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Introduction of diffusing wave spectroscopy to study self-emulsifying drug delivery systems with respect to liquid filling of capsules

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ABSTRACT

The rheology of self-emulsifying drug delivery systems (SEDDS) is not thoroughly characterized these days. Since mechanical rheometers are often not well suited to study this kind of systems, there is need for novel physical methods. Several new optical techniques based on microrheology have recently made significant progress. We apply for the first time a specific microrheological technique called diffusing wave spectroscopy (DWS) to study different SEDDS. The obtained data were then correlated with the dosing precision of automated capsule filling.

As a result, the dynamic viscosities obtained from microrheology were in accordance with data from capillary viscosimetry. The DWS measurements revealed that all formulations had a clearly measurable storage modulus at frequencies >200 rad/s. Thus, all samples were low-viscous, while exhibiting non-Newtonian flow behavior. Obtained values of storage and loss modulus were then successfully correlated with the weight variability of capsules that were filled on a machine. In conclusion, the DWS technique enabled rheological analysis of self-emulsifying systems in a broad frequency range. The good data correlation with a capsule quality attribute was especially promising, since microrheological techniques are typically contact-free. Thus, they have a high potential in a quality by design framework of formulation development and production.

1. Introduction

There is an increasing interest in lipid-based formulations for oral delivery of biopharmaceutically challenging drugs (O'Driscoll and Griffin, 2008; Hauss, 2007; Porter et al., 2008). Different formulations typically range from simple oils to rather complex mixtures (Pouton, 2000, 2006). The more elaborate formulations are often self-emulsifying upon contact with aqueous fluids. Very fine dispersions are formed spontaneously in this way. Depending on the evolving particle size, self-emulsifying drug delivery systems (SEDDS) are differentiated from self-microemulsifying systems (SMEDDS). This dispersion behavior is a characteristic for the formulation even though the final particle size is *in vivo* further affected by the digestion process (Fatouros and Mullertz, 2008; Dahan and Hoffman, 2008).

Apart from the particle size upon dispersion, the rheological properties of the system are important. They define the mechanical behavior of the formulation, which is of technical as well as of biopharmaceutical relevance. Rheological properties are critical for the filling of soft or hard capsules and from a biopharmaceutical perspective, any viscous formulation has to facilitate water penetration sufficiently, before any dispersion can occur (Kuentz, 2011). It is of general interest to correlate such material properties with quality attributes of the final dosage form. This is also an objective of the quality by design (QbD) initiative, in which formulations as well as manufacturing processes are especially designed to ensure a predefined quality (Yu, 2008). A part of the QbD research aims to better characterize materials and formulations to subsequently study their effects on targeted product quality attributes. Specifically new characterization methods such as DWS are needed for a deeper rheological assessment of SEDDS or SMEDDS.

Groves and Galindez (1976) were pioneers in rheological analysis of self-emulsifying oil/surfactant systems. They measured flow curves and found a relation between viscosity and the ease of self-emulsification. Systems with good spontaneity were characterized by a decreasing viscosity at maximal shear rate as the water concentration was increased. In the following years, viscosity was often not considered in studies on self-emulsifying systems. In some cases, only a single viscosity value was reported (Shafiq-un-Nabi et al., 2007; Kadu et al., 2011). Because the formulations may not exhibit Newtonian flow behavior, such results must be handled with care. A more detailed rheological analysis was conducted

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by Biradar et al. (2009), who used oscillatory rheology to study the self-emulsification process. Hydration of the formulation produced intermediate liquid crystalline phases that demonstrated visco-elastic flow behavior. Interestingly, recent studies on diluted O/W microemulsions showed that the evolving low-viscous fluids displayed non-Newtonian flow characteristics with shear thinning (Djekic et al., 2011; Zheng et al., 2011). This was a remarkable result, since a microemulsion with an inner phase of discrete particles would be expected to exhibit Newtonian flow behavior. It was therefore assumed that particle interactions were leading to micelle aggregates or the formation of other colloidal structures that resulted in non-ideal flow behavior.

This differentiation of low-viscous Newtonian from non-Newtonian flow is particularly important for undiluted SEDDS or SMEDDS. Filling of rather low-viscous formulations into hard capsules can lead to loss of filling mass due to splashing around the dosing nozzle of the machine. This typically increases the rate of leaking capsules as well as the weight variability of the units. The phenomenon was recently studied with different pharmaceutical oils to predict a ranking of capsule filling adequacy based on Newtonian viscosity and surface tension (Niederquell and Kuentz, 2011). In a next research step, the capsule filling of simple mixtures and more complex formulations, such as SEDDS or SMEDDS, should be investigated.

Currently, knowledge about the rheological properties of pharmaceutical self-emulsifying systems is rather limited. These fluids are not only mixtures of surfactants, oils, and co-solvents but they often comprise additional polymers. Thus, the characterization of mechanical properties in a broad range of frequencies should be very interesting. However, such oily and low-viscous fluids are not easy to measure using a typical controlled-stress rheometer. Oscillatory measurements of such fluids are conducted at a very low shear to stay in the linear visco-elastic range. Measurement accuracy is one issue in these conditions and another is the typical upper limitation of the angular frequency range in oscillation (~100 rad/s). Higher frequencies are, however, of interest for any process that involves a high shear such as the liquid filling of capsules.

Recent advances in microrheology have improved the capability to investigate complex fluids (Waigh, 2005). Among these techniques, the DWS is particularly interesting and review articles outline the measurement principle (Harden and Viasnoff, 2001; Alexander and Dalgleish, 2007; Corredig and Alexander, 2008; Lopez-Diaz and Castillo, 2011). DWS is an optical technique based on light scattering similar to the well-known dynamic light scattering (DLS). In both cases, intensity fluctuations of scattered light provide information on the dynamics of the light-scattering particles. However, the purpose of DLS is to measure particle size by determining the diffusion coefficient of the particles in a fluid with a given viscosity. DWS, on the other hand, investigates microrheological properties of a fluid based on the mean square displacement of the particles (MSD). The particles can occur naturally in the sample or they are simply added as defined tracer particles. It is important to note that traditional DLS and DWS differ greatly in their number of light scattering events. DLS requires single scattered light for a valid measurement. Therefore only diluted systems are measured or special techniques are employed to suppress "multiple scattering". DWS, in contrast, requires multiple scattering as the calculation of the MSD is based on the assumption that light propagation in the sample can be described as a diffusion process.

Principles of DWS were established in the late 1980s but the development of instrumentation and further theoretical understanding were needed to make the method applicable. To the best of our knowledge, DWS has so far not been used to investigate any pharmaceutical mixture for capsule filling.

The primary aim of this study is to introduce DWS for the characterization of self-emulsifying formulations. Eight typical systems are studied here and DWS microrheological viscosity is compared with values obtained from capillary rheometry. First the dilution behavior is studied by DLS, which allows classification by size. The microrheological method DWS is then used for the first time to characterize the different pharmaceutical formulations. Lastly, an attempt is made to use these rheological results for a correlation with capsule weight variability that is obtained from an automated filling process.

2. Materials and methods

2.1. Materials

The medium-chain triglyceride oil Miglyol® 812 was received from the local vendor Hänseler AG (Herisau, Switzerland). The medium-chain partial glycerides Imwitor® 742 was purchased from Sasol (Witten, Germany), whereas Tween® 80 (polysorbate 80) as well as the hydrophilic polymer polyethylene glycol 400 were obtained from Sigma–Aldrich Ltd. (Buchs, Switzerland). The surfactants Cremophor® RH40 (polyoxyl 40 hydrogenated castor oil), Cremophor® EL (macrogol glycerol ricinoleate 35), and Solutol® HS15 (macrogol 15 hydroxystearate) as well as the viscosity enhancer Kollidon® 30 (1-ethenyl-2-pyrrolidinone homopolymer) were excipients from BASF AG (Ludwigshafen, Germany). Gattefossé (Lucerne, Switzerland) kindly supplied Maisine® 35-1 (glycerylmonolinoleate), Labrasol (caprylocaproylmacrogolglycerides), and Transcutol® P (diethylene glycol monoethyl ether). The emulsifier Capmul® MCM (glyceryl mono-dicaprylate) was obtained from Abitec Corp. (Janesville, USA) and ethanol (absolute) was purchased from J.T. Baker (Deventer, the Netherlands). All excipients were used as supplied without any further purification.

We used Licaps® hard gelatin capsules of size I Capsugel (Bornem, Belgium). Glass cuvettes (5 mm) as well as the titanium dioxide tracer particles (mean diameter 360 nm) were obtained from LS Instruments AG (Fribourg, Switzerland). The polystyrene NIST standard (600 ± 0.30 nm) was purchased from Polysciences Europe Ltd. (Eppelheim, Germany).

Artificial intestinal medium (FaSSGF) was prepared using the digestive enzyme pepsin from Hänselerplc (Herisau, Switzerland) together with sodium taurocholate (PCA S.p.A., Basaluzzo, Italy), sodium chloride from Sigma–Aldrich Ltd. (Buchs, Switzerland) and Lipoid S100 (94% phosphatidyl choline) from Lipoid Ltd. (Ludwigshafen, Germany). Finally, hydrochloric acid (1 N) was added to the medium for adjustment of the pH value (1.6).

2.2. Preparation of self-emulsifying systems

The batches were prepared with a total batch size of 250 g for each formulation and the oily phase was mixed before the surfactant was added. Semi-solid excipients, such as Cremophor® RH40 and Imwitor® 742, were initially melted. One formulation comprised the additive polyvinylpyrrolidone K30 and this mixture required additional stirring for 12 h at 45 °C. Formulations with a co-solvent were prepared by adding this excipient in a last compounding step. The final mixture was visually assessed to assure that a single phase was obtained. Subsequently, the formulations were filled in hermetically sealed vials. All formulation compositions are listed in Table 1 by giving amounts in %(w/w).

2.3. Particle size analysis of diluted samples

A Zeta Sizer Nano-ZS from Malvern Instruments Ltd. (Malvern, United Kingdom) was used to determine particle size by means of DLS. This device employed a 4 mW He–Ne laser with a wavelength

Table 1
Composition of selected SEDDS with excipient amounts in % (w/w).

Composition % (w/w)	CO[a]	PO[a]	PE[a]	SE[a]	PC[b]	PP[b]	CP[c]	MC[d]
Cremophor® RH40	40	–	–	–	–	–	–	–
Miglyol® 812	30	24	20.4	25.5	–	–	–	–
Imwitor® 742	30	24	20.4	34	–	–	–	–
Ethanol	–	–	15	15	–	–	–	–
Solutol® HS15	–	–	–	25.5	–	–	–	–
Polysorbate 80	–	52	44.2	–	10.3	9.8	60	–
PEG 400	–	–	–	–	80.4	76.4	30	–
Capmul® MCM	–	–	–	–	9.3	8.8	10	–
Kollidon® 30 (PVP K30)	–	–	–	–	–	5	–	–
Maisine® 35-1	–	–	–	–	–	–	–	30
Cremophor® EL	–	–	–	–	–	–	–	37.6
Labrasol	–	–	–	–	–	–	–	9.4
Transcutol® P	–	–	–	–	–	–	–	23

[a] Ditner et al. (2009).
[b] Gao et al. (2009).
[c] Dixit et al. (2010).
[d] Spernath and Aserin (2006).

of 633 nm. The signal of the back-scattered laser light was detected at an angle of 173°. From this scattering intensity fluctuation, an autocorrelation function was calculated to finally determine the *z*-average (mean of the measured intensities).

For sample preparation, the different formulations were diluted in 1:100 (w/w) FaSSGF medium (Arnold et al., 2010). Each measurement was conducted in triplicate at 25 °C and 37 °C, while samples were equilibrated for 3 min prior to data collection.

2.4. *Density, surface tension, and kinematic viscosity of the formulations*

The physical properties of each sample (i.e. density, surface tension, and kinematic viscosity) were measured at 25 °C in triplicate. A DA-100M from Mettler-Toledo Ltd. (Greifensee, Switzerland) was used for density determination according to the oscillating body method. For measurements of dynamic surface tension, we employed a SITA DynoTester Tensiometer ("SITA Messtechnik" Ltd., Dresden, Germany). This instrument was based on the bubble pressure method in which air bubbles were introduced into the liquid sample and the differential pressure was measured along a capillary. The obtained surface tension is, however, dependent on the bubble frequency and therefore the age of the generated surface. We selected a surface lifetime of 2500 ms that demonstrated good data reproducibility. Finally, the kinematic viscosity was determined by means of an Ubbelohde viscometer of type II with suspending ball level (SI Analytics Ltd., Mainz, Germany). Prior to the measurements, the filled capillary viscosimeter was equilibrated in a water bath for 15 min at 25 ± 0.1 °C. The obtained kinematic viscosities were then converted to dynamic viscosities by multiplying with the sample densities.

2.5. *Diffusing wave spectroscopy in transmission*

2.5.1. *DWS measurement principle*

DWS is an optical technique that allows microrheological measurements. The intensity fluctuations of light scattered in the sample are measured by a photo detector. The scattering must be sufficient such that the propagation of light in the sample can be approximated by the diffusion equations. Intensity fluctuations of the scattered light, $I(t)$ are then characterized by the autocorrelation function, $g_2(t)-1$ (Pine et al., 1988; Mason and Weitz, 1995; Scheffold et al., 2001).

$$g_2(t)-1=\left\langle I(0)I(t)\right\rangle/\left\langle I(0)\right\rangle^2-1 \qquad (1)$$

The function decay can be expressed in terms of the optical wavenumber k and the ensemble average of the MSD, $k^2\left\langle \Delta r^2(t)\right\rangle$ of the particles. The latter property is related to the complex modulus $G^*(\omega)$ via a generalized Stokes–Einstein relation (Mason et al., 1996; Mason, 2000):

$$G^*(\omega)=\frac{k_BT}{\pi a i\omega\left\langle \Delta\tilde{r}^2(i\omega)\right\rangle}=G'(\omega)+iG''(\omega) \qquad (2)$$

where a is the particle radius, k_B is the Boltzmann constant, T the temperature, and $\left\langle \Delta\tilde{r}^2(i\omega)\right\rangle$ holds for the Laplace transform of $\left\langle \Delta r^2(t)\right\rangle$. The cumulative signal of multiple light scattering allows monitoring the average particle dynamics at very short length scales (Willenbacher and Oelschlaeger, 2007). The method, however, requires that scattering particles have to be sufficiently mono-disperse and that no network is formed within the measurement sample. In line with these considerations, the MSD was used in the generalized Stokes–Einstein relation (Eq. (2)) to estimate the macroscopic loss and storage moduli ($G'(\omega)$ and $G''(\omega)$) of the samples over a broad range of frequencies.

2.5.2. *Experimental setup*

All microrheological samples were measured in transmission mode using a commercial apparatus, DWS ResearchLab (LS Instruments AG, Fribourg, Switzerland). A scheme of the apparatus is depicted in Fig. 1. The instrument generated a laser beam of 685 nm, which was scattered from a rotating ground glass that functioned as a diffuser. The diffuse transmitted light was collected by a single lens and illuminated the turbid probe in the temperature-controlled sample holder with a random speckle pattern. Rotation of the diffuser produced a changing speckle pattern resulting in an independent statistical ensemble. This is important when working with non-ergodic or slowly evolving systems. The photon count rate was the output to a digital correlator, which determined the intensity autocorrelation function, $g_2(t)-1$ from which the software (LS Instruments AG, Fribourg, Switzerland) calculated the rheological sample properties.

To avoid time-consuming measurements at comparatively low frequencies (corresponding to long lag-times in the correlation function), the instrument employed the so-called echo technique. Details of this echo technology can be inferred from Zakharov et al. (2006) or Scheffold and Cerbino (2007). The echo data complemented the dynamic light scattering results to obtain a full intensity autocorrelation function over a broad range of lag times. In this function, the values close to zero or unity were the most critical ones with respect to measurement reproducibility. Thus, artificial noise in the data was reduced by setting adequate cut-off limits, namely $0.15 < g_2(t)-1 < 0.95$.

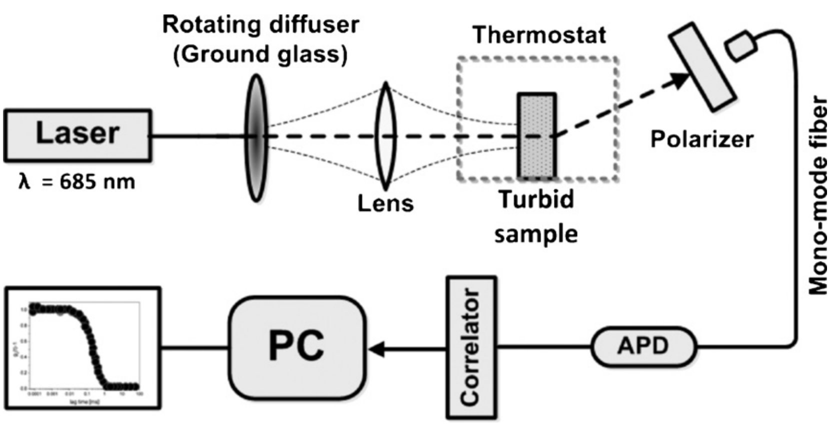

Fig. 1. Schematic representation of the experimental diffusing wave spectroscopic equipment.

2.5.3. Calibration

The DWS ResearchLab was calibrated prior to each measurement. For this purpose, a standard mixture, consisting of an aqueous suspension of polystyrene tracer particles (Polysciences Europe Ltd., Eppelheim, Germany) in purified water (50:50, w/w) was used. The tracer particles (NIST-standard) had a mean size of 600 ± 0.30 nm with a solid content of 1% (w/w) in dispersion. This calibration suspension was filled in cuvettes with a thickness L of 5 mm prior to measuring for 120 s at 25 °C.

Calibration was needed to determine the transport mean free path (l^*) of the standard suspension. The obtained value was on the average 321.21 ± 2.33 μm and therefore much smaller than the cuvette length L, which was required for adequate DWS measurement. Since the intensity of transmitted light is proportional to l^*/L, we linearly estimated the transport mean free path of a sample based on the calibration data and the determined light transmission of the sample (Wyss et al., 2001). The transport mean free path of the sample was needed for the determination of the correlation intensity function and thus for the microrheological characterization.

2.5.4. Sample preparation and microrheology analysis

For sample preparation, 1.68 g of each formulation was filled in 5 mm glass cuvettes. To increase turbidity, titanium dioxide nanoparticles of nominal diameter 360 nm were added in a concentration of 0.26% (w/w). The samples were mixed using a Vortex (IKA® Werke Ltd. & Co. KG, Staufen, Germany) and transferred to an ultrasonic water bath (Bandelin Sonorex Dr. Grogg Chemie plc, Bern, Switzerland). Over at least 15 min of treatment, the transmission count rate was measured several times until a constant value was reached. An optimal count rate was expected to range from 300 kHz to 600 kHz by considering the given detector sensitivity. We targeted a similar count rate for calibration as well as for the sample analysis, so that initial experiments were needed to find a suitable tracer particle concentration.

The average count rate of all measurements was 367 ± 33 kHz and the mean l^*-value yielded 305 ± 24 μm, which was a suitable value for the selected cuvette length. Apart from this transport mean free path, the microrheological analysis needed the refractive index of the samples. This value was determined in triplicate using a digital refractometer RE40D from Mettler Toledo Ltd. (Greifensee, Switzerland).

For the DWS analysis of the samples, the freshly prepared tracer dispersions were equilibrated for 15 min in the measuring chamber. We measured the samples under the same parameters settings as those used for calibration except the measurement time, for which 850 s was selected.

2.6. Capsule filling using the Licaps/LEMS technology

Capsules were filled using a CFS1200™ laboratory-scale filling and sealing machine from Capsugel (Colmar, France). Prior to the filling process, the empty capsules were automatically aligned for separation of the cap by vacuum. We used Licaps™ (Capsugel, Colmar, France) of size I, which were filled with 0.400 ml (80% of the nominal filling volume). The machine was running at 90% of maximum speed, which corresponded to about 1080 capsules per hour. The filling temperature of 25 °C was controlled by three sensors (PT100) and the dosing nozzle had an inner diameter of 1.0 mm. During the entire filling and sealing process, capsules were always maintained in a vertical position. This was different from other manufacturing machines that use a conveyor belt for transferring the filled capsules to the sealing station.

Since the sealing of the capsules is a critical step, the machine employed a clamp system so that capsules were in contact from two opposing sides. An amount of 20 μl sealing solution, consisting of ethanol/water (1:1, w/w), was sprayed through the clamp holes. Capillary forces delivered the solution between the overlapping parts of the two capsule halves to enable a fusion of the material. Excess sealing fluid was at the same time removed by vacuum. Subsequently, the capsules were passing a drying canal with warm air at 45 °C to complete the sealing step.

To manufacture the individual batches, the targeted filling volume was adjusted by fine-tuning the dosing knob. Initial runs of 20 capsules were conducted before we manufactured the main batch of each formulation by rejecting the initial 15 capsules. Three main batches were produced for each formulation and 60 capsules were randomly selected (from a total of about 300 capsules) to check the mean coefficient of weight variability, CV. Capsules were weighed on an analytical balance AB204-S with an accuracy of 0.1 mg (Mettler Toledo, Germany).

2.7. Statistical analysis

The program STATGRAPHICS® Centurion XV ed. Professional from Statpoint Technologies Inc. (Warrenton, USA) was used for statistical processing, whereas Excel V. 2003 (Microsoft Corp., Redmond, USA) was employed for other calculations.

3. Results and discussion

3.1. Characterization of dilution behavior

We selected arbitrarily eight different self-emulsifying drug delivery systems. These model systems exhibited a broad range

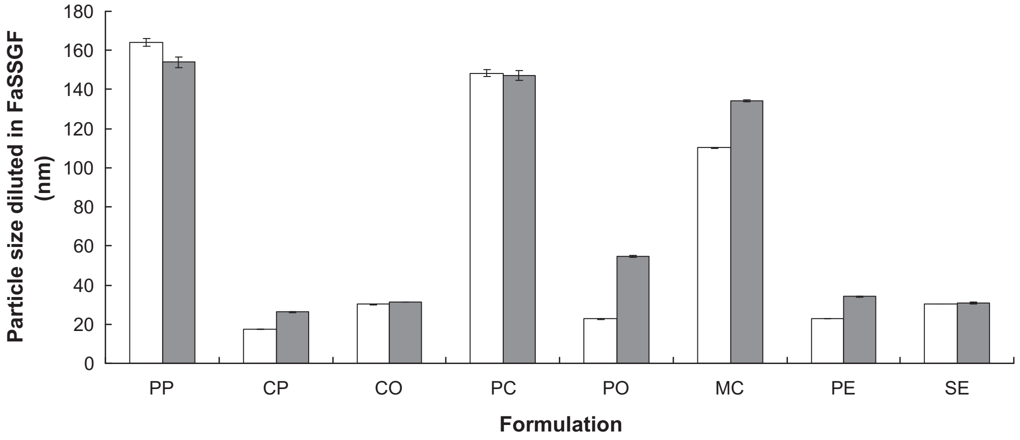

Fig. 2. Particle size (nm) at 25 °C (white bars) and 37 °C (gray bars) of the in FaSSGF-diluted (1:100, w/w) self-emulsifying systems.

of different compositions (Table 1). A first formulation “CO” had Cremophor RH40 as main surfactant and the oil component was a mixture of tri- and partial glycerides of medium-chain length. Similar oil mixtures were used in case of the polysorbate-containing formulations “PO” and “PE”. The latter formulation comprised the additional co-solvent ethanol that was also used in the following Solutol® HS 15-containing formulation “SE”. Further systems “PC”, “PP”, and “CP” were comparatively hydrophilic with some monoglycerides of medium-chain length and a substantial fraction of polyethylene oxide 400. Such polymer-containing SEDDS were deemed as especially interesting with respect to their rheological properties. Finally, another system “MC” was different with a rather high portion of long-chain glycerides even though the composition also included Transcutol P and Labrasol, neither of which were excipients based on long-chain lipids.

The composition of lipid-based systems is one important aspect for categorization according to Pouton’s classification system (Pouton, 2000, 2006). Another aspect is the type of self-emulsification behavior. Thus, some systems are emulsifying as fine droplets, while in other cases an aqueous microemulsion is formed. To differentiate the self-emulsifying (SEDDS) from the self-microemulsifying drug delivery systems (SMEDDS), all formulations were dispersed in aqueous medium at a physiologically relevant level.

We used simulated gastric fluid FaSSGF to dilute the formulations at a ratio of 1:100 (w/w). A mean particle size was then determined by means of dynamic laser light backscattering and the results are shown in Fig. 2. White bars indicate the measurements at 25 °C and the 37 °C results are displayed in gray. In most cases, the mean particle size was in the same order of magnitude so that temperature appeared to have a rather moderate effect. Interestingly, a slight size reduction was noted at 37 °C for PP, whereas the formulations CP, PO, MC, and PE exhibited rather the opposite temperature effect on particle size.

Experiments at both temperatures revealed for PP, PC, and MC a mean particle size (diameter) of more than 100 nm, indicating rather fine emulsions than true microemulsions. These SEDDS were obviously different from the SMEDDS CP, CO, PO, PE, and SE. The latter systems spontaneously formed colloidal dispersions with a mean particle size of about 50 nm and smaller, which was typical for swollen micelles. The observed differences in dilution behavior showed a good diversity of the model formulations. It was subsequently of interest to study how this diversity was reflected by the rheological behavior of the systems.

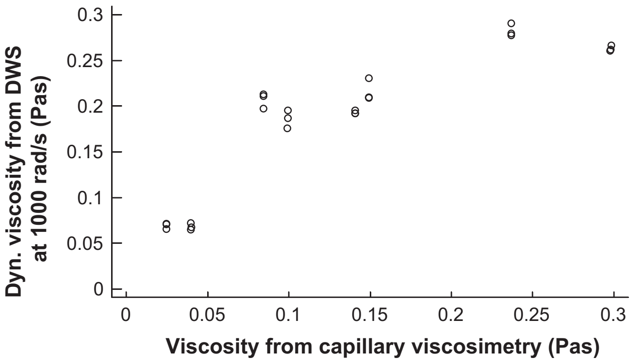

Fig. 3. Comparison of the capillary viscosimetry and DWS-based dynamic viscosity (η’) at 1000 rad/s.

3.2. Capillary viscosimetry and estimates of the microviscosity from diffusing wave spectroscopy

Already the formulation handling indicated different viscous properties among the model systems. Even though all samples were flowing freely, some formulations appeared quite low-viscous, while other systems seemed much more viscous. We first studied the samples by means of capillary viscosimetry. This method is known for its high precision at rather low viscosity, but it has the disadvantage that no quantification of non-Newtonian flow behavior is possible. As a results of the capillary experiments, dynamic viscosity ranged from $0.0247 \pm 3.4 \times 10^{-5}$ Pa s for SE to $0.298 \pm 3.6 \times 10^{-4}$ Pa s in case of the formulation PP. This result indicated how different the formulations were from a mechanical viewpoint. It was remarkable that no significant correlation was found between dynamic viscosity and observed particle size in the dilution experiments. Certainly, the undiluted state has to be differentiated from formulations that already contain some water so that swollen mesophases can form. The viscosity of such liquid crystalline structures was previously shown to correlate with the ease of self-emulsification (Groves and Galindez, 1976). However, the viscosity of the undiluted formulation seems less appropriate to anticipate the type of self-emulsification or even the final particle size upon dispersion.

It was interesting to compare the viscosity from the capillary test with that of the novel microrheological method. The DWS provided a complex shear modulus G^* together with its storage modulus G’ and the loss modulus G” at different frequencies (Eq. (2)). The dynamic viscosity η’ was readily obtained using Eq. (3).

$$\eta' = \frac{G''}{\omega} \tag{3}$$

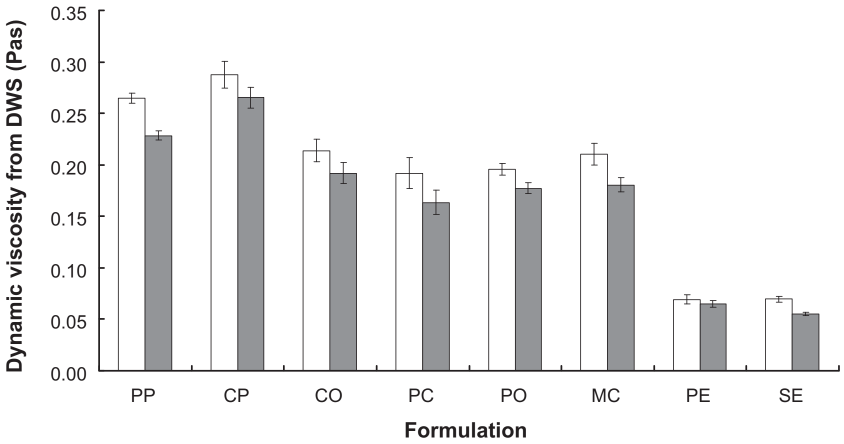

Fig. 4. DWS-based dynamic viscosity (η’) at 1000 (white bars) and 5000 rad/s (gray bars).

Fig. 3 shows a scatter plot of the DWS-based η’ values (at 1000 rad/s) and the dynamic viscosity from the capillary method. The values were in the same order of magnitude. Moreover, a correlation was found with a Pearson product moment correlation of $r = 0.873$ ($p < 0.00001$). Such agreement was in line with recent findings in the physical literature, in which mechanical rheometry of soft matter samples showed almost an overlay with the estimates from DWS (Scheffold et al., 2004; Galvan-Miyoshi et al., 2008). This previous study also highlighted another advantage of DWS. Oscillatory rheometers were limited up to about 100 rad/s, while the DWS method could measure at considerably higher frequencies. Such frequency-dependent analysis was of course not possible with the capillary method that could not differentiate ideal from non-ideal flow behavior.

Considering potential non-ideal flow behavior, we compared dynamic viscosities from DWS at different frequencies. Fig. 4 shows the DWS-based η’ values for 1000 (“light bars”) and 5000 rad/s (“gray bars”). Unlike an ideal-viscous flow regime, the values generally changed. Viscosity decreased at the higher frequency even though the difference was not very pronounced. This result indicated that the studied frequencies were outside of a Newtonian flow regime indicating some visco-elasticity. However, for a more thorough investigation, we studied the mechanical moduli in a wider range of frequencies.

3.3. *Frequency sweeps from DWS*

Visco-elasticity is generally difficult to measure in rather low-viscous formulations. A method has to detect low values of *G*’, if a visco-elastic and ideal flow behavior should be differentiated. In this case, it was an advantage that DWS did not need any mechanical contact because force transmission in oily, low-viscous samples can be erratic due to a loss of grip at higher frequencies. However, the rather low-viscous character of some samples was also challenging for the spectroscopic method. First, a suitable concentration of tracer particles was evaluated and from the obtained autocorrelation functions, it was apparent that reproducibility was not the same at all frequencies. The range was narrowed to a common frequency range of 100–5000 rad/s in which good data reproducibility was given. Table 2 shows the mean and standard deviation values of the loss and storage modulus at two reference frequencies (1000 and 5000 rad/s were considered). The relative standard deviations were in most cases only a few percent indicating a quite robust measurement procedure.

Figs. 5 and 6 depict *G*’ and *G*” as a function of frequency. Interestingly, an existing *G*’ was detected in all formulations. However, there were individual differences. Formulation PP for example demonstrated pronounced *G*’ at higher frequencies, while the storage modulus was less than 1 Pa at 100 rad/s (Fig. 5). Such a low storage modulus in combination with a high ratio of *G*” to *G*’ make it difficult to assess visco-elasticity at low frequencies, which explains the low reproducibility below 100 rad/s. Rising frequencies revealed much higher absolute moduli and in some formulations, the ratio of *G*” to *G*’ was also decreasing (Figs. 5 and 6).

The formulation CP exhibited a similar increase of the storage modulus as PP yielding rather high *G*’ values over 1000 rad/s. These viscous formulations PP and CP had in common that a triglyceride phase was missing. Here, the oil phase was only a low amount of monoglycerides and a substantial fraction of the composition was polyethylene glycol. A similar composition was also given with the system PC, which also showed rather high *G*” values and it was notable that these values increased almost parallel to *G*’ along the different frequencies.

Fig. 6 depicts the results for the formulation PO, MC, PE, and SE. The latter two formulations were apparently different regarding their microrheology. Compared to other formulations, PE and SE could not reach the same high level of *G*” that was corresponding to rather low dynamic viscosity (Fig. 4). Formulation PE did not

Table 2
Overview of the storage and loss modulus (*G*’, *G*”) at 1000 and 5000 rad/s for the different systems. The coefficient of capsule weight variability, CV (%) was determined from $n = 3$ batches.

System	*G*’ (Pa) 1 krad/s	*G*” (Pa) 1 krad/s	*G*’ (Pa) 5 krad/s	*G*” (Pa) 5 krad/s	CV of capsule weight (%)
PP	28.42 ± 1.03	264.53 ± 4.71	240.76 ± 12.52	1141.66 ± 22.55	0.392 ± 0.033
CP	15.31 ± 1.53	287.82 ± 13.04	370.24 ± 94.76	1326.88 ± 50.10	0.402 ± 0.002
CO	20.88 ± 1.25	213.73 ± 10.99	129.29 ± 10.49	959.78 ± 52.11	0.410 ± 0.001
PC	33.17 ± 4.72	191.97 ± 15.22	124.41 ± 16.38	817.25 ± 57.85	0.413 ± 0.004
PO	16.17 ± 0.67	195.52 ± 5.54	113.81 ± 6.36	886.92 ± 25.66	0.529 ± 0.028
MC	27.79 ± 1.96	210.50 ± 10.33	177.72 ± 20.34	903.18 ± 36.27	0.533 ± 0.002
PE	4.85 ± 1.22	69.40 ± 4.11	23.51 ± 3.24	324.07 ± 16.58	1.180. ± 0.117
SE	15.75 ± 2.13	69.44 ± 3.10	60.22 ± 6.00	277.50 ± 8.09	1.332 ± 0.021

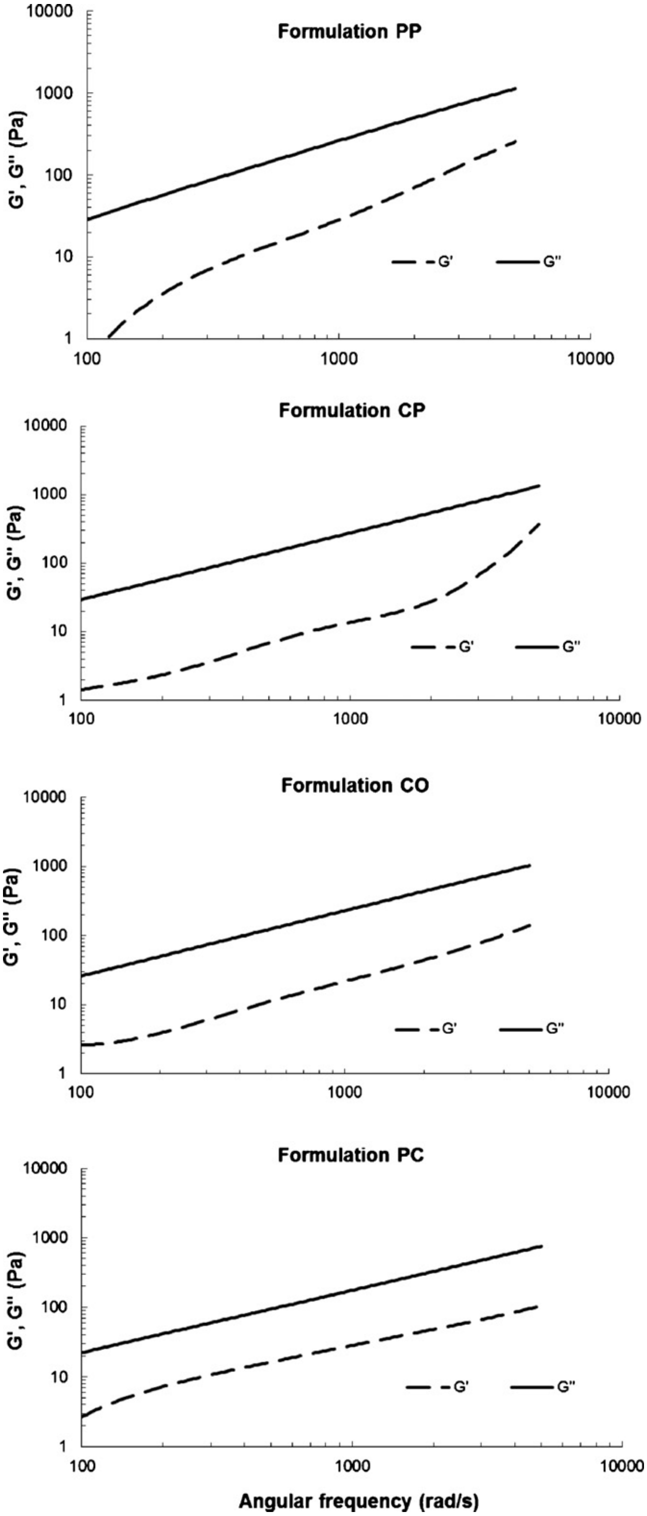

Fig. 5. Frequency sweep at 25 °C using formulations PP, CP, CO, and PC (measurement time 850 s).

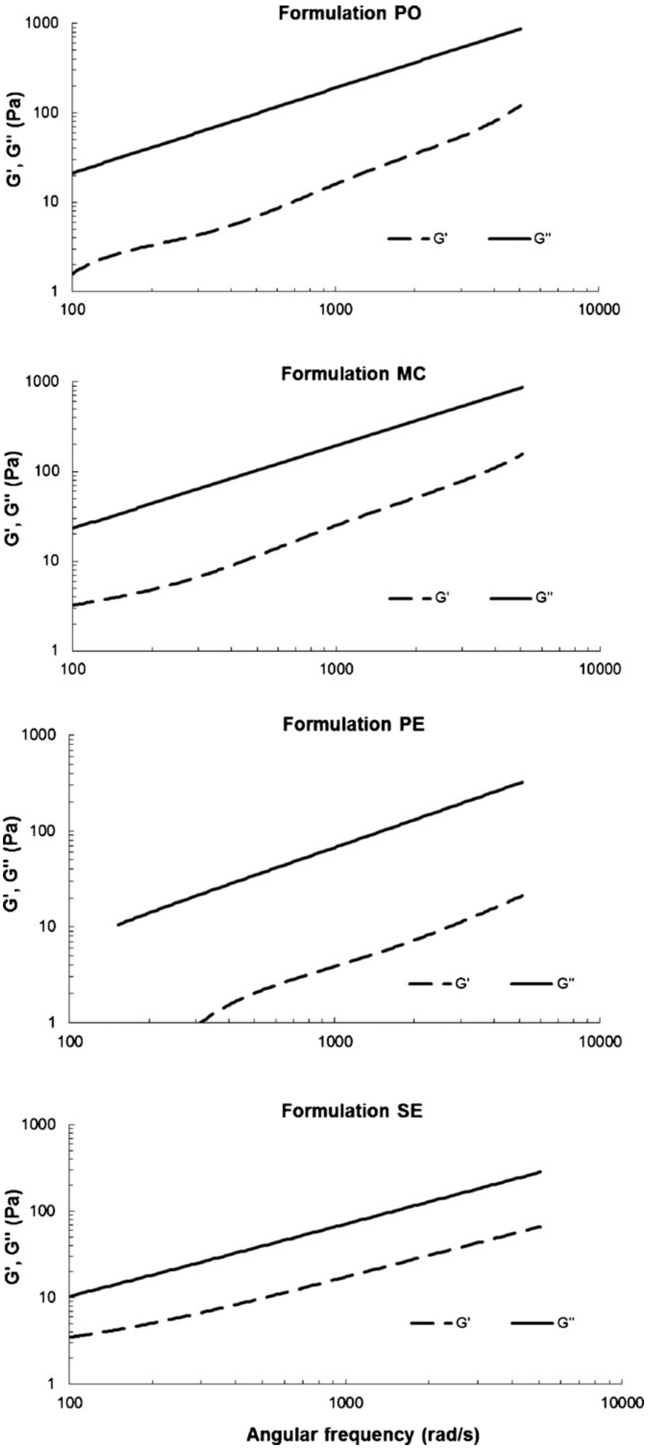

Fig. 6. Frequency sweep at 25 °C using formulations PO, MC, PE, and SE (measurement time 850 s).

reveal a detectable *G*' below about 200 rad/s. This was different in formulation SE that displayed a clear *G*' at 100 rad/s and the ratio *G*''/*G*' was smaller than that in PE. Both formulations had a similar oil phase with ethanol as co-solvent so that the main difference was the choice of surfactant. The semi-solid character of Solutol® HS15 must have caused the observed microrheology of SE compared to PE, which comprised polysorbate 80. Despite these visco-elastic differences among PE and SE, the loss modulus *G*'' was similar for many frequencies. This explains why the dynamic viscosity was similar for both formulations (Eq. (3) and Fig. 4). Care is therefore needed if only dynamic viscosities are compared, since subtle details of individual visco-elastic behavior can remain undiscovered.

In summary, all the tested self-emulsifying systems revealed a marked *G*' at higher frequencies. The moduli dependence on frequency was not the same in the different formulations. Specific for the system and frequency was also the ratio of *G*''/*G*'. However, in all cases, the loss modulus was clearly dominating *G*' so that it

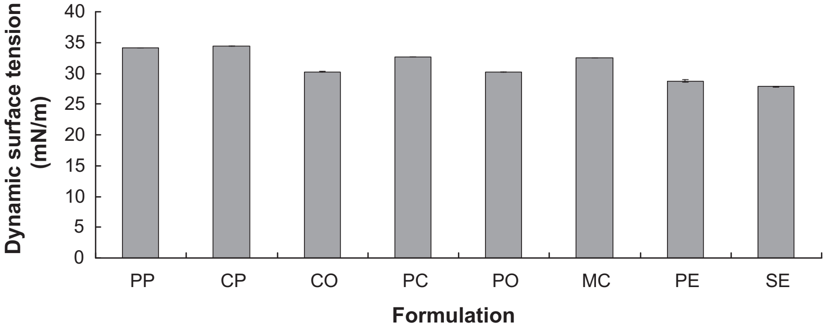

Fig. 7. Dynamic surface tension (mN/m) at 25 °C (surface age of 2500 ms).

contributed the most to the complex modulus *G**. Thus, all self-emulsifying systems were considered as visco-elastic fluids with a predominant viscous component.

3.4. Liquid filling of capsules

We studied the filling precision of the different formulations on an automated capsule filling machine. The mean CV of capsule weight is displayed in Table 2 ($n = 3$ batches). Dosing in general was comparatively precise, as was expected for liquid-filled capsules (Cole et al., 2008). Significant differences were observed with an ANOVA *p*-value of <0.0001. It was remarkable that some formulations, namely PE and SE had especially high capsule weight variability. Both formulations contained ethanol as co-solvent and they showed lowest dynamic viscosity among the different systems (Fig. 4). Low viscosity was a potential factor for limiting the dosing adequacy. This was in line with a recent study on capsule filling of simple oils (Niederquell and Kuentz, 2011). Based on the dynamic viscosity of the oil and its surface tension, it was possible to predict the ranking of oils with regards to dosing precision. Even though the underlying theory was based on Newtonian flow behavior, the findings are also of interest for more complex low-viscous systems as used in this study. Like with pure oils, some splashing was observed with self-emulsifying formulations around the dosing nozzle. However, the effect was mainly seen with the low-viscous formulations, such as PE and SE, while the more viscous systems PP or CP barely showed visible splashing during capsule filling.

Certainly, the dynamic viscosity was not the only parameter that defined the dosing precision of the capsule machine. Having constant machine parameters, it was assumed that surface tension of the formulation was of potential relevance for splashing during the filling step (Niederquell and Kuentz, 2011). The dynamic surface tension γ_s was therefore measured and Fig. 7 shows the obtained values for the individual formulations. Values were scattering in a comparatively narrow range, but differences could still be detected.

Unfortunately, there is currently no theory about formulation splashing and dosing precision of visco-elastic formulations. The situation of such non-Newtonian systems is complex due to the dependence of dynamic viscosity on the shear in the dosing nozzle. Dynamic viscosity under shear is often assigned to a corresponding value from a frequency sweep, but this so-called Cox/Merz relation seems to not hold strictly at a shear > 100 s^{-1} (Metzger, 2011). It was therefore not necessary to exactly determine the shear in the nozzle, but in light of the selected dosing speed and nozzle diameter, a clearly higher shear must result than a previously reported value of ~300 s^{-1} (Kattige and Rowley, 2006). Thus, we arbitrarily selected 1000 rad/s and 5000 rad/s as reference frequencies.

We analyzed Spearman rank correlations between the formulation properties and the CV of the manufactured capsules. This rank correlation *r* had the advantage, that no linear relationship was assumed. Results are shown in Table 3 and a first significant factor was the surface tension. This finding supported the current view of how filling adequacy is limited in low-viscous formulations. Any splashing during filling must be paralleled with generation of droplet surface, which in turn is defined by the surface tension. However, the same mechanism of splashing was in case of pure oils even more dependent on the dynamic viscosity (Niederquell and Kuentz, 2011). Since the latter viscosity and the loss modulus are directly related (Eq. (3)), we used *G*” to correlate with capsule filling CV. The correlation was significant in case of the lower and higher reference frequency. At 5000 rad/s the correlation of *G*’ and CV was interestingly highest. It was this higher reference frequency for which also the storage modulus *G*’ also correlated with the weight variability of the capsules. This was remarkable and can be intuitively explained by the following consideration. If we imagine a droplet that is filled from the dosing nozzle, it will typically impact on the surface of already filled formulation. The added droplet can either fuse with the formulation in the capsule or it comes to splash formation. Such droplet impacts and splash formation constitutes a topic of still ongoing research (Rein, 1993). However, it is evident that only those droplets are critical for dosing adequacy that is splashing out of the capsules. It is likely that *G*’ and thus, the elastic component play a role here if the impact on the liquid surface as well as splash formation happen on a rather short time scale. Such a short time scale corresponds to a high frequency for which the self-emulsifying formulations all exhibited notable *G*’ values.

It should be remembered that only the analysis of higher frequencies was able to demonstrate a correlation of *G*’ with the weight variability of filled capsules. A limited mechanical rheology below 100 s^{-1} (or <100 rad/s angular frequency) seems unsuited to fully characterize the self-emulsifying systems. Care is therefore needed if formulation properties are analyzed with respect to any correlations with quality attributes of a final dosage form. In

Table 3
Spearman rank correlation *r* of capsule weight CV (%) and DWS-based moduli. The *p*-values of the correlations are listed and significance below 0.05 is marked with an asterisk.

System	γ_s[a] (mPa s)	*G*’ (Pa) 1 krad/s	*G*” (Pa) 1 krad/s	*G*’ (Pa) 5 krad/s	*G*” (Pa) 5 krad/s
r (CV(%))	−0.857	−0.405	−0.857	−0.810	−0.881
p-value	0.023*	0.284	0.023*	0.032*	0.020*

[a] Surface tension at 2500 ms.

attempting to find material parameters that are critical to quality, it seemed that DWS filled a gap in adequately studying the rheology of self-emulsifying systems.

4. Conclusions

The novel technique of diffusing wave spectroscopy was applied to study self-emulsifying systems. It was possible to analyze the microrheology with several formulations at comparatively high frequencies. Thus, good reproducibility of storage and loss modulus was obtained in a range of 100–5000 rad/s. These measurements revealed that all systems had a detectable storage modulus indicating visco-elasticity.

Being able to thoroughly characterize the microrheology of self-emulsifying formulations has consequences for the development of formulations as well as later for the manufacturing thereof. A thorough characterization of visco-elastic properties is the basis for better understanding of how these material parameters affect further processing. More research is needed here, but already the current study demonstrated that capsule weight variability was correlated with the mechanical moduli of the formulation. Thus, it was possible to relate a critical material property to a quality attribute of the final dosage form, which is a key objective of the quality by design initiative. Therefore, the DWS technique has a high potential as a tool in the quality by design framework. This starts with the development of formulations for which a fast and reliable measurement is of particular interest. Small quantities can be assessed already in a screening phase of formulations. Apart from the use in early formulation development, DWS is furthermore promising with regards to process analytics. Some formulations do not need tracer particles, which would enable direct measurements. However, even if particles have to be added, like in our study of self-emulsifying systems, the microrheological technique is still faster than any mechanical method so that DWS qualifies for in process controls or for at line analytics. There are many research opportunities in the area of pharmaceutics that can benefit from DWS and thus from recent progress in laser light scattering technologies.

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