The Effect of Solubilized Water on the Size and Shape of Lecithin Micelles in an Apolar Solvent

RICHARD M. EVANS, DAVID ATTWOOD*, SARAH M. CHATHAM† AND STEPHEN J. FARR

*Welsh School of Pharmacy, University of Wales College of Cardiff, Cardiff CF1 3XF, *Pharmacy Department, The University, Oxford Road, Manchester M13 9PL, †Lilly Research Centre, Erl Wood Manor, Windlesham, Surrey GU20 6PH, UK*

Abstract—The changes in the size and shape of the micelles of soya lecithin in trichlorotrifluoroethane following the solubilization of increasing quantities of water have been examined by dynamic and static light scattering and viscometric techniques. Micelles in systems with water/lecithin molar ratios, R, of less than 2·6 were oblate and exhibited no significant changes in asymmetry or aggregation number with changes in the amount of solubilized water. Spherical micelles were evident in systems with R = 2·6 whereas prolate micelles were present in solutions of higher water content ($3·5 \leq R \leq 5·2$). An increase of R in these systems caused a decrease in the axial ratio and an increase in the aggregation number of the micelle.

Amphiphilic molecules in apolar solvents may associate into reversed or inverted micelles which have been visualized as spherical units having a shell composed of hydrocarbon tails surrounding a core of polar headgroups (Eicke & Christen 1974). Small quantities of water may be solubilized within the micelle interior in the form of discrete pools confined by the hydrophilic polar headgroups of the surfactant molecules. Such structures have an increasingly important role in a variety of chemical and biochemical enzymic reactions for the catalytic conversion of water insoluble materials (Fendler & Fendler 1975; Hilhorst et al 1983, 1984; Luisi & Laane 1986). Hydrated, reverse micelles of lecithin (phosphatidylcholine) are also extensively employed to provide information about the structure and function of biomembranes (Montal 1984). For example, the interaction of water with phospholipid choline headgroups has been studied by spectroscopic investigation of micellar solutions of lecithin in a wide variety of solvents such as benzene, carbon tetrachloride and cyclohexane (Kumar & Raghunathan 1986).

Our interest in the association of lecithin in apolar media resides in the production of therapeutic aerosols from pressurized metered dose inhalers (MDIs). These are major devices used in the delivery of drugs to the respiratory tract and traditionally are formulated as suspensions of micronized drug in a chlorofluorocarbon (CFC) blend (Morén 1981). Despite the identification and optimization of factors contributing to the production of a respirable aerosol cloud (Polli et al 1969; Morén 1978a, b; Newman et al 1982, 1984; Havel et al 1985), most of the output immediately after actuation is aerodynamically unfavourable for pulmonary deposition (Morén & Andersson 1980). The design of elaborate actuators to encourage efficient breakup and/or collection of non-respirable aerosol droplets (Byron et al 1989) is restricted by the presence of micronized drug particles within the formulation. Homogeneous systems, where the drug is dissolved in the propellant blend, offer several advantages in this respect which should lead to an improvement in design and subsequent therapeutic efficacy of this dosage form (Dalby & Byron 1988). We proposed,

Correspondence to: S. J. Farr, The Welsh School of Pharmacy, UWCC PO Box 13, Cardiff CF1 3XF, UK.

therefore, to evaluate the potential of lecithin micelles as centres for the solubilization of drugs within CFC blends.

This preliminary investigation examines the association of soya lecithin in a model CFC solvent, trichlorotrifluoroethane. This has similar properties to the commercially used propellants (such as trichlorofluoromethane and dichlorodifluoromethane) but with a higher boiling point making it more suitable for experimental study. The effect of solubilized water on the micellar size and shape was examined by light scattering and viscometric techniques.

Materials and Methods

Soya lecithin was obtained by chromatographic separation (Martin et al 1978) of a 95% commercial source (Epikuron 200; Lucas Meyer, Germany) and stored in nitrogen flushed sealed glass containers at -20 C. Samples were dried immediately before use by repeatedly dissolving in specially dried methanol (Karl Fischer reagent grade, BDH Chemicals, UK) and evaporated to constant weight. The absolute water content of all prepared solutions was determined using a Karl Fischer titrator AF5 (Baird & Tatlock, UK). Trichlorotrifluoroethane (P113; ICI Mond Division, UK) was stored over self-indicating molecular sieve type 4A (BDH Chemicals, UK). Iodine (BDH Chemicals, UK) was used as received. Water was double distilled in an all-glass apparatus.

Iodine solubilization
Samples of a stock solution (2 mmol L⁻¹) of iodine in P113 were added to solutions of soya lecithin in P113 and the final volumes adjusted with solvent to produce a range of concentrations between 10^{-3} and 10^{-1} g L⁻¹. The solutions were shaken in a water bath at $25 \pm 0·1$ C for 24 h and assayed spectrophotometrically at 360 nm for the appearance of the micellar solubilized I_3^- ion (Ross & Baldwin 1966).

Static and dynamic light scattering
Measurements were made at $25 \pm 0·1$ C using a Malvern 7027 digital autocorrelator (Malvern Insturments, UK) equipped with a 3W argon ion laser (Coherent Innova 90)

operating at 488 nm. Dynamic light scattering measurements were made in the single clipped homodyne mode using 60 linearly spaced channels with a far point delay of 1024 sample times. Calibration of the instrument for static light scattering measurements was with a dust-free sample of benzene (Analar; BDH Chemicals, UK). All measurements were at 90° to the incident beam, initial experiments demonstrating no significant dissymmetry of the scattered light. All solutions for light scattering were repeatedly filtered through 0·1 μm membrane filters (Millipore, UK) until particulate free. The refractive index measurements were measured using an Abbs 60 precision refractometer (Bellingham & Stanley, UK) at 25±0·1°C.

Viscosity measurements

A capillary viscometer with a solvent flow time of approximately 150 s was used at 25±0·01°C.

Measurements of partial specific volume

Density measurements were performed at 25±0·01°C using a density meter DMA 60 and density measuring cell DMA 602 (Anton Paar, UK). The partial specific volume, $\bar{v}$, of the micelles was calculated from:

$$d_s = d + (1 + \bar{v}d)C \tag{1}$$

where d_s and d are the densities of solution and solvent respectively and C is the concentration.

Preparation of solubilized systems

Solutions containing various molar ratios of water to lecithin (R) were prepared by the addition of the required amount of water to stock solutions (20 g L⁻¹) of lecithin in P113 using a 20 μL air displacement pipette. All solutions were equilibrated by shaking in a water bath at 25±0·1°C for at least 24 h before measurement.

Results and Discussion

Effect of solubilized water on micellar size

The iodine solubilization data for soya lecithin in P113 are presented as a plot of absorbance at 360 nm as a function of log concentration (Fig. 1). There is clearly no sharp inflection in this plot which can be identified with a critical micellar concentration (CMC) analogous to aqueous systems. The
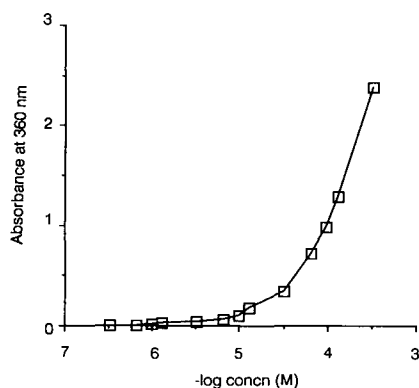
Fig. 1. The appearance of I_3^- in P113 solutions of iodine as a function of log concentration of added soya lecithin.
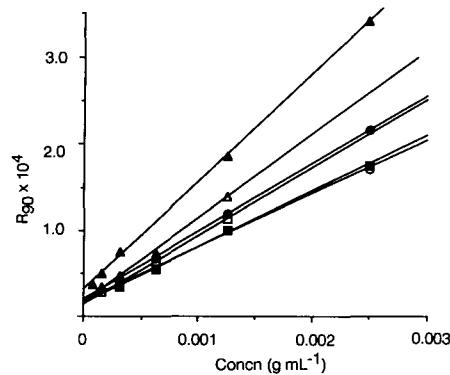
Fig. 2. Rayleigh ratio, R_{90}, as a function of concentration of soya lecithin in P113 containing various amounts of solubilized water. Symbols refer to water/lecithin molar ratios, R, of 0·9 (●), 1·75 (○), 2·61 (■), 3·46 (□), 4·32 (▲) and 5·17 (△).

progressive increase of absorbance with concentration over this concentration range is indicative of an open, rather than micellar association pattern. At much higher concentrations (Fig. 2), the intensity of scattered light was a linear function of lecithin concentration suggesting no significant change of aggregate size with concentration. The precision of the light scattering technique was insufficient to permit meaningful measurement at $C < 10^{-4}$ g mL⁻¹, and hence it was not possible to confirm the association behaviour at low concentration indicated by the solubilization data. Although it is clear that a more detailed study of lecithin/P113 system is required, the results of this present study do show qualitative agreement with the pattern of association proposed by Eicke (1980) in which linear units are formed by the stepwise growth of small subunits at low concentrations and subsequently reorganise to form reversed micelles.

It is also evident from the relative slopes of the plots in Fig. 2 that the size of lecithin micelles alters as a function of the molar ratio water:lecithin (R). No water was added to systems of R = 0·9, this value merely reflecting the residual water present in the lecithin sample used to prepare the solutions. Incremental addition of water to R = 5·17 produced isotropic micellar systems of increasing viscosity. A small further addition of water (R = 5·99) resulted in separation of the system into two distinct phases, one predominantly comprising of the dispersed phase. Kumar et al (1984) reported R values of 15–16 for lecithin/water systems in three different non-polar solvents before the advent of phase separation although, interestingly, systems in cyclohexane were shown to undergo a phase transition at R = 5–6 from an isotropic micellar phase to a highly viscous, anisotropic liquid crystalline phase. The weight average micellar molecular weights, M, of lecithin/P113 systems containing a range of concentrations of solubilized water were derived from:

$$KC/\Delta R_{90} = 1/M + 2BC \tag{2}$$

where K is the optical constant, ΔR_{90} is the Rayleigh ratio of the solution in excess of that from the solvent and B is an interaction coefficient. It should be noted that in all the physicochemical techniques, extrapolation was to infinite dilution rather than to an 'operational CMC'. In view of the low concentration over which any primary aggregates may

Table 1. The influence of solubilized water on the micellar properties of soya lecithin in trichlorotrifluoroethane.

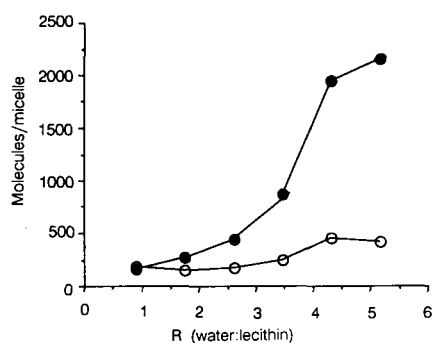| Mol water/ mol lecithin	$M \times 10^{-5}$	D_0 $m^2 s^{-1}$ $\times 10^{11}$	$[\eta]$	Ellipsoid shape	Axial ratio	a nm	δ g solvent / g solute
0·90	1·36	6·51	3·97	Oblate	1·41	3·87	0·82
1·75	1·17	6·63	4·40	Oblate	1·40	3·82	1·08
2·61	1·30	6·83	2·78	Sphere	1·00	4·70	1·33
3·46	1·92	6·72	8·56	Prolate	0·45	7·69	2·60
4·32	3·45	6·18	28·01	Prolate	0·13	14·45	2·76
5·17	3·19	5·91	79·70	Prolate	0·07	18·34	3·69

FIG. 3. The number of molecules per micelle of soya lecithin (O) and water (●) as a function of the water/lecithin molar ratio, R, in lecithin/P113/water solubilized systems.
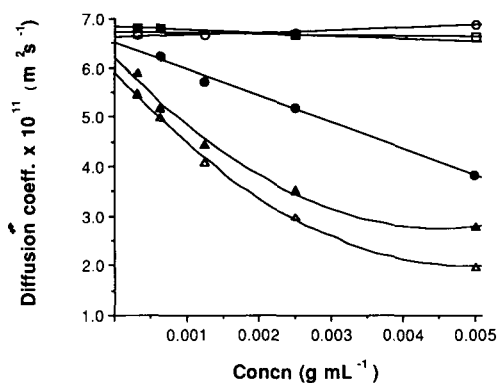

FIG. 4. Apparent diffusion coefficient, as a function of concentration, C, for solubilized systems of soya lecithin in P113 containing water:lecithin molar ratios, R = 0·9 (●), 1·75 (O), 2·61 (■), 3·46 (□), 4·32 (▲) and 5·17 (△).

be formed, this procedure was not thought to introduce any significant error in the micellar parameters determined.

Table 1 shows that the solubilization of water resulted in an increase of molecular weight as might be expected. It is instructive to consider whether this increase is attributable solely to the incorporation of water into the micelles. In view of the extremely low solubility of water in P113 ($< 5\cdot2 \times 10^{-5}$ g mL^{-1}), it may be assumed that most of the water in the system is present within the micellar phase. If it is further assumed that the composition of the micelle is identical to that of the system as a whole, then the mean composition of the micelle may be readily calculated. Fig. 3 shows the number of molecules of water and of lecithin in the micelle plotted as a function of R. It is evident from this figure that low quantities of water ($R \leq 2\cdot6$) can be solubilized without changes in the aggregation number of the lecithin micelles. However, for higher values of R the micellar growth is due not only to the presence of the water molecules but also to an increased number of lecithin molecules per micelle, i.e. there is a restructuring of the micelle to accommodate the solubilized water.

Effect of solubilized water on micellar shape

Apparent diffusion coefficients, D, of micelles in systems with $R \leq 2\cdot6$ were independent of concentration over the concentration range examined (Fig. 4). The pronounced non-linear decrease with C in systems of higher water content is indicative of micellar interaction or growth. Extrapolation of data to infinite dilution by linear or non-linear regression analysis yielded the limiting diffusion coefficients, D_0, of Table 1. Although dissymmetry ratios of scattered light at

angles of 45° and 135° were less than 1·1 in all systems, micelle sphericity cannot be assumed in view of the high aggregation numbers of the micelles. Consequently, D_0 values have been related to the solvated dimensions of the micelles by Perrin's relationships (Zero & Pecora 1985) for ellipsoids:

$$D_0 = \frac{kT}{6\pi\eta a}G(\rho) \tag{3}$$

where k is the Boltzman constant, T the absolute temperature, and η the solvent viscosity. The shape factor $G(\rho)$ is related to the axial ratio ($\rho = b/a$) where a = length of major semi axis and b = length of minor semi axis. For prolate (rod-like) ellipsoids,

$$G(\rho) = (1-\rho^2)^{-\frac{1}{2}}\ln\left[\frac{1+(1-\rho^2)^{\frac{1}{2}}}{\rho}\right] \quad \rho < 1 \tag{4}$$

and for oblate (disk-like) ellipsoids,

$$G(\rho) = (\rho^2-1)^{-\frac{1}{2}}\arctan\left[(\rho^2-1)^{\frac{1}{2}}\right] \quad \rho > 1 \tag{5}$$

The use of equation (3) in the determination of micellar dimensions necessitates the choice of the type of ellipsoid and an estimate of the axial ratio. The intrinsic viscosity (η) was derived by extrapolation of plots of reduced viscosity (η_{sp}/C) against C (Fig. 5) where the reduced specific viscosity is given by:
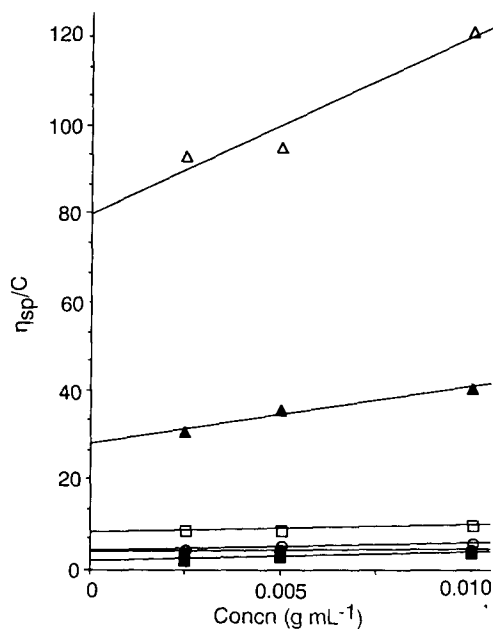
FIG. 5. Variation of reduced viscosity, η_{sp}/C, for solubilizing systems of soya lecithin in P113 containing water/lecithin molar ratios, $R=0.9$ (●), 1.75 (O), 2.61 (■), 3.46 (□), 4.32 (▲) and 5.17 (△).

$$\eta_{sp}/C = \frac{\eta - \eta_0}{\eta_0 C} = \frac{(t - t_0)}{t_0 C} + \left[\frac{1}{\rho_0} - \bar{v}\right]\frac{t}{t_0} \qquad (6)$$

t and t_0 are the flow times for a solution of weight concentration C and for the solvent, respectively. ρ_0 is the density of the solvent and $\bar{v}$ the partial specific volume of the micelles. The intrinsic viscosity is determined both by the axial ratio and the micellar solvation according to:

$$[\eta] = v(\bar{v} + \delta V_1^0) \qquad (7)$$

where δ is the solvation expressed as g solvent/g total solute and V_1^0 is the specific volume of pure solvent. The parameter v has been related to the axial ratio of prolate and oblate ellipsoids (Mehl et al 1940). It is thus necessary to fix either axial ratio or solvation in order to further describe the micelle. Since static light scattering measures non-solvated micellar weight it is, in principle, possible to calculate the solvation by comparison of solvated and non-solvated volumes derived from data from these two techniques. Such a comparison is strictly only valid in systems in which micelles are monosized since the micellar weight from static light scattering is weight average whilst the diffusion coefficient from dynamic light scattering is z average. The non-solvated micellar volume, V_n, may be related to the micellar weight by:

$$V_n = (M/N_A)\bar{v} \qquad (8)$$

where N_A is the Avogadro constant. The solvated micellar volume, V_s, may be derived from each ellipsoid model and a selected axial ratio using equations (3)-(5) and the relevant equation for the volume of the ellipsoid. The solvation as calculated from the difference $V_s - V_n$ and converted to a weight of solvent per unit weight of solute may be compared with the δ value derived from the intrinsic viscosity for the

same axial ratio. Iteration of the axial ratio then permits the determination of the micellar dimensions.

The values of axial ratio and solvation of Table 1 should be treated with some caution in view of the probable range of micellar sizes in the solutions. The table does, however, indicate a clear change of micellar shape; oblate for systems with $R < 2.6$ through to spherical and finally prolate in systems with $R > 2.6$. A significant decrease in the axial ratio of the micelles is observed as the amount of solubilized water was increased. Intermicellar interactions between these asymmetric micelles would be expected to increase with the increase of solution concentration. This would account for the negative gradients on the D against C plots seen in Fig. 4 for the systems of high water content. It should be noted that, despite the pronounced asymmetry of the micelles, the maximum dimension is less than 1/20th of the wavelength of the incident light. Consequently the observed lack of any significant dissymmetry of the scattering envelope would be anticipated.

Similar changes in micellar shape and asymmetry have been reported for several other surfactants in non-aqueous solvents following the solubilization of water (Luisi et al 1988) and are responsible for the high viscosity exhibited by such systems in high concentration. In contrast, Kumar et al (1984) reported the formation of spherical micelles in lecithin/water/carbon tetrachloride and benzene systems. Dissymmetry values ranging from 1.6 to 2.3 were obtained, which were considered indicative of large spherical micelles of dimensions greater than 1/20th wavelength of incident light. The actual radii of these micelles were approximated to 50 nm which may be more accurately ascribed to the structure of microemulsions rather than discrete micelles. Dissymmetry, however, was described in lecithin/water/cyclohexane systems above $R = 6$ due to the formation of elongated aggregates.

In conclusion, the use of a cohort of light scattering and viscometric techniques has permitted description of the size and shape of lecithin micelles in trichlorotrifluoroethane as a function of water incorporation. The findings will have important implications both on the potential of these micelles to solubilize drugs in pressurized CFC based systems and on the ability of these devices to generate microfine, respirable aerosols.

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