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Particle Size Analysis of Concentrated Phospholipid Microemulsions: I. Total Intensity Light Scattering

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ABSTRACT Water-in-oil phospholipid microemulsions prepared from a constant total surfactant/cosurfactant concentration of 25 wt% at four different lecithin/alcohol weight ratios (K_m of 1:1, 1.5:1, 1.77: 1, and 1.94:1) and containing water concentrations (or volume fractions) ranging from 2.0 to 26 wt% (or 0.04 to 0.26) have been examined at 298 K using total intensity light scattering. The data obtained were analysed using the hard-sphere model of Percus-Yevick, modified to account for the partitioning of the alcohol between the various phases. The light-scattering results showed that, regardless of the K_m or the alcohol used, a minimum water concentration of at least 9 wt% was required for the formation of a microemulsion; although this value was reasonably constant for each of the alcohols investigated, there was a tendency for a slightly higher concentration of water to be required for microemulsion formation at higher $K_m$values. Simple calculations suggested that a microemulsion was formed only when sufficient water was present to satisfy the hydration of both the phospholipid head groups and the hydroxyl groups of the cosurfactant associated with the droplet. At water concentrations lower than this minimum value, a cosolvent system was observed. In all systems above this minimum concentration, as the concentration of water increased, the size of the microemulsion droplets also increased. Surprisingly, however, there was little difference in the size of the microemulsion droplets obtained with the different alcohols, regardless of the K_m, although for a particular alcohol there was some indication that the higher K_m systems produced the slightly smaller droplets for an equivalent water concentration. There was also a suggestion that the more hydrophobic alcohols produced slightly smaller droplets than the more polar alcohols at the same K_m.

INTRODUCTION

Microemulsions have recently attracted considerable attention as drug-delivery vehicles, partly because of the successful launch on the market of Neoral, a microemulsion preconcentrate of the drug cyclosporin (1). Unfortunately, as most of the work reported in the literature has used pharmaceutically unacceptable ingredients to formulate the microemulsion, there is a need for the development and study of systems suitable for pharmaceutical use. Of particular interest in the present study are water-in-oil (w/o) microemulsions formulated using lecithin as the primary surfactant.

Because of its high lipophilicity and strong tendency to form liquid crystalline structures, lecithin will not generally form microemulsions with pharmaceutically acceptable oils, such as isopropyl myristate and ethyl oleate, without the aid of a cosurfactant (2-4). We have previously reported on the ability of the commercially available egg lecithin Ovothin 200 (0200) to produce an optically clear, one-phase "microemulsion" in combination with isopropyl myristate and water in the presence of a series of short-chain alcohol cosurfactants at different surfactant/cosurfactant weight ratios, K_m, (5) by means of phase-behavior studies. Unfortunately, these phase studies do not prove the existence of a microemulsion; they prove only that a clear, stable, one-phase system is formed. It is essential to prove the existence of a microemulsion, however, because its presence may influence the release of the drug from the formulation. Indeed we have reported that quite different release patterns of the model drug,

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sodium salicylate, can be obtained from lecithin-based w/o microemulsions of differing composition (6).

Of the few methods available to prove definitively the existence of a microemulsion, the most widely used are scattering techniques or nuclear magnetic resonance (NMR) self-diffusion measurements. In the present study, total intensity (static) light-scattering (TILS) measurements have been used to determine whether or not a microemulsion is indeed produced in our systems. Scattering techniques collectively suffer from the disadvantage that in order to obtain a reliable estimate of particle size, measurements should be made at a range of low-disperse-phase volume fractions and should be extrapolated to infinite dilution in order to avoid the problems encountered as a result of particle-particle interactions. Unfortunately, however, the dilution of a microemulsion containing a cosurfactant is often problematic, due to the partitioning of the cosurfactant between the various phases, which often leads either to a change in the microstructure of the system or, in extreme cases, the disappearance of the microemulsion droplets (7). Consequently, scattering studies on microemulsion systems are often carried out in the concentrated microemulsion region and an appropriate model is then used to correct the results for particle-particle interactions. The most commonly used model is the hard-sphere model of Percus-Yevick (8), which treats the microemulsion droplets under study as hard spheres: not an unreasonable assumption in the case of a w/o microemulsion system where the interactions between droplets arise primarily from the interaction of the hydrophobic surfactant chains expressed on the exterior of the droplet. In the present study, TILS measurements were carried out on concentrated phospholipid microemulsions and analyzed using the Percus-Yevick model to determine the effect of variations of K_m and the nature of alcohol on droplet size. In order to obtain this information, the Percus-Yevick model has been modified to take into account the distribution of cosurfactant in the system. The microemulsions investigated in the present study are those studied in our earlier phase-behavior studies, (5) namely, water-in-isopropylmyristate (IPM) microemulsions stabilized by egg lecithin in combination with one of a series of short-chain alcohols. The alcohols were tested at surfactant-to-alcohol weight ratios (K_m) of 1:1, 1.5:1, 1.77:1, or 1.94:1. In order to ensure a sufficiently large range of water incorporation, a constant total surfactant concentration (including cosurfactant concentration) of 25 wt% was used for the light-scattering study. Obviously, the upper limit of water incorporation varied with the specific system under test. Note that the amount of IPM added varied depending on the amount of water present.

MATERIALS AND METHODS

Materials

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%) was supplied by Lucas Meyer Company (Germany). Isopropyl myristate (EPM) and tert-butanol (tbu) were purchased from Sigma Chemical Company (Dorset, UK). n-Pentanol (npe) was obtained from Aldrich Chemical Company (Dorset, UK). n-Butanol (nbu) and sec-butanol (sbu) were purchased from BDH Limited (Poole, UK), n-propanol (npr) and isopropanol (isp) were from FSA Laboratory Supplies (Loughborough, UK). All reagents were of the highest purity available and, with the exception of the lecithin, 0200, were used as received. Triple-distilled water from a well-seasoned all-glass system was used throughout the study.

In order to avoid possible complications from the absorption of the blue/green laser light (which would lead to a reduction in the scattering of the microemulsion droplets) by the highly yellow/orange-colored microemulsions, 0200 was decolorized. This was achieved by suspending 30 g of 0200 in 100 mL ethyl ether and stirring for a few minutes before removal of the ethyl ether by filtration under vacuum. This process was repeated several times until the lecithin had been decolorized. The decolorized lecithin was dried under vacuum rotatory evaporation, washed with dry acetone, and stored in dry acetone under an atmosphere of nitrogen at 4°C until required. The UV/visible spectra of the decolorized lecithin in chloroform showed no absorption of light in the region of the spectrum of interest (namely 488 nm; data not shown). Comparison of the phase behavior of the decolorized lecithin in the oil-rich part of the phase diagram with that obtained using the original sample of lecithin showed that they were virtually superimposible, suggesting that the decolorization procedure had not significantly altered the composition of the lecithin.

Preparation of Samples for Particle Sizing

Individual samples were prepared by weight using lecithin/IPM/alcohol (6 short-chain alcohols) at a constant total surfactant concentration of 25 wt% but at different surfactant/cosurfactant weight ratios, K_m (1:1, 1.5:1, 1.77:1, 1.94:1), and various water concentrations within the area of microemulsion existence, as determined by earlier phase studies (5). The water concentration studied ranged from 2 wt% up to a maximum of 26 wt% in 2% wt% intervals. Obviously, the maximum level of water incorporation was dependent on the particular system under study. Note that the amount of IPM varied depending on the amount of water present. The code used for sample designation is as follows: the first digit of the sample code shows the K_m used, the next 3 letters denote the cosurfactant used (eg, isp denotes isopropanol), and the last digit indicates the water concentration (in units of wt%).

Refractive Index Measurements

Refractive index measurements were performed using an Abbe 60/ED degree scale precision (5 decimal places) refractometer (Bellingham and Stanley, Sevenoaks, UK) at 298 ± 0.1 K. The refractive index of each sample was determined at 589.6 nm using a D line of a sodium lamp and at 546 and 436 nm employing a high-intensity mercury vapor lamp. The refractive index of each sample at 488 nm (the wavelength of the laser beam) was interpolated from plots of refractive index as a function of $1/\lambda^2$ using the Cauchy formula (9).

Determination of Partition Coefficient of Alcohol

The partition coefficient of each alcohol in IPM and water at 298 ± 1.0 K was determined in triplicate by measuring the refractive index at 589.6 nm of water (containing a given amount of alcohol and previously saturated with IPM) before and after partitioning with an equivalent volume of water saturated with IPM. The refractive index values of IPM-saturated water alone and together with a given amount of alcohol were plotted as a function of the percentage of alcohol in the water phase. The refractive index of water after partitioning was determined and the percentage of the alcohol in the water phase calculated, and from this value the log P was calculated. The error involved in this method of determining log P was estimated to be less than 1.0%.

Light-Scattering Studies

Light-scattering studies were performed using a Malvern 4700c spectrometer, equipped with a 75 mW argon-ion laser (polarized incident radiation of wavelength 488 nm), at 298 ± 0.1 K. The time-average intensity of light scattered by the sample at an angle θ (I_θ) was collected by averaging the individual readings of count rate obtained over a few minutes.

Microemulsions were centrifuged at 298 K at approximately 12,000 *g* for 1 hour and transferred to cylindrical glass-scattering cells. In no case did the centrifugation procedure destabilize the microemulsion. Note that the amount of scattering intensity did not change with time after preparation, with freshly prepared microemulsions exhibiting the same level of scattering as those examined a month or more after preparation. The disymmetry measured as I_{45}/I_{135} was found to be close to unity for all samples examined; therefore, the TILS experiments were usually restricted to scattering angles of 45°, 90°, and 135°. After attainment of temperature equilibrium (10 minutes), the intensity of the scattered light of the sample was routinely measured at the 3 angles and compared to that of a benzene standard. The scattering ratio of each sample relative to benzene at 90°, S_{90} (microemulsion), was determined and then corrected for background (cell and solvent scattering) by the following relationship:

$$S_{90(\text{microemulsion})} = \frac{S_{\text{sample}} - S_{\text{background}}}{S_{\text{benzene}}} \qquad \text{(Equation 1)}$$

The Rayleigh ratio at 90° of each sample relative to benzene was then calculated from

$$R_{90(\text{microemulsion})} = S_{90(\text{microemulsion})}\, R_{90(\text{benzene})} \qquad \text{(Equation 2)}$$

where the value of $R_{90(\text{benzene})}$ at 25°C and $\lambda = 488$ nm was 3.2×10^{-5} cm^{-1} (10).

Analysis of the Light-Scattering Data

The measured Rayleigh ratio of a sample at any scattering angle ($R\theta$) is described by

$$R\theta = K_1 \mathrm{M}c S(Q) P(Q) \qquad \text{(Equation 3)}$$

where $P(\mathrm{Q})$ is the particle-scattering form factor that depends on the shape of the particles and $S(\mathrm{Q})$ is the structure factor that depends on interactions in the system; M is the weight of the scattering unit, c is its concentration, and K_1 is an optical constant that for polarized incident light is given by (11)

$$K_1 = \frac{12\pi^3 n^4}{\lambda_0^4}\left(\frac{n_c^2 - n^2}{n_c^2 + 2n^2}\right)^2 \qquad \text{(Equation 4)}$$

where n is the refractive index of the solvent at 488 nm and n_c is the refractive index of the scattering unit at 488 nm.

For spherical particles, as in the case of the present study, the form factor $P(\mathrm{Q})$ is described by

$$P(\mathrm{Q}) = [3(\sin \mathrm{Q}R - \mathrm{Q}R \cos \mathrm{Q}R)/(\mathrm{Q}^3 R^3)]^2 \qquad \text{(Equation 5)}$$

At $\theta = 90°$, if the radius of the particles is much smaller than λ_0 (ie, $R << \lambda_0$), $P(\mathrm{Q})$ is equal to unity and Equation 3 becomes

$$R_{90} = K_1 \mathrm{M}c S(\mathrm{Q}). \qquad \text{(Equation 6)}$$

For a spherical microemulsion droplet, the weight of the scattering unit is described by

$$\mathrm{M} = 4\pi R_c^3 \rho_c \mathrm{N_A} / 3 \qquad \text{(Equation 7)}$$

where R_c is the radius of the droplet and ρ_c is its density. If the volume fraction of the droplet is

ϕ_c, then $c = \phi_c \rho_c$ and Equation 6 becomes

$$R_{90} = K_1 \phi_c R_c^3 S(90) \qquad \text{(Equation 8)}$$

or

$$\left[\frac{R_{90}}{K_1 \phi_c}\right] = R_c^3 S(90) \qquad \text{(Equation 9)}$$

where $S(\mathrm{Q}) = S(90)$ at $\theta = 90°$.

In order to calculate the interaction parameter, $S(\mathrm{Q})$, the Percus-Yevick hard-sphere model (8) was used. The expression proposed by Ashcroft and Lekner (12) for this parameter is

$$S(\mathrm{Q}) = \frac{1}{[1 - \mathrm{N_d} c(2\mathrm{Q}R_{\mathrm{HS}})]} \qquad \text{(Equation 10)}$$

where $\mathrm{N_d}$ is the number of droplets per cm^3 and

$$c(2\mathrm{Q}R_{\mathrm{HS}}) = -32\pi R_{\mathrm{HS}} \int_0^1 \left(\frac{\sin(2s\mathrm{Q}R_{\mathrm{HS}})}{2s\mathrm{Q}R_{\mathrm{HS}}}\right)\left(\alpha_0 + \beta s + \gamma s^3\right) s^2 ds \qquad \text{(Equation 11)}$$

where R_{HS} is the radius of the hard sphere and the coefficients α_0, β, and γ are defined by

$$\alpha_0 = \frac{(1 + 2\phi_{\mathrm{HS}})^2}{(1 - \phi_{\mathrm{HS}})^4} \qquad \text{(Equation 12)}$$

$$\beta = \frac{-6\phi_{\mathrm{HS}}(1 + 0.5\phi_{\mathrm{HS}})^2}{(1 - \phi_{\mathrm{HS}})^4} \qquad \text{(Equation 13)}$$

$$\gamma = \frac{0.5\phi_{HS}\left(1+2\phi_{HS}\right)^2}{\left(1-\phi_{HS}\right)^4}$$ (Equation 14)

The R_{HS} calculated in this manner is related to the droplet radius, R_c, by the following equation

$$R_{HS} = R_c + t$$ (Equation 15)

where t is the interaction parameter that is a measure of the effective hard-sphere interactions. The hard-sphere volume fraction can then be described by

$$\phi_{HS} = 4\pi R_{HS}^3 N_d / 3V_{total}$$ (Equation 16)

or

$$\phi_{HS} = \frac{R_{HS}^3 \phi_c}{R_c^3}$$ (Equation 17)

In order to analyze the data, an initial estimate of the radius of the droplet, R_c, is required. In this investigation, it was assumed that all the water and all the surfactant went to making up monodisperse, spherical w/o microemulsion droplets, and that the cosurfactant was distributed between the the oil, water, and interfacial regions. As the refractive index of the hydrophobic chains of the surfactant and cosurfactant are similar to that of the continuous phase (ie, IPM plus some alcohol cosurfactant), it was assumed that the scattering units were spheres (of radius R_c) consisting of the dispersed water, some alcohol cosurfactant, and the head groups of the interfacial surfactant-cosurfactant monolayer.

The total surface area of one scattering unit of radius R_c can be obtained from the product of the area occupied by one surfactant molecule, a_s, cosurfactant molecule, a_{co}, and g, the fraction of alcohol in the surfactant monolayers and the number of surfactant, n_s, and cosurfactant molecules, n_{co}, present in one droplet.

$$4\pi R_c^2 = n_{co} a_{co} g + n_s a_s$$ (Equation 18)

The total volume of one scattering unit is the sum of the volumes of the cosurfactant molecules contained in the disperse phase (where h is the fraction of alcohol in the disperse phase), all the water molecules together with the hydrophilic head groups of all the surfactant molecules, $v_{hg(s)}$, and cosurfactant, $v_{hg(co)}$, molecules present in one droplet

$$\frac{4}{3}\pi R_c^3 = g n_{co} v_{hg(co)} + n_s v_{hg(s)} + n_{dp} v_{dp} + n_{co} v_{co} h$$ (Equation 19)

where the subscript dp denotes the dispersed phase. R_c can now be calculated by combining Equations 18 and 19 as

$$R_c = 3\frac{\left(f_{co} g v_{hg(co)} + v_{hg(s)} + f_{dp} v_{dp} + f_{co} v_{co} h\right)}{f_{co} a_{co} g + a_s}$$ (Equation 20)

where f is the molar ratio of each component (the ratio of the number of molecules of each component relative to the number of surfactant molecules). The volume fraction of the scattering unit, ϕ_c, can then be calculated from

$$\phi_c = \frac{f_{co} g v_{hg(co)} + v_{hg(s)} + f_{dp} v_{dp} + f_{co} h v_{co}}{f_{co} v_{co} + v_s + f_{dp} v_{dp} + f_{cp} v_{cp}}$$ (Equation 21)

The analysis used was capable of calculating the surfactant and cosurfactant aggregation numbers, $N_{agg(s)}$ and $N_{agg(co)}$, respectively, and the number of particles per cm^3. An iterative method of calculation was used that altered the value of g (and hence h), keeping the area per surfactant molecule and cosurfactant molecule and t constant, to give the best fit to the experimental light-scattering data. The values of the various parameters used in the analyses are given in Table 1, together with the molecular volumes of the phospholipid and alcohols. In the program, the partition coefficient of the alcohol between IPM and water was used to calculate the fraction of the alcohol in the disperse phase (h), the continuous phase, and, consequently, the interfacial surfactant monolayer. The interaction parameter t in Equation 15 was taken to be the average effective length of the hydrocarbon chains of the phospholipid molecules, which was estimated to be 18 Å; that is, about 80% of the fully extended chain length of the hydrocarbon moiety (16). For each of the selected values of g and h, $S(Q)$ was found. K_1, R_c, and ϕ_c were calculated from Equations 4, 20, and 21, respectively. R_{90} values calculated from Equation 9 were compared with experimental R_{90} values, and iteration was continued until satisfactory agreement between calculated and experimental values was obtained.

RESULTS

Light-scattering studies were performed on a range of w/o lecithin-based microemulsions prepared using IPM at a total surfactant concentration of 25 wt% at various K_m, and containing water concentrations at 2 wt% intervals from 2 wt% up to the limit of the area of microemulsion existence. Figures 1A and 2A show the variation in the measured R_{90}, with water concentration for 2 of the cosurfactants, n-propanol and n-butanol, at each K_m examined; Figures 1B and 2B show the variation in the hard-sphere diameter of the microemulsin droplets with ϕ_c. As little light scattering was seen at water concentrations of less than 8 wt%, these results are not shown. Tables 2-7 give the complete set of data obtained from the analyses of the light-scattering data for all the alcohols studied. The notation used for the sample in the tables consists of the K_m followed first by the 3-letter code for the alcohol and then the concentration (% w/w) of water contained in the system. For the purposes of brevity, the results obtained with each alcohol are not discussed in turn; rather, the general trends are described and comparisons drawn.

Table 1. Parameters used in the analysis of the TILS data.

Component	Molecular weight (g)	Molecular volume (Å^3)	Volume of the head group (Å^3)	Volume of the hydrocarbon moiety(Å^3)	Head group area (Å^2)	Partition coefficient (log P)
Lecithin	765[a]	1295[b]	380[b]	915[b]	70[c]	-
IPM	271	529	-	-	-	-
n-Propanol	60	-	17	108	19	-0.47
Isopropanol	60	-	17	109	19	-0.88
n-Butanol	74	-	17	135	19	0.20
sec-Butanol	74	-	17	136	19	-0.08
Isobutanol	74	-	17	136	19	0.19
tert-Butanol	74	-	17	139	19	-0.33
n-Pentanol	88	-	17	162	19	0.87
Water	18	30	-	-	-	-

[a] *Reference 13.*

[b] *Reference 14.*

[c] *Reference 15.*

Table 2. Analysis of TILS data for w/o lecithin-based microemulsions containing n-propanol (ipr). The first digit of the sample code shows the K_m used, ipr denotes isopropanol, and the last digit indicates the water concentration. h is the fraction of alcohol in the disperse phase, g is the fraction of alcohol in the surfactant monolayer, R_{90} is the Rayleigh ratio at 90° of the sample relative to benzene, d_{HS} is the hard-sphere diameter of the microemulsion droplets, ϕ_{HS} is the hard-sphere volume fraction of the microemulsion droplet, $N_{agg(s)}$ is the surfactant aggregation number, $N_{agg(co)}$ is the aggregation number of the cosuurfactant, N/cm^3 is the number of microemulsion particles per cm^3.

Sample	h	g	R_{90} (10^4 cm^{-1})	d_{HS} (nm)	ϕ_{HS}	$N_{agg(s)}$	$N_{agg(co)}$	N/cm^3 (10^{-17})
1npr9	0.49	0.34	0.467	12.1	0.54	146	638	5.8
-11	0.51	0.31	0.530	13.4	0.52	206	824	4.1
-13	0.54	0.28	2.901	15.1	0.51	302	1066	2.8
-14	0.57	0.24	5.130	16.7	0.49	418	1263	2.1
1.5npr11	0.48	0.36	0.928	12.5	0.54	193	582	5.4
-14	0.54	0.28	2.750	15.0	0.52	353	837	2.9
-16	0.55	0.26	3.560	16.2	0.52	439	988	2.4
-18	0.60	0.19	7.740	18.9	0.51	718	1190	1.5
-20	0.61	0.18	9.480	20.3	0.52	883	1350	1.2
1.77npr11	0.47	0.37	0.499	12.1	0.55	188	505	5.9
-14	0.52	0.30	2.170	14.4	0.53	332	714	3.3
-16	0.53	0.29	2.750	15.5	0.53	407	840	2.7
-18	0.58	0.23	5.180	17.6	0.52	609	995	1.8
-20	0.59	0.22	6.000	18.8	0.54	730	1136	1.5
-22	0.61	0.19	7.900	20.5	0.54	929	1272	1.2
-24	0.64	0.14	11.90	22.9	0.54	1299	1350	0.9
-25	0.65	0.14	12.70	23.7	0.55	1435	1399	0.8
1.94npr11	0.50	0.33	0.358	12.6	0.52	228	498	5.0
-14	0.49	0.34	1.470	13.8	0.54	287	642	4.0
-16	0.52	0.30	2.470	15.2	0.54	394	776	2.9
-18	0.56	0.25	4.280	17.1	0.53	563	913	2.0
-20	0.58	0.23	5.240	18.4	0.54	702	1042	1.6
-22	0.61	0.19	7.600	20.3	0.54	927	1156	1.3
-24	0.61	0.18	8.160	21.5	0.56	1083	1287	1.1
-25	0.63	0.16	9.920	22.7	0.56	1274	1313	0.9

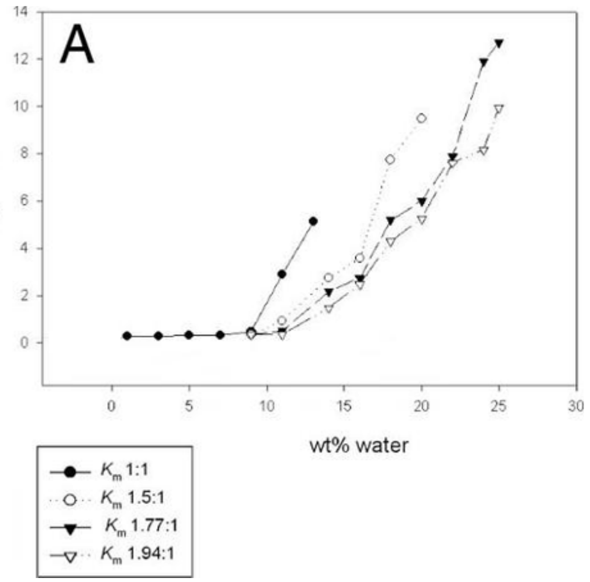
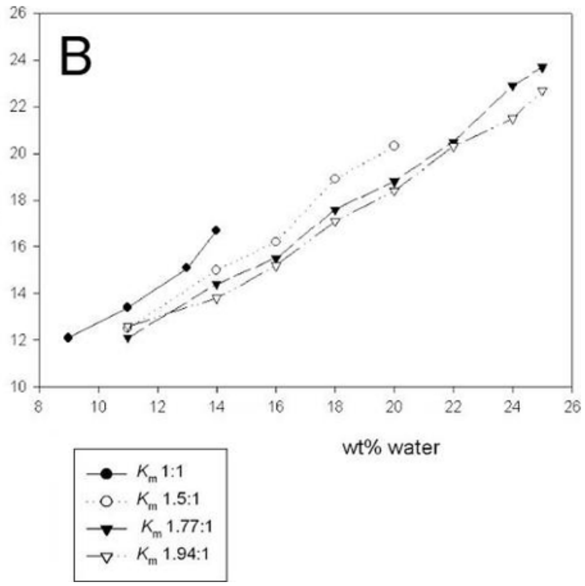


Figure 1. Variation in observed R90 values as a function of water concentration for lecithin/n-propanol microemulsions.



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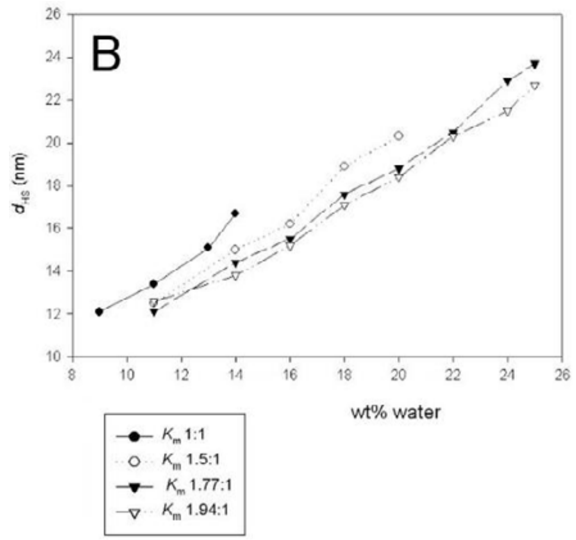

Figure 2. Variation in observed R90 values as a function of water concentration for lecithin/n-butanol microemulsions.

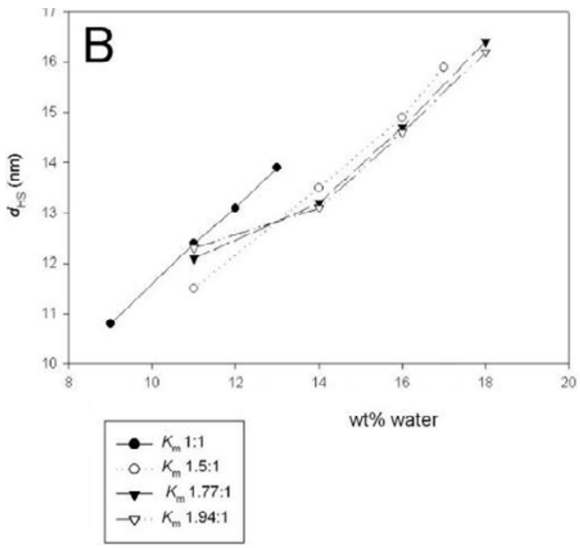

Figure 2. Variation in hard-sphere diameters with volume fraction of the scattering unit for lecithin/n-butanol microemulsions.

At Low Concentrations of Water (<9 wt%)

Regardless of the K_m and nature of the cosurfactant used in preparing the microemulsions, there was little or no change in scattering (ie, R_{90}), with increasing water content at water concentrations lower than a minimum of approximately 9 wt% (with the absolute value of this critical water concentration varying a little with K_m). The lack of an increase in scattering at low added water concentrations is suggestive of the presence of a cosolvent system rather than a particulate microemulsion system. It is also possible, however, that the microemulsion particles in these low-water-content samples are simply "invisible" in the light-scattering experiments because of the absence of any refractive index difference between the continuous and disperse phases (3,17). In order to discount this possibility and thereby confirm the existence of a cosolvent system in these samples, we thus prepared a range of microemulsions containing 10 wt% potassium iodide (a water-soluble material with a high refractive index) in the aqueous phase in an attempt at low water concentrations to increase the refractive index difference of the dispersed phase, thereby avoiding any index matching. In no case, however, was any increase in scattering observed for the low-water-content systems, thereby confirming that these systems are indeed cosolvents and not w/o microemulsions.

Simple calculations assuming a level of hydration per phospholipid of approximately 12 water molecules (14) and 2 water molecules per cosurfactant associated with the droplet indicated that, at water levels of less than 9 wt%, there was insufficient water available to hydrate the head groups. Only when this level of head group hydration had been achieved were microemulsions formed. This result is interesting, as it seems to suggest that free/unbound water is required before formation of a w/o lecithin-based microemulsion droplet is possible. This observation also explains the weak correlation observed between water concentration required for w/o microemulsion formation and the K_m, as the higher K_m systems contain a greater concentration of phospholipid and therefore require a greater amount of water to satisfy the requirement for head group hydration. There is only a weak correlation between the water concentration required for w/o microemulsion formation and K_m because of the large intervals (ie, 2 wt%) in water concentration tested in the study; smaller concentration intervals would be required to determine the exact water concentration required for microemulsion formation.

At High Concentrations of Added Water (>8 wt%)

Figures 1A and 2A show that above the minimum water concentration, there was a nonlinear increase in R_{90} on increasing the amount of water present. Moreover, at a given value of water incorporation, R_{90} decreased as the K_m increased from 1:1 to 1.94:1 (ie, as the proportion of alcohol in the surfactant/cosurfactant mixture decreased). A similar trend in results was seen for all the alcohols tested (see Tables 2-7).

Table 3. Analysis of TILS data for w/o lecithin-based microemulsions containing isopropanol (isp). Details as in Table 2.

Sample	h	g	R_{90} (10^4 cm^{-1})	d_{HS} (nm)	ϕ_{HS}	$N_{agg(s)}$	$N_{agg(co)}$	N/cm^3 (10^{-17})
O1ipr9	0.56	0.35	0.851	12.4	0.54	156	700	5.4
-11	0.61	0.31	1.980	14.2	0.52	244	948	3.5
-13	0.63	0.27	3.500	15.9	0.52	346	1204	2.5
-14	0.66	0.24	5.360	17.2	0.51	449	1391	1.9
O1.5ipr9	0.53	0.39	0.522	11.6	0.56	148	494	6.9
-11	0.57	0.35	1.120	13.0	0.54	216	642	4.8
-14	0.62	0.28	2.830	15.4	0.53	375	908	2.8
-16	0.66	0.24	4.870	17.2	0.52	533	1100	2.0
-18	0.69	0.21	7.150	19.1	0.52	721	1286	1.4
-20	0.71	0.18	9.820	20.9	0.53	946	1463	1.1
O1.77ipr9	0.62	0.29	0.349	12.9	0.50	249	512	4.4
-11	0.55	0.37	0.922	12.6	0.55	209	553	5.3
-14	0.62	0.29	2.600	15.1	0.53	377	781	2.9
-16	0.64	0.26	3.850	16.5	0.53	496	924	2.2
-18	0.68	0.22	6.010	18.4	0.53	684	1074	1.6
-20	0.70	0.19	8.100	20.1	0.53	884	1210	1.3
-22	0.74	0.15	11.200	22.2	0.54	1184	1316	0.9
-24	0.75	0.13	13.700	24.0	0.55	1476	1407	0.8
O1.94ipr11	0.55	0.38	0.698	12.4	0.55	203	510	5.6
-14	0.61	0.30	2.280	14.7	0.53	359	714	3.2
-16	0.62	0.28	3.110	16.0	0.54	454	840	2.5
-18	0.68	0.22	5.820	18.2	0.53	681	979	1.7
-20	0.71	0.18	8.070	19.9	0.53	898	1091	1.3
-22	0.72	0.17	9.160	21.3	0.55	1077	1209	1.1
-24	0.74	0.15	11.100	23.0	0.56	1326	1306	0.9

This trend of increasing R_{90} on increasing the amount of water continued up to the highest water concentration studied for each of the systems tested (which was as close to the phase boundary as possible). As the amount of surfactant/cosurfactant in the system was constant, this increase in size can reasonably be attributed to a growth in size of the microemulsion droplets.

Figures 1B and 2B show the variation in the hard-sphere diameter (d_{HS}) versus ϕ_c calculated for each K_m. At all K_m, d_{HS} was found to increase in an almost linear fashion with increasing water content; this trend was observed for all the alcohols investigated (see Tables 2-7. It is clear from the results that, for an equivalent water concentration, the values obtained for d_{HS} tended to be slightly smaller at the higher K_m, values, with the greatest difference in size being seen to occur between K_m values of 1:1 and 1.5:1. This observation was not surprising because the greatest change in the amount of alcohol present occurs between these two K_m values.

Table 4. Analysis of TILS data for w/o lecithin-based microemulsions containing n-butanol (nbu). Details as in Table 2.

Sample	h	g	R_{90} (10^4 cm^{-1})	d_{HS} (nm)	ϕ_{HS}	$N_{agg(s)}$	$N_{agg(co)}$	N/cm^3 (10^{-17})
1nbu9	0.24	0.38	0.362	10.8	0.50	113	442	7.5
-11	0.26	0.33	1.500	12.4	0.48	180	610	4.8
-12	0.27	0.31	2.060	13.1	0.47	215	693	4.0
-13	0.27	0.29	2.850	13.9	0.46	260	787	3.3
1.5nbu11	0.23	0.40	0.637	11.5	0.51	161	439	6.4
-14	0.26	0.34	1.690	13.5	0.50	266	621	3.9
-16	0.27	0.30	3.140	14.9	0.49	364	757	2.9
-17	0.28	0.27	4.360	15.9	0.49	449	832	2.3
1.77nbu11	0.26	0.34	0.621	12.1	0.49	209	411	5.3
-14	0.25	0.35	1.660	13.2	0.51	266	549	4.2
-16	0.27	0.31	2.800	14.7	0.50	367	663	3.0
-18	0.29	0.25	4.880	16.4	0.49	524	777	2.1
1.94nbu11	0.27	0.31	0.387	12.3	0.48	233	388	4.9
-14	0.24	0.37	1.480	13.1	0.51	261	514	4.4
-16	0.27	0.31	2.750	14.6	0.50	374	619	3.1
-18	0.29	0.26	4.510	16.2	0.50	518	720	2.2

The advantages of using TILS analysis combined with a model for hard-sphere interactions for the investigation of the microemulsion systems under study is that, in addition to the effective hard-sphere radius, information can be obtained about the amount of phospholipid (surfactant) and alcohol (cosurfactant) associated with the droplet in the interfacial region of the dispersed aqueous phase and with the distribution of the cosurfactant between the various phases. The detailed results obtained from the analyses of the light-scattering data at water concentrations above the mininum required for microemulsion formation are given in Tables 2-7, from which the following generalizations can be made.

Table 5. Analysis of TILS data for w/o lecithin-based microemulsions containing sec-butanol (sbu). Details as in Table 2.

Sample	h	g	R_{90} (10^4 cm^{-1})	d_{HS} ^{b} (nm)	ϕHS	$N_{agg(s)}$	$N_{agg(co)}$	N/cm^3 (10^{-17})
O1sbu9	0.37	0.33	0.269	12.3	0.47	174	589	4.9
-11	0.34	0.38	1.160	12.4	0.51	168	653	5.1
-13	0.37	0.33	2.430	14.1	0.49	254	863	3.4
-14	0.38	0.31	3.420	15.0	0.49	311	983	2.8
O1.5sbu9	0.33	0.39	0.090	11.2	0.51	149	403	6.9
-11	0.36	0.35	0.586	12.6	0.49	221	526	4.7
-14	0.36	0.34	2.180	14.0	0.51	294	694	3.5
-16	0.37	0.32	3.210	15.3	0.51	384	838	2.7
-18	0.39	0.28	4.810	16.9	0.51	515	997	2.0

Increasing Water Concentration at Any Given K_m

As expected, as the water concentration increased above the minimum level, the particle size of the microemulsion droplet, d_{HS}, increased, while the number of droplets present per cm^3 decreased. This increase in d_{HS} was the result of several effects; for example, as the water concentration increased, the fraction of alcohol in the water phase increased, although the fraction of alcohol in the interfacial region decreased. Overall, however, the trend was for an increase in the *total* amount of alcohol associated with the droplet. In addition, the number of lecithin molecules per droplet increased, which also led to an increase in d_{HS}. Indeed, the number of lecithin molecules associated with the droplet increased at a faster rate than the number of cosurfactant molecules. Furthermore, not suprisingly, as the water concentration increased, the amount of water associated with the droplet increased. Together, these factors led to an increase in droplet size on increasing water concentration.

Altering the K_m at a Fixed Amount of Water

For a constant amount of added water, as the K_m increased (ie, as the amount of surfactant in the surfactant/cosurfactant mixture increased), the particle size of the droplet, d_{HS}, tended to decrease. This trend was not as clear-cut as the previous one, although in most cases there was a definite reduction in size ongoing from a K_m of 1:1 to 1.5:1, with any further reduction in size on increasing K_m being much less pronounced. Correspondingly, there was also a slight trend toward an increase in the number of droplets present per cm^3 as the K_m increased. Furthermore, as the K_m increased, the amount of water associated with each droplet decreased. The reduction in size observed on increasing K_m corresponded with a decrease in the fraction of total alcohol dispersed in the water phase and an increase in the fraction of alcohol in the interfacial region. Overall, the trend was for the fraction of alcohol associated with the droplet to increase as the K_m increased. Correspondingly, for an equivalent water concentration, there was a decrease in the number of phospholipid molecules associated with the droplet, although the number of lecithin molecules associated with the droplet decreased at a slower rate than the number of cosurfactant molecules. In combination, these factors led to only a very small decrease in droplet size.

Varying the Nature of the Alcohol

Because the proportions of alcohol in the dispersed phase and the continuous phase were fixed by the use of log P in the modelling, it was no surprise to observe that when considering an equivalent K_m and water concentration, the amount of alcohol in the dispersed phase followed the order of log P values: isopropanol > n-propanol > tert-butanol > sec-butanol > n-butanol. Furthermore, the total amount of alcohol associated with the droplet also followed the same trend and decreased in the order isopropanol > n-propanol > tert-butanol > sec-butanol > n-butanol. With respect to the number of particles present, these increased in the order n-butanol = sec-butanol > n-propanol > tert-butanol > isopropanol. Interestingly, however, the overall differences in particle size due to changing the nature of the alcohol used were relatively small; for example, at a water concentration of 14 wt% and a K_m of 1.77, the calculated particle sizes fell within the range 12.8 to 15.1 nm.

General Comments

Interestingly, in spite of increasing the amount of aqueous phase and the varying nature of the cosurfactant, there was no increase in the hard-sphere-volume fraction of the droplets, which remained constant at 0.50 to 0.55. This is explained by the fact that any increase in the amount of oil associated with the droplets is offset by an increase in size of the particle core and by a corresponding expansion of the associated surfactant/cosurfactant interfacial monolayer. At the phase boundary for most of the systems studied, there was between 0.10 and 0.15 volume fraction of oil associated with the particle. Results by Eastoe et al (18,19) using small-angle neutron-scattering data gave similar volume fractions of oil associated with lecithin-based microemulsions, namely 0.05 to 0.20, although it should be noted that these experiments were performed at the phase boundary of the w/o microemulsion region, where different conditions may prevail.

DISCUSSION

Before discussing the results obtained in the present study, it is worth commenting on the suitability of the Percus-Yevick model to interpret the experimental data because of the requirement to make assumptions about the various parameters used in the analysis; in particular, estimates had to be made of the effective head group area of both the lecithin and alcohols used because these values were not determined experimentally in the present study, together with the effective average length of the hydrophobic chains of the lecithin molecules in an aggregate. Because the interpretation of the data using the Percus-Yevick model involves an iterative fitting process, it was essential that meaningful values of the input parameters were used to ensure that the results obtained had physical relevance. A short study was performed to establish how critical the values of the parameters are in influencing the results obtained. In this study the effective lecithin and alcohol head group areas, the log P (determined experimentally in the present study), and the value t were altered in the analysis of systems containing n-propanol and n-butanol at each K_m investigated. The area per lecithin molecule was altered (in 5-$Å^2$ stages) over the range 50 to 75 $Å^2$ because the estimates in the literature for the minimal area per molecule of lecithin generally fall within this region (15,20,21). For the cosurfactants studied, a much larger area per molecule of 30 $Å^2$ was used because there is at least one report in the literature that short-chain alcohols, when present in a mismatched monolayer (ie short-chain alcohols in combination with longer chain molecules), exhibit a larger area per molecule because of thermal motions of the chain (22). Although log P was experimentally determined in the present study, its value was altered to see what effect it had on the results, with values of ±10% of the experimentally determined figure being used. It should be noted, however, that this error was well outside the estimated error of ±1% for the technique. The value of t was altered (in 1-Å steps) in the range 16 to 19 Å because the effective length of a hydrocarbon chain in an aggregate is variously quoted as being 70% to 85% of its fully extended chain length. The results of these various studies showed that there were no differences in the overall trends in the data presented in Tables 2-7, and only (at most) 10% differences in the calculated sizes of the microemulsion droplets.

The results of the present study have several important implications for the use of microemulsions as drug-delivery vehicles (although, in respect to their use as drug-delivery vehicles, it must be remembered that microemulsions cannot be considered inert because the addition of another component, such as a drug, may alter their equilibrium).

Table 6. Analysis of TILS data for w/o lecithin-based microemulsions containing tert-butanol (tbu). Details as in Table 2.

Sample	h	g	R_{90} (10^4 cm^{-1})	d_{HS} (nm)	ϕ_{HS}	$N_{agg(s)}$	$N_{agg(co)}$	N/cm^3 (10^{-17})
O1tbu9	0.44	0.36	0.515	12.5	0.49	175	645	4.9
-11	0.43	0.37	1.570	13.2	0.51	200	760	4.3
-13	0.47	0.31	3.640	15.2	0.49	325	1031	2.6
-14	0.49	0.28	5.260	16.3	0.48	406	1175	2.1
-16	0.51	0.26	7.340	17.9	0.49	528	1413	1.6
O1.5tbu11	0.40	0.42	0.838	12.2	0.53	187	537	5.5
-14	0.44	0.35	2.280	14.4	0.51	313	761	5.3
-16	0.47	0.32	3.590	15.9	0.51	424	923	2.5
-18	0.50	0.27	5.880	17.7	0.51	595	1103	1.8
O1.77tbu9	0.45	0.35	0.173	12.1	0.49	210	422	5.2
-11	0.42	0.38	0.910	12.6	0.51	227	500	4.9
-14	0.45	0.34	2.410	14.4	0.51	338	672	3.3
-16	0.46	0.33	3.080	15.5	0.52	417	795	2.7
-18	0.47	0.31	4.060	16.7	0.53	520	928	2.1
-20	0.50	0.27	5.640	18.3	0.53	675	1071	1.7
-22	0.52	0.24	7.560	20.0	0.54	881	1209	1.3
O1.94tbu9	0.46	0.32	0.230	12.3	0.48	230	396	4.9
-11	0.42	0.39	1.040	12.4	0.51	222	462	5.1
-14	0.45	0.33	2.510	14.4	0.51	352	627	3.3
-16	0.42	0.39	2.030	14.6	0.54	348	715	3.3
-18	0.48	0.30	4.220	16.8	0.52	542	860	2.1
-20	0.50	0.27	5.660	18.3	0.53	694	983	1.7
-22	0.51	0.25	6.390	19.4	0.54	819	1112	1.4

However, the investigation of a simpler drug-free system does greatly aid in the understanding of these systems and will obviously aid in understanding the influence of a drug in the more complex drug-containing systems.

Table 7. Analysis of TILS data for w/o lecithin-based microemulsions containing n-pentanol (npe). Details as in Table 2.

Sample	h	g	R_{90} (10^4 cm^{-1})	d_{HS} (nm)	ϕ_{HS}	$N_{agg(s)}$	$N_{agg(co)}$	N/cm^3 (10^{-17})
1npe9	0.08	0.34	0.358	10.6	0.44	122	358	7.0
-10	0.08	0.31	0.531	11.4	0.43	154	420	5.6
1.5npe9	0.09	0.23	0.122	11.6	0.40	208	277	5.0
-10	0.09	0.28	0.448	11.6	0.42	200	323	5.2
1.77npe11	0.09	0.27	0.432	12.2	0.43	243	317	4.5
-13	0.09	0.25	1.100	13.3	0.43	306	392	3.5
-14	0.08	0.37	1.540	12.8	0.48	253	455	4.4
1.94npe11	0.09	0.23	0.317	12.4	0.42	272	283	4.2
-13	0.08	0.34	0.992	12.5	0.47	253	385	4.5
-14	0.08	0.30	1.450	13.4	0.46	315	417	3.6
-15	0.07	0.37	1.900	13.2	0.49	284	468	4.1

The observation that a minimum amount of water was required before the formation of microemulsion droplets was not surprising and is a function of the solubility of the surfactant in the alcohol cosurfactant/oil solution. What is perhaps surprising, however, is the amount of water the systems can incorporate before forming a microemulsion. The fact that a minimum amount of water is required to form a w/o microemulsion may have very important implications for the use of such systems as sustained release delivery vehicles. Indeed, in a preliminary study, we have noted that the rate of release of sodium salicylate from a w/o microemulsion depends on whether the system is a cosolvent system or a microemulsion.

Above the minimal water concentration, TILS analysis has shown that, as expected, increasing the volume fraction of water results in the formation of larger particles and, as a consequence, an increase in the total interfacial area between the oil and water phase. This result may influence the amount of surface-active drug that can be incorporated into the system, although it should be remembered that a surface-active drug may itself alter the phase diagram of the system, as has been previously shown (23). Furthermore, variance in the amount of alcohol associated with the droplet (in particular the amount present in the disperse phase) may have important implications for the amount of drug it is possible to add. Work is currently underway to determine the influence of drugs on microemulsion droplets.

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