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Investigation of Pharmaceutical Oil/Water Microemulsions by Small-Angle Scattering

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Purpose. Stable oil/water (o/w) microemulsions are very effective vehicle systems for dermal administration of drugs having no or low skin penetration. These systems, consisting of oils, a blend of a high and a low HLB surfactant, and a hydrophilic phase (propylene glycol/water), were developed using pharmaceutically acceptable components.

Methods. In this paper, the droplet size of these microemulsions was characterized by means of dynamic light scattering (DLS) and small-angle neutron scattering (SANS). Furthermore, different size parameters obtained by DLS and SANS experiments were compared and discussed.

Results. Extremely small droplet radii of approximately 10 nm could be observed. A good agreement between the data of DLS and SANS experiments was found. The kind of oil only marginally influences the droplet size.

Conclusions. Particle size determination via scattering techniques is a useful tool to characterize droplets in microemulsions for dermal drug delivery.

KEY WORDS: dynamic light scattering; o/w microemulsions; small-angle neutron scattering.

INTRODUCTION

Microemulsions (MEs) were first described by Hoar and Schulman (1). They are homogeneous, transparent, thermodynamically stable dispersions of water and oil, stabilized by an interfacial film of surfactant, and frequently in combination with a cosurfactant (2). As a consequence of their potential advantages (high solubilization capacity for drugs, penetration enhancement, high stability, and ease of preparation), interest has rapidly grown in the use of microemulsions as pharmaceutical drug delivery systems (3). For selecting a suitable ME system for drug delivery, it is important to know the physicochemical properties of MEs such as drug solubility, area of microemulsion in the phase diagram, and the resulting size of the colloidal phase of the ME. Scattering techniques such as dynamic light scattering (DLS) as well as small-angle neutron scattering (SANS) have been used with microemulsions to characerize their droplet size.

A typical droplet model of the colloidal phase of ME for DLS and SANS is presented in Fig. 1. In the current model, the MEs are considered as *a priori* formed objects: the oil core and surfactant film form a single entity, immersed in a continuous aqueous phase. The geometrical parameters of the model are as follows. The MEs studied here are usually considered as made of an oil core radius, R_{core}. In the region up to R_{core}, the dark black part shows pure oil and the rest shows penetration of surfactant tail into the oil. The region from R_{core} to the position in the continuous aqueous phase where the scattering length density ρ has its maximum forms a propylene glycol/water penetrable shell of surfactants, L_{shell}. The total radius is thus $R_{\text{shell}} = R_{\text{core}} + L_{\text{shell}}$. Hydrodynamic radius, R_{h}, measured via DLS lies somewhere in-between the core radius and the end of the shell.

In the current paper, we report the characterization of droplet size of o/w MEs for dermal administration which have different kinds of pharmaceutical oils, using SANS and DLS to determine the effect of the nature of the pharmaceutical oils on the ME and to explain the uncertainty in droplet size parameters measured using only one experimental method and the advantage of using two or more.

MATERIALS AND METHODS

Chemicals

Tween 80, Tagat O2, Eutanol G, isopropyl palmitate (IPP), propylene glycol (PG), and oleic acid were purchased from Caesar & Loretz (Hilden, Germany). Poloxamer 331 was kindly provided by C. H. Erbsloeh (Krefeld, Germany). The chemical structures of these compounds are presented in Figs. 2–4. Water was used in bidistilled quality. For the SANS experiments, MEs were prepared using 99.9% deuterated water (D_2O; Sigma, Deisenhofen, Germany).

Sample Preparation and Experimental Set-Up of DLS Experiments

For the investigations o/w microemulsions, composed of a mixture of Tween 80 or Tagat O2 (HLB 15.0) and Poloxamer 331 (HLB 1.0) as emulsifying agents, pharmaceutical oils, and a PG/H_2O mixture, were used. The surfactant mixture and the external phase (PG/H_2O) were prepared in advance. The microemulsions were manufactured by simply stirring the components in a special relation (surfactant mixture, oil, hydrophilic phase) together. The composition of the microemulsions used is summarized in Table I.

Prior to the measurements, the samples were filtered through a 1.2-μm pore-size filter (Sartorius, Goettingen, Germany) into dust-clean sample cells.

The light-scattering hardware set-up consists of commercially available equipment for simultaneous static and dynamic experiments made by ALV-Laser Vertriebsgesellschaft mbH. (Langen, Germany). A green Nd:YAG DPSS-200 laser (532 nm) from Coherent (Auburn, California) with an output of 200 mW was used. The thermostated sample cell is placed on a motor-driven precision goniometer (±0.01°) which enables the photomultiplier detector to be moved from 20° to 150° scattering angle. The intensity time-correlation functions

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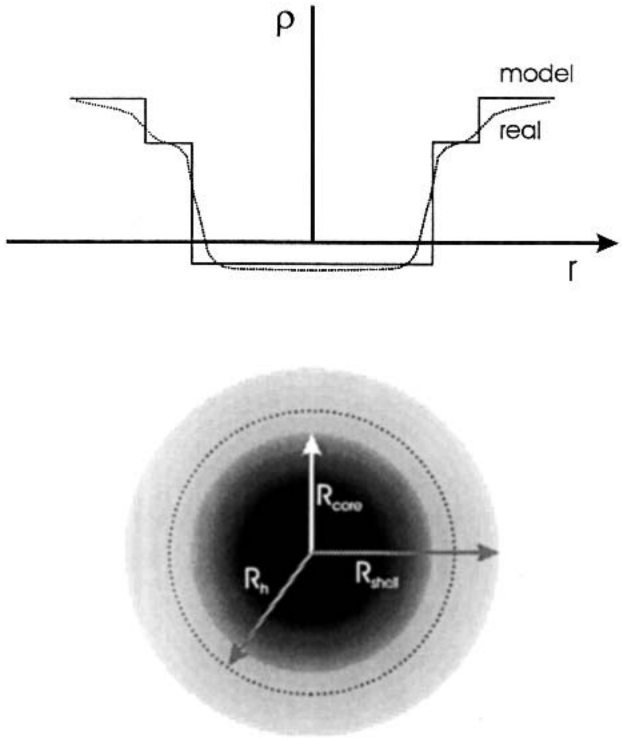


Fig. 1. Model of microemulsion droplet defining size parameters for DLS (R_h) and SANS (R_{core}, R_{shell}) calculations.

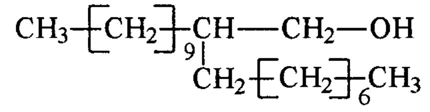
(ITCF) $g^2(\tau)$ are recorded with an ALV-5000E multiple tau digital correlator with fast option. The minimal sampling time of this correlator is 12.5 ns. The cylindrical sample cells are made of Suprasil quartz glass by Hellma (Muellheim, Germany) and have a diameter of 10 mm.

Sample Preparation and Experimental Set-Up of SANS Experiments

For preparing the ME for the SANS experiments, bidistilled water was exchanged to deuterated water. The SANS measurements were carried out at the small-angle scattering spectrometer YUMO at the Joint Institute for Nuclear Research (Dubna, Russia). The pulsed neutron source IBR-2 delivers a spectra of thermal neutrons and the momentum transfer is calculated by time-of-flight methods with an averaged resolution of ~0.10 ($\Delta q/q$). The flat sample cells are made of Suprasil quartz glass and have a path length of 2 mm.

Refractive Index

The refractive index of all samples was measured using an Abbé Refractometer (ABBEMAT, Dr. Kernchen GmbH, Seelze, Germany) at 25.0 ± 0.2°C.



$CH_3-[CH_2]_9-CH(CH_2[CH_2]_6CH_3)-CH_2-OH$

Fig. 2. Structure of Eutanol G (2-octyldodecanol).

Table I. Composition of the Microemulsions Studied by DLS

ME 1	
Tween 80/Poloxamer 331 (2:3)	20%
Oleic acid	5%
PG/H_2O (1:1)	75%
ME 2	
Tagat O2/Poloxamer 331 (2:3)	20%
IPP	5%
PG/H_2O (2:1)	75%
ME 3	
Tagat O2/Poloxamer 331 (2:3)	20%
Eutanol G	5%
PG/H_2O (2:1)	75%

Dynamic Viscosity

The viscosity of the external phase (PG/H_2O mixture) was determined at different shear rates (0.2–200 s^{-1}) using the Couette principle (RHEOMETRICS SCIENTIFIC, Bensheim, Germany) at 25.0°C. Newtonian behavior could be observed. These data are necessary to calculate the hydrodynamic radius (R_h) from the diffusion coefficient.

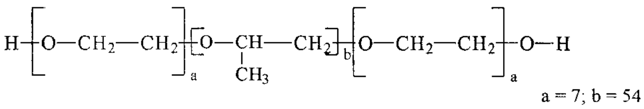
RESULTS AND DISCUSSION

Dynamic Light Scattering

The ITCF of each sample was investigated at different scattering angles between 70° and 100°. Correlation functions corresponding to one set of experimental parameters have been measured five times and the ITCFs used for fitting are averaged over these five measurements. The collective diffusion coefficient has been deduced from a biexponential fitting procedure from the normalized field autocorrelation function $g^1(\tau)$ using Eqs. (2)–(4) (see Appendix, below) and averaged over the angles. To get the free diffusion coefficients of droplets, Eq. (6) was used and the hydrodynamic radius was calculated from that using Eq. (5).

Small-Angle Neutron Scattering

The collected spectra of the three microemulsions were analyzed by fitting Eqs. (8)–(11) as well as using the Guinier approximation (Eqs. 12 and 13). Best fitting obtained using two methods of analysing the data is shown in Figs. 5–8. Assuming that the droplet core only consists of oil, the shell only of surfactants, and the surrounded medium only of PG/D_2O, the scattering length densities necessary for fitting the complete spectra were calculated to be $\rho_{\text{oleic acid}} = 0.235 \times 10^{-14}$ m^{-2}, $\rho_{IPP} = -0.727 \times 10^{-14}$ m^{-2}, $\rho_{\text{Eutanol G}} = -0.327 \times 10^{-14}$ m^{-2}, $\rho_{PG/D2O\ 1:1} = 3.226 \times 10^{-14}$ m^{-2}, $\rho_{PG/D2O\ 2:1} = 2.112 \times 10^{-14}$ m^{-2}, $\rho_{\text{Poloxamer/Tween80}} = 0.515 \times 10^{-14}$ m^{-2}, and $\rho_{\text{Poloxamer/Tagat O2}} = 0.468 \times 10^{-14}$ m^{-2}. An exchange of loose-bounded H and D atoms is incorporated. n_p, R_{core}, and R_{shell} as well as σ are independent fitting parameters. The Guinier region was analyzed by a linear function yielding the gyration radius according to Eq. (12).



$H-[O-CH_2-CH_2]_a-[O-CH(CH_3)-CH_2]_b-[O-CH_2-CH_2]_a-O-H$

$a = 7;\ b = 54$

Fig. 3. Structure of Poloxamer 331.

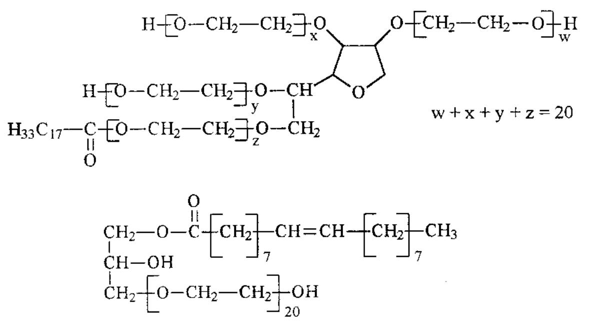


Fig. 4. Structures of Tween 80 (top) and Tagat O2 (bottom).

It is known that the particle size distribution is one of the most important characteristics of microemulsions for the evaluation of stability and penetration mechanism into the skin (4,5). DLS and SANS are proved to be an excellent technique for investigating droplet size of microemulsions yielding different size parameters. These parameters give a detailed overview of how the particle is composed.

DLS yields hydrodynamic radius R_h, which is supposed to consist of the oil core and a stronger-bounded surfactant film, perhaps containing some solvent molecules, too. R_h calculated for each samples from Eq. (5) are listed in Table II.

Very small particle sizes (~10 nm) were observed for our measured samples. With the exception of oleic acid, no remarkable difference is found between Eutanol G or IPP as oils. Probably, the angular structure of oleic acid or the different PG/D_2O ratio causes the greater radii. The fit of SANS data with the polydisperse core shell model yields two values for the radius, R_{core} and R_{shell} (Fig. 1). The first corresponds to the size of the oil droplet, the second to the distance between the center of the particle and a position in the surfactant film where the difference in scattering length density has its maximum. The results from the fit of SANS spectrum are summarized in Table II. From the SANS studies, no remarkable difference is observed between investigated oil except oleic acid. As expected, the outer radius R_{shell} incorporates the looser-bounded surfactant molecules and is substantially bigger than R_h. The outer diameter determines the smallest possible distance between two particles because of its strong influence on the structure factor.

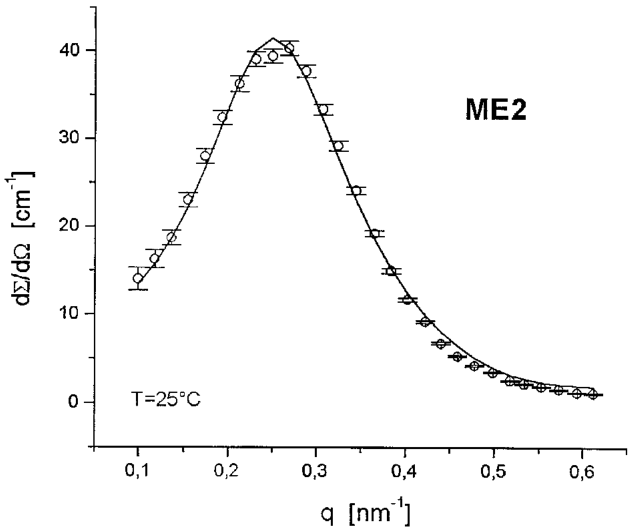


Fig. 6. SANS spectrum from ME 2 (points) and fitted curve (line) vs. *q*.

The hydrophilic groups of Tween 80, Tagat O2, and Poloxamer 331 are polyoxyethylene, formed by polymerization of ethylene oxide (EO). The length of the polyoxyethylene chain is 1.8 Å per monomer at a degree of polymerization of 20 to 40 monomers and 3.5 Å per monomer at a degree of polymerization less than 20 (6). Tagat O2, Tween 80, and Poloxamer 331 have 20, 20 monomers, (maximum possible when $w = 20$ and $x = y = z = 0$) and 7 monomers per molecule in larger chains, respectively. Thus, the length of the hydrophilic chain is 3.6 nm, 3.6 nm, and 2.45 nm for Tagat O2, Tween 80, and Poloxamer 331, respectively.

The shell thickness of loosely bound surfactant for our microemulsions were observed as:

ME 1: Shell thickness of loosely bound surfactant = $R_{shell} - R_{core} = 3.24$ nm

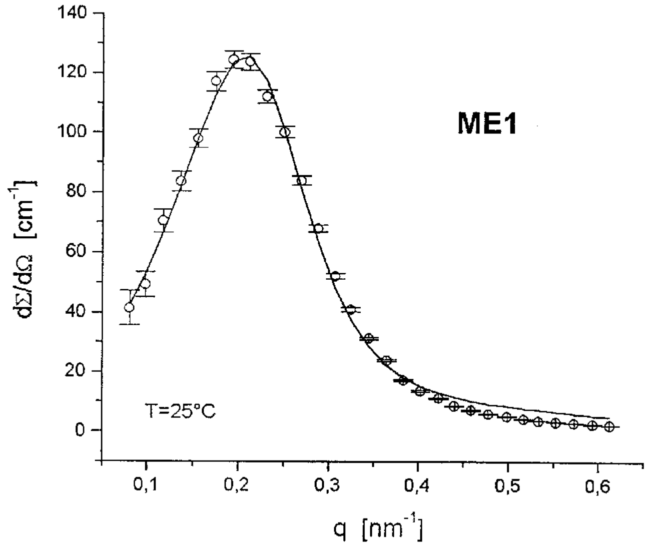


Fig. 5. SANS spectrum from ME 1 (points) and fitted curve (line) vs. *q*.

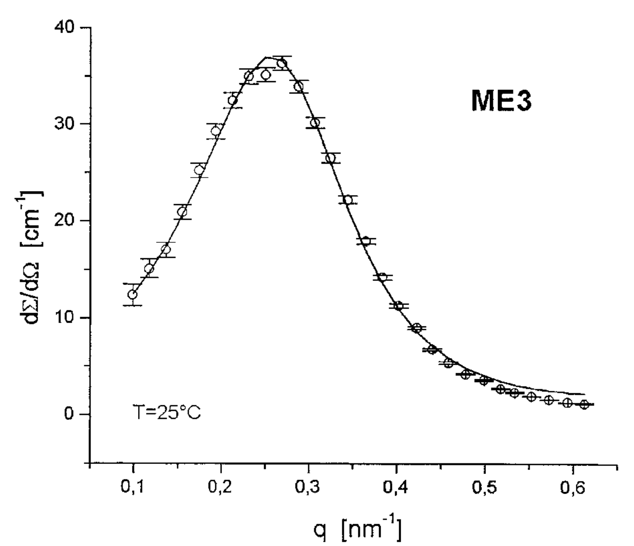


Fig. 7. SANS spectrum from ME 3 (points) and fitted curve (line) vs. *q*.

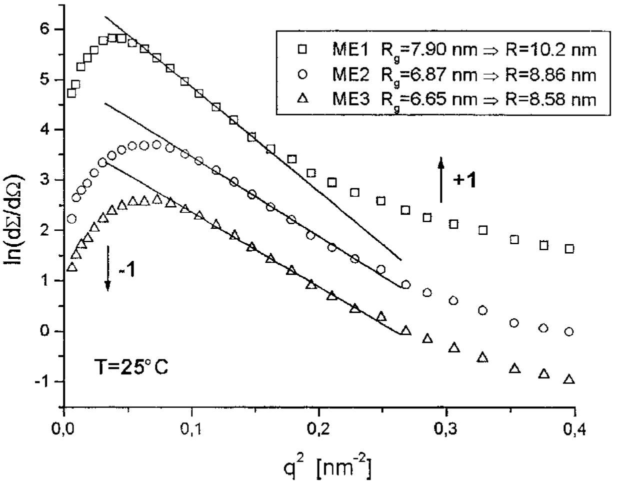


Fig. 8. The logarithmic SANS spectra from different ME vs. q^2 and fitted curves using the Guinier approximation (line), getting the radius of gyration R_g. Corresponding overall size R is calculated using Eq. (13); upper curve is shifted by the value of +1 and the lower curve by −1.

ME 2: Shell thickness of loosely bound surfactant = $R_{shell} - R_{core}$ = 3.74 nm

ME 3: Shell thickness of loosely bound surfactant = $R_{shell} - R_{core}$ = 2.83 nm

From the data, it can be concluded that a monolayer of surfactant is surrounding the oil droplets.

The difference between the values of shell thickness of strongly bound surfactant ($R_h - R_{core}$) cannot be explained only in terms of the surfactant used because different shell thicknesses in ME 2 and ME 3, containing the same kind of surfactant, were observed. Possibly, both kind of surfactant and kind of oil play a role in forming the shell of strongly bound surfactant.

One must consider the surfactant monolayer as being composed of a hydrophilic and a hydrophobic layer. It is reasonable to assume that an oil with a shorter alkyl chain will penetrate into the hydrophobic layer of the interfacial film to a greater extent. The oil penetration will be only in the hydrophobic layer, causing an increase in the oil core, and resulting in a smaller surfactant shell thickness in comparison to a microemulsion having the same surfactant but a different oil with a larger alkyl chain.

The hydrophilic chain of nonionic surfactants (Tween 80 and Tagat O2) is attached to the hydrophobic part of the molecule by polymerization of ethylene oxide (EO), and the number of monomer units per molecule cannot be controlled exactly. For the block copolymer (Poloxamer 331), there also may be a distribution in the hydrophobic domain. This involves polymerization of propylene oxide. The uncertainty in chain length of each surfactant molecule due to their polymerization may also cause different thicknesses of the surfactant layer of MEs containing the same surfactants. Depending on the chemical structure of the surfactant and oil, different thicknesses of strongly bound surfactants were observed as:

ME 1: Shell thickness of strongly bound surfactant = $R_h - R_{core}$ = 1.03 nm

ME 2: Shell thickness of strongly bound surfactant = $R_h - R_{core}$ = 1.83 nm

ME 3: Shell thickness of strongly bound surfactant = $R_h - R_{core}$ = 1.03 nm.

The hydrophilic chain of Tween 80 is shorter in comparison to Tagat O2 because the ethylene oxide monomers are distributed over different chains. This may be the reason for a smaller shell thickness of ME 1 than ME 2.

IPP has a larger alkyl chain than Eutanol G, and uncertainty in chain length of each surfactant molecule due to their synthesis may be the reason that the shell thickness of ME 3 is smaller than that of ME 2.

Noteworthy is the good agreement of the polydispersity indexes calculated from DLS and SANS, which are second-order parameters. This is one hint that the chosen model for fitting is near to the reality.

The SANS data are also fitted in Guinier approximation range, yielding the gyration radius R_g. This size parameter is connected with the moment of inertia of a particle. One can calculate the ratio between R_g and R_h and get the values 0.72 for ME 1, 0.70 for ME 2, and 0.74 for ME 3. These values are quite in agreement for the smallest possible value of 0.77 for hard spheres and justify our calculations, in which we used a hard-sphere model for the structure factor and hard-sphere interaction potential correction for the diffusion coefficient.

CONCLUSIONS

In summary, particle size determination via scattering experiments is a useful tool to characterize droplets in ME used as vehicles for dermal drug delivery, especially when two or more complementary methods are available. Thus, it is possible to get a more detailed description of the colloidal phase of the ME systems. A good agreement between DLS and SANS experiments are found as well as good grounds for supposing that the chosen models are near the reality. Besides, it was found that the kind of oil only marginally influences the droplet size.

ACKNOWLEDGMENTS

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Table II. Particle Size Parameters of Microemulsions[a]

	DLS		SANS				
ME	R_h (nm)	σ	R_{core} (nm)	R_{shell} (nm)	R_g (nm)	σ	R_g/R_h
1	10.9	0.251	9.87	13.11	7.90	0.233	0.72
2	9.58	0.156	7.75	11.49	6.87	0.174	0.71
3	9.01	0.180	7.98	10.81	6.65	0.209	0.74

[a] Errors are smaller than ±2% for the radii and ±10% for the standard deviations σ.

authors wish to thank J. Lange for help in performing the SANS measurements at the Joint Institute for Nuclear Research in Dubna, Russia.

APPENDIX

Theory of Dynamic Light Scattering

In DLS experiments, time-dependent fluctuations of scattered light intensity, $I_s(t)$, due to Brownian motion are measured. These fluctuations can be analyzed in terms of the scattered intensity $I_s(t)$, which contains information about the dynamics of the scattering particles (7). The normalized field autocorrelation function $g^1(\tau)$ has been derived from the scattered intensity via the Siegert relation (7,8).

$$g^1(\tau)=\sqrt{1-\frac{\langle I_s^*(t)I_s(t+\tau)\rangle}{\langle I_s^2\rangle}} \tag{1}$$

The field autocorrelation function $g^1(\tau)$ for monodisperse solutions (all particles are identical in size) where particles are spherical is (9)

$$g^1(\tau)=e^{-\Gamma\tau} \tag{2}$$

The relaxation rate Γ is connected with the translational diffusion coefficient D according to

$$\Gamma=D\times q^2 \tag{3}$$

with the scattering wave vector $q = 4\pi n/\lambda \times \sin(\theta/2)$. Wave vector q depends on the wavelength λ of the incident light, the refractive index of ME n, and scattering angle θ.

For dense, polydisperse, interacting systems of colloidal particles, Eq. (2) is split into two components depending on collective motion (and present even for a truly monodisperse system) and self or tracer motions. In the small q-limit (10),

$$g^1(\tau)=A_1e^{-D_cq^2\tau}+A_2e^{-D_sq^2\tau} \tag{4}$$

Two exponentials in Eq. (4) are attributed to the existence of two modes: fast mode is due to collective diffusion D_c (i.e., total number density fluctuations) whereas the slow mode is due to self-diffusion D_s (i.e., concentration fluctuations that decay by the exchange of species by single particle diffusion). A_1 and A_2 are amplitude factors and can be used as a measure of the polydispersity σ. For small, homogeneous hard spheres, size polydispersity can be calculated in terms of relative amplitude of slower decay mode A_2 for a known value of volume fraction (10,11).

Particle size R_h (hydrodynamic radius) can be determined using the Stokes-Einstein equation,

$$R_h=\frac{k_BT}{6\pi\eta D_0} \tag{5}$$

In the Stokes-Einstein equation, D_0 is the free particle diffusion coefficient, k_B is 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 microemulsion). For spherical particles interacting through essentially hard-sphere or excluded-volume forces, one can deduce the free particle diffusion coefficient D_0 from the collective diffusion coefficient D_c using Eq. (6):

$$D_c\,D_0(1+1.56\varphi+0.91\varphi^2+\ldots) \tag{6}$$

where φ is the volume fraction of particles (12).

Theory of Small-Angle Neutron Scattering

The principle of SANS can be understood due to the similarities to those of dynamic light scattering. The scattering of thermal neutrons from soft matter is describable via coherent scattering cross-section per unit volume, which is given by

$$\frac{d\Sigma}{d\Omega}(q)=\frac{1}{V}\left\langle\sum_{i=1}^{N}\sum_{j=1}^{N}f_if_je^{iq(r_i-r_j)}\right\rangle, \tag{7}$$

where N is the total number of particles in the scattering volume V, and r_i and r_j are the space positions of the particles i and j. f_i is the scattering amplitude of the particle i. The angular bracket represents an ensemble average.

The importance about SANS is the small wavelength λ of the incident neutrons, which is of the order 1 to 10 Å. Thus, microemulsion droplets even at the lower limit of colloidal size cannot be considered as point scatterers. In neutron scattering, a form factor must be used except at zero scattering angle. If the scattering unit is itself a collection of objects (e.g., microemulsion droplets), the summations of Eq. (7) are to be taken for all the pairs i and j, both in the particles and between the particles. It is therefore convenient to group these contributions into two parts. One obtains Eq. (7) as follows (13):

$$\frac{d\Sigma}{d\Omega}(q)=n_pP(q)S(q). \tag{8}$$

represents the average number density of droplets in scattering volume V. The form factor $P(q) = \langle|F(q)|^2\rangle$ is a function that describes how $d\Sigma/d\Omega$ is modulated by interference effects between radiation scattered by different parts of the same scattering body. Consequently, it is very dependent on the shape of the scattering body.

$$F_i(q)=\int_{\text{Droplet i}}dr^3[\rho_i(r)-\rho_s(r)]e^{-iqr} \tag{9}$$

Here, $\rho_i(r)$ and $\rho_s(r)$ are the scattering length densities of the droplet i and solvent s, respectively. For fitting, the form factor of spherical core shell particles is used. The form factor of a spherical particle with an internal core of radius R_{core} and a scattering length density ρ_{core} surrounded by a shell with an outer radius R_{shell} and a scattering length density ρ_{shell} is written as (14)

$$F(q)=\frac{4}{3}\pi R_{core}^3(\rho_{core}-\rho_{shell})\frac{3j_1(qR_{core})}{qR_{core}}+\frac{4\pi}{3}R_{shell}^3(\rho_{shell}-\rho_s)\frac{3j_1(qR_{shell})}{qR_{shell}} \tag{10}$$

where $j_1(x) = (\sin x - x\cos x)/x^2$. $F(q)$ is integrated during the fitting procedure over a Schultz distribution function to get the polydispersity σ concerning the overall size R_{shell}. The structure factor $S(q)$ is a function that describes how $d\Sigma/d\Omega$ is modulated by interference effects between radiation scattered by different scattering bodies. Consequently, it is dependent on the degree of local order in the sample such as might arise in an interacting system, for example. For fitting the structure factor, a hard-sphere system is used, which is described by Percus and Yevick and Janich (8,15):

$$S_{\mathrm{PY}}(K) = \frac{1}{1 - n_{\mathrm{p}}c(K)} \quad K = dq \tag{11}$$

$$\begin{aligned} c(K) = -\frac{4\pi d^3}{K^3}\{&\alpha_0 K^3(\sin K - K\cos K) + \beta_0 K^2 \\ &[2K\sin \mathrm{K} - (K^2 - 2)\cos K - 2] + \gamma_0[\sin K(4K^3 - 24K) \\ &- (K^4 - 12K^2 + 24)\cos K + 24]\} \end{aligned}$$

$$\alpha_0 = \frac{(1 + 2\varphi)^2}{(1 - \varphi)^4} \quad \beta_0 = -\frac{6\varphi\left(1 + \frac{\varphi}{2}\right)^2}{(1 - \varphi)^4}$$

$$\gamma_0 = \frac{\varphi\alpha_0}{2} \qquad \varphi = \frac{\pi}{6} n_{\mathrm{p}} d^3$$

where $d = 2 \times R_{\mathrm{shell}}$ and n_{p} are the particle diameter and particle number density, respectively.

The asymptotic behavior of the form factor is general and independent of the shape of the particle. This is because of small q (more exactly in the so-called Guinier region $qR \leq 2$); the spatial resolution is not sufficient to determine the shape, and only information about the size can be obtained. Under these conditions, scattering cross-section per unit volume is given by the Guinier relation (16)

$$\frac{d\Sigma}{d\Omega}(q) = Ae^{\frac{-R_{\mathrm{g}}^2}{3}} \Rightarrow \ln\left[\frac{d\Sigma}{d\Omega}(q)\right] = A' - \frac{R_{\mathrm{g}}^2 q^2}{3} \tag{12}$$

where A and A' are constants and R_{g} is the so-called radius of gyration. Regarding the radius of a hard sphere, it is related to radius of gyration as

$$R = \sqrt{\frac{5}{3}}R_{\mathrm{g}} \tag{13}$$

REFERENCES

1. T. P. Hoar and J. H. Schulman. Transparent water-in-oil dispersions: The oleopathic hydro-micelle. *Nature* **152**:102–103 (1943).
2. P. G. de Gennes and C. Taupin. Microemulsions and the flexibility of oil/water interfaces. *J. Phys. Chem.* **86**:2294–2304 (1982).
3. D. O. Shah. *The Micelles, Microemulsions and Monolayers*, Marcel Dekker, New York, 1998.
4. P. P. Constantinides and S. H. Yiv. Particle size determination of phase-inverted water-in-oil microemulsions under different dilution and storage conditions. *Int. J. Pharm.* **115**:225–234 (1995).
5. B. W. Mueller and R. H. Mueller. Particle size distributions and particle size alterations in microemulsions. *J. Pharm. Sci.* **73**:919–922 (1984).
6. V. Degiorgio. Nonionic micelles. In V. Degiorgio and M. Corti (eds.), *Physics of Amphiphiles: Micelles, Vesicles and Microemulsions*, Elsevier, Amsterdam, 1995, p. 303.
7. R. Pecora. *The Dynamic Light Scattering*, Plenum Press, New York, 1985.
8. M. Janich, J. Lange, H. Graener, and R. Neubert. Extended light scattering investigations on dihydroxy bile salt micelles in low-salt aqueous solutions. *J. Phys. Chem. B* **102**:5957–5962 (1998).
9. R. Pecora. Doppler shifts in light scattering from pure liquids and polymer solutions. *J. Chem. Phys.* **40**:1604–1614 (1964).
10. Y. D. Yan and J. H. R. Clarke. Dynamic light scattering from concentrated water-in-oil microemulsions: The coupling of optical of size polydispersity. *J. Chem. Phys.* **93**:4501–4509 (1990).
11. P. N. Pusey, H. M. Fijnaut, and A. Vrig. Mode amplitudes in dynamic light scattering by concentrated liquid suspensions of polydisperse hard spheres. *J. Chem. Phys* **77**:4270–4281 (1982).
12. P. N. Pusey. Diffusion of spherical particles in concentrated dispersion. In V. Degiorgio and M. Corti (eds.), *Physics of Amphiphiles: Micelles, Vesicles and Microemulsions*, Elsevier, Amsterdam, 1995, p. 152.
13. M. Kotlarchyk and S.-H. Chen. Analysis of small angle neutron scattering spectra from polydisperse interacting colloids. *J. Chem. Phys.* **79**:2461–2469 (1983).
14. S. H. Chen. Small angle neutron scattering studies of the structure and interaction in micellar and microemulsion systems. *Ann. Rev. Phys. Chem.* **37**:351–399 (1986).
15. J. K. Percus and G. J. Yevick. Analysis of classical statistical mechanics by means of collective co-ordinates. *Phys. Rev.* **110**:1–13 (1958).
16. A. Guinier and G. Fournet. *Small Angle Scattering of X-Rays*, Wiley, New York, 1955.