolution of the microscope.

The good agreement between the values of d_N obtained by these two optical procedures (difference of only 0.05 μm) is a direct confirmation that our optical procedure for single drop-size determination is sufficiently accurate for our purpose and its experimental error does not exceed 0.3 μm.

3.2. Effect of the Acquisition Parameters on the Results Obtained by Low-Resolution NMR. *3.2.1. Determination of the Self-Diffusion Coefficient of the Molecules in Bulk Oil.* The self-diffusion coefficient of the oil molecules, D_O, is an important parameter for interpretation of the NMR data, and its value should be precisely determined prior to the drop-size measurements. To determine D_O, we used the "diffusion stimulated echo.app" acquisition program (Bruker Optics, Germany). The dependence of the echo attenuation ratio on the gradient field strength, $R(G)$, was measured, and the experimental data were fitted by eq 2 (see Figure 5). From the best linear fit of the data, plotted as ln R versus G^2, we obtained $D_O = 1.15 \times 10^{-11}$ m^2/s for soybean oil at 23 °C. The latter value was used for interpretation of the NMR data from the studied emulsions.

3.2.2. Effect of Diffusion Time, Δ, on the NMR Results. The accuracy of the NMR method depends on the appropriate choice of certain acquisition parameters. In the PGSE sequence used, these parameters are the gradient field strength, G, and the diffusion time, Δ. Several series of experiments were performed to find the optimal ranges of values for G and Δ and to check how sensitive the NMR results are with respect to these values.

The range of possible values for the diffusion time, Δ, is bound by several limitations.[7,24,25] On one hand, Δ must be sufficiently long, so that a significant fraction of the oil molecules encounter the drop surface during the time interval, Δ. If Δ is shorter, the echo attenuation ratio approaches the value for bulk oil and is slightly dependent on drop size. On the other hand, Δ cannot be too long because the signal becomes strongly attenuated by longitudinal relaxation and the signal-to-noise ratio is very poor. Thus, measurements are only possible using a limited range of Δ values. For our emulsions, the upper boundary of usable Δ values, determined by the signal-to-noise ratio, varied between 300 and 350 ms.

An estimate for the optimal magnitude of Δ can be made[25] from the Einstein law for translation molecular diffusion by comparing the mean-square displacement of the molecules along one direction, $2D_O\Delta$, with the square of the mean drop radius, $(d_{33}/2)^2$. This estimate shows that Δ should be $\geq (d_{33})^2/(8D_O)$. For $d_{33} \approx 4$ μm, which corresponds to the smallest mean drop size in the studied emulsions and $D_O \approx 10^{-11}$ m^2/s, this estimate gives $\Delta \geq$ 200 ms. Note that if we take larger drop diameters, $d_{33} \geq 5$ μm, this estimate predicts $\Delta \geq 300$ ms, which could not be used in our systems because of the poor signal-to-noise ratio. One can conclude from this estimate that (1) the range of Δ values which can be used in our experiments is very narrow and (2) for most of the studied samples, we have to use shorter values of Δ than the optimal ones, because $d_{33} > 5$ μm (see section 3.3.3 for further discussion).

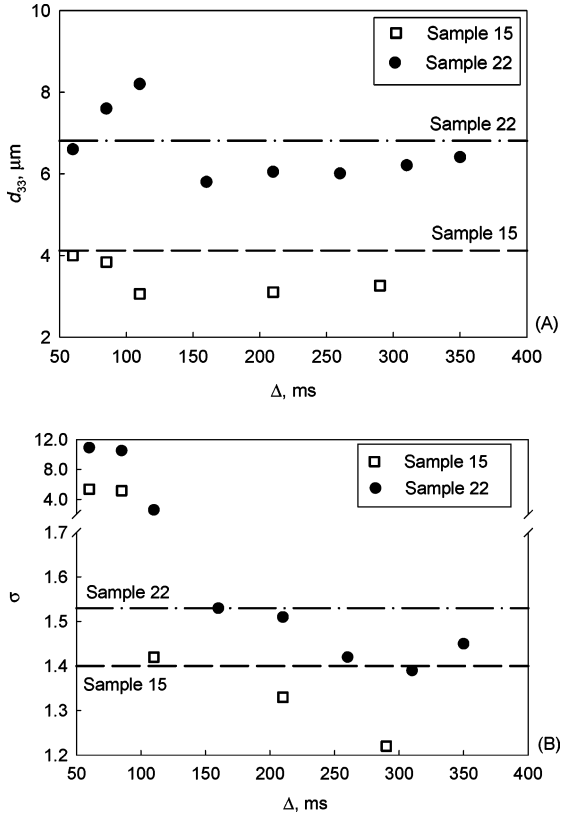

Figure 6. Dependence of (A) d_{33} and (B) σ, as measured by NMR, on the value of diffusion time, Δ, for samples 15 and 22. $G = 2$ T/m in these experiments. The horizontal lines indicate the respective values, as measured by optical microscopy.

To check experimentally how the final results for d_{33} and σ depend on the chosen value of Δ, we performed measurements on a given sample by varying Δ in the range between 50 and 350 ms at $G = 2$ T/m. Experiments on several emulsions showed that the final results are not stable for $\Delta < 150$ ms; the measured values of d_{33} and σ vary significantly upon small changes of Δ. Moreover, the values of σ were unrealistically large (typically, above 4) at $\Delta < 150$ ms. Figure 6 shows the results for emulsion nos. 15 and 22 as two examples (similar results were obtained with several other emulsions). As explained in the previous paragraphs, the reason for the inaccurate measurements at short Δ is that the echo attenuation becomes insensitive to drop size.

At $\Delta > 150$ ms, the obtained results for d_{33} were stable and slightly dependent on the particular value of Δ. The results for σ also varied in a reasonable and relatively narrow range when Δ was between 150 and 210 ms (see Figure 6B). At $\Delta \geq 250$ ms, the measured values of σ were still reasonable, though systematically lower than those determined by optical microscopy.

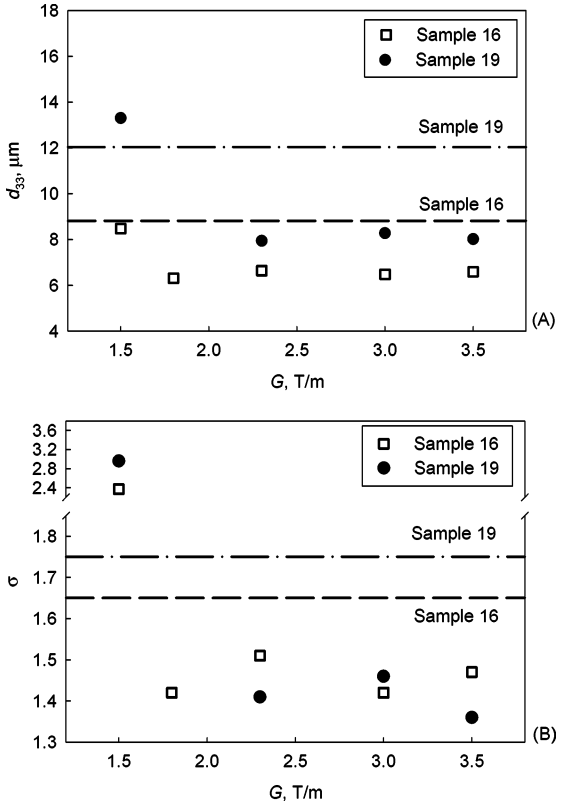

Figure 7. Dependence of (A) d_{33} and (B) σ, as measured by NMR, on the value of gradient strength, G, for samples 16 and 19. $\Delta = 210$ ms in these experiments. The horizontal lines indicate the respective values, as measured by optical microscopy.

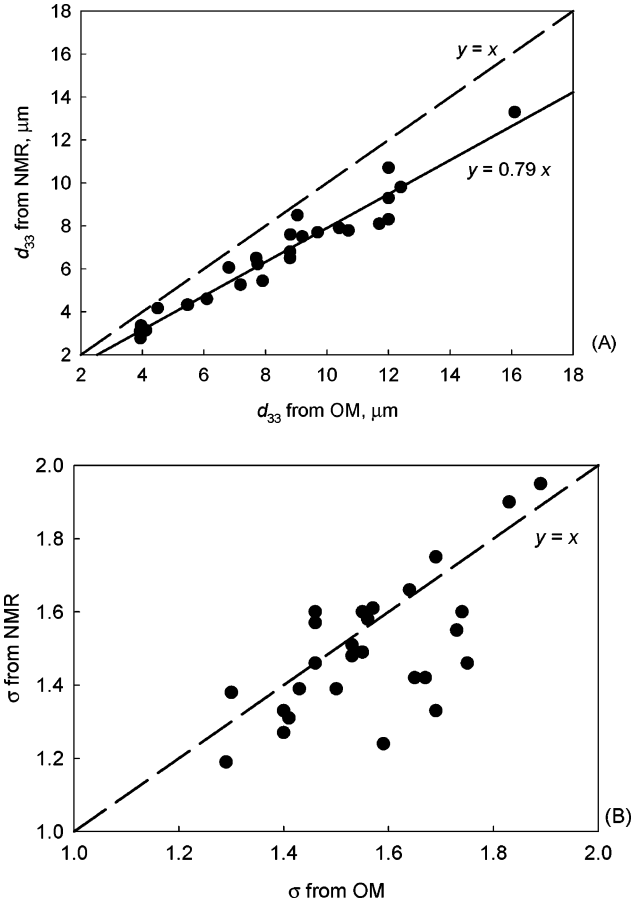

Figure 8. Correlation plots for (A) d_{33} and (B) σ, as determined from NMR and optical microscopy (OM) for the emulsions listed in Table 1. The dashed lines on both plots correspond to d_{33}(NMR) = d_{33}(OM) and σ(NMR) = σ(OM), respectively. The solid line in part A is a best linear fit, according to the equation d_{33}(NMR) = 0.79d_{33}(OM).

As seen from Figure 6, the results for d_{33}, obtained by NMR at $\Delta > 150$ ms, are slightly lower than those determined by optical microscopy. Similar results were obtained with the other emulsions as well; see section 3.3.1 below. Moreover, the values of d_{33}, measured by NMR, exhibited a slight but steady trend in the possible range of Δ values (between 150 and 300 ms)—the measured mean drop size slowly increased with the increase of Δ and became closer to the values measured by optical microscopy. Therefore, longer values of Δ would be preferable if the signal-to-noise ratio was sufficiently good. However, in most of the emulsions, the signal-to-noise ratio at $\Delta =$ 300 ms was relatively poor and the measurements were difficult.

In conclusion, the possible range for Δ values in our measurements was practically limited between 150 and 300 ms (for example, measurements at $\Delta > 300$ ms were impossible with sample 15 in Figure 6 due to poor signal-to-noise ratio). We found $\Delta \approx 200$ ms to be an optimal value because both d_{33} and σ were measured with reasonable accuracy and very good reproducibility. For the further systematic comparison of the NMR method and optical microscopy (section 3.3), we used $\Delta = 210$ ms, which was also used in ref 7. Note that the results for d_{33} obtained at $\Delta = 210$ ms are similar to those obtained at slightly larger values of Δ (see Figure 6). Therefore, the main conclusions of the present study would be similar if we had chosen $\Delta = 250$ ms (instead of 210 ms) for the systematic comparison of NMR and optical microscopy.

3.2.3. Effect of the Gradient Pulse Strength, G, on the NMR Results. Illustrative results for the dependence of d_{33} and σ on the selected value of G are presented in Figure 7 for two of the studied emulsions (nos. 16 and 19 in Table 1) at $\Delta = 210$ ms. The results obtained with other samples were qualitatively similar. As seen from Figure 7, the measurements at $G < 1.8$ T/m gave results for σ which were unrealistically high and strongly dependent on the particular value of G. The reason is that the echo attenuation strongly depends on the gradient strength (see eqs 2 and 3) and the effect of diffusion becomes negligible at small values of G. On the other hand, the results for d_{33} and σ did not depend on the value of G, when the latter was varied between 1.8 and 3.5 T/m. In conclusion, the recommended range of values for G is between 2 and 3.5 T/m.

Note that NMR measurements again showed lower values for d_{33}, as compared to the microscopy data (see Figure 7A). In the particular examples shown in Figure 7B, the values of σ, measured by NMR, were also lower than the values measured by optical microscopy, but the opposite dependence was observed for some of the other samples (see section 3.3.1 and Figure 8).

3.3. Comparison of the NMR Results with Those from Optical Microscopy. *3.3.1. Correlation Plots.* To compare the results from the NMR method to those obtained by optical microscopy (OM), we constructed the respective correlation plots for d_{33} and σ, as measured by these two methods. The NMR results, presented in Figure 8 and discussed in this section, were obtained at $\Delta = 210$ ms and $G = 2.0$ T/m. These values were chosen on the basis of the tests described in sections 3.2.2 and 3.2.3. The same values of Δ and G were used in some of the experiments by van Duynhoven et al.[7] as well. One sees from Figure 8A that the values of d_{33}, determined by the

NMR method, were systematically lower than those determined by optical microscopy. Averaged over all studied samples, this difference was 21 ± 7%; see the solid line in Figure 8A, which is a least-squares fit with a straight line, corresponding to the relation $d_{33}(\mathrm{NMR}) = 0.79 d_{33}(\mathrm{OM})$. The reproducibility of the measured values of d_{33} for a given sample by both methods is represented approximately by the size of the symbols used in Figure 8A. Therefore, the discrepancy between the two methods cannot be explained by poor reproducibility (see section 3.3.2 for further discussion of this issue).

The results for the width of the distribution, σ, were more scattered. As seen from Figure 8B, the values of σ, determined by NMR, were significantly smaller for five of the samples than those measured by optical microscopy (by 0.3−0.4 dimensionless units). For most of the samples, however, the difference was smaller (<0.2 units) and random; both smaller and larger values were measured by NMR. Note that the reproducibility in measuring σ for a given sample was very good—about ±0.05 with the NMR method and about ±0.02 using optical microscopy. Therefore, the observed scattering in the results, shown in Figure 8B, cannot be explained by poor reproducibility of the measurements. The most probable explanation for the observed scattering is that the results for σ (which corresponds to a second moment of the drop-size distribution curve) are very sensitive to the experimental NMR data, $R_{\mathrm{res}}(\delta)$. Indeed, by artificially shifting up or down some of the experimental points, $R_{\mathrm{res}}(\delta)$, we found that the calculated values of σ are affected strongly by very small imperfections in the experimental data, which can be caused by possible artifacts during measurement, such as transient B_0 inhomogeneities, gradient imperfections, and temperature variations.

The observed difference between the NMR and microscopy results was not found to depend in a systematic way on any of the properties of the studied emulsions, such as mean drop size, width and shape of the drop-size distribution, oil volume fraction, or emulsifier. Since the observed difference is larger than the accuracy of the optical microscopy (0.3 μm), one can conclude that this difference is related to this NMR method. As explained in the previous section, this difference cannot be removed by a better choice of the acquisition parameters of the NMR experiment. The possible reasons for the observed discrepancy between the two methods are discussed in the following section.

3.3.2. Possible Reasons for the Observed Difference between NMR and Optical Microscopy. The observed difference between the results for d_{33}, obtained by NMR and optical microscopy, is not very large (≈20%). This agreement is rather satisfactory for the intended application of low-resolution NMR as a routine method with simple sample preparation. Note that the method is noninvasive and relatively fast and the results are robust with respect to the acquisition parameters, if the latter are chosen in their optimal ranges, and with respect to the emulsion properties (mean size, width of the distribution, and oil volume fraction). Moreover, by knowing that the NMR method gives slightly lower values for d_{33} (Figure 8A), one can make a correction of the value, measured by NMR, and obtain a very good estimate of the actual mean drop size in the studied emulsion. If we make this correction, that is, if we increase by 20% all values of d_{33}, measured by NMR in our study, we end up with a very good agreement between NMR and optical microscopy for all samples (random deviation ±7%). Note that such an artificial correction is justified only for systems similar to those from the present study (O/W emulsions of vegetable triglyceride oils) and for the same conditions in the NMR experiment. Therefore, the NMR method is a convenient tool for the comparison of such emulsions and for measuring the main drop-size characteristics with reasonable accuracy. For some specific emulsions (e.g., with gelled aqueous phase, like in cheese emulsions), which are very difficult for drop-size analysis, the NMR method is certainly among the most appropriate methods.

On the other hand, the observed difference between the NMR and optical microscopy data is systematic and larger than the estimated accuracy of the optical microscopy. The reasons for this systematic difference are not entirely clear at the present moment. Two possible reasons are briefly described below:

(1) According to eq 7 in section 3.4.1, the optimal drop-size range for application of the PGSE NMR method in the case of oil-in-water emulsions with $D_{\mathrm{O}} \approx 10^{-11}$ m^2/s is below a diameter of $d_{\max} \approx 4$ μm. On the other hand, the mean drop size in the studied emulsions varied between 4 and 16 μm, which appears to be above the optimal range. For such relatively large drops, a problem arises from the impossibility of using a sufficiently long diffusion time, Δ, to ensure a mean-square displacement of the oil molecules comparable to the drop size ($D\Delta/a^2 \sim 1$). In fact, the studied triglyceride oils exhibit an unfavorable combination of relatively slow diffusion and fast relaxation. The slow diffusion requires long Δ values, while the short relaxation time limits the actual possible range of diffusion times by the value $\Delta_{\max} \approx 300-350$ ms.

(2) The theoretical description of the spin−echo (eqs 3 and 5) implies that the effect of surface relaxation, M, on the spin−echo attenuation is negligible.[25] In the so-called "fast diffusion regime" ($Ma/D \ll 1$), the effect of surface relaxation can be neglected because the echo attenuation, caused by diffusion, is dominant.[25] However, the effect of surface relaxation is not necessarily negligible in systems containing slowly diffusing molecules. Theoretical simulations of the spin−echo attenuation under the combined influence of molecular diffusion and surface relaxation showed that ignoring surface relaxation can lead to apparently smaller drop sizes.[42,43] The effect of surface relaxation is more pronounced for small drops, which have a larger surface-to-volume ratio.

In conclusion, the two effects mentioned above could explain qualitatively the observed discrepancy between the results obtained by NMR and by optical microscopy. Effect 1 is expected to be more important for larger drops (with $d > 4$ μm), whereas effect 2 is expected to be more important for smaller drops. Note that these effects are specific for the oil/water NMR method used here and are not expected to affect the low-resolution NMR determination of drop size in water-in-oil emulsions.

Additional checks were performed to clarify how the NMR results are affected by the value of D_{O}, which is an important parameter in data interpretation (as explained in section 3.2.1, $D_{\mathrm{O}} = 1.15 \times 10^{-11}$ m^2/s was measured with bulk soybean oil). For this purpose, we simulated the NMR echo attenuation signal for several emulsions, using their actual drop-size distributions as determined by optical microscopy, by considering D_{O} as a free adjustable parameter for the given emulsion. The comparison of the simulated echo attenuations with the measured ones showed that the results could agree only if the oil diffusion coefficient, used in the numerical

(42) Kuchel, P. W.; Lennon, A. J.; Durrant, C. *J. Magn. Reson.* **1996**, *112*, 1.
(43) Codd, S. L.; Callaghan, P. T. *J. Magn. Reson.* **1999**, *137*, 358.

simulations, is substantially smaller in magnitude than the measured one. For emulsions with a diameter of $d_{33} \geq 7\,\mu$m, this difference was around 20%. For smaller drops, the difference was even more significant; for example, we should use $D_O \approx 0.4 \times 10^{-11}$ m^2/s (which seems to be an unrealistically small value) to describe the data for emulsions with $d_{33} \approx 4\,\mu$m (samples 12−15 in Table 1). This comparison of the simulated and actual NMR results shows that the discrepancy between the NMR and microscopy results cannot be resolved by simply changing the value of D_O in the NMR data interpretation. Furthermore, the fact that the discrepancy does not depend on the emulsifier used (SDS, whey protein concentrate, or egg yolk) indicates that the diffusion coefficient of the oil, inside the actual emulsion drops, is not specifically affected by the emulsifier.

3.3.3. Limitations of the NMR Method Used. Some limitations of the NMR method are discussed in the original papers.[7,24] Further discussion of the main assumptions in the PGSE method and of the range of drop sizes, which can be resolved by this method, is presented in the recent review by Pena and Hirasaki.[25] In this section, we summarize some of the limitations of the PGSE method, taking into account also our own experience with the studied food emulsions.

(a) Range of Drop Diameters in Which the Method is Applicable. The following estimate was suggested in ref 25 for the maximum drop size, d_{max}, for which the used PGSE method can be applied:

$$d_{max} = 2(D_O\Delta_{max})^{1/2} \quad (7)$$

where D_O is the oil diffusion coefficient and Δ_{max} is the maximum diffusion time at which the echo signal is sufficiently larger than noise. As explained in sections 3.2.1 and 3.2.2, $D_O = 1.15 \times 10^{-11}$ m^2/s and $\Delta_{max} \approx 300$ ms for the studied triglyceride oils. Thus, one estimates $d_{max} \approx 4\,\mu$m from eq 7.

On the other hand, our experiments showed that the method of Goudappel et al.[24] can be applied to emulsions with a mean drop size of $d_{33} \approx 20\,\mu$m (though systematically lower values for d_{33} are measured, Figure 8A). For typical emulsions with a distribution width of $\sigma \approx 1.5$, this limitation corresponds to a distribution peak for which most of the drops are below $\sim 30\,\mu$m. Indeed, experiments with other emulsions (not shown in Table 1 and Figure 8) showed that the method fails to give reasonable results for emulsions containing a significant fraction of drops with a diameter above $30\,\mu$m. These experimental results show that eq 7 underestimates d_{max} by a factor of at least 5. Most probably, the reason is that all molecules in the drop are actually at a distance which is smaller than (d/2) from the nearest drop boundary. Therefore, most of the molecules encounter the drop boundary without travelling distances as large as the drop diameter, d. On the basis of our experiments, we suggest modifying eq 7 by increasing the numerical factor 5 times

$$d_{max} \approx 10(D_O\Delta_{max})^{1/2} \quad (7')$$

keeping in mind, however, that underestimated values of d_{33} can be measured in the range of large drops (section 3.3.1).

For estimation of the minimal drop size, d_{min}, Pena and Hirasaki[25] suggested the following equation:

$$d_{min} = \left(525\lambda\frac{D_O}{\gamma^2 G^2 \delta_{max}}\right)^{1/4} \quad (8)$$

where $\lambda = [1 - R(\delta_{max})]$ is the smallest reduction of the attenuation signal which is reliably detected by the instrument. Due to the particular pulse sequence Goudappel et al.[24] used to suppress the signal from the continuous aqueous phase, the first ≈10% of the echo attenuation is not detected, which means that $\lambda \approx 0.1$ in this method. As explained in section 2.4, $\delta_{max} \approx 2.5$ ms in our experiments. Thus, one estimates $d_{min} \approx 2.3\,\mu$m from eq 8. As seen from Figure 8, we were able to measure emulsions with $d_{33} \approx 4\,\mu$m, in which most of the drops had a diameter above $\sim 2.5\,\mu$m. Therefore, the diameter of the smallest drops in the studied emulsions was close to the theoretical limit of d_{min} (eq 8).

The limitations of this NMR method can be illustrated by considering the theoretical simulations of the curves $R(\delta)$ for soybean-oil-in-water emulsions (see Figure 3). The simulations show that the theoretical curves for large drops ($d \geq 10\,\mu$m) have very similar shapes and gradually approach the curve for free diffusion of the oil molecules. The curve for $d = 50\,\mu$m becomes practically indistinguishable from that corresponding to free diffusion. Note that the actual experimental data are affected by the experimental noise as well as by the polydispersity of the real emulsions. As a result, it becomes impossible to interpret precisely the experimental points from the NMR measurements with emulsions containing a significant volume fraction of drops with $d > 30\,\mu$m.

One can conclude that the accessible size range for triglyceride-in-water emulsions, as in this study, is between 2 and 30 μm (with a mean drop size of up to 20 μm). The same estimates are expected to be valid for other oil-in-water emulsions prepared with oils with a similar diffusion coefficient, $D \approx 10^{-11}$ m^2/s, and studied by low-resolution NMR.

(b) Oil Phase Volume Fraction. The oil volume fraction in the studied emulsions varied in the range between 28 and 92%. For all emulsions, we were able to adjust the amplitude of the NMR echo signal to be between 85 and 95% of the maximum signal output (the recommended optimal range) by changing the receiver gain of the instrument. For the range of oil fractions, mentioned above, the adjusted receiver gain was between 85 and 70 dB. We did not perform a systematic experimental investigation of the lower limit of detectable oil in emulsions. Instead, from the upper limit of the receiver gain of the minispec instrument (100 dB), we estimated that the minimum oil volume fraction which can be studied by this instrument is around 10 vol %. The latter value is in agreement with the estimate quoted by Duynhoven et al.[7]

Note that other experimental problems could sometimes arise in diluted emulsions with a nongelled aqueous phase of low viscosity, viz., drop sedimentation and/or diffusion could affect the NMR signal; see the discussion and estimates in ref 24.

(c) Type of Oil. There are several important requirements of the oil phase for successful application of the method of Goudappel et al.[24] First, the oil should be in a liquid state because solid particles, if present in the oil drops, can impede the diffusion of the oil molecules and lead to misinterpretation of the NMR data.[7]

Another important requirement for the oil phase is that the ratio between the self-diffusion coefficients of the oil and water molecules should differ by at least 3 times. This requirement restricts the application of the method (as implemented in the minispec instrument used here) to oils with diffusion coefficients smaller than 10^{-10} m^2/s because the value used for the calculation of δ_{min} in the

Bruker software is $D_W \approx 6 \times 10^{-10}$ m^2/s (to make the measurement possible even in the case of a gelled aqueous phase).[24] By direct experimental checks, we found that it was impossible to determine the drop-size distribution for tetradecane-in-water ($D_O = 5.4 \times 10^{-10}$ m^2/s) and hexadecane-in-water ($D_O = 4.3 \times 10^{-10}$ m^2/s) emulsions—it was impossible to acquire an oil signal of detectable amplitude at any values of the acquisition parameters in the commercial software package.

Theoretical simulations similar to those plotted in Figure 3 showed that, if the water phase is not gelled (viz., $D_W = 2.30 \times 10^{-9}$ m^2/s is used for the estimate of δ_{min}) and the acquisition parameters are optimized, one could efficiently suppress the water signal and measure the drop-size distribution in oil-in-water emulsions, with $D_O \approx 5 \times 10^{-10}$ m^2/s. Therefore, measurements with tetradecane-in-water and hexadecane-in-water emulsions should be possible if most appropriate acquisition parameters are selected. However, Balinov and Soderman[23] found that the method is not applicable to oils with $D_O < 10^{-12}$ m^2/s, due to the slow molecular diffusion and short relaxation times of such oils. From the above consideration, one can conclude that the method[24] is applicable to liquid oils with a diffusion coefficient between $\sim 10^{-12}$ and $\sim 5 \times 10^{-10}$ m^2/s.

4. Conclusions

The accuracy of the low-resolution NMR method of Goudappel et al.,[24] for the determination of drop-size distribution in oil-in-water emulsions, was evaluated by comparing results obtained on a commercial minispec mq20 instrument (Bruker Optics, Germany) with data from optical microscopy. The experimental error of the microscopy measurements was estimated to be below 0.3 μm, which allows us to use these data as a reference. The results can be summarized as follows:

The low-resolution NMR method gave lower values for the mean drop size, d_{33}, by $\approx$20%. The results for the distribution width, σ, were more scattered. The values of σ, measured by NMR, were significantly smaller than the actual ones (by 0.3−0.4 dimensionless units) for 20% of the samples. For the remaining samples, the values of σ were almost randomly scattered around the reference within $\pm$0.1 to 0.2 units. No systematic relation was found between the main properties of the studied emulsion (mean drop size, polydispersity, oil volume fraction, and emulsifier) and the relative difference between the microscopy and NMR results.

The experiments are highly dependent on the selection of appropriate acquisition parameters. We showed that the possible range of values for the diffusion time, Δ, is between 150 and 300 ms. Values of Δ around 200 ms are optimal with respect to the accuracy in measurement of both d_{33} and σ. The recommended gradient strength for obtaining reproducible results is 2 T/m $\leq G \leq$ 3.5 T/m. Even if the values of Δ and G are chosen to fall in the recommended ranges, the NMR method underestimates d_{33}, as described in the previous paragraphs.

The method[24] is applicable to oils with a diffusion coefficient between $\sim 10^{-12}$ and $\sim 5 \times 10^{-10}$ m^2/s. The size range of all emulsion droplets which can be resolved by this method is between $\sim$2 and $\sim$30 μm for vegetable triglyceride oils with $D_O \approx 10^{-11}$ m^2/s.

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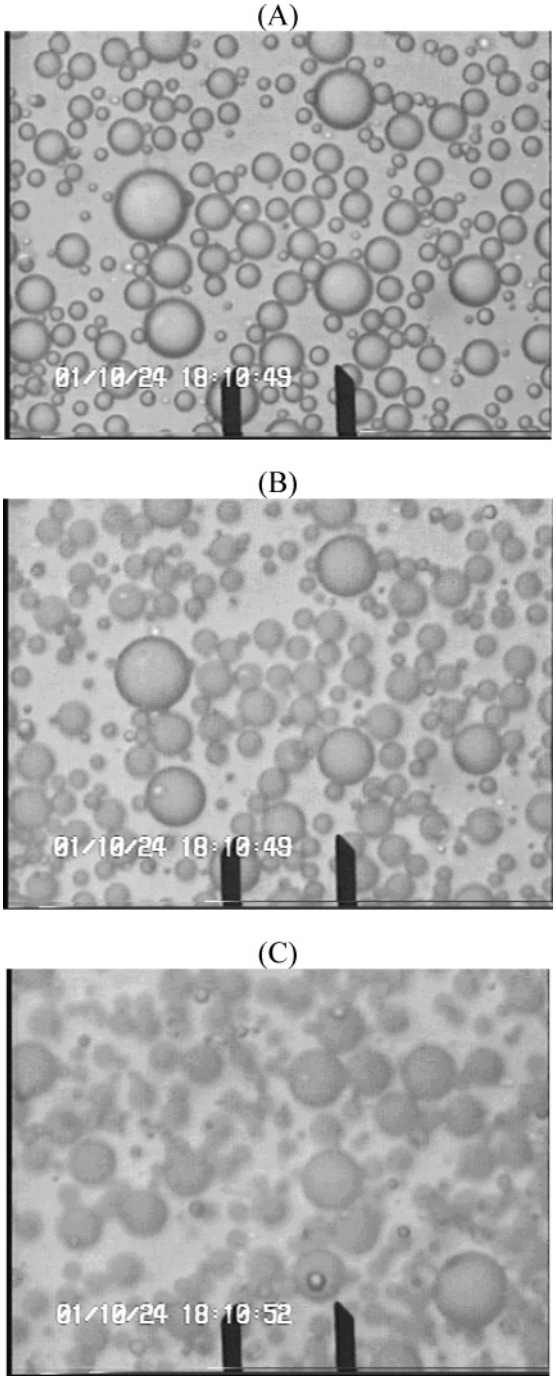

Figure 9. Images of oil drops at different focal planes, in a fixed region of the emulsion sample. The focus plane is gradually changed, scanning the entire depth of the capillary, and the size of the drops is measured from the image showing sharpest drop boundary. The distance between the bars is 20 μm.

Appendix. Estimates of the Errors in the Microscopy Determination of Drop Size.

The theoretical resolution limit of the optical microscopy, Res, is determined[5] by the wavelength of the illuminating light, λ, and by the numerical aperture of the objective, A:

$$\text{Res} = \frac{0.61\lambda}{A} \tag{A1}$$

In our experiments, $\lambda = 540$ nm and $A = 0.5$, which corresponds to Res ≈ 0.66 μm. However, Jokela et al.[6] found experimentally that, when video-enhanced optical microscopy is used (which is the case in our experiments), the actual resolution limit is about half of the theoretical value, that is, around Res/2 ≈ 0.3 μm.

One important factor which can affect the measurement is the adjustment of the focal plane of the microscope to determine the drop diameters at their respective equators.[5] In our experiments, the microscope focus and the light intensity were carefully controlled and optimized to obtain the sharpest possible boundaries between the oil drops and the surrounding aqueous medium. As an example, in Figure 9, we show a series of consecutive drop images, obtained by changing the vertical position of the focal plane. The size of a given drop was measured from the image where it exhibited the sharpest boundary. A comparison of the drop sizes measured at the sharpest focus to those in slightly defocused images showed that the error created by imperfect focus is <0.2 μm.

The image digitization by the video system (768×576 pixels) is another factor that could affect the accuracy, due to the finite size of the pixels. The pixel size corresponded to 0.13 μm in our experiments, which is significantly smaller than the resolution limit of the microscope. Note that the finite pixel size creates a random, rather than systematic error. Hence, this error is relatively small and can be neglected in comparison with the error caused by the optical resolution limit.

Large drops can be deformed by the buoyancy force, which could cause a systematic error in the drop-size measurement. The importance of this effect can be evaluated by considering the Bond number:[5]

$$B = \frac{\Delta\rho g a^2}{\sigma_{\text{OW}}} \tag{A2}$$

which is a ratio of the gravity and capillary pressures. Here, $\Delta\rho$ is the difference between the mass densities of the drop and continuous phases ($\Delta\rho = 0.08$ g/cm^3 in our systems), g is the acceleration of gravity, and σ_{OW} is the interfacial tension. For our emulsions, the lowest value of the interfacial tension was $\sigma_{\text{OW}} \approx 6$ mN/m, which corresponded to $B \approx 3 \times 10^{-5}$ for drops with a diameter of 30 μm (the upper drop-size limit in the studied emulsions). The value of B rapidly decreases with decreasing drop size (see eq A2). The estimated small values of Bond number show that the drop deformation, driven by gravity, was negligible in our systems.

One can conclude from the above consideration that the most significant error in the procedure used for the optical determination of drop size is caused by the microscope resolution, and it is around 0.3 μm (see also section 3.1).

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