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PHYSICAL CHEMICAL STUDIES OF SHORT-CHAIN LECITHIN HOMOLOGUES. PHASE SEPARATION AND LIGHT SCATTERING STUDIES ON AQUEOUS DIOCTANOYLLECITHIN SOLUTIONS

R.J.M. TAUSK, C. OUDSHOORN and J.Th.G. OVERBEEK
Van 't Hoff Laboratory, State University, Transitorium III, Padualaan 8, Utrecht – De Uithof, The Netherlands

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Some phase separation phenomena in aqueous dioctanoyllecithin solutions including the effects of NaCl and on the phase diagram are reported. At low electrolyte concentrations (below 0.2 M) both salts cause the upper c solute temperatures (u.c.t.) to decrease, probably due to a decrease of electrostatic attraction between the lipid r cules. At higher salt concentrations the effect of LiI continues in the same direction (salting-in) but NaCl leads t increase of the u.c.t. (salting-out).

Micellar weight determinations could be performed at room temperature in homogeneous lecithin solutions c taining 0.2 M LiI. An attempt was made to interpret the light scattering data with the help of the open associatic model (equal association constants for aggregation steps beyond a certain step) and the Flory–Huggins type of thermodynamic nonideality. The angular dependence of the light scattering points to very large and extended mi celles. The radii of gyration are approximately proportional to the square root of the micellar weights.

1. Introduction

Characterisation of the association properties of dioctanoyllecithin (diC_{8-}) is important for the understanding of certain biochemical and physical chemical phenomena. In the course of the study [1] of the kinetics of the hydrolysis of different lecithin homologues by pancreatic phospholipase A it was found that the enzyme activity is strongly dependent on the chain length of the lecithin, probably through a dependence on the lipid aggregation structure. The highest activities were encountered with dioctanoyllecithin as the substrate. Dissolved in water this lecithin associates, at concentrations just above the the critical micelle concentration (CMC) into thermodynamically stable aggregates (micelles), whereas the next higher homologue (diC_{9-}) forms liquid crystalline dispersions (liposomes). At slightly higher concentrations of diC_{8-} a phase separation sets in. The enzyme kinetics were actually studied in these inhomogeneous solutions.

Pugh [2] has performed light scattering measurements to obtain the micellar weight of diC_{8-}. In his thesis he reported a micellar weight of but gave no further details. Furthermore he mention a phase separation.

Even in homogeneous solutions the inter of the aggregation phenomena will be hamp the strong thermodynamic nonideality conn with the proximity of the phase separation. ficulties have also arisen during studies on o factants [3–5].

We first studied the demixing of dioctan in aqueous solutions. We were interested in fluence of inorganic electrolytes on the dem two reasons. In the first place the enzymati ysis by phospholipase A reveals large salt ef Moreover salt effects on the demixing migh formation on the electrostatic contribution free energy of mixing. The influence of the out agent NaCl and the salting-in electrolyte the CMC [6] have been discussed in part I c series [7] (hereafter referred to as I). The ir of NaCl on the micellar weights of diC_{6-} an

lecithin has been described in II [8]. Correlations between effects on the CMC's and the phase diagrams are to be expected.

To study the association phenomena we performed light scattering experiments in homogeneous solutions aiming in particular at the influence of temperature changes when approaching the phase separation. The study of the influence of the lecithin concentration was facilitated by the finding that the phase separation at room temperature could be suppressed by addition of LiI.

2. Methods and materials

The synthesis and purification of dioctanoyllecithin as well as the preparation of the aqueous solutions, containing variable concentrations of electrolytes have been described in I [7]. The lecithin was not completely free of surface active impurities since the γ versus log c curve (γ = surface tension) showed a minimum around the CMC [7].

2.1. Phase diagram

Phase separation was determined in 1 ml graduated pipets in a regulated constant temperature bath. A glass joint with a stopper was fused to the lower end of each pipet. This spoiled the calibration of the lower part, which was therefore filled with mercury. The open upper end of the pipet was sealed with parafilm (Marathon). To homogenise the solution at elevated temperatures a small piece of iron sealed in a plastic tube was moved up and down in the pipet by a magnet. The temperature was slowly lowered and the cloud points were noted visually. A few times the solutions were left for 12 to 24 hours at temperatures below the cloud points in order to determine the phase volumes and concentrations in both phases. However, with increasing duration of the experiments reproducibility of the diagrams decreased and the cloud point curves tended towards lower temperatures, probably due to decomposition of the diC_{8}-. Since rather limited amounts of material were available dilution series were carried out in the pipets. Lecithin monohydrate concentrations were determined from phosphor analysis [9].

2.2. Light scattering

The light scattering technique and the calibration of the Fica 50 instrument of the Société Française d'Instruments de Contrôle et d'Analyse have been described in II [8]. The angular dependence of the scattering was checked with aqueous solutions of fluorescein, bovine serum albumin and polystyrene latex (diameter: 109 nm) and with a solution of poly-α-methylstyrene [10] (molecular weight: 1×10^6) in toluene. The temperature of the toluene outer vessel was regulated and the temperature in the light scattering cells was recorded with a thermistor. We never waited for full temperature equilibrium since the experiments had to be performed rather quickly due to the decomposition of the lecithin. The solutions were filtered under pressure through millipore filters (0.45 or 0.1 μ). Before measuring the light scattering of homogeneous solutions at high dilutions and room temperature (25.0°C, 0.2 M LiI) the cuvettes were centrifuged for 1 hour at 20000 rpm. Before studying the temperature dependence of the scattering in systems showing phase separation at 45°C the solutions were filtered at elevated temperatures (ca 80°C).

2.3. Refractive index increments

The increment at room temperature was measured in a Rayleigh interferometer (Aus Jena). The temperature dependence of the increment was obtained with a Brice-Phoenix differential refractometer. The instrument was calibrated with sucrose solutions.

3. Light scattering equations

The light scattering, in excess over the solvent scattering, from a solution containing various macromolecules with different molecular weights M_i, weight fractions f_i and equal refractive index increments dn/dc is given [11] by the equation

$$Kc(1+\cos^2\theta)/R_\theta = \{M_w\langle P_1(\theta,c)\rangle\}^{-1} + c\langle Q(\theta,c)A_{ij}(c)\rangle + \ldots . \quad (1)$$

R_θ stands for the excess Rayleigh ratio at an angle θ to the incident beam, if no polariser or analyser are used. $K = 2\pi^2 n_0^2 (dn/dc)^2 \lambda_v^{-4} N_0^{-1}$, where n_0 is the

refractive index of the solvent, λ_v is the wavelength in vacuum and N_0 is Avogadro's constant. c is the total solute concentration in mass per unit volume. $A_{ij}(c)$ is a concentration dependent interaction parameter, discussed in more detail in II. $\langle P_1(\theta, c)\rangle$ is an average of angle and concentration dependent factors, resulting from intramolecular (internal-) interferences. $Q(\theta, c)$ also corrects for interferences but contains contributions from both intra- and intermolecular (external-) interferences. M_w equals the weight average molecular weight given by

$$M_w = \sum_i f_i M_i = \int_0^\infty M f(M)\,dM \qquad (2)$$

where $f(M)$ stands for the weight distribution function of the macromolecules.

By using the "single contact approximation" Zimm [12] showed that for large molecules at low concentrations in a two component system $Q(\theta, c)$ equals unity. Vrij and van den Esker [13] obtained the same result by quite a different method. $Q(\theta, c)$ is very difficult to estimate in our case. As an approximation we will also assume $Q = 1$, although our micelles might in fact have structures very different from flexible polymers. For particles with rather small radii of gyration, r_g, or small angles, θ, the light scattering equation reads:

$$Kc(1 + \cos^2\theta)/R_\theta = A + B\sin^2(\theta/2) \qquad (3)$$

with

$$A = (RT)^{-1}\,d\Pi/dc = 1/M_{w,app.} = 1/M_w + A_2 c \qquad (4)$$

and

$$B = 16\pi^2(3\lambda^2)^{-1}\langle r_g^2\rangle_z/M_w = 16\pi^2(3\lambda^2)^{-1}q^2. \qquad (5)$$

Π is the osmotic pressure and λ equals the wavelength in solution ($\lambda = \lambda_v/n$). For simplicity's sake we abbreviated $\langle A_{ij}(c)\rangle$ by A_2 as is common practice. $\langle r_g^2\rangle_z$ stands for the z-average of the radius of gyration squared and is defined by

$$\langle r_g^2\rangle_z = M_w^{-1}\int_0^\infty M f(M) r_g^2\,dM. \qquad (6)$$

Considering the fact that our system consists of only two components the use of $d\Pi/dc$ in eq. (4) is justified. The interpretation in terms of several lecithin species (second equality in eq. (4)) implies that relations between concentrations and activity coefficients (incorporated in A_2) exist [8]. Eqs. (3) and (4) form the basis of the well known "Zimm plots" [12].

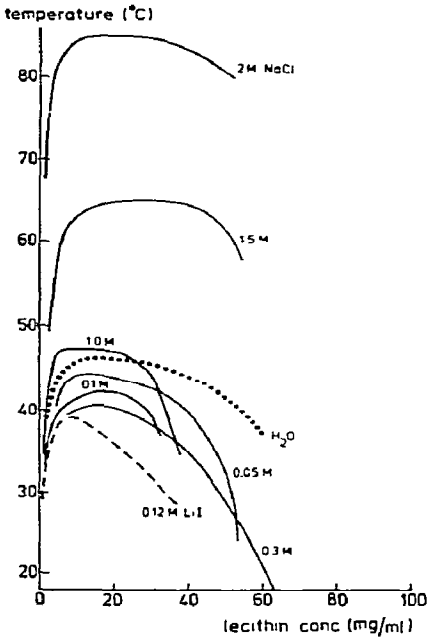

Fig. 1. Phase separation diagrams of dioctanoyllecithin in aqueous selutions. The dotted line (...) was obtained in electrolyte free solutions. The fully drawn lines (——) and the broken line (– – –) refer to solutions containing NaCl and LiI respectively.

Contrary to the lecithins with shorter chains, studied in our previous paper [8], dioctanoyllecithin forms very large micelles. The critical micelle concentration is rather low (0.12–0.16 mg/ml). These two properties enable us to use the "Debye approximation" [14], in which micellar concentrations (c_m = total concentration – CMC) are substituted in eqs. (3) and (4).

In systems close to a phase separation ($d\Pi/dc \to 0$) [15] or composed of very large particles [16] the scattering becomes so large that one should take the attenuation and the multiple scattering into account. The former correction is easily performed. For systems whose scattering can be described by eq. (3) one can use the relations given by Debye [15]. The correction for multiple scattering is more difficult to evaluate

exactly. It can, however, be shown that this correction is small compared to other experimental accuracies and was therefore disregarded.

4. Results

4.1. Phase separation diagrams

In fig. 1 the influence of various concentrations of NaCl on the phase separation diagram are shown. In electrolyte free solutions and at room temperature the demixing sets in at about 0.8 mg/ml. The CMC (0.12–0.16 mg/ml [7]) was virtually temperature independent up to 50°C (the highest temperature used). Addition of the salt in relatively low concentrations leads to a lowering of the cloud point curve. At higher concentrations the demixing region rises again. The temperatures in the maxima of the diagrams (critical temperatures) as a function of the NaCl concentration are plotted in fig. 2. The cloud point curves were not very reproducible from one lecithin sample to the other. We found for another separately synthesized sample a maximal temperature of 50.6°C in NaCl free solutions. The general behaviour on increasing NaCl concentrations remained the same.

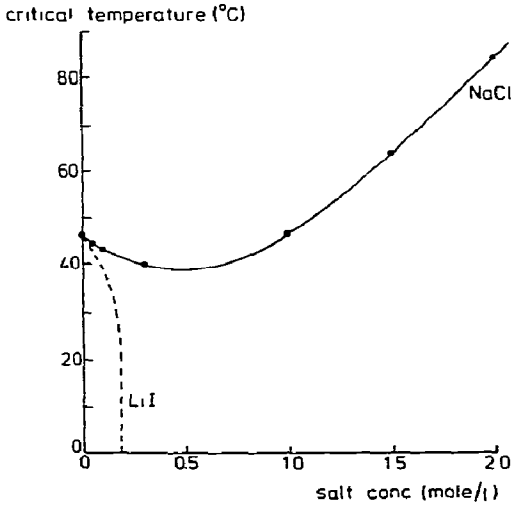

Fig. 2. Critical temperatures of the phase separations as a function of the salt concentrations. ——: NaCl, – – –: LiI.

The influence of 0.12 M LiI on the phase diagram is also shown in fig. 1. Addition of higher concentrations of LiI brings about a further lowering of the curve (as can be seen from fig. 2). It appeared to be impossible to obtain phase separation at high LiI contents.

The combined effects of NaCl and LiI were qualitatively investigated. Continuous addition of NaCl to a solution containing 0.12 M LiI first led to a decrease and then to an increase of the cloud point temperatures. The cloud point curve lowered again upon a further addition of LiI. The same phenomena can be obtained with KCNS instead of LiI.

The critical concentration was difficult to estimate due to the flatness of the upper part of the curves. At the critical concentration and temperature the volumes of both phases should be finite. By investigation of the phase volumes in salt free solutions we obtained a critical concentration of about 21 mg/ml.

4.2. Refractive index increments

The refractive index increments measured at room temperature in 0.2 M LiI and with an accuracy of ± 0.0005 ml/g were 0.118^0, 0.118^3 and 0.119^5 ml/g at wavelengths of 578, 546 and 436 nm respectively. The results of studies of the temperature dependence of the increments in 10^{-2} M phosphate buffer (pH = 6.9 ± 0.1) and at temperatures above the phase separation are shown in fig. 3. Linear extrapolations of these

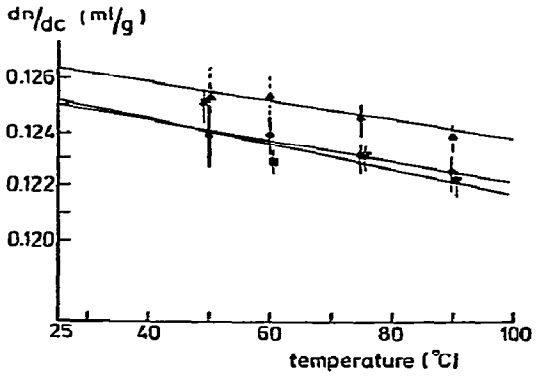

Fig. 3. Temperature and wavelength dependence of the refractive index increments in 10^{-2} M phosphate buffer (pH = 6.9 ± 0.1). ■: λ_v = 578 nm, ●: λ_v = 546 nm, ▲: λ_v = 436 nm.

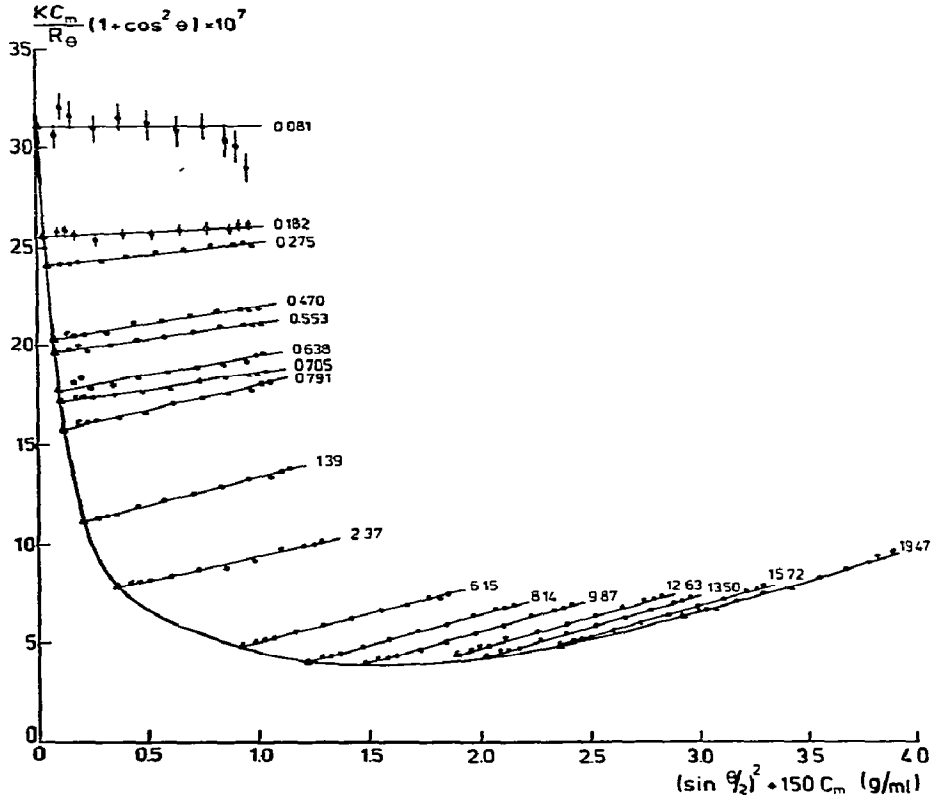

Fig. 4. "Zimm plot" of dioctanoyllecithin at 25.0°C, 0.2 M LiI and λ_v = 546 nm. The numbers on the right hand side of the lines refer to micellar concentrations in mg/ml.

increments to 25°C yield 0.125 ml/g for 578 nm and 546 nm and 0.126 ml/g for 436 nm. The accuracy of these extrapolated values is about 0.001 ml/g. The differences between these values and the increments directly measured at room temperature can be explained by the increase in the solvent refractive index due to the presence of LiI [8]. Our values are significantly smaller than the value reported by Pugh (0.138 ml/g) [2].

4.3. Concentration dependence of the light scattering in 0.2 M LiI

The results of the scattering experiments in 0.2 M LiI at 25°C and λ_v = 546 nm are given in fig. 4. The concentrations c_m used in this graph are the micellar concentrations. A CMC value of 0.128 mg/ml was used. This value was obtained by extrapolation of the results at low micellar concentrations in a manner explained in part II of this series [8]. The lines of constant concentration were extrapolated to $\theta = 0°$. The values of $R_0/(2Kc_m) = M_{w,app.}$ as a function of the micellar concentration are replotted in fig. 5. Within the measuring accuracy of about 2% the same data were obtained with the two other wavelengths.

The values of $q = [\langle r_g^2\rangle_z/M_w]^{1/2}$ as calculated from the slopes with the help of eqs. (3) and (5) at various concentrations are plotted in fig. 6. At low concentrations ($c_m < 2$ mg/ml) these slopes are of course rather inaccurate. The values of q at λ_v = 578 nm and λ_v = 546 nm were equal within the experimental error. The values at λ_v = 436 nm, however, were always about 4% smaller. Part of this effect can be explained by taking the depolarisation into account. This

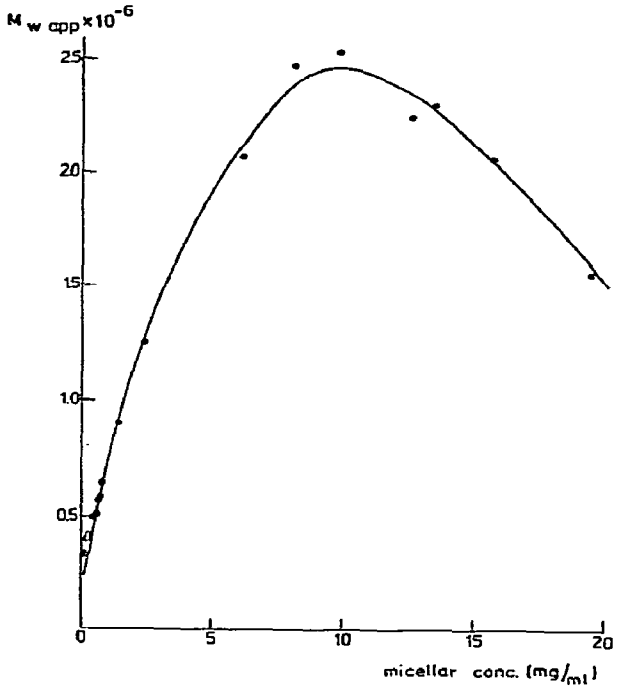

Fig. 5. Apparent weight average micellar weights as a function of micellar concentrations. The curve was obtained by least square curve fitting with eqs. (9) and (10).

depolarisation ($\rho_u = U_h/U_v$) was wavelength dependent and increased notably at lecithin concentrations above 13 mg/ml. The values of ρ_u at $\theta = 90°$ in the whole concentration range increased at $\lambda_v = 578$ nm from 0.005 to 0.009, at $\lambda_v = 546$ nm from 0.006 to 0.009 and at $\lambda_v = 436$ nm from 0.01 to 0.018.

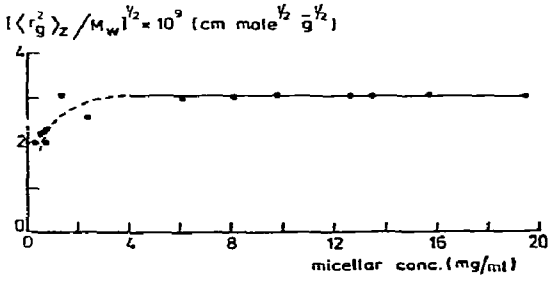

Fig. 6. $[\langle r_g^2 \rangle_z / M_w]^{1/2}$ from eqs. (3) and (5) as a function of the micellar concentrations.

4.4. Temperature dependence of the light scattering in salt free solution

A few preliminary experiments at $\lambda_v = 546$ nm on the temperature dependence of the light scattering near phase separation have been performed at total lecithin concentrations of 13.6, 16.7, 19.1 and 22.5 mg/g. From the angular dependence we determined the scattering at $\theta = 0°$ and the slope of the regression line representing the reciprocal value of the scattering versus $\sin^2(\theta/2)$. The results are given in figs. 7, 8 and 9. The scattering envelopes at high temperatures were not so well behaved as those obtained at room temperature and 0.2 M LiI. This is very likely due to the

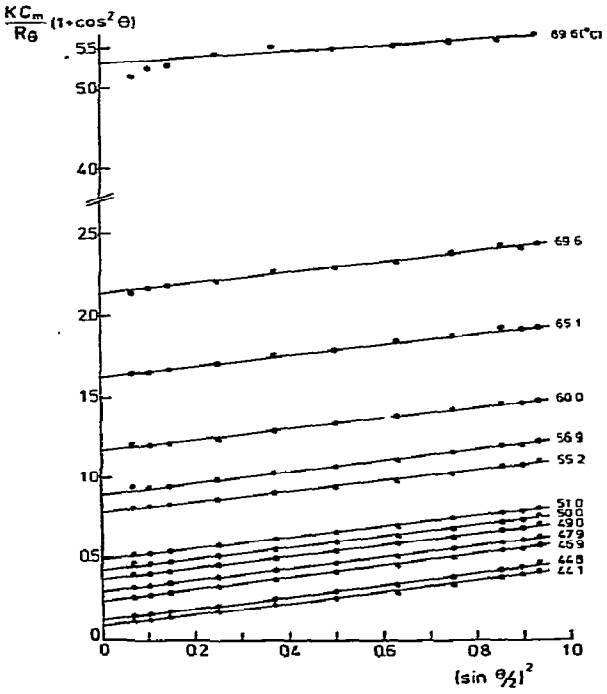

Fig. 7. Temperature dependence of the reciprocal scattering at $\lambda_V = 546$ nm and a lecithin concentration of 22.5 mg/g in salt free solutions. The numbers on the right-hand side of the lines refer to the temperatures. Phase separation occurred at 44.0°C.

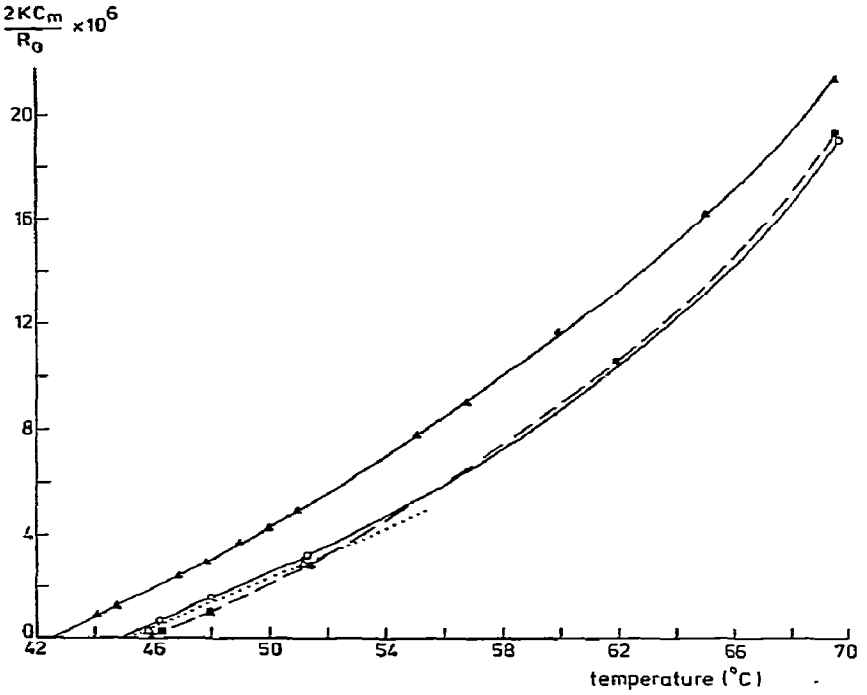

Fig. 8. Temperature dependence of $2Kc_m/R_0$. The four lecithin concentrations in mg/g are o: 13.6, ■: 16.7, △: 19.1 and ▲: 22.5.

presence of dust, which is difficult to remove quantitatively at high temperatures. The depolarisation strongly increased when approaching the phase separation region (at 10°C from the spinodal ρ_u was 0.01, at 3°C ρ_u was 0.03 whereas at 0.5° from the spinodal ρ_u rose to about 0.08). This effect could be due to multiple scattering.

If the lines of fig. 8 are extrapolated to $2Kc_m/R_0 = 0$ we obtain points from the spinodal [17]. Except for the critical point this curve lies below the binodal, that is the cloud point curve. In fig. 10 we have plotted parts of the binodal (visually determined) and the spinodal obtained from the light scattering experiments. The whole cloud point curve from these experiments

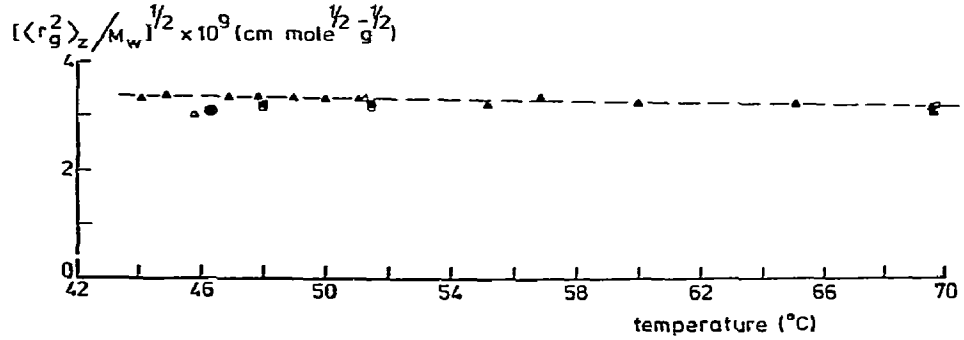

Fig. 9. Temperature dependence of $[\langle r_g^2\rangle_z/M_w]^{1/2}$ in salt free solutions. The lecithin concentrations in mg/g were o: 13.6, ■: 16.7, △: 19.1 and ▲: 22.5

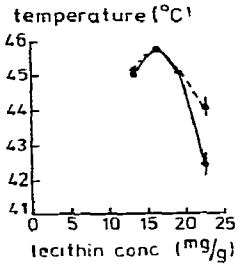

Fig. 10. The broken line (– – –) represents a part of the binodal (phase separation curve). The fully drawn line (——) is a part of the spinodal.

lies somewhat below and has a smaller width than the curve obtained in the experiments shown in fig. 1 (dotted line).

5. Discussion

5.1. Light scattering at room temperature in 0.2 M LiI

The aggregation properties of dioctanoyllecithin at room temperature in homogeneous solutions could only be obtained by increasing the solubility by addition of salt. We thus dissolved the lecithin in 0.2 M LiI. This leads to a lowering of the upper consolute temperature below 0°C.

5.1.1. Micellar weight

In fig. 5 we see a very strong increase of the apparent micellar weight already at low micellar concentrations. Normal thermodynamic nonideality effects can be expected to play a minor role in that region. In particular it would be difficult to visualise such a large negative virial coefficient. We therefore have to assume that the real micellar weight strongly increases with the concentration. This also implies a polydisperse micellar system [8]. In association phenomena a large polydispersity is obtained when all equilibrium constants (K_i) for successive aggregation steps (beyond a certain point) are equal [8, 18].

$$K_i = K = C_i/(C_{i-1} \times C_1) \qquad (7)$$

where C_i equals the molar concentration of the i-mer. In nonideal solutions eq. (7) also holds if the chemical potentials of the different species are related [19] according to

$$\mu_i = \mu_i^0(P, T, c') + RT[\ln c_i + M_i(B_1 c + B_2 c^2 + \ldots)]. \qquad (8)$$

Eq. (7) leads to an approximately linear increase of the weight average micellar weight with the square root of the micellar concentration c_m (in mass per unit volume) according to

$$M_w = k(c_m)^{1/2}. \qquad (9)$$

In order to interpret the apparent micellar weights more in detail we need a theory for estimating the thermodynamic nonideality in eqs. (4) and (8), that is to say we need values for the second and higher virial coefficients. The micellar weights are, however, so high that the calculation of the second virial coefficient on the basis of noninteracting rigid molecules, as performed in II [8], becomes very questionable. One can easily show that the use of that theory, in conjunction with the experimental data is unjustified above concentrations of 6 to 8 mg/ml since the excluded volume of cylinders of reasonable diameters would be larger than the volume of the whole solution. Higher virial terms should then be taken into account, but they are difficult to estimate. We therefore considered another model. We assumed the real micellar weight to increase according to eq. (9) and expanded the apparent micellar weight concentration dependence into virial terms according to

$$1/M_{w,app.} = 1/M_w + A_2 c_m + A_3 c_m^2. \qquad (10)$$

Such an expansion into constants is in agreement with eq. (8), which yields virial coefficients that are independent of the molecular weight. We next performed least square curve fitting procedures [20] to obtain the value for k from eq. (9) and A_2 and A_3. Taking $A_3 = 0$ we obtained a very bad fit. Including A_3 as an adjustable parameter a good fit could be obtained. We found $k = 2.38 \times 10^7$ ml$^{1/2}$g$^{1/2}$mole^{-1}, $A_2 = -2.36 \times 10^{-5}$ mole ml g^{-2} and $A_3 = 2.1 \times 10^{-3}$ mole ml^2 g^{-3}. The accuracy of these values depends almost entirely on the accuracy of the CMC. We estimated the maximal error in the CMC to be ± 0.014 mg/ml. The systematic errors in the parameters k, A_2 and A_3 then are $\pm 0.1 \times 10^7$, $\mp 0.5 \times 10^{-5}$ and $\mp 0.2 \times 10^{-3}$ respectively.

We interpreted these parameters in terms of the

classical Flory–Huggins theory [21] developed for the interaction of large polymer molecules. In this case we use

$$2Kc_m/R_0 = 1/M_w + v_1^2(M_0v_0)^{-1}(1-2\chi)c_m + v_1^3(M_0v_0)^{-1}c_m^2 \quad (11)$$

where M_0 and v_0 are the solvent molecular weight and specific volume respectively. v_1 is the specific volume of the solute (assumed to be 0.90 ml/g) and χ is an interaction parameter which is concentration dependent. In order to calculate χ from the parameters A_2 and A_3 we have to expand χ according to

$$\chi = \chi_0 + gc_m. \quad (12)$$

This procedure leads to $\chi_0 = 0.50025 \pm 6 \times 10^{-5}$ and $g = 0.428 \pm 0.002$ ml/g. Fitting eq. (11) to the experimental data with χ independent of the concentration gave $\chi = 0.506$. This would, however, imply a phase separation ($2Kc_m/R_0 \leq 0$) at concentrations between 5 and 13 mg/ml. In the theories on the thermodynamics of macromolecules a value for χ above 0.5 indicates that the system is near a phase separation, which in our system is indeed the case.

5.1.2. Micellar shape

A remarkable feature of our Zimm plot (fig. 4) is the parallelism of the lines at constant concentration. This implies that $q = [\langle r_g^2\rangle_z/M_w]^{1/2}$ as calculated with the help of eqs. (3) and (5) is also constant (fig. 6). The value for q is 3.1×10^{-9} cm mole$^{1/2}$g$^{-1/2}$. At low micellar concentrations q tends to lower values, but the accuracy is of course less in that region. Using the association model (M_w is dependent on the concentration) this implies that q is also independent of M_w.

We will now discuss the constant value for q with the help of three alternative models for the geometry of the micelles: a) the disk, b) the rigid rod, c) the random coil.

a) *Disk.* The z-average radius of gyration is connected with the z-average radius (R) of a circular disk according to [22]

$$\langle r_g^2\rangle_z = \tfrac{1}{2}\langle R^2\rangle_z. \quad (13)$$

Since $\langle R^2\rangle_z$ is proportional to the z-average particle weight (and also to the weight average weight), q is independent of the average micellar weight if the system consists of disks of equal thickness and different radii. The open association model with $M_w/M_n = 2$ leads to $M_z = \frac{3}{2}M_w$ [8]. Using a thickness of the disks of 2 × 20 Å we find $q = 0.95 \times 10^{-9}$ cm mole$^{1/2}$ g$^{-1/2}$, whereas the experimentally observed value ($q = 3.1 \times 10^{-9}$) would be consistent with disks of thickness 2 × 1.8 Å. The area per molecule would be 440 Å^2. The disk model would thus lead to absurd dimensions of the lecithin molecules and can therefore be discarded.

b) *Rigid rod.* The z-average radius of gyration squared is proportional to the ρ-average rod length L_ρ squared according to [23]

$$\langle r_g^2\rangle_z = \tfrac{1}{12}\langle L^2\rangle_\rho \quad (14)$$

where $\langle L^2\rangle_\rho$ is connected with the ρ-average molecular weight $\langle M^2\rangle_\rho$ according to [24]

$$M_\rho = [(1/M_w)\int_0^\infty M^2 f(M)\,dM]^{1/2}. \quad (15)$$

The "most probable distribution" ($M_w/M_n = 2$) [8, 25] leads to

$$M_\rho = \sqrt{3}M_w.$$

q^2 is therefore proportional to M_w. The open association model of rigid rods is therefore incompatible with our constant value of q. If the micellar system were monodisperse a value for q of 3.1×10^{-9}, together with a rod radius of 20 Å, would be consistent with a micellar weight of 800 000 and a rod length of 960 Å. For several reasons this monodisperse model is also not satisfactory. It would in the first place be very unlikely for a rod of 960 Å length and 40 Å diameter to be rigid. Moreover in the low concentration range we have measured micellar weights well below 800 000 (our lowest value was 320 000). In that concentration range q tends to lower values which can be interpreted as arising from a concentration dependent micellar weight. There seems, however, no justification for the hypothesis that the micellar size first increases and then remains constant at 800 000. A further increase would then be expected. In the previous section (5.1.1) we also gave another reason for postulating a polydisperse system.

c) *Random coil.* The z-average radius of gyration squared of a random coil is proportional to the z-average end to end distance squared ($\langle h^2\rangle_z$) and thereby

proportional to the z-average micellar weight [23], implying q independent of the micellar size

$$\langle r_g^2 \rangle_z = \tfrac{1}{6} \langle h^2 \rangle_z. \qquad (16)$$

If we assume the real micellar weight to increase according to eq. (8), with $k = 2.38 \times 10^7$ ml$^{1/2}$g$^{1/2}$ mole^{-1}, we can calculate the end to end distance and compare it with the contour length (length L of a rigid rod with a radius of 20 Å). We now for instance obtain at a concentration of 10 mg/ml, $M_w = 2.38 \times 10^6$, $\langle h^2 \rangle_z^{1/2} = 1170$ Å and $L_z = 4260$ Å. The end to end distance is too large compared to the contour length to apply the random coil statistics.

The most likely picture of the micellar shapes, that emerges from the considerations given above, is that of a very extended structure, probably wormlike with an increasing flexibility with increasing size (the more classical wormlike structure yields q values that still depend on the micellar size). Perhaps we should not think of unbranched rods but visualize the micelles more as an extended network.

5.2. Temperature dependence of the scattering in salt free solutions

As can be seen from figs. 7 and 8 the scattering increases very much on approaching the phase separation. The complete interpretation of this decrease in $d\Pi/dc$ can not yet be given. Probably two effects operate at the same time: the average micellar weight and thermodynamic nonideality (concentration fluctuations) both increase with decreasing temperature.

It is remarkable that in this case too $\langle r_g^2 \rangle_z / M_w$ remains approximately constant (see fig. 9). Since the value for q equals $(3.2 \pm 0.15) \times 10^{-9}$ cm mole$^{1/2}$ g$^{-1/2}$ we may conclude that the micellar structures in this case closely resemble the structures obtained at room temperature and 0.2 M LiI ($q = 3.1 \times 10^{-9}$, fig. 6).

5.3. Phase separation diagrams

We propose the rise of the upper consolute temperature at high NaCl concentrations as shown in figs. 1 and 2 to be due to a general salting-out effect [6,7], which is also reflected by the decrease of the CMC. This salting-out is most likely based upon two effects that both lead to an increase of the demixing temperatures. Addition of NaCl will probably lead to an increase of the average micellar weight (as was the case with the shorter homologues [8]) and to an increase of the interaction parameter χ (reduction of the solvent quality).

The initial decrease of the critical temperature can be explained by a decrease in electrostatic attractions between the lecithin molecules. This is quite reasonable if we assume Coulomb attraction forces between the phosphate group of one molecule and the choline group of a neighbouring molecule. The exact orientations and the resulting interactions between the polar groups are still very much debated (see, e.g., refs. [26,27]).

The opposite effect of LiI and NaCl in mixtures of these salts at high concentrations, as described in section 4.1 has its analogue in the change of the CMC in mixtures of the same electrolytes [7]. Again we assume a general salting-in effect due to LiI. Although the CMC of the lecithin in water is only slightly influenced by addition of LiI the micellar properties are probably greatly effected.

It is worthwhile mentioning that other theories exist for the appearance of demixing in solutions of macromolecules. Flory [28] and Onsager [29] proved that a phase separation in solutions of asymmetrical molecules can also appear even without taking χ into account. A strict application of their theories, however, is rather difficult in our associating system. Moreover these theories require an anisotropic concentrated phase, which in our case is not formed.

5.4. General conclusions on the aggregation properties of the short-chain lecithin homologues and the phospholipase A enzyme kinetics

In summarising the results from the micellar weight determinations on dihexanoyl-, diheptanoyl- and dioctanoyllecithin we arrive at our main conclusion concerning the general aggregation properties: The average micellar weight at a certain micellar concentration increases strongly with elongation of the chain length. This effect is most likely due to an increase of the micellar polydispersity, which accompanies the micellar weight-concentration dependence. Simple inorganic electrolytes, added in high concentrations, affect the micellar properties by general salting-out and salting-in (change of the hydrophobic bond). In this case too

we postulate an influence through a change of the polydisperisyt. In part IV of this series [31] we will present a simple association model which is in agreement with these observations.

This research project was initiated mainly to find correlations between the micellar aggregation properties and the phospholipase A enzyme kinetics [1,30]. Our thermodynamic data of the general behaviour of these lecithins do not provide detailed information of the local orientations and interactions of the lecithin molecules in the micelles. It is therefore impossible to test different hypotheses concerning the influence of the local environment of the lipid molecules on the enzyme kinetics. If hydrophobic bonds play an important role, we would expect the enzyme activity to increase with increasing chain length of the lecithins and with increasing concentrations of salting-out agents. A salting-in agent (for instance LiI) should in principle lead to a lowering of the activities. These effects have indeed been observed [1,30]. The "enzyme-substrate complex dissociation constants" change in the directions in agreement with this hydrophobic bond hypothesis. The details of the influences of the chainlength of the lipids and the salt concentrations are, however, still obscure, since the main effects on the enzyme activities stem from changes in the "maximal velocity" and not from changes in the dissociation constant. We therefore have to assume the maximal velocity to depend also on the hydrophobic bond, through changes in the environment of the lipid molecules bound at the active site of the enzyme.

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