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International Journal of Pharmaceutics 312 (2006) 187–195
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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Pharmaceutical Nanotechnology

Light scattering measurements on microemulsions: Estimation of droplet sizes

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Received 14 November 2005; received in revised form 14 November 2005; accepted 11 January 2006
Available online 24 February 2006

Abstract

Different scattering methods were used as tools to assess the size of droplets in highly diluted microemulsions. These were obtained after dilution of a self-emulsifying system made up of an oil, a surfactant and ethanol. Typical methods, often used in size and shape determination of particles, such as SAXS and USAXS suffer in the present case from a lack of electrondensity contrast. It becomes clear from our extensive use of dynamic light scattering that one should be careful in interpreting the latter data as well. Sample preparation and the subsequent handling of the samples during the experiments strongly affect reproducibility of the results. There is a need for well-defined protocols at the level of sample preparation and data handling. In the present research one uses extensively dynamic light scattering (DLS) in the back scattering mode and strengths and pitfalls, inherent to the backscattering technique, are discussed. It is crucial to be aware of droplet size distributions (monomodal/bimodal/multimodal) while reporting mean radii (R_h) as this radius is only relevant in the case of well-defined monomodal distributions. Moreover, one should asses the shape of the droplets prior to data interpretation, as usual in scattering methods, by an independent method. Anyway the shape of the time correlation functions of the scattered intensity should be reported or at least inspected as they provide information on the reproducibility of the experiments hence safegarding the value of the physical meaning of the final value of droplet size (R_h). Preferentially static light scattering (SLS) measurements should always support DLS experiments as the angular dependence is very sensitive to the presence of large particles.

Keywords: Self-emulsifying systems; Microemulsion; Dynamic light scattering; Droplet radius determination

1. Introduction

An increasing number of recently discovered drug substances exhibit poor water solubility and hence low absorption after oral administration. Technology Catalysts International reported in 2002 that approximately 35–40% of all new chemical compounds suffer from poor aqueous solubility.

To overcome this problem, modification of the drug's physicochemical properties, such as salt formation and particle size reduction may be an approach to improve the dissolution rate of the drug. Unfortunately, these methods have their limitations and various other formulation strategies have been developed including the use of cyclodextrins and solid dispersions. In a number of cases the latter strategy appeared to have been successful (Liu, 2000).

For drug substances that exhibit sufficient lipophilic properties, it will be beneficial to dose them in a predissolved state (e.g. formulation of a lipid solution, lipid emulsion, microemulsion, self-emulsifying drug delivery system (SEDDS) or self-microemulsifying drug delivery system (SMEDDS)) (Charman et al., 1992; Humberstone and Charman, 1997; Craig, 1993; Porter et al., 2004), thereby reducing the energy associated with a solid–liquid phase transition. The application of self-emulsifying systems has gained considerable interest after the commercial success of lipid-based formulations of cyclosporine A (originally marketed as 'Sandimmune™' and now as the improved product 'Neoral Sandimmun™') (Uede et al., 1984; Grevel et al., 1986; Tarr and Yalkowsky, 1989) and two HIV protease inhibitors, saquinavir ('Fortovase™') and ritonavir (Norvir™).

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doi:10.1016/j.ijpharm.2006.01.037

SMEDDS are isotropic mixtures made up of oil, surfactant and sometimes cosurfactant or cosolvent. In an aqueous environment a homogeneous, transparent (or at least translucent), isotropic and thermodynamically stable dispersion will result, the formation of which is improved by gentle agitation, in vivo provided by gastrointestinal motility (Greiner and Evans, 1990; Shah et al., 1994; Constantinides, 1995). The large amount of small droplets (submicrometer size) leads to a considerable increase of surface area and hence improved absorption. It has been found that the microemulsion structure plays an important role in the rate of drug release (Armstrong and James, 1980; Trotta, 1999; Kossena et al., 2004; Podlogar et al., 2004). Furthermore, these formulations can be used to protect peptides and are known to reduce inter- and intra-individual variations in biological availability. The emulsions formed can be of three types: water in oil (W/O), oil in water (O/W) or bicontinuous. Upon dilution, the droplet structure can pass from a reversed spherical droplet to a reversed rod-shaped droplet, hexagonal phase, lamellar phase, cubic phase and various other structures until, after appropriate dilution, a spherical droplet will be formed again.

Although the formulation and preparation of these lipid systems is relatively simple, the physicochemical background is extremely complex and fundamental knowledge of these systems is still very limited. It has been demonstrated that the influence of the properties and structure of the different components (oil–surfactant–drug) on the emulsification and phase behaviour is a practically unknown territory. Hence, formulating new systems is still done by trial and error.

In order to clarify the current situation and to gain some insight in the physicochemical principles underlying the formation of SMEDDS, characterisation of the influence of every component on the size of the oil droplets in the microemulsion is necessary, as this size has an impact on the bioavailability of the incorporated drug (Tarr and Yalkowsky, 1989). Dynamic light scattering (DLS or photon correlation spectroscopy, PCS) has been described as an appropriate method for measuring droplet size in microemulsions (Craig et al., 1995; Constantinides and Scalart, 1997; Khoo et al., 1998; Porter et al., 2004). Screening methods for the formulation of SMEDDS are often solely based on such measurements. Although this experimental approach provides a good indication of the droplet size in the case of a dilute monodisperse solution, interpretation of the results becomes more complex in a concentrated or polydisperse system. For concentrated systems, two distinct issues are involved: the possible physical interaction between particles, which of course has an effect on the DLS results, and purely theoretical aspects regarding scattering behaviour of concentrated systems (such as multiple scattering, etc.). The combination of these two factors makes it difficult to obtain reliable data using light scattering. Furthermore, other limitations of this scattering method, for instance, the use of the cumulant analysis for very polydisperse systems or potential errors inherent to scattering measurements of microemulsions, might affect the final results, thus resulting in unreliable mean radii.

The present research focuses on the droplet size analysis in microemulsions, prepared after dilution of a SMEDSS, more precisely on the factors to be considered when carrying out and reporting DLS experiments on microemulsions. The influence of various preparation protocols, as observed by DLS in the backscattering mode, are analyzed and DLS results will be compared not only with SLS experiments, but also with DLS results obtained at much smaller angles.

2. Materials and methods

2.1. Materials

Table 1 lists the oils and surfactants used in this study. Ethanol p.a. was used as the cosolvent.

2.2. Methods

2.2.1. Sample preparation

Visual inspection and particle size determination by light scattering techniques were carried out in series of 2%, 1%, 0.5% and 0.25% (w/v) oil in water microemulsions. Two different preparation methods are used in this study. Most samples were prepared at room temperature by addition of water to a specific amount of an oil–surfactant–ethanol mixture blended for 5 min with a magnetic stirrer. After dilution, the microemulsion was additionally stirred for 1 min (“immediate preparation method”). A number of formulations that are discussed in the second paragraph of Section 3, were prepared by dilution of a concentrated 2% stock solution prepared as described above. The resultant diluted microemulsion was subsequently stirred for 1 min with a magnetic stirrer (“stock solution method”). Water used to dilute the SMEDDS was filtrated using 0.45 μm pore size filters

Table 1
Oils and surfactants used in this study

Trade name	Supplier	Compound
Miglyol 812	SASOL (Witten, Germany)	Capric triglyceride
Imwitor 642	SASOL (Witten, Germany)	Glyceryl hexanoate
Capmul MCM C8	ABITEC CORP (Janesville, WI)	Glyceryl monocaprylate
Imwitor 308	SASOL (Witten, Germany)	Glyceryl monocaprylate
Tween 80	VWR INT (Leuven, Belgium)	Polyoxyethylene-20-Sorbitan monooleate
Cremophor RH40	BASF (Ludwigshaven, Germany)	Polyoxyl 40 hydrogenated castor oil
Simulsol 1285	SEPPIC (Paris, France)	Polyoxyl 60 castor oil
Simulsol 1292	SEPPIC (Paris, France)	Polyoxyl 25 hydrogenated castor oil
Simulsol 4000	SEPPIC (Paris, France)	Polyoxyl 40 hydrogenated castor oil

(Millipore). Light scattering analysis was performed within 4 h after preparation. Samples for SESANS analysis were for reasons of contrast prepared with deuterated water.

2.2.2. Dynamic light scattering

The theory of dynamic light scattering, also called photon correlation spectroscopy, is well known and will not be discussed in this article. Briefly, the original measurement is a time correlation function of the scattered intensity. The decrease of this correlation function with displacement time (called "lag time") can be used to extract information about the diffusion coefficient of a particle or droplet in solution. The measured diffusion coefficient can be used to calculate a hydrodynamic radius (R_h) of the droplet using the Stokes–Einstein equation:

$$R_h = \frac{kT}{6\pi\eta D}$$

where k is the Boltzmann constant, T the absolute temperature, η the viscosity of the continuous phase and D is the diffusion coefficient.

The data are first analysed by cumulant analysis to obtain an average diffusion coefficient and subsequently by CONTIN analysis in order to obtain information about the entire distribution of the particle size (monomodal or multimodal) (Koppel, 1972; Provencher, 1979). Two sets of data are obtained: one measurement was carried out at least 4 h after preparation of the sample, while a second one was performed after 72 h. This second measurement is intended to provide information on the long-term stability of the microemulsion.

2.2.2.1. Backscattering (bs) dynamic light scattering spectrometer. Light scattering measurements were performed using an ALV-NIBS High Performance Particle Sizer (ALV, Langen, Germany) equipped with a He–Ne laser with approximately 3 mW output power at 632.8 nm, a digital correlator (ALV-5000/E Multiple Tau Digital correlator) and a single photon detector module (PMT). Detection was carried out in a backscattering mode (scattering angle 173°). Viscosity of the solutions was set at 0.89 mPa s, temperature at 25 °C.

2.2.2.2. Multi-angle light scattering spectrometer. Multi-angle light scattering experiments were performed on a CGS-3 spectrometer (Malvern Instruments, Worcestershire, UK) equipped with a goniometer, a uniphase 22 mW He–Ne laser operating at 632.8 nm, an avalanche photodiode and detector and an ALV-5000/EPP multi-angle tau correlator. The CGS-3 has an angular range of 12–152° and temperature can be controlled using an external water bath circulation.

2.2.3. Static light scattering

Static light scattering measurements were performed on an Amtec MM 1000 photometer (Amtec, Nice, France) equipped with a Spectra-Physics Stabilite, model 124B, He–Ne laser operating at 632.8 nm (Spectra-Physics, Darmstadt, Germany) (Slootmaekers et al., 1988; Cuppo et al., 2002). For all measurements, cylindrical cells were used, immersed in a toluene-index matched container. Intensities were recorded at different angles: 30°, 40°, 50°, 60°, 70°, 80°, 90°, 100°, 110°, 120°, 130°, 140° and 150° and normalised to $I_{\text{toluene},\theta=90^\circ}$.

2.2.4. Spin-echo small-angle neutron scattering (SESANS)

The SESANS method is based on the Larmor precession of polarised neutrons in magnetic fields with inclined faces, which essentially encodes the neutron trajectory. The measured polarisation is a Fourier transform of the scattering cross-section and thus closely related to the scattering length density correlation function, which facilitates data interpretation (Krouglov et al., 2003). The sensitivity can be tuned by varying the applied magnetic field, the wavelength, the length of the set-up and the tilt angle of the interfaces. All these parameters can be combined into a single parameter, the spin-echo length, z, which is both an experimental scan parameter and the length cord between two scattering volumes in the sample. The technique is sensitive in the range between 10 nm and 10 μm.

The SESANS technique was used as previously reported (Bouwman et al., 2004). To summarize, a set of six pyrolytic graphite monochromators focuses a beam with a wavelength of 0.21 nm on the sample position. A set of supermirrors polarises the beam. A similar set at the end of the set-up acts as an analyser before the ^{3}He-detector. Two sets of slits, one after the polariser and one before the analyser, define the dimensions of the incoming and outgoing beam, respectively, to 8 mm high and 22 mm wide. The four electromagnets with the π-flip foils are positioned on an aluminium table to avoid disturbance of the surroundings of the neutron path. The sign of the magnetic field changes from the second to third magnet and for this purpose a field stepper is installed. The samples are mounted on a sample changer on a translation stage that moves the samples between the third and fourth magnets that are separated over a distance of 1.34 m. The magnetic fields are set and controlled to values between 0.5 and 100 mT. The basic component in the set-up that creates the triangular shaped precession regions are 3 μm thick perm alloy films deposited on silicon wafers, positioned with an angle of 5.5° to the central axis of the neutron beam in the centre of the rectangular poles of electromagnets.

3. Results and discussion

Self-emulsifying systems consist of a mixture of at least an oil and a surfactant, which spontaneously form an emulsion when water is added. In this paper, we will focus on SMEDDS that result in O/W microemulsions upon dilution with water. Numerous combinations of the oils and surfactants, as listed in Table 1, were formulated and droplet sizes investigated with DLS. In order to use the previously stated CONTIN and cumulant analysis, the latter of which is often used to report mean R_h values in microemulsions, one assumes the droplets to be spherical.

Therefore, it is recommended to check the droplet shape, e.g. by an independent method such as SAXS, SANS or spin-echo small-angle neutron scattering. SESANS uses the usual principles of neutron spin echo to encode the scattering angle of the neutron irrespective of the collimation of the neutron beam

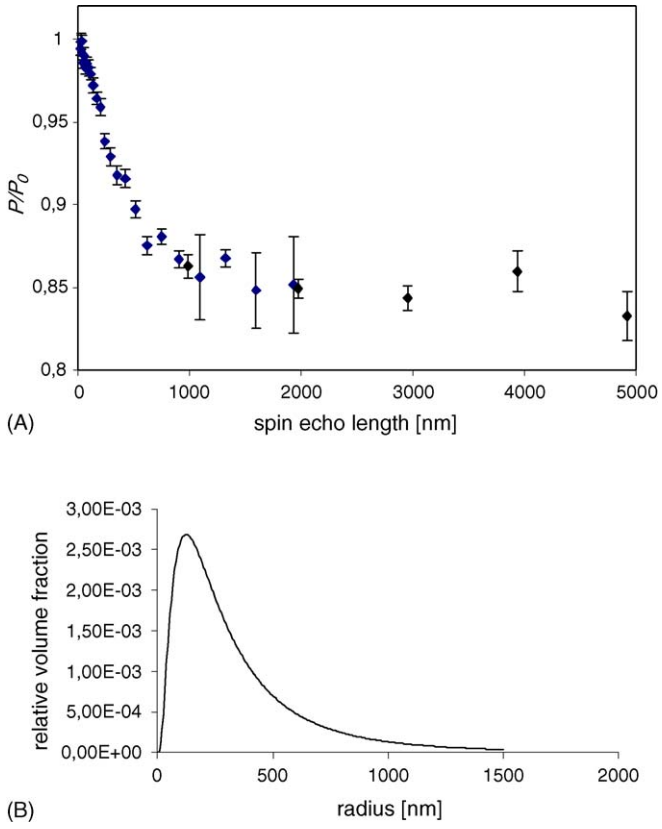

Fig. 1. (A) SESANS results of a 10% aqueous dilution of a formulation consisting of 15% Imwitor 642, 30% Tween 80 and 55% ethanol. The measured polarisation is plotted as a function of the spin-echo length. The drawn line represents a fit to the data with a log-normal distribution of solid spheres which is also represented in (B) as a distribution function of the droplet sizes.

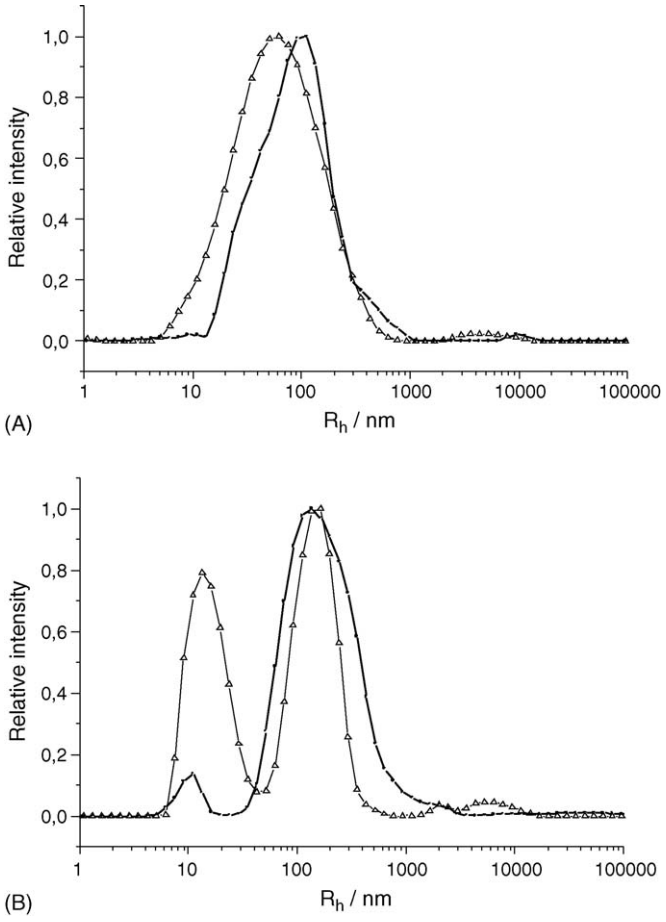

Fig. 2. Distribution functions of two formulations, prepared by dilution of a concentrated stock solution ("stock solution method", △) or by immediate addition of the correct amount of water ("immediate preparation method", ●) and measured using a backscattering DLS method (detection angle = 173°). The microemulsion consists of 1 g of the following combination: 30% Imwitor 642, 61.5% Tween 80 and 8.5% ethanol, in 100 ml solution (A, microemulsion A) or 200 ml solution (B, microemulsion B).

as explained in Section 2. An example of the SESANS method on a fairly concentrated system (10%), together with a plot of the droplet size distribution is shown in Fig. 1. Remind that under such conditions different shapes can be simultaneously present. The SESANS correlation function can be well described by a log-normal distribution of solid spheres with as a radius 255 ± 21 nm and a standard deviation of 0.85 ± 0.11. SESANS cannot discriminate between extreme shapes and polydispersity (Uca et al., 2003), thus the value for the width of the distribution is an upper limit.

3.1. Preparation and experiment variations

As there is at present no standard procedure, different preparation methods of SMEDDS are commonly used leading to variances in the structures – size and shape – of the resulting microemulsions. Changing the preparation method, including differences in shear force and temperature, can largely influence the droplet size, shape and stability of the droplets (Salager et al., 1996).

Fig. 2 represents distribution functions of the hydrodynamic radius of two microemulsions A and B of different dilution containing the same components, but prepared in two different ways: the first preparation (full circle, ●) is carried out by addition of water to a combination of oil and surfactant, after mixing this combination for 5 min with a magnetic stirrer. The second microemulsion (open triangle, Δ) was made by dilution of a concentrated 2% stock solution prepared under the same conditions as the first sample. DLS (bs) results show a difference in particle size distribution for both samples making clear that sample preparation plays an important role in the size determination of the droplets.

Not only differences in preparation methodology will cause droplet sizes to differ, also a given preparation procedure will not always result in the same distribution as is illustrated in Fig. 3. Based on DLS analysis of the pure surfactant (Fig. 4), the first peak can most likely be attributed to predominantly pure surfactant micelles. The observed bimodality of samples A and B is an important issue as to the determination and the physical meaning represented by a single R_h value. Consequently, it seems obsolete to obtain a mean R_h by using a cumulant analysis in the case of systems showing multimodal behaviour. Unfortunately, results reported in the literature often neglect to pinpoint the nature of the droplet size distribution.

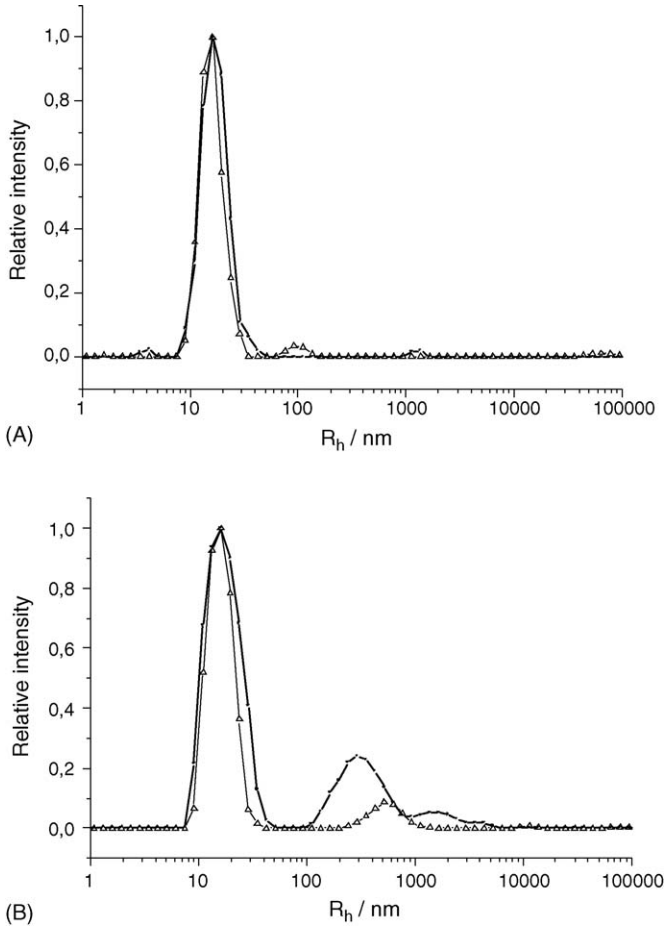

Fig. 3. Distribution functions of two formulations prepared two times under the same conditions ("immediate preparation method") and measured using a backscattering DLS method (detection angle = 173°). The first formulation (A) is a 2% aqueous dilution of a combination of 20% Imwitor 642, 70% Simulsol 1285 and 10% ethanol. The second formulation (B) is a 0.25% aqueous dilution of a combination of 20% Imwitor 642, 70% Simulsol 4000 and 10% ethanol.

The reproducibility of the DLS (bs) measurements can easily be checked by multiple repetitions on the same sample. Fig. 5 shows the variation in the detection of droplet sizes of larger particles.

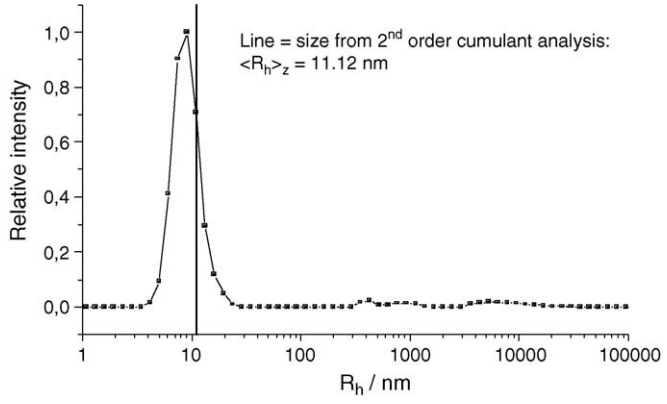

Fig. 4. Backscattering DLS result (detection angle = 173°) of a 1% aqueous dilution of a combination of 80% Tween 80 and 20% ethanol.

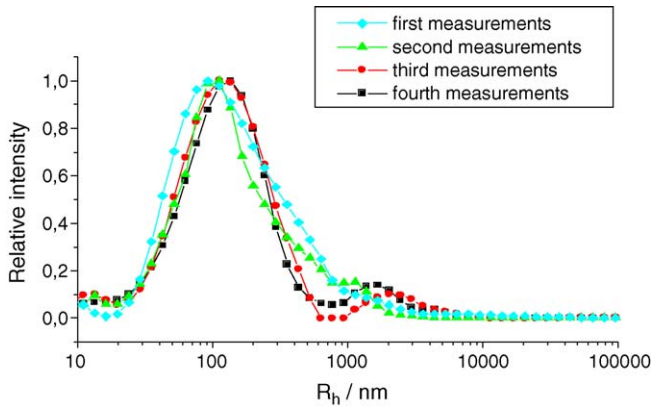

Fig. 5. DLS results of a 0.5% aqueous dilution of a combination of 30% Imwitor 642, 61.5% Tween 80 and 8.5% ethanol. Measurements were performed using a backscattering DLS method (detection angle = 173°) and carried out immediately one after the other, without touching the cuvette.

Measurements of a sample using a multi-angle DLS at a detection angle of 30°, where the measurement is more sensitive for large particles better reflects the distribution of the larger particles compared to backscattering DLS of the same sample

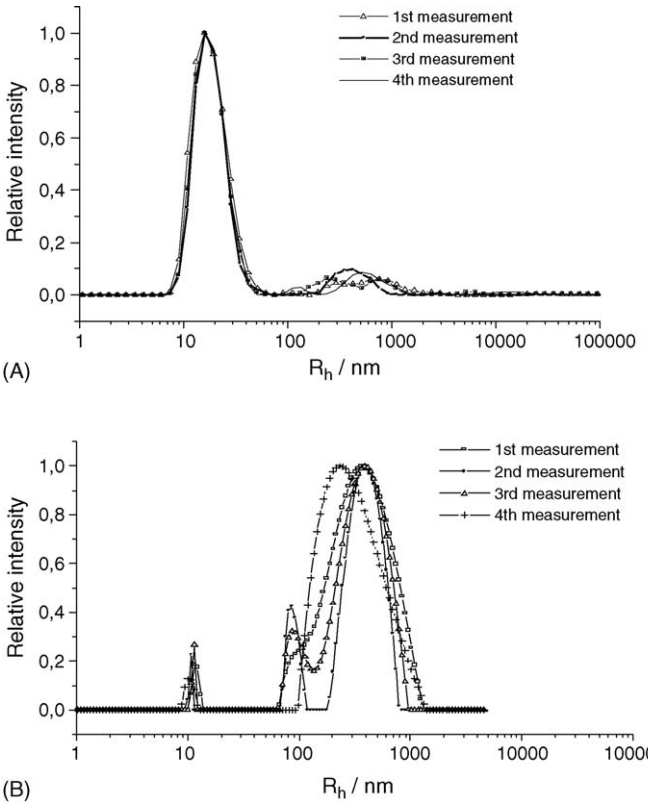

Fig. 6. (A) Measurements of a 0.25% aqueous solution of a combination of 15% Capmul MCM C8, 60% Simulsol 1292 and 25% ethanol. Measurements were performed using a backscattering DLS method (detection angle = 173°) and carried out immediately one after the other, without touching the cuvette, 6 days after preparation of the sample. (B) Measurements of a 0.25% aqueous solution of a combination of 15% Capmul MCM C8, 60% Simulsol 1292 and 25% ethanol. Measurements were performed using multi-angle DLS at a detection angle of 30° and carried out immediately one after the other, without touching the cuvette, 6 days after preparation of the sample.

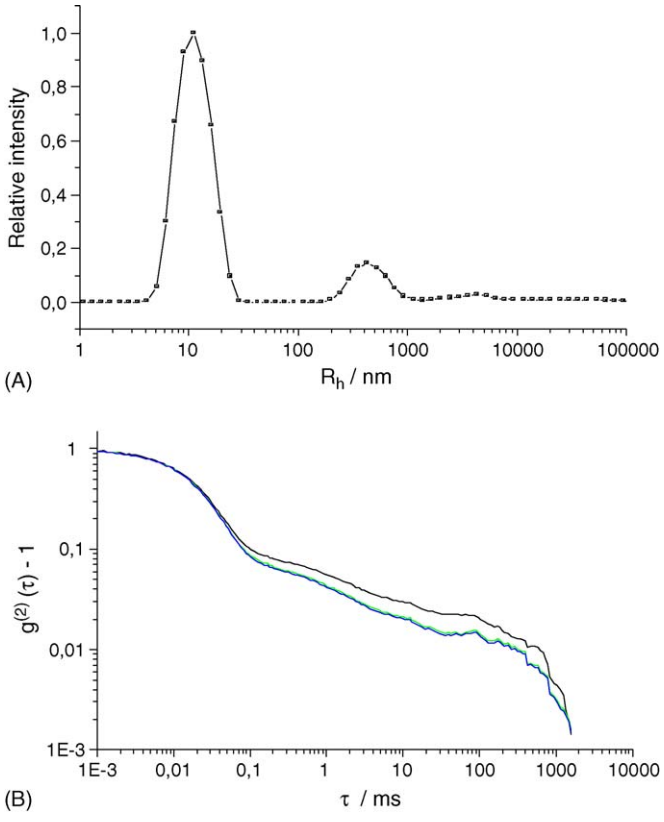

Fig. 7. Distribution function (A) and autocorrelation function (B) of a 0.25% aqueous dilution of a combination of 20% Imwitor 642, 70% Tween 80 and 10% ethanol that was measured using a backscattering DLS method at a detection angle of 173°. The sample was prepared by dilution of a concentrated 2% solution.

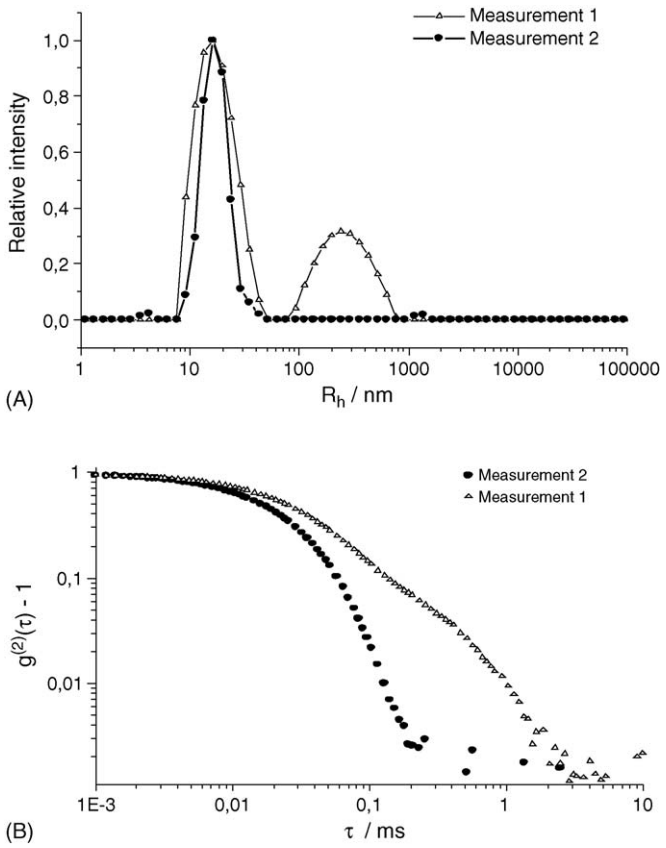

Fig. 8. Distribution functions (A) and autocorrelation functions (B) of two measurements of two identical samples, made under equal conditions and measured using a backscattering DLS method at a detection angle of 173°. The samples are 2% aqueous solutions of a mixture of 20% Imwitor 642, 70% Simulsol 1285 and 10% ethanol.

(Fig. 6A and B). Consequently, one should always specify the angle of detection when DLS analyses of microemulsions are reported.

3.2. Interpretation of backscattering experiments

If problematic variations in DLS results occur, than it is worthwhile to inspect the correlation functions more closely. In the present case we recorded for each measurement, five autocorrelation functions. In order to obtain a reliable distribution function, the latter should all be similar. Fig. 7 describes the distribution function and autocorrelation functions of a typical scattering measurement. There is only one deviating correlation function present, described by the upper curve. The lowest line represents four quasi-identical correlation functions that nicely superimpose. The deviating correlation function is most probably due to a dust particle in the laser beam. One has to realize that the quality of the correlation functions determines the accuracy of the final result for R_h. In any way, it is advisable that correlation functions and distribution curves are inspected rather than just listing mean values of hydrodynamic radii.

Fig. 8 introduces a more complex phenomenon. The microemulsion was prepared twice using exactly the same experimental conditions. There was no aberrant correlation function in either measurement (each line represents five data sets obtained on the same sample), but the correlation functions of the two samples are quite different. This means that the measurements were as precise as possible and that bigger droplets are present in the first measurement, as can be seen in the distribution function.

A factor, often neglected during interpretation of DLS results, originates from the fact that a distribution function displays "relative intensity" data. A possible consequence is the apparent absence of peaks representing larger droplets in the distribution curve. Fig. 9 shows static light scattering (SLS) results of three microemulsions along with two SLS results of an aqueous Tween 80 solution. The intensity increase at the lower angles, points for the two micellar samples to the presence of droplets with a much larger diameter. A computer simulation of these micelles (using RASWIN software) (www.openrasmol.org) describes a Tween 80 micelle to have a R_h of approximately 10 nm. The observed large aggregates appear to form spontaneously and rapidly since also SLS results of a sample, previously filtered through a Millipore filter (pore size 0.22 μm) using a peristaltic pump in a closed circuit, indicate this angular dependence. Not only the surfactant solutions, but also all three microemulsions strongly

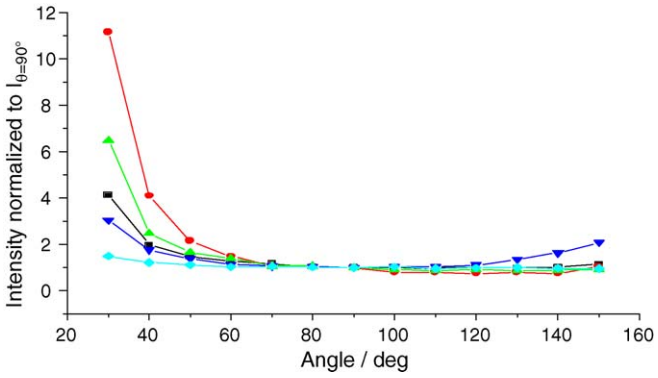

Fig. 9. SLS results of three different aqueous dilutions of a mixture of 20% Imwitor 642, 70% Tween 80 and 10% ethanol: 2% (■), 0.5% (●) and 0.25% (▲) dilution. (▼) An 1% aqueous solution of a combination of 80% Tween 80 and 20% ethanol, while (♦) describes the same combination in a 0.02% dilution after filtration of the microemulsion.

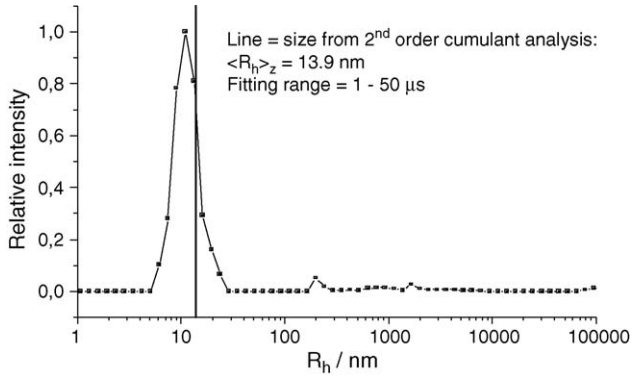

Fig. 10. Backscattering DLS result (detection angle = 173°) of a 1% aqueous dilution of a mixture of 20% Imwitor 642, 70% Tween 80 and 10% ethanol.

scatter in an angular dependent mode at the lowest angles available in the SLS instrument and therefore point to the presence of even larger droplets. Because one is probably out of the sensitivity range of the instrument, neither DLS results of the same 1% microemulsion (Fig. 10), nor scattering results of the micellar samples (Fig. 4) show the presence of these larger droplets; the baseline oscillations reported in Figs. 4 and 9 are still a matter of debate. However, these droplets or aggregates are definitely present, as detected by SLS. Most probably the micellar peak obscures the second microemulsion peak, because the scattering capacity of the micelles is too high. To verify this statement, DLS tests were performed on different microemulsions similar to the previous formulation, but with a different amount of surfactant (Fig. 11). As the concentration of Tween 80 is reduced, the microemulsion will contain less micelles, the population of these micelles will consequently decrease. As this intensity is expressed relatively to the light scattered by the oil droplets, the height of the second oil droplet peak will increase and a more reliable estimate of the oil droplet size is possible. Therefore, if DLS does not detect any larger droplets, it is recommended to accompany these measurements with SLS experiments, to verify the absence of larger oil droplets. If there is in fact an angular dependence at low angles, surfactant concentration should be lowered until DLS results reveal a clear vision of the oil droplet peak. One has to keep in mind that the minimum surfactant concentration is reached when the oil droplet peak changes its position on the *X*-axis, as in that case the maximum emulsification of the oil phase has not been reached yet.

One could also have a better estimate of the sizes of the larger oil droplets by measuring scattered light at a smaller angle, as in

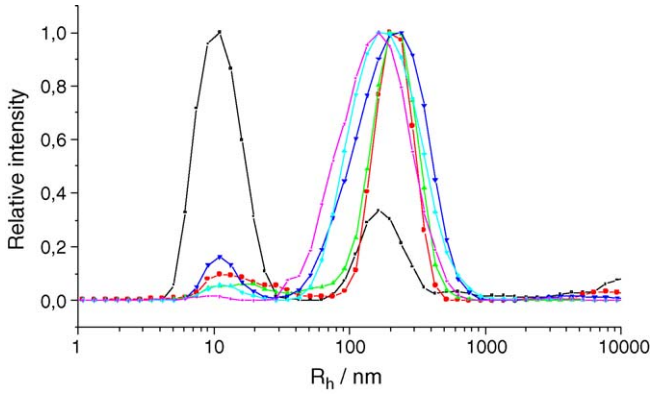

Fig. 11. Backscattering DLS result (detection angle = 173°) of 0.25% aqueous dilutions of a formulation of Imwitor 642, Tween 80 and ethanol. Different combinations are shown: (■) a combination of 20%, 63% and 17%; (●) a combination of 20%, 53% and 27%; (▲) a combination of 20%, 48% and 32%; (▼) a combination of 20%, 45% and 35%; (♦) a combination of 20%, 40% and 40%; (+) a combination of 20%, 30% and 50% of Imwitor 642, Tween 80 and ethanol, respectively.

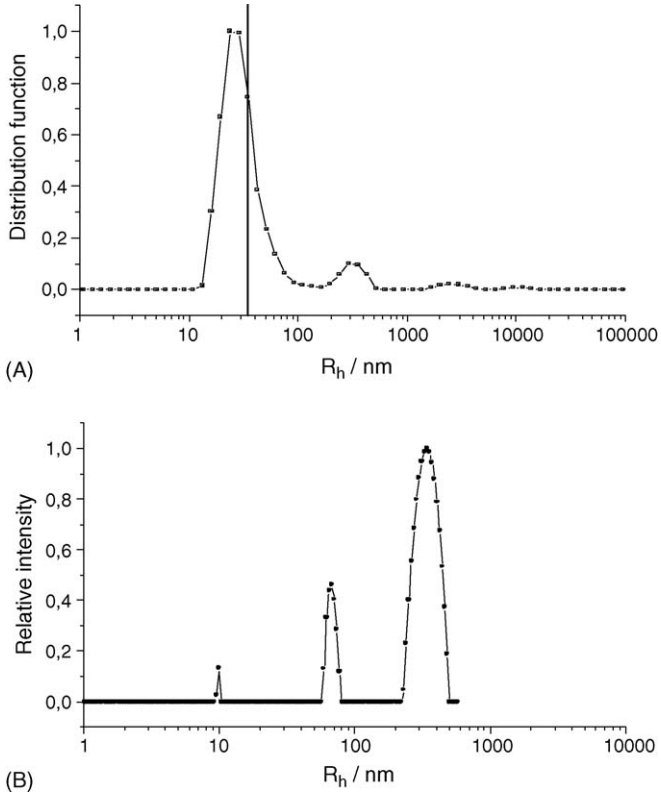

Fig. 12. Comparison of DLS experiments using the method in a backscattering mode (detection angle = 173°) (A) and in a multi-angle mode with detection at an angle of 30° (B) of a 0.5% aqueous dilution of a combination of 19% Miglyol 812, 61% Cremophor RH40 and 20% ethanol.

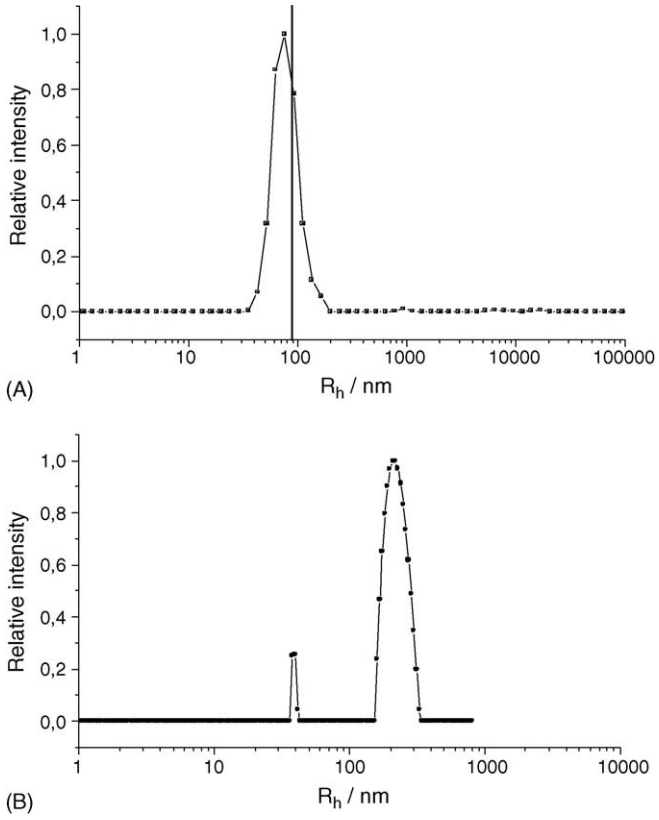

Fig. 13. Comparison of DLS experiments using the method in a backscattering mode (detection angle = 173°) (A) and in a multi-angle mode with detection at an angle of 30° (B) of a 0.5% aqueous dilution of a combination of 33% Imwitor 308, 6% Simulsol 1285 and 61% ethanol.

that case one focusses more on the detection of larger particles. Figs. 12 and 13 compare distribution functions as determined by DLS in a backscattering mode and by multi-angle DLS at a detection angle of 30°. This detection at a lower angle seems to greatly enhance resolution: while in Fig. 12 the first method shows a broad oil droplet peak, detection at 30° seems to distinguish different size populations within this peak. The increased resolution together with the previously stated reduction of method variations, confirms that one should always select the optimal detection angle for the particle sizes that are to be analysed with a DLS method. This detection angle should in any way be specified when DLS results of microemulsions are reported to correctly interpret scattering results.

4. Conclusion

Light scattering measurements are often routinely used to determine droplet sizes in microemulsions (R_h as measured by dynamic light scattering). It is shown in the present research that one has to be very careful as to the reproducibility of the preparation procedures and the scattering methods used. Awareness of the shape of the droplets prior to analysis of the scattering data (e.g. with SANS or SESANS) is necessary because commercial instruments assume spherical shapes. Mean values should always be interpreted with caution; it is crucial to check whether or not the R_h values result from monomodal distributions, multimodal distributions, or strongly polydisperse systems. In the case of uncertainties or irregularities, the reproducibility of the full correlation functions needs to be checked. Variations, inherent to the DLS method itself, can be reduced by using a multi-angle DLS instrument and by selection of the detection angle appropriate for the droplet sizes under consideration. Hence, it is essential to report all experimental details proper to the DLS measurements (type of instrument, angle of detection, monomodality or multimodality of the droplet size distribution functions, reproducibility of correlation functions).

It is recommended to parallel DLS measurements with static light scattering observations as occasionally the micelles scatter so strongly that the oil droplet peak becomes obscured in the distribution function. Failure to observe the oil droplet peaks in a backscattering procedure is due to the fact that distribution curves show only relative intensities.

When the presence of larger oil droplets is detected by SLS, but not by DLS, surfactant concentrations should be lowered in order to reduce the amount of micelles and subsequently the micellar scattering. Another way to avoid the obscurance of the larger droplets, is to use multi-angle DLS. By lowering the detection angle, it is possible to focus on larger droplets, regardless the amount of micelles present.

One has to be aware that micellar aggregates can be mistaken for larger oil droplets as these aggregates form spontaneously and give rise to structures with diameters of several hundreds of nanometers. To distinguish such aggregates from oil droplets of similar diameter, USANS experiments may offer a solution.

Acknowledgements

The authors would like to thank Prof. J. Vermant and Prof. J. Martens for providing access to their light scattering facilities. J. Plomp is also gratefully acknowledged for his dedicated assistance during the SESANS experiments. H. Reynaers and F. Cuppo are indebted to BOF K.U. Leuven and FWO-Vlaanderen for a research grant (F.C.) and continuous support.

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