Particle Size Analysis of Concentrated Phospholipid Microemulsions: II. Photon Correlation Spectroscopy

Submitted April 11, 2000; accepted June 19, 2000; published July 14, 2000

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ABSTRACT The solvated droplet size of concentrated water-in-oil (w/o) microemulsions prepared from egg and soy lecithin/water/isopropyl myristate and containing short-chain alcohol cosurfactants has been determined using photon correlation spectroscopy (PCS). The effect of increasing the water volume fraction (from 0.04 to 0.26) on the solvated size of the w/o droplets at 298 K has been investigated at 4 different surfactant/cosurfactant weight ratios (K_m of 1:1, 1.5:1, 1.77:1, and 1.94:1); in all cases the total surfactant/cosurfactant concentration was kept constant at 25% w/w. In the case of the microemulsions prepared from egg lecithin, the diffusion coefficients obtained from PCS measurements were corrected for interparticle interactions using a hard-sphere model that necessitated estimation of the droplet volume fractions, which in the present study were obtained from earlier total intensity light-scattering (TILS) studies performed on the same systems. Once corrected for hard-sphere interactions, the diffusion coefficients were converted to solvated radii using the Stokes-Einstein equation assuming spherical microemulsion droplets. For both egg and soy lecithin systems, no microemulsion droplets were detected at water concentrations less than 9 wt% regardless of the alcohol and K_m used, suggesting that at low concentrations of added water, cosolvent systems were formed. At higher water concentrations, however, microemulsion droplets were observed. The changes in droplet size followed the expected trend in that for a fixed K_m the size of the microemulsion droplets increased with increasing volume fraction of water. At constant water

concentration, droplet size decreased slightly upon increasing K_m . Interestingly, only small differences in size were seen upon changing the type of alcohol used. The application of the hard-sphere model to account for interparticle interactions for the egg lecithin systems indicated that the uncorrected diffusion coefficients underestimated particle size by a factor of slightly less than 2. Reassuringly, the corrected droplet sizes agreed very well with those obtained from our earlier TILS study.

INTRODUCTION

Microemulsions are monophasic, thermodynamically stable, transparent (or slightly translucent) dispersions of oil and water. As a consequence of their perceived advantages (in particular their clarity, high stability, ease of preparation, and ability to incorporate a range of drugs of varying physico-chemical properties), they have attracted much attention as drug delivery systems (1). In contrast to their ease of preparation, however, it is a far from trivial matter to characterize their microstructure, yet such knowledge is essential for their successful commercial exploitation. For example, we have shown that the rate of release of sodium salicylate from lecithin-based microemulsions, similar to the ones studied here, depends on their microstructure (2). Very similar findings have also been reported by Trotta and coworkers (3,4).

Scattering techniques (in particular, light and neutron scattering) are routinely used in the determination of the droplet size of a microemulsion. However, although these techniques provide a good indication of size in the case of dilute monodisperse spheres, when a concentrated and/or polydisperse system is examined, interpretation is more difficult because of the presence of interactions between individual particles (5). Note that in respect to size

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determination by light scattering, a system is considered concentrated if more than a few volume percent of dispersed phase is present. Unfortunately, it is not generally possible to remove these interparticulate interactions by dilution of the microemulsion, as this frequently results in a change of the microstructure of the microemulsion and possibly even the disappearance of the droplets (6), especially if any cosurfactant present partitions between each of the phases used (as in the present study, in which short-chain alcohols have been used as cosurfactants). It is often necessary, therefore, to work with systems containing a relatively high dispersed phase concentration and to account for interparticulate interactions using a model (7).

Surprisingly, despite the requirement to make these corrections, many of the reported studies on concentrated microemulsion systems have used *uncorrected* particle sizes obtained using photon correlation spectroscopy measurements (8-11); probably because it is not trivial to make such corrections. Furthermore, in addition to neglecting the need for correction, some workers have even attempted to correlate these uncorrected droplet sizes with the oral bioavailability of the drug in the microemulsion, not surprisingly without success (12). It is therefore the aim of this paper to show that the corrections can be significant for high disperse phase microemulsions. In order to do this in the present study, we have compared the sizes obtained for concentrated water-in-oil (w/o) microemulsions prepared from egg lecithin/water/isopropyl myristate and containing short-chain alcohol cosurfactants using photon correlation spectroscopy (PCS) with those after correcting for droplet interactions using a hard-sphere model.

MATERIALS AND METHODS

Materials

Epikuron 200 (E200; soy lecithin, minimum. 95 wt% phosphatidylcholine; fatty acid content palmitic, stearic 16-20%, oleic acid 8-12%, linoleic acid 62-66%, linolenic acid 6-8%) and Ovothin 200 (0200; egg lecithin, minimum. 92 wt% phosphatidylcholine; fatty acid content palmitic, stearic 39-47%, oleic acid

28-32%, linoleic acid 13-17%, linolenic acid 6-8%, arachidonic acid 3-6%, palmitoleic acid 1-2%) were supplied by Lucas Meyer Company (Germany). Note that the sample of 0200 used was either decolorized as described in Aboofazeli and Lawrence (13) or used as received. E200 was used as received. All other materials were used as in Aboofazeli and Lawrence (13).

PREPARATION OF SAMPLES FOR PARTICLE SIZING

W/o microemulsions were prepared as previously described (14) using lecithin/IPM/alcohol (6 short-chain alcohols) at a total surfactant/cosurfactant concentration of 25 wt% and containing different surfactant/cosurfactant weight ratios, K_m (1:1, 1.5:1, 1.77:1, 1.94:1). The volume fraction of water present varied between 0.04 to 0.26.

Light-Scattering Studies

Light-scattering studies were performed at 298 ± 0.1 K using a Malvern 4700c spectrometer (Malvern, UK), equipped with a 75-mW argon-ion laser (vertically polarized incident radiation of wavelength 488 nm), a digital correlator (Malvern 7032 Mutli 8), and a computer-controlled, stepper-motor-driven variable angle detection system. The PCS measurements were conducted using the 128-channel correlator, and the data were analyzed by a cumulants analysis (15) to obtain a diffusion coefficient.

Microemulsions were clarified as described previously (13). The ratio of the measured diffusion coefficient at 45° to the diffusion coefficient at 135° (D_{45}/D_{135}) was, in all cases, between 0.95 and 1.05, suggesting the absence of significant droplet asymmetry; consequently, subsequent PCS measurements were restricted to scattering angles of 45° , 90° , and 135° . Because the droplet sizes were identical at each angle, only the results obtained at 90° are reported. Measurements were performed in triplicate at each angle.

For analysis of the PCS measurements, the external/continuous phase of the microemulsion was considered to be a 3-component system comprising IPM/alcohol/lecithin at a total surfactant/cosurfactant

level of 25% w/w and at the appropriate surfactant/cosurfactant K_m . The refractive index and viscosity of the “continuous phase” were experimentally determined at 298 K using an Abbe ED/60 precision refractometer (Bellingham and Stanley, Sevenoaks, UK) and a calibrated U-tube viscometer (Ubbelohde), respectively. The refractive index of the “continuous phase” at the wavelength of the laser was obtained using the Comou approximation (16) as described previously (13).

Analysis of the Light-Scattering Data

The theory of PCS is well known and will therefore not be discussed in detail. For further details, readers are referred to Finsey (17). PCS measurements enable the determination of a diffusion coefficient (D) of a particle or droplet in solution. By assuming spherical aggregates, the measured D can be used to calculate the hydrodynamic radius, d , of the particle/droplet using the Stokes-Einstein equation:

$$\bar{d} = \frac{kT}{3\pi\eta D} \quad (\text{Eq. 1})$$

where k and T have their usual meaning and η is the viscosity of the continuous phase. Note that, because neither the PCS nor the earlier TILS experiments (13) showed any evidence of particle asymmetry, it was considered acceptable to use the Stokes-Einstein equation in the form given.

RESULTS AND DISCUSSION

In the present study, the influence of lecithin type, water concentration, and surfactant/cosurfactant weight ratio (K_m) on the particle size of lecithin-based w/o microemulsion droplets have been examined using PCS. Tables 1, 2, 3, 4, 5, and 6 show the uncorrected (measured) droplet size ($d_{\text{uncorrected}}$) obtained from the diffusion coefficient data for systems stabilized by soy or (decolorized) egg lecithin and using as cosurfactant one of the alcohols examined. Note that the sizes quoted are a mean of 3 measurements and that in all cases the standard deviation obtained for droplet size was less than ± 0.3 nm. It should also be noted that, because of the presence of slightly differing upper water solubilization phase boundaries in the systems

prepared with the 2 types of lecithin, it was not always possible to examine exactly the same range of compositions. This impediment was particularly notable in samples prepared from sec-butanol (Table 4) and tert-butanol (Table 6).

As can be seen, very little difference was generally observed between the droplet sizes obtained with either egg or soy lecithin; in general, the variation was within the standard deviation recorded for these experiments. The only exception tended to be the samples prepared at the highest (and sometimes lowest) water content. This effect was particularly noticable for the samples containing n-propanol, where the difference undoubtedly arose from the close proximity of the upper limit of the water solubilization phase boundary. This similarity in sizes in the systems prepared with the different alcohols was also seen in our earlier TILS study and suggests that the nature of the low molecular hydrophilic cosurfactant used is not critical in determining size. Furthermore, the results suggest that because the differences seen when the different types of lipid used are small, that the differences in lipid content again are not critical.

Note that no differences were found between the sizes obtained by PCS for the undecolorized egg lecithin (data not shown) and the decolorized lecithin, whose results are reported here and which was also used for the earlier TILS study (13). It is worth explaining that the decolorized egg lecithin was used for the TILS study because the samples prepared using undecolorized lecithin were bright orange. Consequently, it was felt that problems may be encountered in the TILS measurement because of absorption of the blue/green laser by the sample, leading to a reduction in the intensity of the light scattered and thereby underestimating size. Note that, as it did not prove possible (in our hands) to decolorize soy lecithin, no TILS studies were performed on the systems prepared with this lipid. Because PCS relies on the variation in the intensity of scattered light, rather than changes in the absolute scattering intensity as with TILS, as long as sufficient light is scattered by the sample, no problem exists because of loss of intensity from the sample absorbing some of the light.

Table 1. Photon Correlation Spectroscopy Results for W/O Microemulsions Containing Egg Lecithin (or, Where Indicated, Soy Lecithin) and N-Propanol

Sample ^a	Hard-Sphere Volume Fraction ϕ_{HS}	Measured Diffusion Coefficient D (10^7)(cm^2/s)	Corrected Diffusion Coefficient D_o (10^7)(cm^2/s)	Uncorrected Diameter (nm)	Droplet $d_{uncorrected}$	Uncorrected Droplet Diameter $d_{corrected}$ (nm)	Uncorrected Droplet Diameter $d_{hard\ sphere}$ (nm)
				Egg Lecithin	Soy Lecithin		
1npr9	0.54	1.37	0.75	5.1	4.9	9.5	12.1
-11	0.53	1.21	0.67	5.9	5.7	10.7	13.4
-13	0.51	1.03	0.57	6.9	7.0	12.4	15.1
-14	0.50	0.93	0.53	7.6	7.3	13.5	16.7
1.5npr9	-	-	-	-	-	-	-
-11	0.54	1.05	0.57	5.8	5.5	10.7	12.5
-14	0.52	0.90	0.50	6.7	6.4	12.2	15.0
-16	0.52	0.84	0.46	7.3	7.3	13.2	16.2
-18	0.51	0.69	0.38	8.8	8.7	15.9	18.9
-20	0.52	0.60	0.33	10.2	10.2	18.5	20.3
1.77npr9	-	-	-	-	-	-	-
-11	0.55	1.01	0.55	5.7	5.4	10.5	12.1
-14	0.53	0.86	0.47	6.4	6.7	12.2	14.4
-16	0.53	0.80	0.44	7.1	6.8	13.0	15.5
-18	0.52	0.72	0.40	8.0	8.2	14.5	17.6
-20	0.54	0.65	0.35	8.8	9.5	16.2	18.8
-22	0.54	0.57	0.31	10.1	10.1	18.6	20.5
-24	0.54	0.48	0.26	12.0	13.6	22.3	22.9
-25	0.55	0.46	0.25	12.3	OA	23.0	23.7
1.94npr9	-	-	-	-	-	-	-
-11	0.52	0.97	0.53	5.8	6.3	10.5	12.6
-14	0.54	0.88	0.48	6.3	6.2	11.7	13.8
-16	0.54	0.79	0.43	7.1	7.6	13.1	15.2
-18	0.53	0.72	0.39	7.8	7.7	14.3	17.1
-20	0.54	0.63	0.34	8.8	8.9	16.3	18.4
-22	0.54	0.56	0.31	9.9	10.0	18.3	20.3
-24	0.56	0.52	0.28	10.8	12.7	20.2	21.5
-25	0.56	0.48	0.26	11.7	OA	21.9	22.7

^a The first digits show the K_m , npr denotes n-propanol and the last digit indicates the water concentration (wt%).

-, no particle size obtained; OA, outside region of microemulsion existence.

When performing a sizing by PCS, it is essential to use the value of the viscosity and refractive index (at the wavelength of the laser) of the continuous phase of the microemulsion because the refractive index is used in the calculation of the scattering vector required for determining diffusion coefficient and the viscosity for the conversion of the diffusion coefficient to droplet size using the Stokes-Einstein equation. Generally, the required values can be taken simply as those for either water (in the case of oil-in-water microemulsions) or the organic solvent used as the external phase (in the case of w/o microemulsions). Unfortunately, because

cosurfactants were present in this study, it is not possible to assume that the continuous phase was solely isopropyl myristate because it undoubtedly contains some cosurfactant. As a consequence, therefore, in the present study it was decided to use the experimentally determined refractive index and viscosity values obtained at zero water concentration, as the results suggest that at water concentrations below about 9 wt% a cosolvent rather than a microemulsion system is present and that all the added water is associated with the droplets once formed.

Table 2. Photon Correlation Spectroscopy Results for W/O Microemulsions Containing Egg Lecithin (or Where Indicated Soy Lecithin) and Isopropanol

Sample ^a	Hard- sphere volume fraction ϕ_{HS}	Measured diffusion Coefficient D (10 ⁷) (cm ² /s)	Corrected Diffusion Coefficient D ₀ (10 ⁷) (cm ² /s)	Uncorrected Droplet Diameter d _{uncorrected} (nm)		Uncorrected Droplet Diameter d _{corrected} (nm)	Uncorrected Droplet Diameter d _{hard sphere} (nm)
				Egg Lecithin	Soy Lecithin		
O1ipr9	0.54	1.12	0.61	6.5	6.4	11.9	12.4
-11	0.52	0.98	0.54	7.4	7.6	13.4	14.2
-13	0.52	0.89	0.49	8.2	8.1	14.8	15.9
-14	0.51	0.81	0.45	8.9	9.3	15.9	17.2
-15	-	-	-	-	10.7	-	-
O1.5ipr9	0.56	0.99	0.53	6.3	5.4	11.8	11.6
-11	0.54	0.93	0.51	6.6	5.7	12.3	13.0
-14	0.53	0.80	0.44	7.8	7.3	14.3	15.4
-16	0.52	0.71	0.39	8.8	8.1	16.0	17.2
-18	0.52	0.63	0.35	9.8	9.8	17.9	19.1
-20	0.53	0.55	0.30	11.2	10.9	20.5	20.9
O1.77ipr9	0.50	0.93	0.52	5.9	4.7	11.2	12.9
-11	0.55	0.92	0.50	6.4	5.8	11.8	12.6
-14	0.53	0.78	0.43	7.5	7.3	13.7	15.1
-16	0.53	0.69	0.38	8.4	8.5	15.5	16.5
-18	0.53	0.61	0.33	9.6	9.2	17.6	18.4
-20	0.53	0.55	0.30	10.6	10.0	19.4	20.1
-22	0.54	0.49	0.27	11.9	12.4	21.8	22.2
-24	0.55	0.45	0.24	13.0	13.7	24.2	24.0
O1.94ipr9	-	-	-	-	-	-	-
-11	0.55	0.92	0.49	6.2	6.0	11.4	12.4
-14	0.53	0.78	0.43	7.2	6.4	13.2	14.7
-16	0.54	0.73	0.40	7.7	7.6	14.2	16.0
-18	0.53	0.64	0.35	8.9	8.9	16.2	18.2
-20	0.53	0.55	0.30	10.3	9.6	18.9	19.9
-22	0.55	0.52	0.28	10.8	11.0	20.0	21.3
-24	0.56	0.46	0.24	12.4	11.9	23.2	23.0

^aThe first digits show the K_m ; ipr denotes isopropanol; and the last digit indicates the water concentration (wt%).

- No particle size obtained.

Interestingly, a survey of the pharmaceutical literature reveals that some researchers have used the refractive index and viscosity of the *whole* microemulsion for their PCS analysis (18) rather than those of the continuous phase. This method is obviously not correct and will undoubtedly introduce errors into the analysis. For example, the use of microemulsion viscosity, particularly for concentrated systems, may lead to a large underestimation of droplet size.

Even if the appropriate viscosity and refractive index are used in the analysis, it must be remembered that the calculation of droplet diameter from the measured diffusion coefficient obtained by PCS measurements is only valid for dilute, noninteracting systems, obviously not the case in the present study. It was therefore necessary to correct the experimentally obtained diffusion coefficients for these interactions to enable calculation of the “true” radius of the microemulsion droplets.

Table 3. Photon Correlation Spectroscopy Results for W/O Microemulsions Containing Egg Lecithin (or Where Indicated Soy Lecithin) and *N*-Butanol

Sample ^a	Hard-Sphere Volume Fraction ϕ_{HS}	Measured Diffusion Coefficient D (10^7) (cm^2/s)	Corrected Diffusion Coefficient D_o (10^7) (cm^2/s)	Uncorrected Droplet Diameter $d_{\text{uncorrected}}$ (nm)		Uncorrected Droplet Diameter $d_{\text{corrected}}$ (nm)	Uncorrected Droplet Diameter $d_{\text{hard sphere}}$ (nm)
				Egg Lecithin	Soy Lecithin		
1nbu9	0.50	1.19	0.67	5.7	6.4	10.3	10.8
-11	0.47	0.99	0.57	6.9	6.7	12.0	12.4
-12	0.47	0.92	0.53	7.4	6.9	12.8	13.1
-13	0.46	0.86	0.50	8.0	OA	13.8	13.9
1.5nbu9	-	-	-	-	-	-	-
-11	0.51	0.93	0.52	6.5	6.2	11.7	11.5
-14	0.50	0.84	0.47	7.2	6.8	12.8	13.5
-16	0.50	0.72	0.41	8.4	6.9	14.9	14.9
-17	0.49	0.69	0.40	8.9	OA	15.6	15.9
1.77nbu9	-	-	-	-	-	-	-
-11	0.49	0.88	0.50	6.4	6.1	11.3	12.1
-13	ND	ND	ND	ND	6.6	ND	ND
-14	0.51	0.84	0.47	6.7	6.6	12.0	13.2
-16	0.50	0.75	0.42	7.6	OA	13.5	14.7
-18	0.49	0.67	0.38	8.4	OA	14.9	16.4
1.94nbu9	-	-	-	-	-	-	-
-11	0.47	0.85	0.49	6.4	6.7	11.2	12.3
-14	0.51	0.78	0.43	7.0	6.6	12.6	13.1
-16	0.50	0.76	0.43	7.2	6.7	12.8	14.6
-18	0.50	0.67	0.38	8.1	7.4	14.4	16.2

^a The first digits show K_m ; nbu denotes *n*-butanol; and the last digit indicates the water concentration (wt%).

-, no particle size obtained; OA, outside region of microemulsion existence; ND, not determined.

In the present study, the experimentally determined diffusion coefficients were corrected assuming the presence of hard-sphere interactions only, using the following equation:

$$D = D_o[1 + \alpha\phi] \quad (\text{Eq. 2})$$

where D is the measured diffusion coefficient, D_o is the corrected diffusion coefficient, ϕ is the droplet volume fraction, and α is a factor that takes into account both the thermodynamic and hydrodynamic components of the hard-sphere correction and in the present study was assumed to be 1.56 after the work of Cheng et al (7). Note that although in the form shown, Equation 2, corrects for the presence of hard-sphere interactions only, it has been expanded to include a term to account for electrostatic interactions (7). However, because the microemulsion systems

studied here are composed of droplets of water and cosurfactant surrounded by surfactant and cosurfactant orientated in such a manner that the hydrocarbon chains are expressed on the exterior of the droplet and are therefore in contact with an oily environment, it is reasonable to assume the presence of hard-sphere interactions only.

It should also be noted that, in addition to the need for an assumption of the nature of the interactions (ie, hard sphere or electrostatic) present in the system under study, the method of correction used obviously also requires an estimate to be made of the volume fraction of the microemulsion droplets, ϕ . The value of ϕ chosen is important because it is critical in determining the final size of the corrected droplets. Generally, in order to make an estimate of ϕ , a number of assumptions are made; these include the

assumption that all the water and surfactant go to make up monodisperse, spherical w/o microemulsion droplets and that the area per molecule occupied by the surfactant in the interfacial monolayer is the same as that occupied by the molecule at the air-water interface. Using these assumptions together with a knowledge of the group volume contributions and the effective length of the hydrophobic chain of the surfactant, it is possible to estimate ϕ using simple geometric considerations. In the present system,

however, the situation is more complex because of the presence of the alcohol cosurfactants, which partition throughout the system and for which it is hard to make an estimate of the amount associated with each of the phases and surfactant monolayer. Also, estimating the volume fraction in the manner outlined does not take into account any repulsive interactions between the hydrophobic chains on the exterior of the droplets, therefore possibly underestimating droplet size.

Table 4. Photon Correlation Spectroscopy Results for W/O Microemulsions Containing Egg Lecithin (or Where Indicated Soy Lecithin) and Sec-Butanol

Sample ^a	Hard-sphere volume fraction ϕ_{HS}	Measured Diffusion Coefficient D (10^7) (cm^2/s)	Corrected Diffusion Coefficient D_o (10^7) (cm^2/s)	Uncorrected Droplet Diameter $d_{\text{uncorrected}}$ (nm)		Uncorrected Droplet Diameter $d_{\text{corrected}}$ (nm)	Uncorrected Droplet Diameter $d_{\text{hard sphere}}$ (nm)
				Egg Lecithin	Soy Lecithin		
O1sbu9	0.47	1.10	0.63	6.4	6.4	11.1	12.3
-11	0.51	0.94	0.52	7.5	7.1	13.4	12.4
-13	0.49	0.82	0.47	8.9	7.9	15.1	14.1
-14	0.49	0.80	0.46	8.8	8.4	15.5	15.0
-16	0.51	0.97	0.54	6.3	10.1	11.3	11.2
O1.5sbu9	0.49	0.85	0.50	7.2	-	12.3	12.6
-11	0.51	0.73	0.41	8.3	6.9	14.9	14.0
-14	0.51	0.73	0.41	8.4	7.4	15.1	15.3
-16	0.51	0.68	0.40	9.0	8.2	16.2	16.9
-18	NM	NM	NM	NM	9.2	NM	NM
O1.77sbu9	NM	NM	NM	NM	-	NM	NM
-11	NM	NM	NM	NM	6.8	NM	NM
-14	NM	NM	NM	NM	7.3	NM	NM
-16	NM	NM	NM	NM	7.6	NM	NM
-18	NM	NM	NM	NM	8.2	NM	NM
O1.94sbu9	NM	NM	NM	NM	-	NM	NM
-11	NM	NM	NM	NM	-	NM	NM
-14	NM	NM	NM	NM	7.3	NM	NM
-16	NM	NM	NM	NM	7.9	NM	NM
-18	NM	NM	NM	NM	8.0	NM	NM
-20					8.7		

^a The first digits show the K_m ; sbu denotes sec-butanol; and the last digit indicates the water concentration (wt%).

-, no particle size obtained; NM, no microemulsion formed.

In the present study, therefore, a value of ϕ was obtained from the results of our earlier TILS study (13) in which an estimate of the hard-sphere volume of the *whole* microemulsion droplet was made using the Percus-Yevick model (19). This estimate takes into account hard-sphere interactions between the microemulsion droplets.

Once the experimentally determined diffusion coefficients (D) were corrected using Equation 2, the corrected diffusion coefficient (D_o) was substituted into Equation 1 and a corrected droplet size obtained. Tables 1, 2, 3, 4, 5, and 6 detail the complete set of uncorrected droplet sizes ($d_{\text{uncorrected}}$) obtained using (decolorized) egg lecithin together with the corrected

droplet sizes ($d_{\text{corrected}}$) obtained using Equation 2. Note that it was only possible to correct the data obtained with egg lecithin because TILS studies had been performed only with the samples prepared using this lipid (12). It is clear from Tables 1, 2, 3, 4, 5, and 6 that in most cases the diffusion coefficient increases by approximately twice its original value. Interestingly, the correction value is relatively constant because the hard-sphere fraction obtained from the TILS was in the region 0.4-0.57 (13). As a consequence, therefore, the use of a correction in this case did not change the trend of the results observed with the uncorrected data in Tables 1, 2, 3, 4, 5, and 6. Also given in Tables 1, 2, 3, 4, 5, and 6 is the

droplet size ($d_{\text{hard sphere}}$) obtained from the earlier TILS analysis. Significantly, it can be seen that the correction used in the present study brings the size determined by PCS satisfyingly into line with that obtained from the TILS results. However, with the exception of the pentanol systems, it should be noted that the droplet sizes obtained by PCS still tended to be slightly lower than those obtained from the TILS study. This result was not as expected because the droplet sizes from PCS include any solvation shell and should therefore be the same or possibly slightly larger than those obtained from the TILS, which gives the anhydrous droplet size.

Table 5. Photon Correlation Spectroscopy Results for W/O Microemulsions Containing Egg Lecithin (or Where Indicated Soy Lecithin) and *Tert*-Butanol

Sample ^a	Hard-Sphere Volume Fraction ϕ_{HS}	Measured Diffusion Coefficient D (10^7) (cm^2/s)	Corrected Diffusion Coefficient D_0 (10^7) (cm^2/s)	Uncorrected Droplet Diameter $d_{\text{uncorrected}}$ (nm)		Uncorrected Droplet Diameter $d_{\text{corrected}}$ (nm)	Uncorrected Droplet Diameter $d_{\text{hard sphere}}$ (nm)
				Egg Lecithin	Soy Lecithin		
O1tbu9	0.49	1.07	0.61	6.0	6.5	10.7	12.5
-10	-	-	-	-	7.0	-	-
-11	0.51	0.90	0.50	7.2	7.25	13.0	13.2
-13	0.49	0.81	0.46	8.0	OA	14.0	15.2
-14	0.48	0.74	0.42	8.7	OA	15.3	16.3
-16	0.49	0.66	0.38	9.8	OA	17.3	17.9
O1.5tbu9	-	-	-	-	6.5	-	-
-11	0.53	0.88	0.48	6.5	6.6	11.9	12.2
-13	-	-	-	-	6.9	-	-
-14	0.51	0.77	0.43	7.5	7.4	13.5	14.4
-16	0.51	0.70	0.39	8.3	OA	14.9	15.9
-18	0.51	0.64	0.36	9.0	OA	16.2	17.7
O1.77tbu9	0.49	1.16	0.66	4.8	-	8.4	12.1
-11	0.51	0.86	0.48	6.4	6.0	11.5	12.6
-13	-	-	-	-	6.6	13.5	14.4
-14	0.51	0.73	0.41	7.5	6.9	-	-
-16	0.52	0.66	0.37	8.2	OA	15.1	15.5
-18	0.53	0.62	0.34	9.0	OA	16.3	16.7
-20	0.53	0.57	0.31	9.6	OA	17.6	18.3
-22	0.54	0.53	0.29	10.5	OA	19.2	20.0
O1.94tbu9	0.48	1.09	0.62	4.8	-	8.4	12.3
-11	0.51	0.85	0.47	6.2	-	11.2	12.4
-13	-	-	-	-	6.8	-	-
-14	0.51	0.75	0.41	7.0	6.8	12.8	14.4
-16	0.54	0.71	0.39	7.4	7.1	13.7	14.6
-18	0.52	0.61	0.34	8.7	OA	15.8	16.8
-20	0.53	0.56	0.31	9.4	OA	17.2	18.3
-22	0.54	0.55	0.30	9.7	OA	18.9	19.4

^a The first digits show the K_m ; tbu denotes *tert*-butanol; and the last digit indicates the water concentration (wt%).

-, no particle size obtained; OA, outside region of microemulsion existence.

In summary, the results in the tables show that regardless of the alcohol and K_m used, no microemulsion droplets were detected at water concentrations less than 9 wt%, suggesting that at low concentrations of added water, cosolvent systems were formed. At higher water concentrations, however, microemulsion droplets were observed. The changes in droplet size followed the expected trend in

that for a fixed K_m the size of the microemulsion droplets increased with increasing volume fraction of water. At constant water concentration, droplet size decreased slightly upon increasing K_m . Furthermore, there were only small differences in size seen upon changing the type of alcohol used. Exactly the same observations were recorded in our earlier TILS studies of these systems.

Table 6. Spectroscopy Results for W/O Microemulsions Containing Egg Lecithin (or Where Indicated Soy Lecithin) and *N*-Pentanol

Sample ^a	Hard-Sphere Volume Fraction ϕ_{HS}	Measured Diffusion Coefficient D (10^7) (cm^2/s)	Corrected Diffusion Coefficient D_o (10^7) (cm^2/s)	Uncorrected Droplet Diameter $d_{uncorrected}$ (nm)		Uncorrected Droplet Diameter $d_{corrected}$ (nm)	Uncorrected Droplet Diameter $d_{hard\ sphere}$ (nm)
				Egg Lecithin	Soy Lecithin		
1npe7	-	-	-	-	-	-	-
-8	-	-	-	-	-	-	-
-9	0.44	0.95	0.56	7.0	7.1	11.7	10.6
-10	0.43	0.96	0.58	6.9	OA	11.5	11.4
1.5npe7	-	-	-	-	-	-	-
-8	-	-	-	-	-	-	-
-9	0.40	0.78	0.48	7.5	-	12.2	11.6
-10	0.42	0.87	0.53	6.6	6.2	11.1	11.6
1.77npe7-	-	-	-	-	-	-	-
-9	-	-	-	-	-	-	-
-11	0.43	0.79	0.47	7.0	6.7	11.6	12.2
-13	0.43	0.79	0.47	7.0	OA	11.8	13.3
-14	0.48	0.77	0.44	7.1	OA	12.8	12.8
1.94npe9-	-	-	-	-	-	-	-
-10	-	-	-	-	8.4	-	-
-11	0.42	0.76	0.46	7.0	OA	11.7	12.4
-13	0.47	0.79	0.46	6.8	OA	11.7	12.5
-14	0.46	0.75	0.44	7.1	OA	12.2	13.4
-15	0.49	0.76	0.43	7.0	OA	12.4	13.3

^aThe first digits show K_m ; npe stands for *n*-propanol; and the last digit indicates the water concentration (wt%).

-, no particle size obtained; OA, outside region of microemulsion existence; ND, not determined.

CONCLUSION

It is clear that the interpretation of scattering experiments on concentrated microemulsions is by no means simple. In our analysis, the use of a comparatively simple hard-sphere model provides one possible approach to the interpretation of data obtained from concentrated systems. It is clear, however, that the interpretation of PCS data should only be attempted when it is possible to obtain a

reasonable estimate of the hard-sphere volume fraction of the microemulsion droplets, either from TILS or neutron-scattering studies. The combined use of these techniques should allow a description of the size and shape of the phospholipid microemulsions as a function of water incorporation. In the absence of such a correction, the value of droplet size obtained should be taken as indicative only of the presence of microemulsion droplets and used for assessment of particle size stability.

ACKNOWLEDGMENTS

We are grateful to the Ministry of Health, Treatment and Medical Education of I.R. Iran for supporting this research.

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