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SHORT COMMUNICATION

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Diffusion behavior of pharmaceutical O/W microemulsions studied by dynamic light scattering

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Abstract Dynamic light scattering experiments have been performed at various concentrations, of pharmaceutical oil-in-water microemulsions consisting of Eutanol G as oil, a blend of a high (Tagat O2) and a low (Poloxamer 331) hydrophilic–lipophilic balance surfactant, and a hydrophilic phase (propylene glycol/water). We probe the dynamics of these microemulsions by dynamic light scattering. In the measured concentration range, two modes of relaxation were observed. The faster decaying mode is ascribed classically to the collective diffusion D_c (total droplet number density fluctuation). We show that the slow mode is also diffusive and suggest that its possible origin is the relaxation of polydispersity fluctuations. The diffusion coefficient associated with this mode is then the self-diffusion D_s of the droplets. It was found that D_c and D_s had opposite volume fractions of oil plus surfactants (ϕ) dependence and a common limiting value D_0 for ϕ=0. Average hydrodynamic radius (R_h=10.5 nm) of droplets was calculated from D_0. R_h is supposed to compose the inner core, a surfactant film including possible solvent molecules, which migrate with the droplet. The concentration dependence of diffusion coefficients reflects the effect of hard sphere and the supplementary repulsive interactions which arises due to loss of entropy, when absorbed chains of surfactant intermingle on the close approach of the two droplets. This mechanism could also explain the observed stability of our systems. The estimated extent of polydispersity is 0.22 from the amplitude of slower decaying mode. The polydispersity in microemulsion systems is dynamic in origin. Results indicate that the time scale for local polydispersity fluctuations is at least three orders of magnitude longer than the estimated time between droplet collisions.

Keywords O/W microemulsions · Dynamic light scattering · Dermal drug delivery

Introduction

Microemulsions (MEs), thermodynamically stable dispersions of oil and water stabilized by surfactants and in some cases additionally by cosurfactants [1, 2], have attracted much interest in recent years in terms of their drug delivery potential and other great practical importance because of their interesting physical properties [3, 4]. We have recently studied the dynamical behavior of model ternary microemulsions in the large concentration range [5]. Therefore, we considered it as interesting to carry out a detailed study of the decay rates for more complicated pharmaceutical MEs. In the present study, MEs were formulated using only pharmaceutically acceptable components. It is important to know something about the physico-chemical properties of ME to select a suitable ME system for drug delivery. Key ingredients in a physico-chemical characterization of ME systems are (1) phase

stability and phase behavior, (2) microstructure, dimension (size and distribution), shape (or conformation), and surface features (specific area, charge and distribution), and (3) local molecular arrangements, interactions and dynamics. Of these properties, particle sizes, interactions and dynamics of ME systems are of fundamental importance because they control so many of the general properties of these systems.

Information about the dynamic behavior of colloidal systems can be obtained with dynamic light scattering (DLS) by measurements of the intensity auto-autocorrelation functions [6]. In the case of diluted monodisperse systems these functions can be described by single exponentials and from the observed relaxation time the apparent diffusion coefficient of the particles can be obtained [7, 8]. With increasing particle concentration, the autocorrelation functions are no longer single exponentials. This is due to several effects such as direct and hydrodynamic particle interactions, formation of clusters, polydispersity in scattering amplitudes, and memory effects [8]. These two modes were observed in concentrated colloidal dispersions [9, 10], in charged dispersions [11], and in water-in-oil droplet MEs [5, 12]. In bicontinuous MEs, two relaxation modes were reported [13], which have a different origin related to the local fluctuations of the topology of the bicontinuous network. Two relaxation modes have recently been observed in the dynamics of charged MEs. The authors claim the second mode to be due to the relaxation of local charge fluctuations via the local exchange of droplets bearing different numbers of charges [11]. Also in viscoelastic fluids, complex DLS spectra are observed with two or three relaxation modes (see [14] and [15] and references therein). One of these modes is directly related to the transient elasticity of the system. This list is not exhaustive, but it illustrates the variety of systems where DLS spectra display two or more relaxation modes, the origin of which depends strongly on the type of system studied.

In the present paper, we consider concentrated pharmaceutical oil-in-water MEs. DLS spectra of these ME systems clearly exhibit two relaxation modes. These two modes are attributed to the existence of two relaxation modes: one mode is due to collective diffusion and another due to self-diffusion [5, 16, 17]. A particular difficulty arises in the direct and unambiguous determination of droplet radius from relaxation modes as it is expected to be a strong function of droplet–droplet interactions. To obtain a reliable estimation of particle size, measurement should be made at a range of low-disperse-phase volume fractions and should be extrapolated to infinite dilution to avoid the problems encountered as a result of droplet–droplet interactions [18]. These systems could not be diluted below ϕ=0.10 without phase separation, as is common to many MEs. As a result, DLS data cannot be used without making suitable corrections for droplet–droplet interactions to allow a meaningful calculation of droplet size at finite droplet concentration.

In the majority of pharmaceutical ME systems, it is necessary to work in relatively high particle volume fraction regimes and account for interparticle interactions and polydispersity when interpreting light scattering data. The previous investigators have used either uncorrected [19–21] or hard sphere models [22, 23] to correct the results obtained for droplet–droplet interactions. Some workers have even attempted to correlate these uncorrected droplet sizes with the oral bioavailability of the drug in the ME, unsurprisingly without success [24]. Much less effort have been done to study experimentally the nature of interparticle interactions and polydispersity using dynamic light scattering in comparison. It is therefore the aim of this paper to show that the interparticle interactions and polydispersity play very important roles in interpreting light scattering data for high disperse phase pharmaceutical MEs. We have reported detailed analysis of the experimental data of water/PG-Tagat O2/ Poloxamer 331-Eutanol G over the large range of ϕ (0.10−0.36) at T=25°C to do this in the present study. A dilution procedure was used in the region of the phase diagram, where surfactant-covered oil droplets were formed, allowing us to deduce the concentration dependence of both collective and self-diffusion coefficients from experimental data. From the concentration dependence of these diffusion coefficients, droplet size, polydispersity, and interaction potential between the droplets has been unambiguously estimated. To our knowledge, this is the first time this methodology was used to unambiguously characterize the droplet size, size distribution, and interparticle interactions in concentrated pharmaceutical MEs.

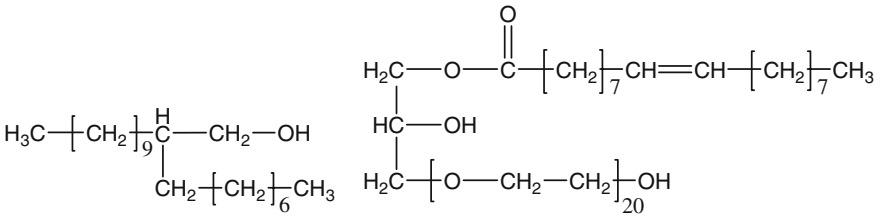
Materials and methods

Chemicals and sample preparation

Tagat O2 (hydrophilic–lipophilic balance, HLB=15), Eutanol G, and propylene glycol (PG) were purchased from Caesar & Loretz, Hilden, Germany. Poloxamer 331 (HLB=1) was kindly provided by C. H. Erbsloeh, Krefeld, Germany. The chemical structures of these compounds are presented in Fig. 1. Water was used in bidistilled quality.

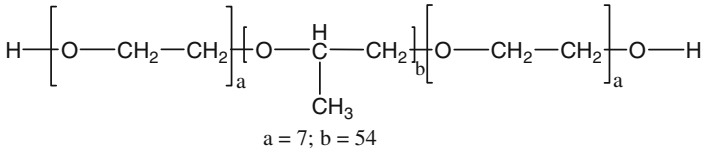
The basic ME consists of a quaternary mixture of Eutanol G, 5 wt.%, Poloxamer 331/Tagat O2 (3:2), 20 wt.%, and PG/H_2O (2:1), 75 wt.%. The basic ME was diluted with the continuous phase keeping constant the ratio $\mu = \frac{[\text{wt.\% of surfactants}]}{[\text{wt.\% of oil}]}$ and varying oil weight percentage α in the range $2<\alpha<7$ to preserve a constant droplet radius. MEs are considered as a priori formed objects: The oil core and surfactant films form a single entity, immersed in a continuous water phase. Therefore, the volume fraction (ϕ) of the ME droplets can be defined as $\phi=\phi_o+\phi_s$, where

Fig. 1 Chemical structure of species used in the formation of o/w microemulsions



Eutanol® G (*2-Octyl-1-dodecanol*)

Tagat® O2 (Polyoxyethylene glycerol monooleate)



a = 7; b = 54

Poloxamer 331

ϕ_o and ϕ_s are the volume fractions of oil and surfactants phase, respectively.

Before the measurements were taken, the samples were filtered through a 0.45-μm pore size filter into dust-free sample cells. The cylindrical sample cells are made of Suprasil quartz glass by Hellma, Muellheim, Germany and have a diameter of 10 mm.

Dynamic light scattering experiment

Dynamic light scattering experiments were performed on a standard commercial apparatus (ALV) using a green Nd: YAG DPSS-200 mW laser emitting vertically polarized light at a wavelength of 532 nm. The thermostated sample cell (temperature controlled to±0.1°C) is placed on a motor-driven precision goniometer (±0.01°) that enables the photomultiplier detector to be moved from 20° to 150° scattering angle. The intensity time autocorrelation functions g_2 (τ) are recorded with an ALV-5000E multiple tau digital correlator with fast option. The minimal sampling time of this correlator is 12.5 ns.

The light-scattering process defines a wave vector $q = \frac{4\pi n}{\lambda} \sin(\theta/2)$, where λ is the wavelength of the incident light in a vacuum, θ is the scattering angle, and n is the refractive index of droplet. The value of n was measured for each sample using an Abbe refractometer.

Results and discussion

It is known that the droplet size distribution and interdroplet interactions are the most important characteristics of MEs for the evaluation of its stability and penetration mechanism into the skin [25, 26]. DLS was proven to be an excellent technique for investigating size distribution and interdroplet interactions of MEs.

In a DLS experiment, intensity–intensity time correlation function g_2 (τ) is measured. If the scattered field obeys Gaussian statistics, the measured correlation function g_2 (τ) can be related to the theoretically amenable first-order field correlation function g_1 (τ) by the Siegert relationship [27] $g_2(\tau)-1=\beta|g_1(\tau)|^2$, where β is the coherence factor of the experiment and it should ideally be 1. Coherence factor of the setup was proven to be better than 0.9. All DLS measurements were made at different scattering angles between 40° and 140° at a temperature of 25°C. The intensity time autocorrelation function corresponding to one set of experimental parameters have been measured five times, and data used for fitting are averaged over these five measurements. In all cases, the decay of g_2 (τ)−1 was followed up to values of τ large enough to reach the base line. Fig. 2 shows the two exponential and inadequate single exponential fitting of the g_2 (τ)−1 at a scattering angle of 90° for the water/PG-Tagat O2/ Poloxamer 331-Eutanol G at ϕ=0.36. Fig. 2 also shows the residuals obtained when fitted to a stretched exponential as was done by Chen and Huang (1985) [28] for concentrated MEs. As it can be seen, the residuals for the fit to two exponentials are substantially smaller than that for a stretched exponential or single exponential. Therefore, we fitted g_2 (τ)−1 by two exponentials as did Shukla et al. (2004) [5], Peter et al. (2001) [13], Yan and Clarke (1990) [12], and Kops-Werkhoven and Fijnaut (1982) [8]

$$g_1(\tau) = A_{\text{fast}} e^{-\Gamma_{\text{fast}}\tau} + A_{\text{slow}} e^{-\Gamma_{\text{slow}}\tau} \quad (1)$$

where A_i are the amplitudes and Γ_i are the characteristic relaxation rate of a mode i.

Figure 3 shows the decay rates of the fast and slow mode for the samples corresponding to volume fractions ϕ=0.10,

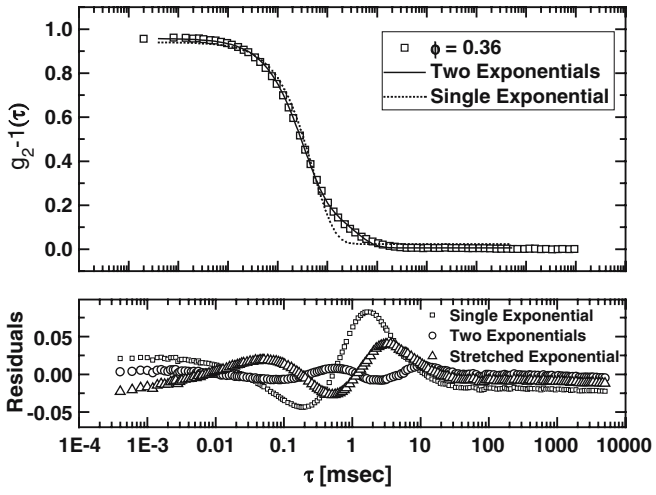


Fig. 2 **a** The correlation function $g_2-1(\tau)$ for microemulsion system at ϕ=0.36, θ=90°, and T=25°C for the systems water/PG-Tagat O2/Poloxamer 331-Eutanol G. A two exponential fit is plotted through the *solid line*. The inadequate two exponential fit is shown as a *broken line*. **b** Residuals obtained from the fits single, two and stretched exponential. The only second point of the autocorrelation data is plotted

0.23 and 0.36. In all cases, relaxation rates seem to be diffusive, i.e., the characteristic decay rate varies to a good agreement with the square of the wave vector, indicating a hydrodynamic origin of modes. The solid lines in Fig. 3 represent a fit of $\Gamma_i=D_iq^2$, where D_i are the diffusion coefficients of a mode i. In case of the hydrodynamic origin, fast mode represents collective diffusion (fluctuations in the number density of the droplets) and slow mode represents self-diffusion (polydispersity in scattering amplitude of the droplets) [8]. In the following equations, the indexes c and s will stand for "collective" and "self" respectively.

Figure 4 shows a corresponding plot of diffusion coefficients obtained by the relaxation rates. As shown in Fig. 4, the diffusion coefficient D_s decreases in value with increasing volume fraction, whereas D_c shows an increase. This is consistent with the interpretation that D_s describes polydispersity fluctuations by self-diffusion and that D_c arises from mutual diffusion of ME droplets [5]. Each of the two diffusion coefficients (D_c and D_s represented in Fig. 4) should fit to the same value, D_0, as $\phi\rightarrow 0$, so the use of two-exponentials method of data analysis permits to estimate the droplet size quite accurately using the Stokes–Einstein equation, $R_h=\frac{k_BT}{6\pi\eta D_0}$ where k_B is the Boltzmann's constant, T is the absolute temperature and η is the coefficient of viscosity of the solvent (the continuous phase in the case of ME). Same D_0 is indeed observed from both diffusion coefficients as shown in Fig. 4, D_c and D_s satisfactorily fit to a linear $D_c=D_0\ (1+\alpha_1{}^c\phi)$ and quadratic $D_s=D_0\ (1+\alpha_1{}^s\phi+\alpha_2{}^s\phi^2)$ with $D_0=2.2\times10^{-8}$cm^2/s, $\alpha_1{}^c=2.78$, $\alpha_1{}^s=-4.84$ and $\alpha_2{}^s=6.71$. Mean droplet hydrodynamic radius R_h, which is supposed to consist of the oil core (R_{core}), a penetrable surfactant film (L) and perhaps some solvent molecules too, was calculated using D_0 from Stokes-Einstein equation is ~10.5 nm. This is consistent with the Small Angle Neutron Scattering (SANS) result [4]. If we assume that the droplet size does not vary significantly over the concentration range, then these results can be compared with theoretical predictions to first order in the volume fraction. This is not an unreasonable assumption as it is recognized that droplet size is dominantly characterized by the molar ratio of dispersed phase to surfactant rather than actual concentration. Felderhof (1978) [29], using theoretical approach, obtained that the difference of the collective diffusion of Brownian particles with hydrodynamic interaction from its hard sphere value $\delta\alpha_1{}^c=\alpha_1{}^c-(\alpha_1{}^c)^{HS}$ is negative if there are supplementary attractive interactions

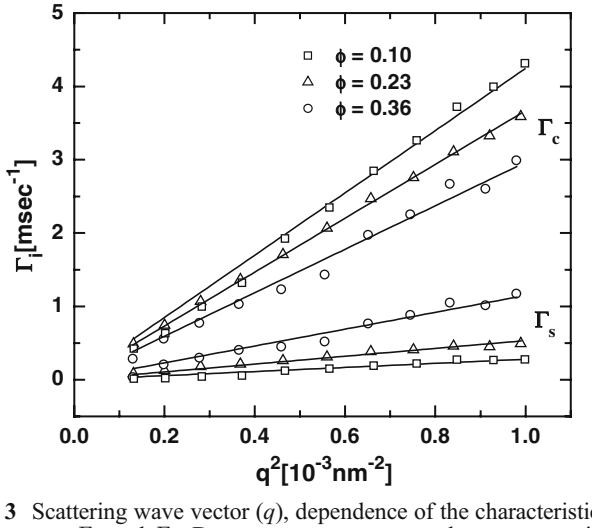


Fig. 3 Scattering wave vector (q), dependence of the characteristics decay rates Γ_c. and Γ_s. Decay rates vary to a good agreement with the square of the q (*fitted solid line*), indicating diffusive processes

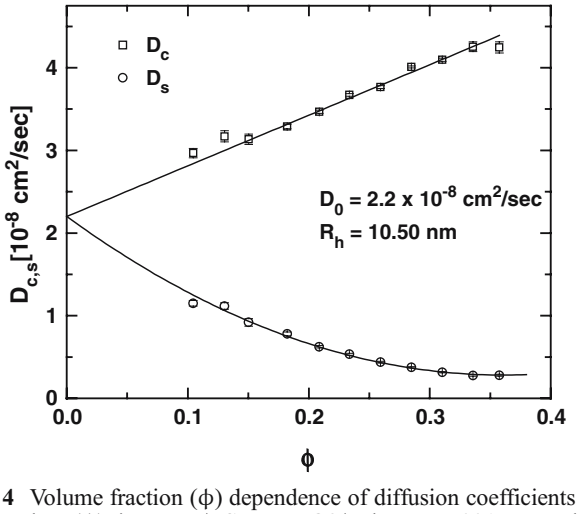


Fig. 4 Volume fraction (ϕ) dependence of diffusion coefficients D_c (ϕ) and D_s(ϕ) in water/PG-Tagat O2/Poloxamer 331-Eutanol G microemulsions at 25°C. D_0 is the infinite dilution (extrapolated) value

and positive in the case of additional repulsive interactions. For hard spheres $(\alpha_1^c)^{HS}$=1.56 [30]. For studied systems, $\delta\alpha_1^c=\alpha_1^c-(\alpha_1^c)^{HS}$=1.22 is positive, indicating that droplets are interacting via hard sphere interaction with perturbation of repulsive potential. Concentration dependence of self-diffusion is highly dependent on the ratio of hard core radius and a penetrable surfactant film (L) [31]. Cichoki and Felderhof (1990) [31] also predicted that $\delta\alpha_1^s=\alpha_1^s-(\alpha_1^s)^{HS}$ may be positive or negative depending on the value of the hard core radius and L for additional repulsive interactions. The parameters needed for the calculations for concentration dependence of self-diffusion are unknown. Nevertheless, the concentration dependence of self-diffusion has theoretically been calculated to the volume fraction for hard spherical particles: $(\alpha_1^s)^{HS}=-1.73$ and $(\alpha_2^s)^{HS}=0.88$ [30]. However, these theoretical considerations are based upon the situation that the change in particle configuration during the relaxation time of autocorrelation function is negligible. In the hydrodynamic regime however, the relaxation occurs over greater distances compared to the particle separation. In this situation, $(\alpha_1^s)^{HS}$ is equal to −2.68 [8] and is in accordance with the measured value −4.8. This is consistent with our finding that droplets interact via hard sphere interaction with perturbation of repulsive potential.

The origin of this repulsive interaction is thought to be due to the loss of entropy when adsorbed hydrated chains of nonionic polyether surfactants intermingle on close approach of two similar droplets. Deviation of the entropy from its equilibrium maximum value creates forces whose effects are every bit as real as those arising from the gradient of a potential. Therefore, the stability of water-in-oil MEs or o/w MEs, stabilized by adsorbed nonionic surfactants where the electrical considerations are often of secondary importance, can be explained by the steric and hydrational forces. Hydration force, which is essentially responsible for observed repulsive interactions, may arise because of the breaking of hydrogen bonds due to removal of water molecules from overlapping volume [32]. Observed repulsive energy barrier prevents the droplets from coming close enough and ensure the identity of an ME. This is consistent with observed long time stability (more than 3 years) of our MEs.

A value of size polydispersity index $\sigma_s = \sqrt{\frac{\overline{R^2}}{\overline{R}^2} - 1}$ can be obtained from relative amplitude of the slow decay mode using theoretical results [33], which are valid for narrow size distribution. Fig. 5 shows the relative amplitude of the slow decay mode as a function of the volume fraction ϕ. In low concentration region, the difference between D_c and D_s is expected to be small due their opposite ϕ dependence. Therefore, resolution in the low volume fraction region of the measured correlation function by a sum of two exponents will be difficult. In Fig. 5, large statistical error bars over low volume fraction region represent this fact. We obtained a value σ_s=0.22, a result

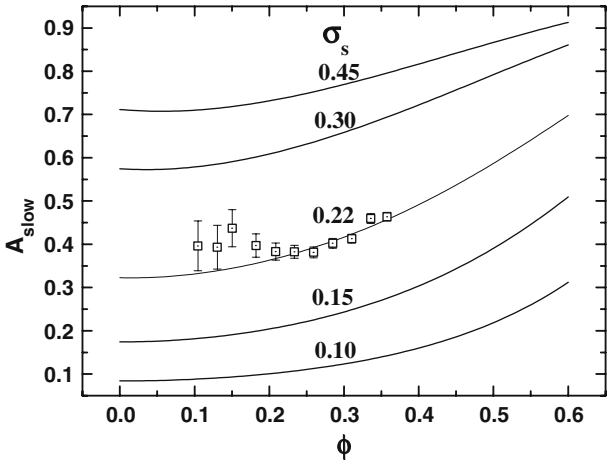


Fig. 5 Relative amplitude of slow relaxation caused by polydispersity fluctuations plotted as a function of volume fraction for water/PG-Tagat O2/Poloxamer 331-Eutanol G microemulsions at temperature 25°C. The *solid lines* are the predictions from [33] for different size polydispersity

which is close to the value of 0.21 deducted by approximate analysis of the SANS and in agreement with the values expected theoretically [34] for stable MEs.

In interpreting the above results, it is useful to distinguish spatial- and collision-induced concentration or polydispersity fluctuations. The observation of the particle self-diffusion implies that polydispersity is dynamic in origin and characterized by polydispersity fluctuation time $\tau_I > 10^{-4}$ s [5]. Therefore, this indicates that the polydispersity fluctuations is at least three orders of magnitude slower than the particle collision frequency.

Conclusions

It is clear that the interpretation of scattering experiments on concentrated MEs is by no means simple. In our measurements, two diffusive relaxation modes in concentrated MEs are observed. Fast mode may be associated with droplet number density fluctuations and slow modes with polydispersity fluctuations. The mean hydrodynamic radius (~10.5 nm) is obtained precisely from collective and self-diffusion coefficients extrapolated to infinite dilution. The concentration dependence of collective diffusion reflects the effect of hard sphere and the supplementary repulsive interactions. The origin of these repulsive interactions is thought to be because adsorbed hydrated chains of nonionic polyether surfactants allow penetration of the hydrated chains of the other droplets during collisions. Observed repulsive force should arise, because of the breaking of hydrogen bonds due to removal of water molecules from overlapping volume. This mechanism could also explain the observed stability of our systems. The polydispersity in ME systems is dynamic in origin. It is

characterized by a local fluctuation time $\tau_I > 10^{-4}$ s. From the amplitudes of the two exponentials, the estimated extent of size polydispersity is 0.22.

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