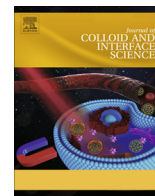




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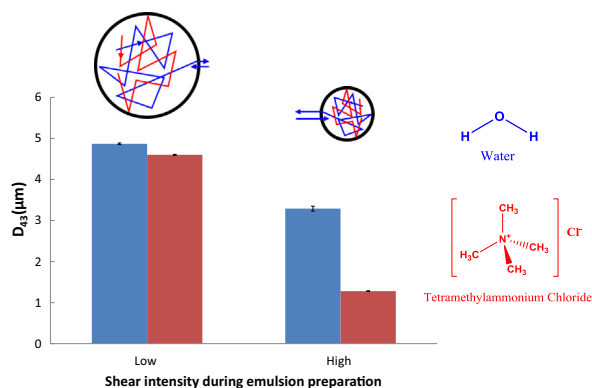
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Regular Article

Increasing water solubility with decreasing droplet size limits the use of water NMR diffusometry in submicron W/O-emulsion droplet size analysis

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GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: Water droplet size analysis of water-in-oil emulsions using water NMR diffusometry yielded values that were, from a certain shear intensity onwards, independent from the shear which was used during production. It was assumed that the constant water droplet size, obtained for samples prepared at higher shear, were only apparent droplet diameters. Considering the well-known increased solubility of the dispersed phase in the continuous phase at smaller droplet sizes, it is hypothesized that water diffusion in the oil phase was responsible for the fact that apparent rather than real sizes were obtained.

Experiments: W/O-emulsions, prepared with a varying shear intensity, were characterized using dynamic light scattering, light microscopy, T₂-relaxometry and PFG-NMR diffusometry. The latter measurements were conducted on both a low- and a high-resolution device and was based on either water (LR- and HR-NMR) or a water-soluble marker (HR-NMR).

Findings: Low-resolution PFG-NMR is incapable of accurately determining the droplet size of W/O-emulsions containing (sub)micron sized droplets. On the other hand, using high-resolution PFG-NMR diffusometry and the addition of an oil insoluble marker to the water phase, the application window could

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be extended towards smaller droplet sizes. Finally, it was shown that T_2 -relaxometry was capable of detecting differences in droplet size between (sub)micron sized W/O-emulsions.

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1. Introduction

Emulsions occur in a wide variety of industrial branches such as the food, cosmetic, pharmaceutical, chemical and refinery industries. Most often, they consist of a lipophilic and a hydrophilic phase, with one phase being dispersed as droplets in the other phase, stabilized using emulsifiers [1].

The droplet size distribution of an emulsion has a big influence on its properties: it influences the gravitational, coalescence and microbiological stability upon storage as well as the rheology, appearance, texture, flavor and compound release rate [1–4]. Moreover, the droplet size distribution provides insight into the efficiency of the used emulsifier and production process of the emulsion [4].

A lot of droplet size characterization techniques require dilution of the sample, which might have an influence on the droplet size distribution [1,5]. When W/O-emulsions are characterized, the used dilution liquid often differs from the used oil phase as dilution in a viscous phase is difficult. For example, hexadecane [6,7], dodecane [8] or dodecane containing 0.5 wt% sorbitan mono-oleate [9], have been used to dilute and subsequently analyze W/O-emulsions.

Analysis of these diluted emulsions is usually performed using laser light scattering techniques. Static laser light scattering measurements are quite insensitive to the used refractive indices for larger droplets ($>25\ \mu\text{m}$). However, the smaller the droplets get, the higher the sensitivity of the result towards the used refractive indices of the continuous and dispersed phase [10]. In contrast, using dynamic light scattering (DLS), only the viscosity of the continuous phase has to be known in order to determine the droplet size distribution. In DLS measurements, the Brownian motion of droplets is followed. Therefore, these droplets have to be sufficiently small (typically $<1\ \mu\text{m}$) in order to avoid very long measurements. The fact that the Brownian motion is followed is an additional reason to dilute W/O-emulsions in an organic solvent instead of the oil phase: diffusion of droplets in this viscous oil phase would be too slow [11].

For some of the other less frequently used droplet size determination techniques, each with their own advantages and shortcomings, reference is made to Bernewitz et al. [1] and van Duynhoven et al. [5].

In contrast to most droplet size determination techniques, NMR-based techniques do not require dilution of the sample. Moreover, the size of individual droplets is determined, even if these droplets are flocculated whereas light scattering techniques would measure the size of the flocs [12].

A frequently used NMR technique to determine droplet size distributions is pulsed field gradient (PFG)-NMR diffusometry [1,5]. Using echo decay data, diffusion of protons (^1H) can be followed and diffusion coefficients determined. Murday and Cotts [13] introduced a data analysis procedure whereby the droplet size distribution is estimated from the restricted diffusion coefficient of molecules in the dispersed phase, assuming the droplet boundaries to be impermeable [4].

Next to PFG-NMR diffusometry, T_1 - or T_2 -relaxometry can be used to characterize the droplet size (in fact the specific surface area) of W/O-emulsions [14–16]. Relaxometry has mainly been used in the characterization of oilfield emulsions [14–16] and in the determination of the specific surface area of porous sedimen-

tary rocks [17,18]. In this context, it can be mentioned that Peña and Hirasaki [15] measured low values for the surface relaxivity, a parameter which links a change in specific surface area to a change in relaxation rate, for W/O-emulsions with an oil phase free of paramagnetic impurities and stabilized using non-ionic surfactants. As far as we know, the applicability of relaxometry for emulsions with a potentially low surface relaxivity, such as most edible emulsions, has not been fully ascertained.

Finally, it can be mentioned that both the aforementioned NMR techniques can be combined in order to obtain a droplet size distribution without prior assumptions of the shape of the droplet size distribution [15,16]. In contrast, unless regularization methods are applied, a lognormal distribution of the droplets is usually assumed when PFG-NMR diffusometry is used to determine the droplet size distribution [4].

Vermeir et al. [4] showed that during PFG-NMR water diffusometry measurements on W/O-emulsions, increasing temperatures led to increasing water diffusion through the oil phase. Due to the increased diffusion of water between droplets, a higher apparent droplet size was measured at higher temperatures. Therefore, in order to minimize the influence of water exchange between droplets, PFG-NMR measurements are usually conducted at a low temperature, typically $5\ ^\circ\text{C}$ [4].

In this work, it was examined whether measuring at a low temperature indeed leads to satisfactory results, even in case (sub)micron sized water droplets are present in the W/O-emulsion. Indeed, small droplets have a higher solubility than larger droplets or the bulk material, which is called the Kelvin effect [19]. In addition, also an exploratory test was performed to determine whether a difference in droplet size in the produced edible W/O-emulsions could be detected using T_2 -relaxometry.

2. Materials and methods

2.1. Materials

The hydrophobic emulsifier polyglycerol polyricinoleate (PGPR 4175) was kindly provided by Palsgaard A/S (Juelsminde, Denmark). Sunflower oil was acquired from a local supermarket while high oleic sunflower oil (hoso; Iodine Value = 87; 82% C18:1) was acquired from Contined BV (Wageningen, The Netherlands).

The water phase contained 0.1M NaCl (VWR Chemicals: BDH Prolabo, Leuven, Belgium) in the emulsions analyzed using only low-resolution NMR while 0.1M tetramethylammonium chloride (TMACl, Sigma-Aldrich, Steinheim, Germany), in a mixture (50/50; m/m) of $\text{D}_2\text{O}/\text{H}_2\text{O}$, was used in the emulsions analyzed using both low- and high-resolution NMR. In the high-resolution tests, TMACl was used instead of NaCl because this salt is an oil-insoluble compound which can be detected using high resolution-NMR [4]. Finally, all water phases also contained 0.02 wt% of the anti-microbial agent NaN_3 (Sigma-Aldrich, Steinheim, Germany).

2.2. Preparation of the emulsions

2.2.1. Samples analyzed using low-resolution NMR

2.2.1.1. Samples analyzed using both PFG-NMR diffusometry and T_2 -relaxometry. 5 wt% PGPR 4175 was added to hoso after which this phase was heated to $60\ ^\circ\text{C}$ and stirred to obtain a homoge-

neous solution. The water phase was also heated to 60 °C after which the emulsions were prepared.

In a first production scheme, the 50/50 (m/m) emulsions were prepared using a rotor-stator homogenizer (Ultra-Turrax: type S25-10G, IKA®-Werke, Germany). Hereby, the water phase was gradually added during mixing. Three different mixing intensities were used namely 6500, 17500 and 24000 rpm; these emulsions are referred to as UT 6500-5wt, UT 17500-5wt and UT 24000-5wt, respectively.

In a second scheme, a premix was prepared using a bigger rotor stator homogenizer (Ultra-Turrax: type S50N-G45F, IKA®-Werke, Germany). Hereby, the water phase was gradually added during mixing at 5200 rpm. Afterwards, the emulsions were further processed using 1 pass through a Microfluidizer (type M110S, Microfluidics) at 140, 420 or 840 bar during which the temperature was kept at 60 °C using a heating bath. The aforementioned emulsions are referred to as MF 140-5wt, MF 420-5wt and MF 840-5wt, respectively.

2.2.1.2. Samples analyzed using T_2 -relaxometry at different temperatures. In order to determine the influence of the measurement temperature on the T_2 -relaxation time of the water phases in the W/O-emulsions, two samples which contained 10 wt% PGPR 4175 in hoso were prepared at a different shear intensity.

In a first production scheme, the 50/50 (m/m) emulsion was prepared using a rotor-stator homogenizer (Ultra-Turrax: type S25-10G, IKA®-Werke, Germany) at 6500 rpm; this emulsion is referred to as UT 6500-10wt

In a second production scheme, a premix was prepared using the same rotor stator homogenizer (Ultra-Turrax: type S25-10G, IKA®-Werke, Germany). Hereby, the water phase was gradually added during mixing at 24000 rpm. Afterwards, the emulsion was further processed using 1 pass through a Microfluidizer (type M110S, Microfluidics) at 840 bar during which the temperature was kept at 60 °C using a heating bath; this emulsion is referred to as MF 840-10wt

2.2.2. Samples analyzed using both low- and high-resolution NMR

10 wt% PGPR 4175 was added to sunflower oil after which this phase was heated to 60 °C and stirred until a homogeneous solution was obtained. The water phase, containing 0.1M TMACl in a 50/50 (m/m) mixture of D_2O and H_2O , was also heated to 60 °C after which the emulsions were prepared, according to the production schemes described in Section 2.2.1.2.

The emulsion obtained using the first production scheme is referred to as UT 6500-HR. The premix obtained in the second scheme is referred to as UT 24000-HR, whereas the emulsion obtained after one pass through the Microfluidizer at 840 bar is referred to as MF 840-HR.

2.3. Low-resolution NMR

2.3.1. PFG-NMR diffusometry

Low-resolution (LR) pulsed field gradient (PFG) NMR diffusometry measurements were performed at 5 °C using a benchtop Maran Ultra spectrometer (Oxford Instruments, UK) operating at a frequency of 23.4 MHz. The samples of about 2.5 g were filled in 18 mm outer diameter glass NMR-tubes (Oxford Instruments, UK). A monopolar rectangular gradient pulse was used. Thus, the standard Stejskal-Tanner equation was used in the determination of free diffusion coefficients [20]. As described in the latter work, the shape factor σ equals 1 while Δ' equals $(\Delta - \delta/3)$ for this gradient pulse shape. For protons, the gyromagnetic ratio (γ) is equal to $2.675 \times 10^8 \text{ s}^{-1} \text{ T}^{-1}$.

The free self-diffusion coefficient of the water phase (D) was measured using the DSD script (Oxford Instruments, UK) and vary-

ing the gradient duration (δ) in ten steps between 0.05 and 2.75 ms while keeping the gradient strength (G) and the diffusion delay (Δ) constant at 0.14 T/m and 200 ms, respectively. The free self-diffusion coefficient of the water phase was measured in triplicate and amounted to $1.34 \pm 0.04 \times 10^{-9} \text{ m}^2/\text{s}$ for the water phase (D_2O/H_2O ; 50/50; m/m) containing 0.1 M TMACl while a diffusion coefficient of $1.29 \pm 0.01 \times 10^{-9} \text{ m}^2/\text{s}$ was measured for the water phase (H_2O) containing 0.1 M NaCl.

Pulsed field gradient-NMR experiments on the W/O-emulsions were performed using the stimulated echo pulse (STE) sequence, which was preceded by an inversion recovery sequence for suppression of the NMR-contribution from the oil phase in the emulsion. The inversion recovery experiment on the sunflower oil phase containing 10 wt% PGPR led to a τ_0 of 80 ms while a τ_0 of 50 ms was measured for the high oleic sunflower oil containing 5 wt% PGPR. The measurements were performed varying the gradient strength (G) between 0 and 3.17 T/m and, unless stated otherwise, using a diffusion delay (Δ) of 180 ms and a gradient pulse duration (δ) of 2.5 or 8 ms, for the first and second series of PFG-NMR diffusometry tests, respectively.

To determine the volume weighed average droplet size of the water droplet size distribution in the W/O-emulsions, the Murday-Cotts model was fitted to the obtained echo decay curves using the diffusion coefficient as determined for the water phase. In order to estimate the experimental uncertainty, the standard error of the point estimate was calculated by taking the square root of the reciprocal of the Hessian [21].

2.3.2. T_2 -relaxometry

T_2 -relaxation (transversal relaxation) measurements were conducted using the Carr-Purcell-Meiboom-Gill (CPMG) sequence while keeping the receiver gain constant. Unless stated otherwise, the temperature was kept at 5 °C and T_2 -distributions were obtained by CONTIN analysis of the relaxation curve using the WinDXP 1.8.1.0 software (Oxford Instruments, UK).

As described by Godefroy et al. [14], there is a relationship between the T_2 -relaxation time of the water phase and the droplet size given by Eq. (1):

$$\frac{1}{T_2} = \frac{1}{T_{2B}} + \frac{3 * \rho_2}{a} \frac{1}{1 + \frac{\rho_2 * a}{2 * D}} \quad (1)$$

In which ρ_2 is the surface relaxivity, a is the radius of the droplets, T_{2B} is the bulk T_2 -relaxation time and D is the translational diffusion coefficient of the liquid inside the droplets. Thus, what should in fact be considered is the difference between $1/T_2$ and $1/T_{2B}$:

$$\frac{1}{T_2} - \frac{1}{T_{2B}} = \frac{1}{T_{2,corr}} = \frac{3 * \rho_2}{a} \frac{1}{1 + \frac{\rho_2 * a}{2 * D}} \quad (2)$$

2.4. High-resolution PFG-NMR diffusometry

High-resolution (HR) pulsed field gradient (PFG) NMR diffusometry measurements were performed using a Bruker Avance III spectrometer operating at a 1H frequency of 500.13 MHz and equipped with a 5 mm DIFF30 high-gradient probe with a maximum gradient strength of 18 T/m. Measurements were performed at 5 °C and the temperature was controlled with a Eurotherm 3000 VT digital controller. The samples were filled in glass NMR-tubes with a diameter of 5 mm (Euriso-top SAS, France).

PFG-NMR experiments were performed using a stimulated echo pulse sequence with bipolar sine bell shaped gradients and a δ and Δ of 2.4 ms and 180 ms, respectively. The free self-diffusion coefficients of water and TMACl in the water phase were measured upon varying G while keeping δ and Δ constant at 2.4 ms and 180 ms,

respectively. Taking into account the shape factor (σ) of $\pi/2$ and a Δ' of $(\Delta - 5 * \delta/16 - \tau/2)$ for the used gradient pulse [20], $D_{s,water}$ and $D_{s,TMACI}$ were measured to be $1.17 * 10^{-9} \text{ m}^2/\text{s}$ and $5.60 * 10^{-10} \text{ m}^2/\text{s}$, respectively.

To determine the volume weighed average droplet size of the water droplet size distribution in the W/O-emulsions, the Murday-Cotts model was fitted to the obtained echo decay curves using the diffusion coefficient as determined for either the water or the TMACI-molecules. In order to estimate the experimental uncertainty, the standard error of the point estimate was calculated by taking the square root of the reciprocal of the Hessian [21].

In order to determine the diffusion coefficient of PGPR in sunflower oil, a solution of 10 wt% PGPR in sunflower oil was prepared. The sample was filled in a glass NMR-tube with a diameter of 5 mm (Euriso-top SAS, France), equipped with a NI5CCI-B coaxial insert which contains D-aceton (Euriso-top SAS, France). PFG-NMR experiments were performed using a stimulated echo pulse sequence with bipolar sine bell shaped gradients and a δ and Δ of 2.4 ms and 180 ms, respectively. The peak corresponding to the proton next to polymerized OH-groups of ricinoleate was followed. This peak was found at 3.6 ppm when PGPR was dissolved in chloroform [22]. Due to the use of a different solvent (sunflower oil) in our experiment, the ^1H -peaks shifted to higher values and the peak of interest was found at 4.1 ppm.

2.5. Dynamic light scattering

Measurements were conducted using a Zetasizer 3000Hs (Malvern Instruments, UK) or a spectrometer PCS-100 SM (Malvern Instruments, UK) at a scattering angle of 150° and aperture of 50 μm . The latter spectrometer was equipped with a digital correlator K7032CN (Malvern Instruments, UK). Samples were diluted in 1-bromododecane (Alfa Aesar, viscosity at $25^\circ\text{C} = 3.37 \text{ mPa}\cdot\text{s}$) in order to prevent multiple scattering after which 3 subsequent measurements were conducted. Data analysis was performed using the monomodal option (cumulant analysis).

2.6. Light microscopy

The emulsions were diluted ten times in high oleic sunflower oil and observed under the microscope. The diluted emulsions were brought on a microscopy slide using a capillary after which a cover slip was gently applied. Image acquisition was conducted using an optical microscope (Olympus Cx40) with 100-fold objective coupled with a digital camera (AxioCam ERc 5S).

3. Results

3.1. Samples examined using low-resolution NMR

Six W/O-emulsions were prepared with the same composition but with a varying shear intensity during production. Light microscopy, dynamic light scattering and two low-resolution NMR techniques, namely PFG-NMR diffusometry and T_2 -relaxometry (part 3.3.), were used to determine the water droplet size distribution.

3.1.1. Light microscopy

As shown in Fig. 1, a decreasing water droplet size was obtained with increasing shear intensity during production of the W/O-emulsion. Light microscopy thus indicates that, as expected, the droplet size is reduced when higher shear intensities were used during production of the W/O-emulsion.

3.1.2. Dynamic light scattering

Samples were diluted in 1-bromododecane because this solvent has a polarity which is similar to oil and a density which is similar to water. Moreover, the viscosity is smaller than that of the used oil phase [23,24]. Due to the fact that the density difference between the continuous and dispersed phase is small, sedimentation is retarded during the measurement. Table 1 shows the obtained Z-average diameters and polydispersity indices of the emulsions as determined using cumulant analysis of the dynamic light scattering data.

These measurements clearly show that DLS after diluting the W/O-emulsions in 1-bromododecane could also detect a difference in droplet size between the samples.

3.1.3. PFG-NMR diffusometry

Fig. 2 shows the echo decay curves which were obtained for the W/O-emulsions, prepared using different shear intensities. For the samples that were prepared using the lowest shear intensities (UT 6500-5wt and UT 17500-5wt), a decrease to about 17 and 42% of the initial value was obtained, respectively. In contrast, a similar decay profile was obtained for the other 4 samples in which the signal decreased to about 60% of its initial value.

As the obtained echo decay was similar for the four samples which were prepared using the highest shear intensities, the obtained average droplet size and standard deviation of the droplet size distribution were similar when the Murday-Cotts model was fitted to these echo decay curves (Table 2).

The results shown in Table 2 indicate that low-resolution PFG-NMR diffusometry, with the used parameter settings, did not detect a difference between the four samples that were produced under the highest shear intensities (UT 17500-5wt, and MF 140-5wt, MF 420-5wt and MF 840-5wt) whereas both light microscopy and DLS clearly pointed out a difference between these samples.

Hence, these results point to the fact that droplet size analysis by PFG-NMR water diffusometry has a lower boundary, whereby all smaller droplets give the same response, and hence the same apparent droplet size distribution.

Using a (lower) diffusion delay (Δ) of 60 or 120 ms, the curves of the 4 samples prepared at the highest shear intensity still coincided while the UT 6500-5wt and UT 17500-5wt samples did not. For the UT 6500-5wt and UT 17500-5wt samples, the echo decay curves were influenced by the used diffusion delay, which is expected for hindered diffusion. Fitting of the Murday-Cotts model to the echo decay curves of these samples led to similar volume weighed average diameters as compared to when a diffusion delay of 180 ms was used. However, for the 4 samples which were prepared at the highest shear intensity, the echo decay curves coincided, regardless of the used diffusion delay. This kind of behavior is typical for free diffusion.

3.2. Comparison of low- and high-resolution PFG-NMR diffusometry

As described in the previous section, low-resolution PFG-NMR diffusometry using $\delta = 2.5 \text{ ms}$ and $\Delta = 60, 120$ or 180 ms , was not capable of detecting a difference between samples that did, however, contain droplets with a different droplet size distribution indicated by light microscopy, dynamic light scattering as well as T_2 -relaxometry (part 3.3.). Moreover, variation of the used diffusion delay indicated that free instead of hindered diffusion occurred in the 4 samples which were prepared at the highest shear intensity. Therefore, a set of new experiments were conducted in which low- and high-resolution PFG-NMR diffusometry were compared to determine the cause of this effect.

Fig. 3 shows that the echo decay curves of the samples, prepared using a higher shear intensity (and thus containing smaller droplets), coincided for small values of the ordinate. These small

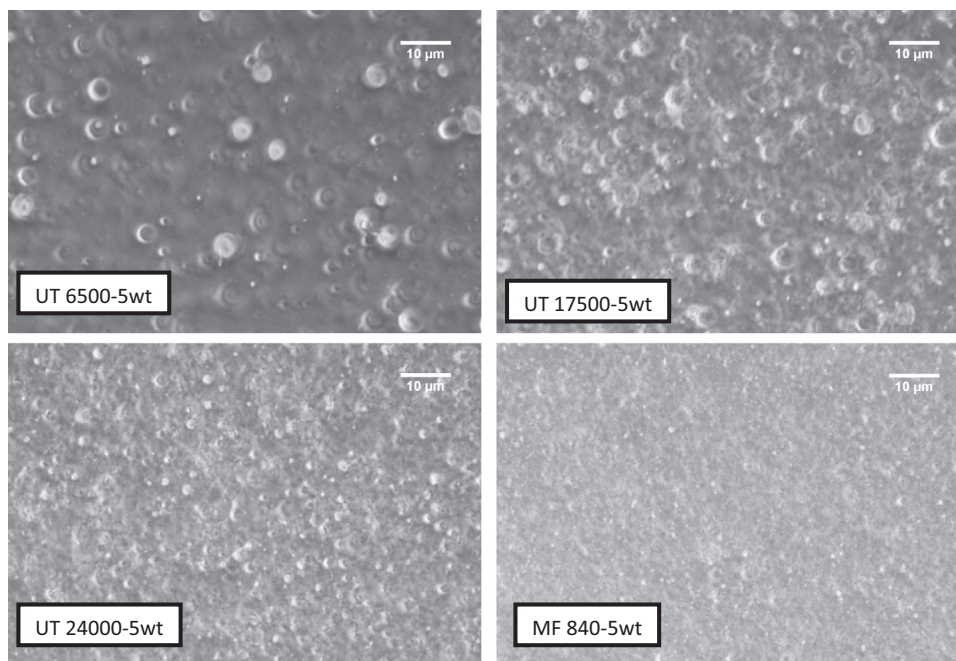


Fig. 1. Pictures of the emulsions after 10× dilution in *hoso* obtained from light microscopy using a 100× objective.

Table 1

Z-average diameter and polydispersity index of the W/O-emulsions; the average value and standard deviations were determined based on 3 repetitions after dilution in 1-bromododecane.

Sample	Z-average diameter (nm)	Polydispersity index (-)
UT 6500-5wt	1072 ± 30	0.399 ± 0.284
UT 17500-5wt	849 ± 17	0.535 ± 0.022
UT 24000-5wt	586 ± 8	0.444 ± 0.038
MF 140-5wt	493 ± 16	0.294 ± 0.115
MF 420-5wt	355 ± 3	0.312 ± 0.053
MF 840-5wt	272 ± 2	0.159 ± 0.023

values may be either obtained by weak gradient strengths at long gradient duration (i.e. 8 ms) or by stronger gradient strengths at short gradient duration (i.e. 2.5 ms). As such, the indicated region corresponds to the whole x-axis interval that was considered during the LR-experiments shown in Fig. 2. Hence, using the LR-NMR settings ($\delta = 2.5$ ms) the two decay curves would coincide, as was the case with four of the six samples in Fig. 2. However, using a δ of 8 ms, the decay was more pronounced and a difference between both samples could be distinguished, even with LR-NMR. Independency of the echo decay curve from the used gradient pulse duration is also a typical property of free diffusion, which

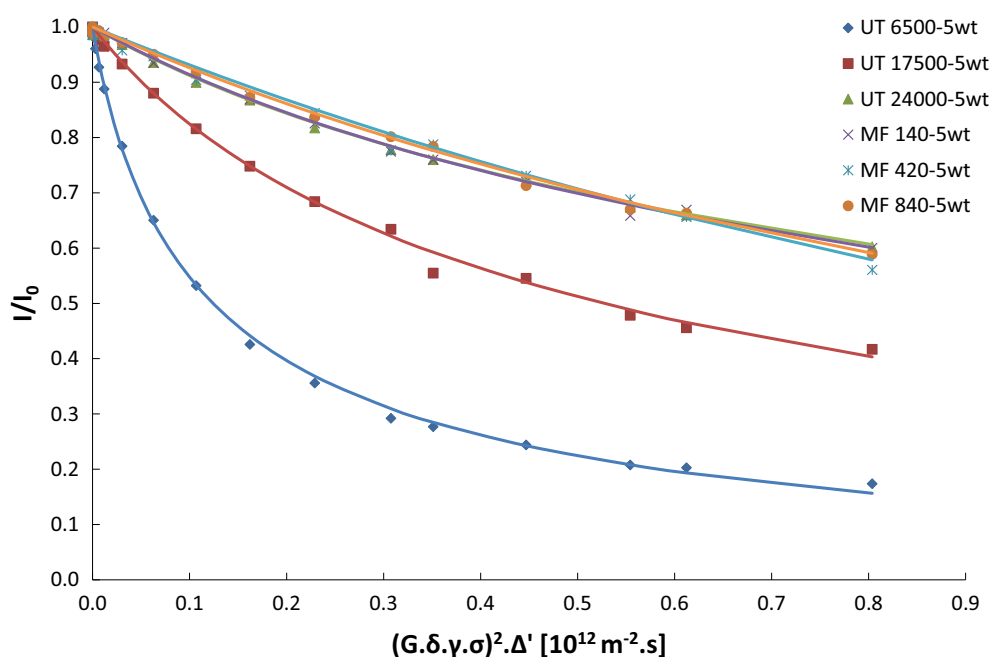


Fig. 2. Echo decay curves of the W/O-emulsions prepared using different shear intensities; the used gradient pulse duration was equal to 2.5 ms while the diffusion delay was set to 180 ms. Lines represent the fit of the Murday-Cotts model to the data.

Table 2

Volume weighed average diameter (d_{43}) and standard deviation of the volume weighed distribution (σ_v) of the W/O-emulsions (together with their standard error) as obtained from fitting the Murday-Cotts model to the echo decay data.

Sample	d_{43} (μm)	σ_v (μm)
UT 6500-5wt	6.36 ± 0.05	3.39 ± 0.10
UT 17500-5wt	3.82 ± 0.04	1.53 ± 0.16
UT 24000-5wt	2.95 ± 0.01	1.18 ± 0.08
MF 140-5wt	2.96 ± 0.03	1.11 ± 0.16
MF 420-5wt	3.01 ± 0.03	0.37 ± 0.27
MF 840-5wt	2.97 ± 0.02	0.71 ± 0.13

implies free diffusion dominated the echo decay curve of fine emulsions for small values of the ordinate. For coarse emulsion samples, the obtained echo decay curves depended on the used gradient pulse duration for every value of the ordinate, as expected for hindered diffusion. When the Murday-Cotts model was fitted to these curves, similar volume weighed average diameters were determined, regardless of the used gradient pulse duration (Supplementary material).

As shown in Fig. 3, a faster echo decay was recorded at bigger values of the ordinate for the sample which was prepared using the highest shear (MF 840-HR). This indicates that free diffusion contributed more to the signal of this sample as compared to the sample produced at lower shear (UT 24000-HR). In order to examine this effect, high-resolution PFG-NMR was conducted on the same samples, during which the diffusion behavior of water as well as an oil insoluble marker (TMACI) was followed separately (Fig. 4).

As shown in Fig. 4, the echo decay curves of the water phase, as determined using low-resolution NMR, and of the water molecules, as determined using high-resolution NMR, nearly coincided for the samples under consideration. This close resemblance proves that the inversion recovery sequence which was applied to remove the oil contribution from the LR-NMR data was effective and hence the LR-NMR data indeed reflected the behavior of water. Moreover,

it shows that the echo decay curves which were obtained using different devices (high- and low resolution NMR) were comparable.

Fig. 4A shows that in the sample containing the biggest water droplets, TMACI and water have a similar decay profile, which indicates that their diffusion behavior is determined by the size of the restriction within which they can diffuse. The most interesting observation, however, is that for smaller droplets (Fig. 4B and C), the echo decay curves of TMACI strongly deviate from the echo decay curves of the water molecules. This shows that, for these samples, the average molecular diffusion of the water molecules is not restricted to the same extent as the diffusion of the TMACI-molecules.

Table 3 shows the obtained volume weighed average diameter (d_{43}) and standard deviation of the volume weighed distribution (σ_v) for the different samples when the Murday-Cotts model was fitted to the obtained echo decay curves. It should be mentioned that for the MF 840-HR sample, fitting to the echo decay of TMACI did not yield satisfactory results as almost no decay of the echo signal was recorded (Fig. 4C). However, from simulations of the decay profile as a function of droplet size (not shown), it follows that all droplets in this sample have to be smaller than about $0.5 \mu\text{m}$ in diameter.

Table 3 thus shows that similar results were obtained when the diffusion of either TMACI or water was considered for the emulsion with the largest water droplet size. However, for the samples subjected to higher shear, a significant difference in resulting droplet size distribution parameters was observed based on either the water or TMACI echo decay curves. Indeed, the water decay and thus derived droplet size was hardly affected by further processing whereas the TMACI decay and derived size was. Moreover, an apparent lower limit of about $3 \mu\text{m}$ is observed based on water diffrusometry. This is not in line with light microscopy, where droplets can hardly be observed in the samples processed at higher shear, indicating submicron droplets. On the other hand, the size information derived from the TMACI decay curve is much more in line with other observations. Indeed, dynamic light scattering measure-

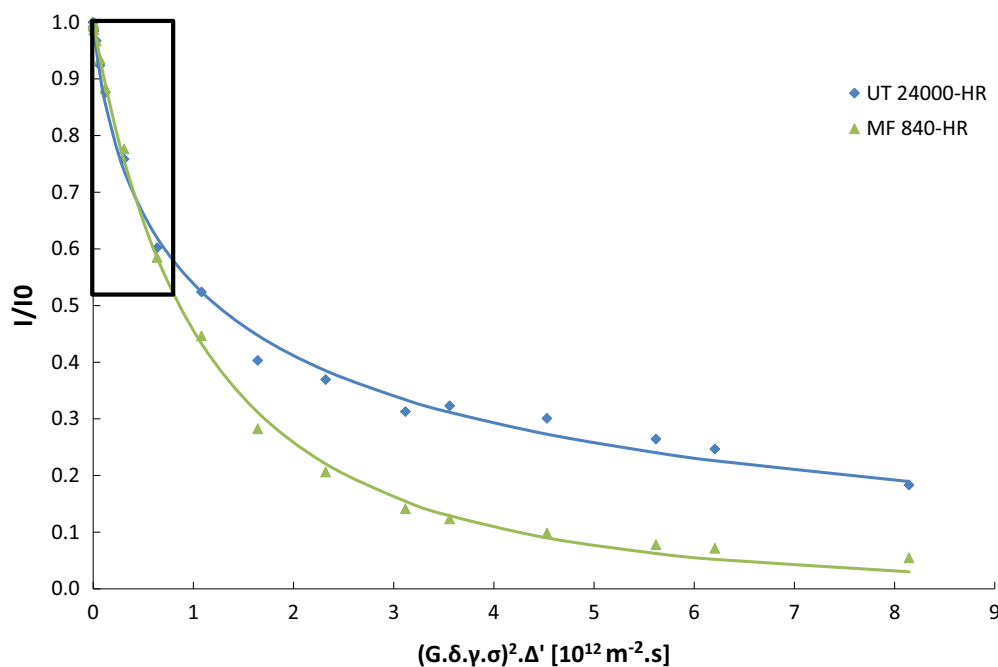


Fig. 3. Echo decay curves of the water phases (LR-NMR) of the W/O-emulsions prepared using different shear intensities. The used gradient pulse duration was equal to 8 ms while the diffusion delay was set to 180 ms. The rectangle indicates the experimentally accessible window for LR-NMR measurements at $\delta = 2.5$ ms. Lines represent the fit of the Murday-Cotts model to the data.

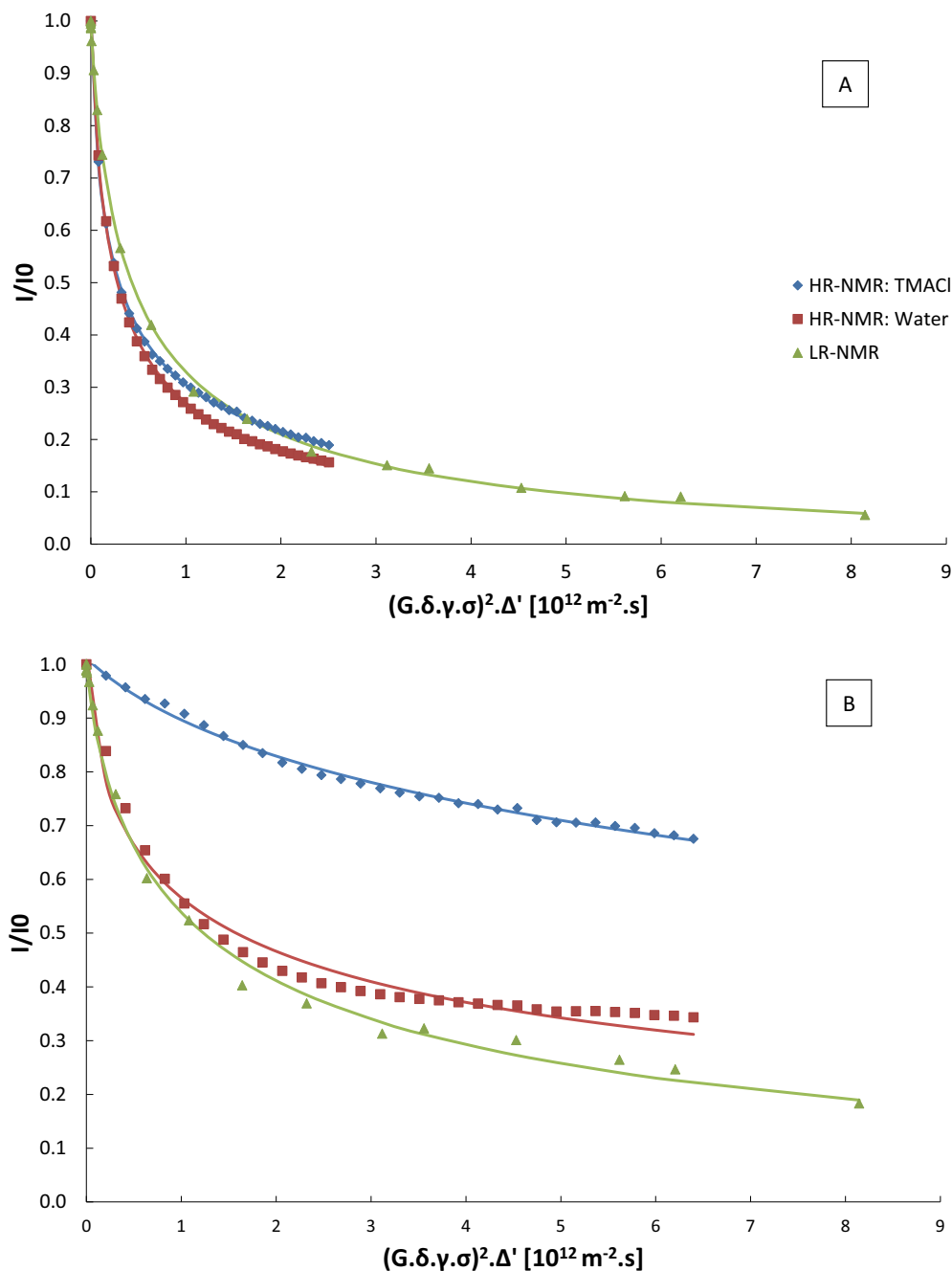


Fig. 4. Echo decay curves of water and TMACI (HR-NMR) or of the water phase (LR-NMR) of the W/O-emulsions prepared using different shear intensities. The used gradient pulse duration was equal to 8 ms while the diffusion delay was set to 180 ms for the LR-NMR measurement. (A) UT 6500-HR (B) UT 24000-HR and (C) MF 840-HR. Lines represent the fit of the Murday-Cotts model to the data.

ments show a difference between all of the samples (Table 4). Hereby, it should be mentioned that the results of the UT 6500-HR sample are less reliable due to the high polydispersity.

The observed difference in diffusion behavior between water and TMACI in small emulsion droplets might be explained by the higher solubility of water in the surrounding oil phase for smaller water droplets, called the Kelvin effect [19]. Moreover, smaller droplets have a higher surface area, therefore a higher amount of collisions will occur between the water molecules inside the droplets and the droplet interface during the diffusion delay. Indeed, the amount of collisions is directly proportional to the specific surface area of the droplets. The Murday-Cotts model assumes that the droplet boundaries are impermeable

which implies that all collisions between water and the interface should lead to reflection of the water molecules. However, part of these collisions might be non-reflecting. As a conclusion, it can be stated that the smaller the water droplets are, the higher the number of collisions between water and the interface will be and the higher the solubility of water in oil. The combination of both effects strongly increases the chance of the occurrence of a non-reflecting collision during the diffusion delay. When a non-reflecting collision occurs and water molecules leave the water droplet, they diffuse freely in the oil phase which leads to the observation of echo decay curves typical for free diffusion (i.e. independent from diffusion delay and gradient pulse duration).

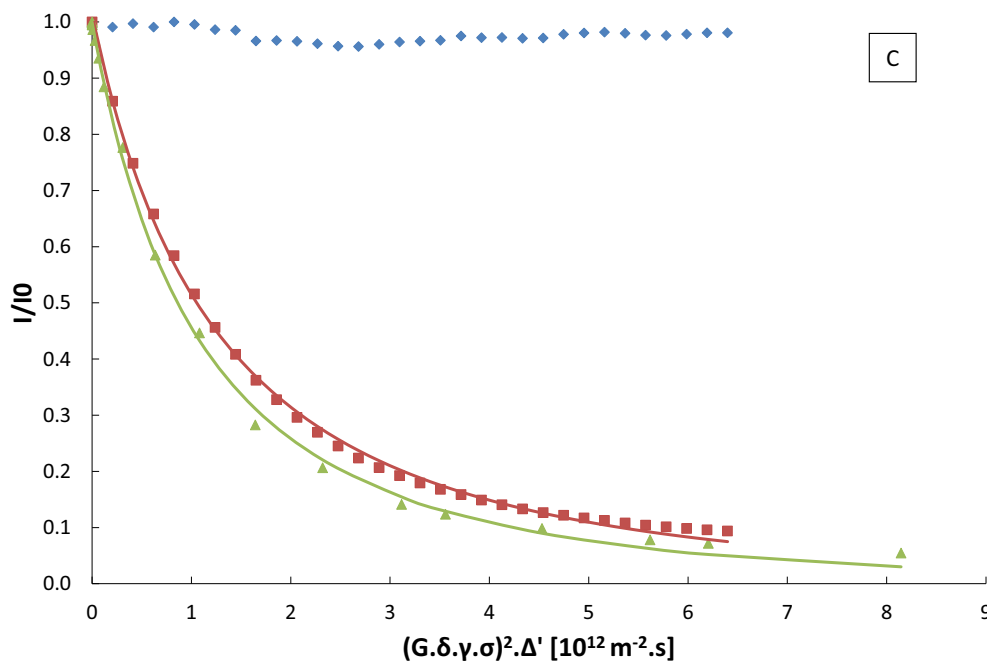


Fig. 4 (continued)

Table 3

Volume weighed average diameter (d_{43}) and standard deviation of the volume weighed distribution (σ_v) of the W/O-emulsions, each \pm standard error, as obtained from fitting the Murday-Cotts model to the echo decay data of either water or TMACI in HR-NMR.

	d_{43} (μm)		σ_v (μm)	
	Water diffusion	TMACI diffusion	Water diffusion	TMACI diffusion
UT 6500-HR	4.87 ± 0.02	4.60 ± 0.01	2.90 ± 0.03	3.65 ± 0.02
UT 24000-HR	3.29 ± 0.06	1.28 ± 0.01	2.70 ± 0.14	0.74 ± 0.07
MF 840-HR	2.94 ± 0.02	<0.50	0.67 ± 0.03	–

Table 4

Z-average diameter and polydispersity index of the W/O-emulsions, each \pm standard deviation, determined after dilution in 1-bromododecane.

Sample	Z-average diameter (nm)	Polydispersity index (–)
UT 6500-HR	630 ± 7	0.775 ± 0.083
UT 24000-HR	360 ± 6	0.424 ± 0.003
MF 840-HR	172 ± 2	0.147 ± 0.004

From previous research, it is known that reduction of the diffusion delay might help in getting less contribution of the freely diffusing compound [4]. However, similar (apparent) average droplet diameters were obtained at a diffusion delay of 60 ms. Hence, this value still seemed to be insufficient to solve the problem. Moreover, using this shorter diffusion delay, a contribution due to eddy currents became visible in the decay curves at higher gradient strengths (more than ± 1.5 T/m): at high ordinate values, the slope of the decay curves increased (Supplementary material). In addition, to get to a decay of the same extent, the gradient pulse strength and/or duration will have to be increased. Finally, reducing the diffusion delay leads to a reduction in size of the biggest droplets that can be determined.

Trying to correct for free diffusion (fast decay in the echo decay curve) to get a droplet size estimate from the part with slow decay is also not feasible as the slow decay will be governed by the bigger droplets in the sample and hence will give rise to an overestimation. The reason is twofold, firstly, as described by Balcaen et al. [25], the amplitude of the echo decay signal is linked to the

relaxation time. As water inside bigger droplets has a higher relaxation time (Fig. 6), this will lead to a larger contribution of these droplets to the echo decay curve. Moreover, as discussed previously, the number of collisions between the water molecules and the interface is lower in bigger droplets as compared to smaller droplets, which decreases the contribution of free diffusion to the echo decay curve of bigger droplets.

Diffusion of water in the emulsions containing small droplets thus seems to be a combination of free diffusion through the oil phase and restricted diffusion within the droplet. Free diffusion seems to be the dominating effect in the first 5 points of the echo decay curves of the MF 840-HR and UT 24000-HR samples, as indicated by the fact that both the diffusion delay and gradient pulse duration do not influence the obtained echo decay in this region. Based on the data in this region, a diffusion coefficient (D) can be determined using linear regression of the natural logarithm of the echo decay signal and the appropriate Stesjkal-Tanner equation [20]. This leads to a diffusion coefficient of $6.50 \times 10^{-13} \text{ m}^2/\text{s}$ and $6.14 \times 10^{-13} \text{ m}^2/\text{s}$, for the MF 840-HR and UT 24000-HR samples, respectively. If the same principle is used for the decay profiles of the 4 smallest W/O-samples shown in Fig. 2, a similar diffusion coefficient of $6.75 \pm 0.01 \times 10^{-13} \text{ m}^2/\text{s}$ could be calculated.

Interestingly, when the diffusion coefficient of PGPR in a 10 wt% PGPR in sunflower oil solution was determined, it was found to be equal to $6.16 \times 10^{-13} \text{ m}^2/\text{s}$. Thus, as shown in Fig. 5, the echo decay curves of PGPR in sunflower oil and water in either the MF 840-HR or UT 24000-HR sample coincided for low gradient pulse strengths. This shows that in fact the water molecules are moving at the

speed of PGPR and thus hydrated PGPR molecules are leading to the observed free-diffusion component in the echo decay curve of water.

Considering the viscosity of the used oil phase at 5 °C (about 0.25 Pa·s), and using the Stokes-Einstein equation, the hydrodynamic diameter of the diffusing PGPR-species is calculated to be about 2.6 nm. This small diameter implies that the PGPR-diffusion speed is mainly governed by monomers of PGPR as this size corresponds better to the size of a PGPR molecule than of a micelle. Indeed, Orfanakis et al. [22] measured a hydrodynamic diameter of PGPR monomers in toluene of about 8.8 nm. The fact that the measured hydrodynamic diameters do not completely correspond can be attributed to the different solvent system (toluene instead of sunflower oil) and measurement technique.

3.3. T_2 -relaxometry

The T_2 -distributions of the emulsions clearly separate the contribution of the oil protons (T_2 -relaxation time <120 ms) from the contribution of the water protons (T_2 -relaxation time >700 ms). Fig. 6 shows the cumulative T_2 -relaxation time distributions for the water droplets in the W/O-emulsions, which were prepared using different shear intensities. The shift of the T_2 -relaxation time distribution towards lower values increased with increasing shear intensity during production, thus with decreasing droplet size. The fact that the sensitivity of the T_2 -relaxation time distribution towards the droplet size distribution strongly increased with decreasing droplet size is typically observed for emulsions with a low surface relaxivity. Indeed, the relaxation time does not vary linearly as a function of the droplet size but is only highly influenced by the droplet size from a certain size downwards, as can be observed from the limits of Eq. (2). Considering large values of the radius (a), the right-hand side of Eq. (2) approaches 0 and hence the T_2 -relaxation time of coarse emulsions approaches the T_2 -relaxation time of the bulk water phase. As the droplet radius becomes smaller, the right-hand side approaches $3 * \rho_2/a$, which means that T_2 -relaxation time tends to zero for extremely small

droplets and linearly increases with droplet radius with a slope of $1/(3 * \rho_2)$.

Eq. (2) indicates that, using the T_2 -relaxation time distribution of the samples, it is possible to determine the droplet size distribution. However, the surface relaxivity (ρ_2) has to be known for the specific emulsion composition which requires independent sizing experiments. The use of PFG-NMR diffusometry in combination with T_2 -relaxometry in order to obtain the surface relaxivity parameter (ρ_2) has been described in literature [15,16]. However, our results indicate that for our samples, only from a certain size downwards, T_2 -relaxometry is capable of accurately determining a difference in droplet size distribution. In contrast, PFG-NMR is capable of determining a difference in droplet size only from a certain size upwards. The combination of both T_2 -relaxometry and PFG-NMR diffusometry in order to determine the surface relaxivity of the samples is therefore not feasible for these samples as the region of sensitivity towards the droplet size does not sufficiently overlap between both techniques. However, increasing the surface relaxivity might increase the droplet size range for which T_2 -relaxometry is sensitive, which might enable an overlapping droplet size range for T_2 -relaxometry and PFG-NMR diffusometry.

The addition of paramagnetic compounds to the oil phase might aid in increasing the surface relaxivity. Also, measuring at a higher temperature may increase the difference in T_2 -relaxation time between bulk and restricted water.

As shown in Fig. 7, the difference in T_2 -relaxation time between the water droplets in either a UT- or MF-processed W/O-emulsion and the bulk water phase indeed increased with increasing temperature. Hereby, the mode of the T_2 -distribution became less sensitive towards a temperature increase when the water droplets were smaller.

However, the droplet size is linked to $1/T_{2,corr}$ and there is no strong effect of temperature on $1/T_{2,corr}$, which indicates that the surface relaxivity is not strongly temperature dependent (Fig. S4). The average $1/T_{2,corr} \pm$ standard deviation in the temperature interval under consideration was 0.32 ± 0.04 and 0.13 ± 0.04 1/s for the MF 840-10wt and UT 6500-10wt samples, respectively.

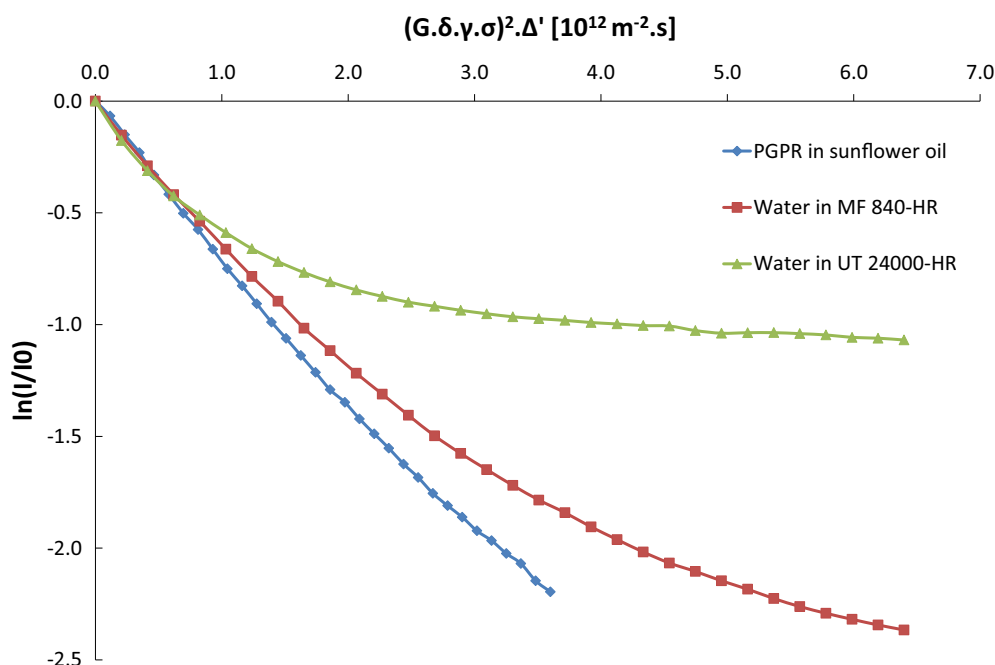


Fig. 5. HR-NMR echo decay curves of 10 wt% PGPR in sunflower oil, water in the MF 840-HR sample and water in the UT 24000-HR sample.

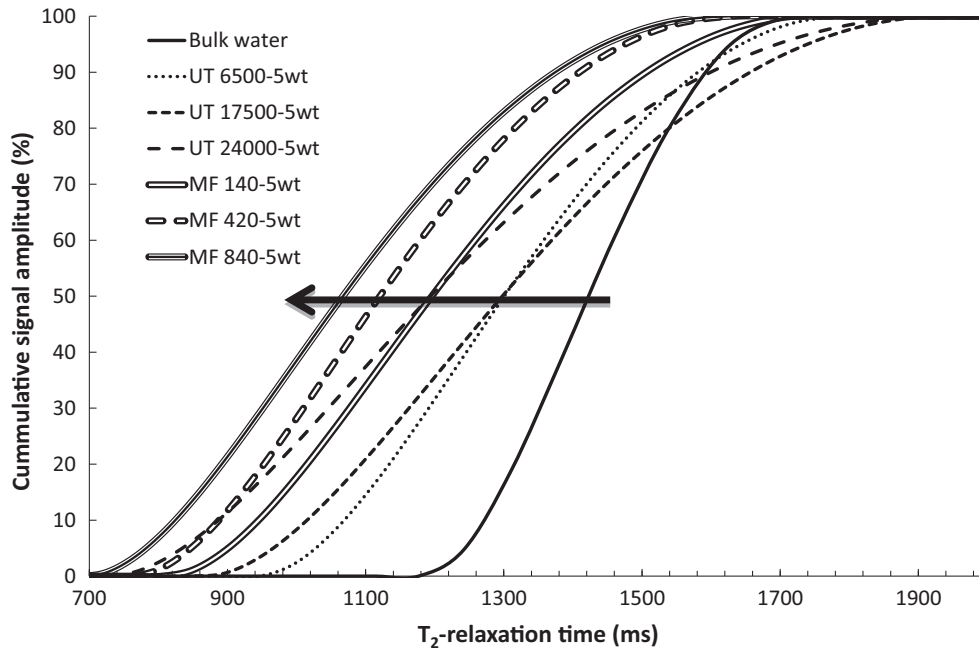


Fig. 6. T_2 -relaxation time distributions of the water droplets in the W/O-emulsion samples prepared using different shear intensities.

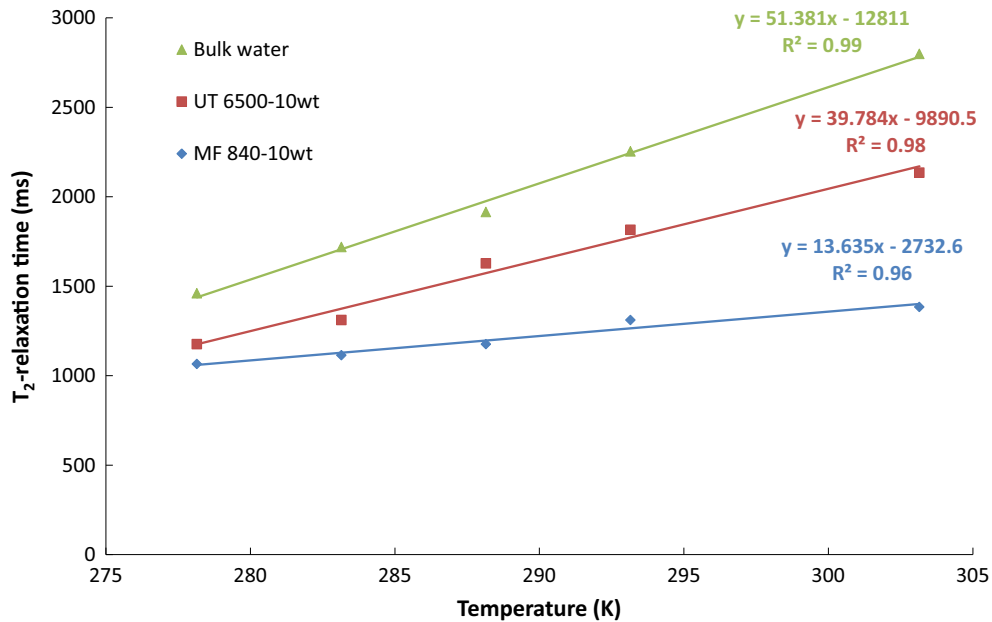


Fig. 7. Influence of temperature on the mode of the T_2 -relaxation time distribution of the water phase and of the water droplets in the UT 6500-10wt and MF 840-10wt emulsions.

From the aforementioned relaxometry measurements, it can be stated that conducting measurements at a higher temperature will increase the shift of the T_2 -relaxation time distribution towards lower values, relative to the T_{2B} -relaxation time, especially for small droplets. Therefore, the detection of droplet size distribution differences using T_2 -relaxometry should be conducted at a sufficiently high temperature. However, the droplet size itself is linked to $1/T_{2,corr}$ which is less sensitive towards changes in temperature.

It should be noted that, for the W/O-emulsions which contain small water droplets, the ratio between their surface weighed average droplet sizes (d_{32}) can be calculated using the ratio between the $1/T_{2,corr}$ values. Indeed, these samples have the same

composition and thus the same ρ_2 . Moreover, it is reasonable to use the surface limited scenario ($\frac{\rho_2 r_0^3}{2 \times D} \ll 1$) for droplet radii smaller than $3 \mu\text{m}$, translational diffusion coefficients of the water molecules in the order of $10^{-9} \text{m}^2/\text{s}$ and typical values for the surface relaxivity in the order of some $\mu\text{m}/\text{s}$. The surface weighed average droplet size ratio between two samples (a and b) can therefore be calculated based on their average (corrected) T_2 -relaxation time using Eq. (3).

$$\frac{d_{32,a}}{d_{32,b}} = \frac{\frac{1}{T_{2,corr,b}}}{\frac{1}{T_{2,corr,a}}} = \frac{T_{2,corr,a}}{T_{2,corr,b}} \quad (3)$$

Using Eq. (3), the surface weighed droplet size (d_{32}), relative to the surface weighed droplet size of the MF 140-5wt sample, can be calculated for the MF 420-5wt and MF 840-5wt samples. This gives a ratio of 0.77 and 0.63 for the MF 420-5wt and MF 840-5wt samples, respectively. This corresponds quite well to the ratio between the Z-average diameters of these samples relative to the Z-average diameter of the MF 140-5wt sample (Table 2), which equal 0.72 and 0.55, respectively.

4. Conclusions

In a previous study [4] the effect of the measurement temperature on the accuracy of droplet size determination of W/O-emulsions, based on water NMR diffusometry, was determined. It was shown that when the temperature increased, also the measured droplet size increased when water diffusion was followed. In contrast, a constant droplet size distribution was determined when the diffusion of the ionic marker tetramethylammonium chloride was followed instead. This difference in diffusion behavior was attributed to an increasing contribution of water diffusion through the oil phase when increasing the measurement temperature. Therefore, it was concluded that PFG-NMR water diffusometry should be conducted at a sufficiently low temperature. Because, like temperature, the water droplet size also influences the solubility of water (Kelvin effect [19]), we examined whether a decreasing droplet size affects the droplet size distribution as determined using water NMR diffusometry.

It was shown that from a certain droplet size downwards, water exchange at the W/O-interface is substantial enough to lead to the observation of free diffusion of water through the oil phase using PFG-NMR diffusometry. Under the experimental conditions used in this manuscript, the lower limit was found at about 3 μm . However, this limit will probably depend on the composition of the W/O-emulsion. This implies that the generally used low-resolution PFG-NMR technique [1,4,5] is incapable of accurately determining the droplet size distribution of samples that contain (sub)micron sized water droplets. Therefore, care should be taken when small droplets (<3 μm) are expected in a sample because the echo decay might be substantially influenced by free diffusion through the oil phase whereby fitting to this echo decay leads to erroneous (i.e. overestimated) results.

High-resolution NMR revealed that water diffusion occurred at a speed similar to that of PGPR-monomers. This indicates that (hydrated) PGPR is not irreversibly adsorbed at the W/O-interface but is exchanging and diffusing through the oil phase. Therefore, the concentration of PGPR in the oil phase will probably influence the contribution of free water diffusion through the oil phase.

Determination of the echo decay curve of the oil insoluble marker tetramethylammonium chloride in the water phase extends the application window of PFG-NMR diffusometry towards smaller droplet sizes. However, in order to obtain a sufficient echo decay for (sub)micron droplets, very high gradient pulse durations and/or strengths are required. Moreover, the marker has to be added before preparation of the W/O-emulsion which renders the use of PFG-NMR in the determination of droplet sizes in existing W/O-emulsions, containing small droplets, impossible.

Another NMR-technique which has been used in the determination of W/O-emulsion droplet size distributions is relaxometry [14–16]. However, this technique has not been tested on edible W/O-emulsions and Peña and Hirasaki [15] noted that these emulsions usually have low surface relaxivities, which makes the relaxation time distribution of the dispersed phase less sensitive towards changes in specific surface area (and thus droplet size). We measured the T_2 -relaxation time distributions of edible W/O-

emulsions containing droplets with different sizes and were able to show that T_2 -relaxometry could detect a difference between these samples.

It was also shown that, in order to increase the influence of the water droplet size on the T_2 -relaxation time distribution, measurements should be conducted at a higher measurement temperature. Measuring at a higher temperature increases the shift of the T_2 -relaxation time distribution towards lower values, relative to the bulk T_2 -relaxation time. However, the droplet size is linked to $1/T_{2,\text{corr}}$ which is less sensitive to changes in temperature. The addition of paramagnetic compounds to the oil phase might increase the surface relaxivity parameter and therefore increase the sensitivity of the relaxation time distribution towards changes in the droplet size distribution.

Finally, if T_2 -relaxometry is used to determine the droplet size distribution, an independent sizing technique is still necessary to determine the surface relaxivity parameter. A possible approach would be to determine the surface relaxivity for a W/O-composition using an emulsion of which the droplet size should be chosen in a region where both PFG-NMR and T_2 -relaxometry are sensitive towards droplet size changes. This region might also be increased by increasing the surface relaxivity using paramagnetic compounds. However, as shown in this work, the determination of the surface relaxivity can be omitted by dividing the corrected surface relaxation times of two samples by each other. This ratio equals the ratio of the surface weighed average droplet sizes of these samples. This approach can for example be used in quality control, during which the determination of the absolute droplet size is less important.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jcis.2017.12.049>.

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