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Investigation in W/O developed microemulsions with DMSO as a cosurfactant

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In this study the effect of dimethylsulfoxide (DMSO) radii on the droplets and on the required non-ionic surfactant volume for preparing water-in-oil microemulsions (MEs) was investigated. Five series of MEs were prepared either with the aid of isopropylpalmitate or medium chain triglycerides (MCTG) as continuous phases. The MEs were stabilized via either Tween®80 or Span®20 or a mixture of both. A mixture of water:DMSO (W:DMSO) with different ratios formed the colloidal phase of the first four MEs series. Only DMSO was used as colloidal phase of the fifth series. Dynamic light scattering (DLS) was used for measuring the radii of the droplets of MEs. The results showed that the consumed volumes of the surfactants were related to the W:DMSO ratio and the surfactant type. Moreover, the consumed surfactant volumes increased with decreasing radii of the droplets of the MEs. The MEs stabilized with Span®20 had lower radii of the droplets (4-15 nm) than MEs stabilized by Tween80 (25-65 nm). It was evident that DMSO changed the interfacial tension which is reflected by changing the the volume of the surfactant consumed and by altering the droplets sizes. Consequently, DMSO acted as co-surfactant in stabilizing the MEs by reducing the required volumes of the surfactant which is important for reducing their toxicity.

1. Introduction

Microemulsions (MEs) are thermodynamically stable dispersions of oil in water (having an oily colloidal phase) or water in oil (having an aqueous colloidal phase) stabilized by surfactants and co-surfactants. They have attracted much interest in recent years in terms of their drug delivery potential and other substantial practical importance because of their interesting physical properties such as their low droplets size and thermodynamical stability (Neubert 2011; Madhav and Gupta 2011). The high concentrations of surfactant and co-surfactant which are necessary for stabilizing the MEs may cause toxicity or irritancy (Jelinek 2001; Warisnoicharoen et al. 2003; He et al. 2010).

It is also known that the radii of the droplets distribution is one of the most important characteristics of MEs for the evaluation of its stability and penetration mechanism into the skin (Mahrhauser et al. 2015; Naoui et al. 2011). Many studies were done using dynamic light scattering (DLS) to identify which factors affect the radii of the droplets of the MEs. A study was made for water in oil phospholipid MEs which were made with different (lecithin:alcohol) ratios (Km). The MEs showed a tendency for a slightly higher concentration of water to be required for ME formation at higher Km values. Furthermore, for a fixed Km the size of the ME droplets increased with increasing the volume fraction of water. At constant water concentrations, radii of the droplets decreased slightly upon increasing Km (Aboofazeli et al. 2000a- b).

O/W MEs prepared by non-ionic surfactant C18:1E10 and containing one of the oils having a higher molecular mass (that is, either a medium chain triglyceride, Miglyol 812, or soybean oil) or the ethyl ester of fatty acid such as ethyloleate. These MEs exhibited first a decrease and then an increase in the hydrodynamic size and in the surfactant aggregation number, suggesting that the asymmetric C18:1E10 micelles became spherical upon the addition of a small amount of oil and grew thereafter because of further oil being incorporated into the core of the spherical ME (Warisnoicharoen et al. 2000).

Shrestha et al. (2011) explained the increase in the nonionic reverse micellar size with an increase in the hydrophilic head group size of the surfactant, in terms of a decrease in the critical packing parameter. On the other hand, an opposite trend was observed in the same study with an increase in the lipophilicity of the surfactant and with a decrease in the chain length of the oil (increasing in the polarity of the oil).

In another study, Sharma et al. (2015), reported that the developed growth of nanostructures of the ME was linearly upon increasing the water content up to a critical value beyond which the finite bending modulus of surfactant film triggers the structural rearrangement of ME droplets and the linear plot shows deviation (Sharma et al. 2015).

It is necessary to correct scattering measurement in high concentration regions and an appropriate model is then used to correct the results for particle particle interaction to allow meaningful calculation of the radii of the droplets (Goddeeris et al. 2006). The most commonly used model is the hard sphere model which prevents the overlap of different hard sphere droplets (Shukla et al. 2002; Zackrisson et al. 2004).

DMSO is used usually as a penetration enhancer in semisolid formulations (Marren 2011; Notman et al. 2007). Elles and Levinger (2000) reported studying aerosol OT/cyclohexane reverse micelles solubilizing DMSO and DMSO/water mixtures utilizing dynamic light scattering that the polarity of DMSO increases inside of the micelles because the intramicellar DMSO/water polarity depends both on the ratio of the solvents and on micellar size (Elles and Levinger 2000).

The aim of this article is studying the effect of DMSO as a cosurfactant. Fixed volumes of different water:DMSO ratios as a colloidal phase were used for measuring the required volume of non-ionic surfactants (Span® 20 and Tween® 80) which are

necessary for preparing transparent and stable W/O MEs (having a colloidal aqueous phase). DLS was used for estimating the radii of the droplets and for relating it to the volume surfactant which is needed to stabilize the MEs. IPP and medium chain triglycerides (MCTG) were used as continuous phases they are pharmaceutically acceptable components with acceptable daily intake (ADI) (values of the components DMSO, Span® 20 and Tween® 80 used in the formulation of MEs assigned by the Food and drug administration (FDA) are 17.4 mg/kg, 254 mg/kg, 254 mg, respectively). The hard sphere model was used for calculating the sizes of the colloidal droplets of the MEs.

2. Investigations and results

For each ME series, one typical example of the fit of the auto correlation function is shown in Fig. 1.

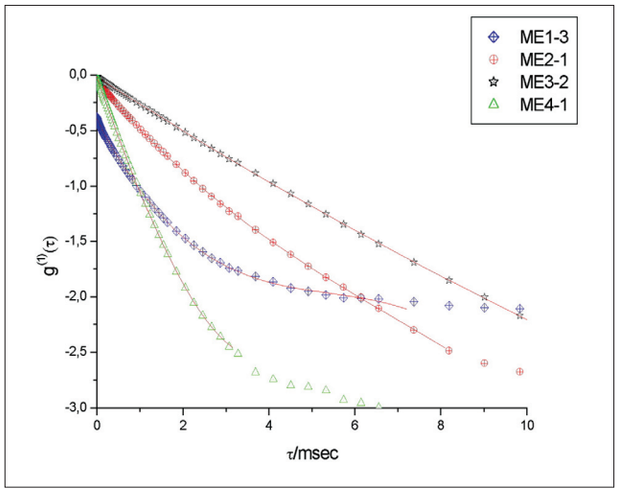

Fig. 1: Typical cumulant fit for the w/o MEs of series 1 & 2 at scattering angle 60° and for series 3 & 4 at scattering angle 90°.

2.1. Measurements of DLS and required Tween®80 for Series 1 (IPP Tween®80 MEs)

Series1 was prepared as mentioned in Section 4.2. The radii of MEs of the colloidal droplets and the consumed Tween®80 are presented in Figure 3.

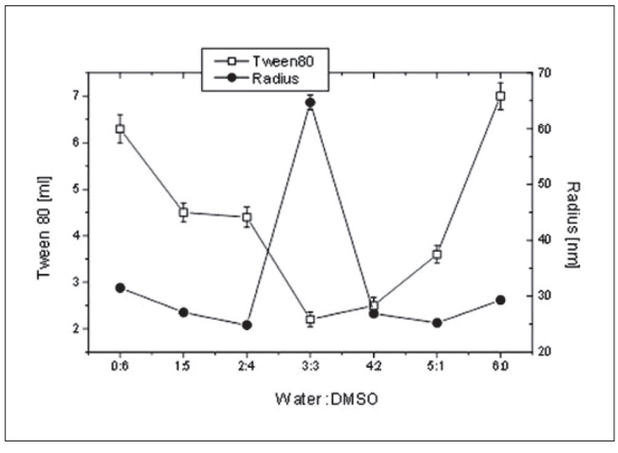

Fig. 2: Radii of the droplets and required amounts of Tween® 80 for MEs each of them consists of (1ml) W: DMSO with different ratios (0:6, 1:5, 2:4, 3:3, 2:4, 5:1, 6:0) and 8 ml IPP.

Figure 2 shows that the required volume of the surfactant Tween80 which was used to get a transparent and stable MEs decreased with the decrease of the DMSO fraction to a W:DMSO 3:3 ratio then increased again with a decrease of the DMSO fraction. ME with a W:DMSO 3:3 ratio exhibited the largest radii of the droplets and the lowest required volume of the surfactant. The radii of the droplets of MEs were decreased from 65 nm in case of 3:3 ratio of 25 and 27 nm for 2:4 and 4:2 ratios, respectively, with increasing in the volume of the surfactant from 2.2 ml for the 3:3 ratio to 4.4 and 2.5 ml for 2:4 and 4:2 ratios, respectively. A slight increase in the radii of the droplets was observed with an increase in the volume of the surfactant from 4.4 to 7 ml and from 2.5 to 6.5 ml representing DMSO fractions between 2:4 and 4:2, respectively.

2.2. Measurements of DLS and required volume of Span®20 for Series 2 (IPP Span®20 MEs)

The preparation of Series 2 is described in Section 4.2. The radii of the colloidal droplets and consumed volumes of Span®20 are presented in Fig. 3.

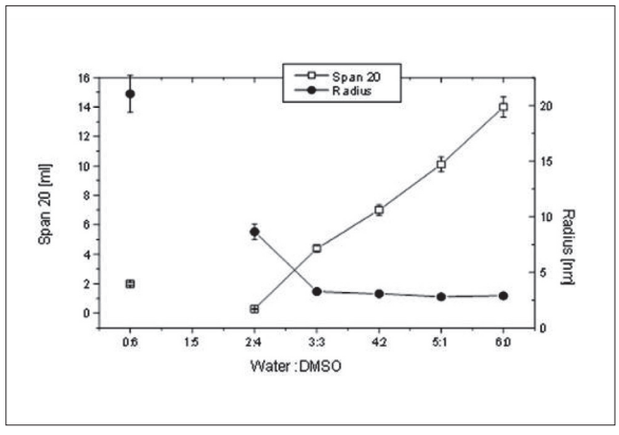

Fig. 3: Radii of the droplets and required amounts of Span®20 for MEs each of them consists of (1ml) W: DMSO with different ratios (0:6, 1:5, 2:4, 3:3, 2:4, 5:1, 6:0) and 8 ml IPP.

The lowest volume of surfactant (see Fig. 3) was required for the ME with a W:DMSO ratio of 2:4 using Span®20 which showed a large radii of the droplets (8.7 nm). The ME with a ratio of 1:5 was unstable. The required volume of surfactant increased with decreasing DMSO fraction in MEs from W:DMSO ratio of 2:4 to 6:0 while the radii of the droplets decreased with increasing the volume of surfactant. The largest radii of the droplets (21 nm) in comparison with the other MEs in this series were measured when only DMSO (0:6) was used despite of the required volume of surfactant (2 ml) which was more than for the ME with 2:4 ratio.

2.3. Measurements of DLS and required volume of surfactant (T: S 3:2) for Series 3 (MCTG T: S (3:2) -MEs)

The different radii of the colloidal droplets of prepared MEs (see unit 4.2) of series3 and the consumed volume of T:S (3:2) are presented in Fig. 4.

The third series (see Fig. 4) which is stabilized using T:S of 3:2 ratio and the ME with 3:3 ratio of W: DMSO needs the lowest volume of surfactant and shows the largest radius of the droplets (72 nm). In contrast, the MEs with (6:0, 4:2, 5:1, 6:0) ratio were unstable. Also, the MEs requires a decreased volume of the surfactant and an increase in the radii of the droplets with decreasing DMSO fraction.

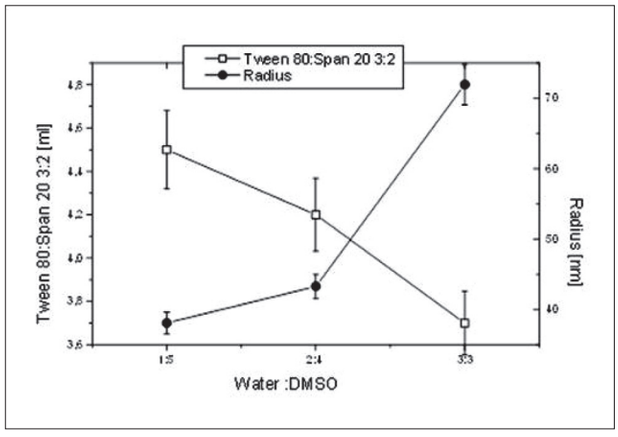

Fig. 4: Radii of the droplets and required amounts of Tween®80:Span®20 (3:2) for MEs each of them consists of (1 ml) W: DMSO with different ratios (0:6, 1:5, 2:4, 3:3, 2:4, 5:1, 6:0) and 8 ml MCTG.

2.4. Measurements of DLS and required volume of surfactant (T: S 2:3) for Series 4 (MCTG T: S (2:3) -MEs)

The results of DLS as well as consumed volume of the surfactant for preparing of series 4 are shown in Fig. 5.

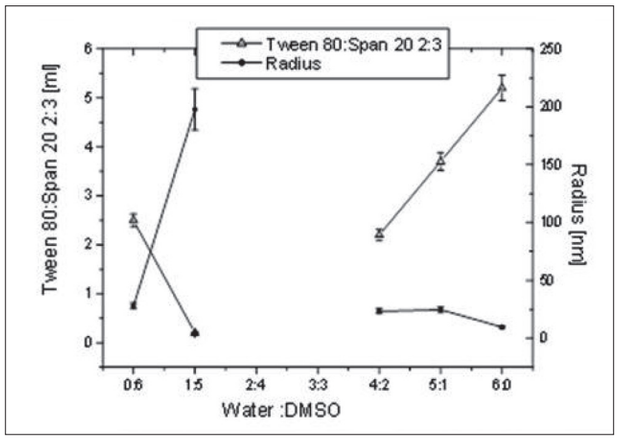

Fig. 5: Radii of the droplets and required amounts of Tween®80:Span®20 (2:3) for MEs each of them consists of (1ml) W: DMSO with different ratios (0:6, 1:5, 2:4, 3:3, 2:4, 5:1, 6:0) and 8 ml MCTG.

Figure 5 shows that the stability of MEs which are stabilized using T:S with 2:3 ratio is changed inversely regarding W:DMSO ratios in comparison to MEs which are stabilized using T:S with 3:2 ratio. The droplets radii were in general lower than those using T:S 3:2 except for the ME with W:DMSO ratio of 1:5 which had the largest droplets size of 198 nm and the lowest required volume of surfactant. ME with W:DMSO 2:4 and 3:3 ratios were unstable. The required volume of surfactant increased with a decrease in the DMSO fraction from W:DMSO 4:2 to 6:0 ratio. However, the required volume of surfactant increased obviously connected with a slightly decrease in droplets size.

2.5. Measurements of DLS for Series 5 (DMSO IPP MEs)

Span®20 formed MEs with small radii of the droplets. The series was prepared with an increase in the volume of the surfactant (Span®20) (2.2, 2.4, 2.6, 2.8, 3 ml) and with fixed volumes of DMSO and IPP (1 ml and 8 ml, respectively), in order to examine the volume of surfactant on the radius of the droplets.

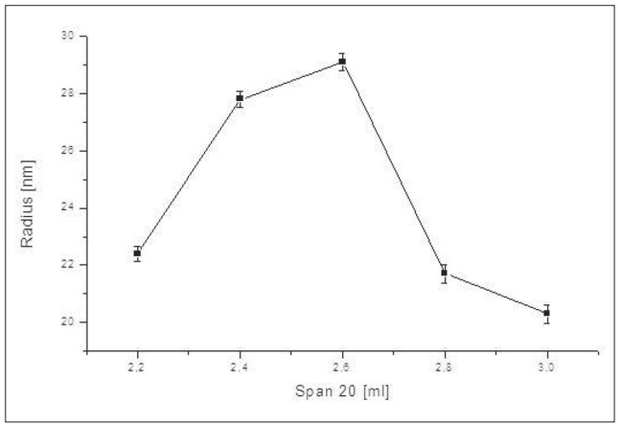

Fig. 6: Radii of the droplets of MEs consist of 1ml DMSO, 8 ml IPP, and different amounts of Span®20 (2,2, 2,4, 2,6, 2,8, 3 ml)

The results of the DLS measurements of series5 (see unit 4.2.) are shown in Fig. 6.

Figure 7 exhibits an increase in the radius of the droplets up to a ME with a volume of surfactant of 2.6 ml then a decrease is observed.

3. Discussion

The results of measurements of consumed volume of the surfactant which is necessary for preparation of the different MEs are summarized in Fig. 7. The results show a clear, systematic influence of DMSO on the required volume of surfactant.

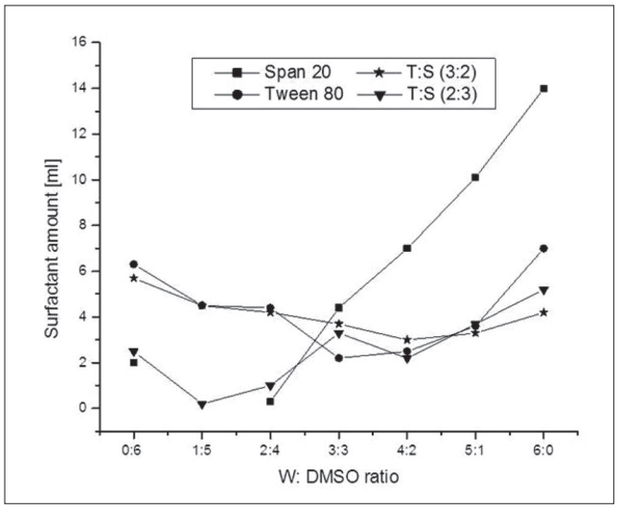

Fig. 7: The required amounts of different surfactants for preparing different meals which has a fixed amount (1 ml) of W: DMSO with different ratios (0:6, 1:5, 2:4, 3:3, 2:4, 5:1, 6:0) and either IPP or MCTG as outer phase.

It was observed that a higher volume of surfactant caused smaller droplet sizes. If the radius of the droplets is large, the separation surface between the hydrophilic phase and lipophilic phase will be small and small separation surface needs only a small quantity of the surfactant to surround ME droplets. The relationship between the radii of the droplets and interfacial tension is given in Eq. (8) Davis 1994.

It is suggested that DMSO changes the interfacial tension by changing the hydrophilicity of the colloidal phase. Lower or higher

interfacial tension can change the radius of the droplets which requires different volumes of the surfactant (Elles and Levinger 2000).
MEs with Span®20 only (see Fig. 4) shows the lower radii of the droplets, but higher volumes of the surfactant are required and lower stabilities were observed in comparison to MEs with Tween®80 (see Fig. 3). It is assumed that this increase in the required volume of surfactant t which is accompanied by an increase in the radii of the droplets using Tween®80 (see Fig. 3) is related to an accumulation of the surfactant on the interface. The noted slight decrease in the droplets size using Span®20 (Fig. 4) appears to caused by the larger molecular volume of Tween®80 (Shrestha et al. 2011).
The increase in Span®20 from 2:3 to 3:2 ratio led to changes in the stability and reduction of the radii of the droplets (Fig. 5 and 6). The higher hydrophilicity of Tween®80 in comparison to Span® 20 may cause the larger radii of the droplets (Fig. 1), because of the hard sphere model, which supposes that MEs to consist of the core of the colloidal phase surrounded by a monolayer of the surfactant. This study assumes that an increase of volume of the surfactant in series 5 led to an accumulation of the surfactant on the interface. The accumulation of the surfactant caused an increase in the droplets size up to a saturation of the surface. Then there was no more space for more surfactant (2.8 ml) on the interface. This led to an increase of the total interface area due to a decrease in the droplets size up to 3 ml of Span®20 was used. The ME showed no more capacity to reduce its radii of the droplets because this would lead to a distraction of the ME.
Dimethylsulfoxide (DMSO) acts as a cosurfactant and reduced the consumed volume of the surfactant that is required for forming the MEs. This effect is accompanied by an increase in the droplets radii. This may be seen as a result of reduction of the interfacial tension caused by a reduction of the hydrophilicity of the colloidal phase. DMSO in the MEs formulation as a cosurfactant reduced the volume of surfactant which is necessary for the preparation of stable MEs. Therefore, it would be useful to use DMSO for reducing their toxicity which is related to high volumes of surfactant incorporated.

4. Experimental

4.1. Materials

Dimethylsulfoxide (DMSO), sorbitanmonolaurate (Span® 20), poloxyethylenesorbitan mono oleate (Tween® 80), isopropylpalmitate (IPP) and medium chain triglycerides (MCTG) were purchased from Fluka, Buchs, Switzerland. Water was used in bi-distilled quality.

4.2. MEs preparation

Five ME series were prepared. The components of each ME series are summarized in the Table.
The MEs in each series are numbered (M_1, M_2, M_3,...). The MEs in the different series are prepared as follows.

Table: Compositions of MEs (M_1, M_2,....) in the five series (Series 1, 2, ...,5) according to consumed surfactant and lipophilic phase.

Mn	Series 1 and 2 stabilized using s.q. either of Tween®80 or Span®20		Series 2 and 4 stabilized using s.q. either of T:S 3:2 or T:S 3:2		Series 5		
	IPP Ml	W: DMSO 1 ml	MCTG ml	W: DMSO 1 ml	Span 20 Ml	DMSO Ml	IPP M l
M_1	8	0:6	8	0:6	2,2	1	8
M_2	8	1:5	8	1:5	2,4	1	8
M_3	8	2:4	8	2:4	2,6	1	8
M_4	8	3:3	8	3:3	2,8	1	8
M_5	8	4:2	8	4:2	3	1	8
M_6	8	5:1	8	5:1			
M_7	8	6:0	8	6:0			

s.q.: Sufficient volume, $M_{n...}$: Number of the microemulsion

4.2.1. Series 1 (IPP Tween®80 MEs)

8 ml IPP and 1 ml W:DMSO in different ratios (0:6, 1:5, 2:4, 3:3, 2:4, 5:1, 6:0) were added in each beaker, then Tween® 80 was added dropwise with continuous stirring on a magnetic stirrer until a clear ME was formed. The consumed volume of Tween®80 (T) for each ME was recorded.

4.2.2. Series 2 (IPP Span®20 MEs)

MEs were prepared as described in Section 4.2.1 but Span®20 (S) was used instead of Tween®80.

4.2.3. Series 3 (MCTG T: S (3:2) MEs)

8 ml MCTG and 1ml W:DMSO with different ratios (0:6, 1:5, 2:4, 3:3, 2:4, 5:1, 6:0) were added into each beaker. Then, Tween®80:Span®20 in a ratio of 3:2 was dropped with continuous stirring over a magnetic stirrer until transparent MEs were formed. The consumed volumes of the surfactants were recorded.

4.2.4. Series 4 (MCTG T:S (2:3) MEs)

It was prepared as in series 3 but Tween®80:Span®20 with a ratio of 2:3 was used instead of 3:2.

4.2.5. Series 5 (DMSO IPP MEs)

1 ml DMSO, 8 ml IPP and increased volumes of Span®20 (2.2, 2.4, 2.6, 2.8, 3 ml) were added.

4.3. Dynamic light scattering measurements

The light scattering hardware setup consists of commercially available equipment for simultaneous static and dynamic experiments made by ALV-Laser Vertriebsgesellschaft m.b.H., Langen, Germany. A green Nd:YAG DPSS-200 laser (532 nm) from Coherent (Auburn, USA) with an output of 200 mW was used. The thermostated sample cell is placed on a motor-driven precision goniometer (± 0.01°) which enables the photomultiplier detector to be moved from 20° to 150° scattering angle. The cylindrical sample cells are made of Suprasil® quartz glass by Hellma (Mühlheim, Germany) and have a radius of 10 mm. The minimal sampling time of this correlator is 12.5 ns. The intensity time-correlation functions (ITCF) $g^2(t)$ was recorded using an ALV-5000E multiple tau digital correlator with fast option at scattering angle between 50 and 60 at the step of 5° for series of MEs 1 and 2 and at scattering angle between 80 and 90 at the step of 5° for series of MEs 3 and 4.
The field autocorrelation function $g^1(t)$ was determined according the Siegert relation

$$\beta = \sqrt{\frac{\overline{R^2}}{\overline{R}^2} - 1} = \frac{\sqrt{K_2}}{\Gamma_1} \tag{1}$$

where A is the average uncorrelated scattering intensity and B an instrument parameter.
The field autocorrelation function of the scattered light of mono disperse solution is given by (Pecora 1964)

$$\left|g^{(1)}(\tau)\right| = \exp(-\Gamma\tau)$$
$$\textit{Where} \tag{2}$$
$$\Gamma = Dq^2$$

where D is the translational diffusion coefficient of the molecules, and q is the magnitude of the scattering vector.
For polydisperse solutions Eq. (1) must be generalized to a sum or distribution of exponentials:

$$\left|g^{(1)}(\tau)\right| = \int_0^\infty G(\Gamma)\exp(-\Gamma\tau)d\Gamma,$$
$$\textit{with} \tag{3}$$
$$\int_0^\infty G(\Gamma)d\Gamma = 1.$$

The distribution function of the decay rates, G(Γ), can be a broad continuous distribution, a series of discrete delta functions or some combination of two. G(Γ)=dr is the function of the total intensity scattered, on average, by molecules for which Dq^2= Γ, within dΓ
The correlation function of the polydisperse solutions can be analysed in terms of moments or cumulants (Koppel 1972).

$$\ln\left|g^{(1)}(\tau)\right| = K(-\tau;\Gamma) \tag{4}$$

Logarithms of $g^{(1)}(\tau)$ are then plotted as a function of the delay time τ and fitted by a polynomial K_0-$K_1\tau$+1/2$K_2\tau^2$ from which first cumulant K_1 and second cumulant K_2 was extracted. The term $K_1 = \Gamma_1$ is defined as the initial decay rate of the density correlation function.
The coefficient in this series, which are known as the cumulant describe some of the properties of the G(Γ). Collective diffusion coefficient D can be obtained from first cumulant.

$$< \Gamma_1 > \Rightarrow < D > = \frac{K_1}{q^2} \quad (5)$$

Another quantity, which is often used to specify the degree of polydispersity, β is the normalized variance defined as

$$\beta = \sqrt{\frac{\overline{R^2}}{\overline{R}^2} - 1} = \frac{\sqrt{K_2}}{\Gamma_1} \quad (6)$$

Free particle diffusion coefficient D_0 can be deduced for spherical particles interacting through hard sphere force from collective diffusion D as

$$D_c = D_0\left(1 + 1.56\phi + 0.91\phi^2 + ...\right) \quad (7)$$

where ϕ is the volume fraction of particles (Degiorgio and Corti 1985).
Knowing the value of D_0, the hydrodynamic radius R_h can be calculated by the Stokes-Einstein equation (8)

$$R_h = \frac{k_B T}{6\pi\eta D_0} \quad (8)$$

where η the viscosity of the continuous phase of ME.
The collective diffusion coefficient and polydispersity index have been deduced using cumulant fitting procedure from the normalized field auto correlation function $g^1(\tau)$ using Eq. (4), Eq. (5) and Eq. (7) and averaged over the angles. To get the free diffusion coefficient of droplets Eq. (7) was used, then the hydrodynamic radius calculated from that using Eq. (8).
Prior to the measurements the samples were filtered through 1.2 μm pore size filter (Sartorius, Goettingen, Germany) into dust clean sample cells.
The refractive index of all samples was measured an Abbe Refractometer from ABBEMAT; (Dr. Kernchen GmbH, Seelze, Germany) at 25.0 ± 0.2 °C. The viscosity of the external phase IPP oil was determined using Couette principle from Rheometrics scientific (Bensheim, Germany) at 25 °C. These data are necessary to calculate the hydrodynamic radius from diffusion coefficient. Results of DLS data were fitted using FORTRAN program package, CONTIN by Provencher. "Probability to Reject" = 0.5, used for Fisher F-test. Furthermore, all measurements were repeated three times and the median were used for further calculations.

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