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# PHYSICAL CHEMICAL STUDIES OF SHORT-CHAIN LECITHIN HOMOLOGUES. I. INFLUENCE OF THE CHAIN LENGTH OF THE FATTY ACID ESTER AND OF ELECTROLYTES ON THE CRITICAL MICELLE CONCENTRATION

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The critical micelle concentration (CMC) of four synthetic phosphatidylcholines (containing two hexanoyl, heptanoyl, octanoyl or nonanoyl residues respectively) in aqueous solutions have been determined by surface tension measurements. The dependence of the CMC on the chain length is discussed on the basis of the mass action model for micelle formation. For the three higher homologues a contribution of 1.08 $kT$ per $CH_2$ group to the standard free energy of micellisation is found. The change in this free energy in going from the dihexanoyl- to the diheptanoyllecithin is somewhat larger (1.2 $kT$ per $CH_2$ group).

The influence of high concentrations (several moles per liter) of simple electrolytes on the CMC is interpreted as a salting-out of nonpolar solutes in water. Contrary to expectations the effects of NaCl and LiI on the CMC of dioctanoyllecithin are not additive.

## 1. Introduction

Studies of the enzymatic breakdown of lecithins continue to yield information on protein–lipid interactions of great potential value for the study of living systems [1–8]. From the recent work of de Haas and co-workers [1, 2] it appeared that the mode of aggregation of lecithins plays an essential role in their interaction with porcine pancreatic phospholipase A. This enzyme catalyses the hydrolysis of the 2 fatty acid ester bonds. It was found to be very weakly active on dispersions of natural or synthetic lecithins, when aggregated in the form of smectic liquid crystals. The activity is greatly increased when these lecithins form micelles after addition of soap-like substances, such as deoxycholate. A similar effect is produced by organic solvents which by solubilisation may profoundly change the aggregate structure.

With lecithins containing shorter acyl chains normal micellisation processes occur. Dioctanoyllecithin ($diC_8$)*, the highest homologue to show this phenomenon, is an excellent substrate for the enzyme, even in the absence of additives. At concentrations slightly above the critical micelle concentration (CMC) a phase separation occurs by formation of a coacervate (in the terminology of Bungenberg de Jong [9]: a unicomplexcoacervate). Shorter lecithin homologues behave like normal soaps. They form small Hartley micelles [10] at least at low concentrations. When aggregated in small micelles they are hydrolysed more slowly by the enzyme than the dioctanoyl homologue. When dissolved as single molecules they are broken down extremely slowly. It was found, that the activity of the enzyme does not primarily depend on the chain length of the lecithins but on their state of aggregation. Such conclusions could also be drawn from monolayer studies [2, 11].

Apart from this special aspect, the study of micelle formation of short-chain lecithins ($diC_6$–$diC_9$) is important from a more classical physical chemical point of view. The molecules contain two nonpolar carbon chains and a zwitter-ionic polar group. Few studies have been published on surfactants with two carbon chains [12–14]. Molecules with a zwitter-ionic head group have received much less attention than the more common ionic- or nonionic surfactants. The most important contributions come from Swarbick, Daruwala and coworkers

* Abbreviation for 1,2-dioctanoyl-sn-glycero-3-phosphorylcholine. This type of abbreviation will be used throughout this paper.

[15], Tori and Nakagawa [16, 17], Hermann [18] and Corkill and coworkers [19, 20]. Roholt and Schlamowitz [21] studied the CMC and the micellar weight of $diC_6$-lecithin. The micellar weight of $diC_7$-lecithin was reported by Smink [22]. Pugh measured the micellar weight of the $diC_8$ homologue [23].

In this paper we are mainly interested in the CMCs and in the standard free energy of micelle formation. One of the questions is: Does each carbon chain in the monomer molecule, containing two acyl chains, contribute independently to the micellisation energy? Moreover, the knowledge of the CMCs is very useful for the interpretation of micellar weight determinations.

For several reasons we became interested in the possible effects of electrolytes on our systems. These effects may provide information on the interactions between the polar groups in the micellar interface and thereby on the orientation of these groups, which has been debated [24–28]. Electrolytes can also produce salting-out or salting-in [29–33]. Finally by addition of salt we might be able to change the micellar structure and interactions between solute molecules without changing the lecithin molecule at all. This might open another way to study the factors, which control lipid–protein interactions. In the specific case of the hydrolysis by phospholipase A, large salt effects have been observed [1].

The first paper in this series will be devoted to the CMC of the short-chain lecithin homologues ($diC_6$–$diC_9$). The CMC of the dinonanoyl lecithin (which forms a liquid crystalline dispersion) is defined by the break-point in the plot of the free monomer concentration versus the total lipid concentration.

In later publications micellar weights of the $diC_6$-, $diC_7$- and $diC_8$-lecithin system and some peculiarities of the phase separation in the $diC_8$-lecithin–water system will be discussed.

## 2. Materials and methods

The short-chain lecithins* were prepared from egg yolk lecithin according to the procedure of Cubero Robles and DeJongh [34]. The egg lecithin was extracted from chicken eggs with $CHCl_3$–MeOH (2:1) and purified according to the procedure of Pangborn [35]. The 3-sn glycerylphosphorylcholine, obtained after hydrolysis of the natural lecithin with tetrabutylammonium hydroxide [36], was purified by repeated precipitation by diethylether from a methanol solution. Next the glycerylphosphorylcholine was esterified with the appropriate acid anhydride.

The resulting lipids were purified by the following steps:

(a) Column chromatography on silicic acid (Merck 70–230 mesh or Malinckrodt 60–100 mesh), elution with chloroform and increasing concentrations (up to 70%) of methanol.

(b) Column ion exchange chromatography with mixed-bed amberlite (IR 45, IRC 50 from BDH), elution with methanol–water (75:25). The ion exchange resins were purified extensively with 1M acetic acid, 1M ammonia and hot and cold methanol [37].

(c) Silicic acid chromatography, at least twice.

(d) Column chromatography on aluminum oxide (Woelm) with chloroform and chloroform–methanol (90:10) elution.

Depending on the results obtained with thin layer chromatography an extra batchwise ion exchange treatment was introduced between the two treatments in step (c). Between step (c) and (d) we often performed an extraction of the lecithin in methanol and water with hexane. When we used large amounts in the esterification reaction (for ten grams resulting lecithin or more) the main purification difficulty resulted from the formation of byproducts. These were extremely difficult to eliminate by column chromatography or other purification methods, such as $CdCl_2$ complex precipitation, charcoal treatment and chromatography with sephadex LH 20 in methanol. Fractional crystallisation was never successful. One of the main drawbacks of column chromatography is the need of large elution volumes which inevitably contain contaminants from the solvents, even when we used spectroscopic quality, which sometimes had been passed through an aluminum oxide column for further purification. The aluminum oxide step (d) was largely intended to remove these solvent contaminants. Traces of fatty acids are also removed in this step. Silicic acid may cause hydrolysis of the lecithin and ion exchange resins will nearly always release contaminants. The lipid obtained was usually colorless and was stored in ethylalcohol at −20°C. On thin layer chromatography (elution with chloroform–

* The $diC_9$-lecithin was kindly supplied by dr. W.A. Pieterson of the Department of Biochemistry, University of Utrecht.

methanol–water, 65:35:4) often a small spot at the elution front showed up, when large quantities of lecithin were applied.

All chemicals used were of p.a. or equivalent quality except for the organic solvents in the preliminary steps of the synthesis and in step (d) (where spectroscopic quality was used). Sodium chloride was heated at 500°C for at least 5 hours. Lithium iodide was heated under vacuum to 120°C. Aqueous solutions were filtered through millipore filters (0.05$\mu$), which were washed with boiling water. Water was double distilled, the second time from an all quartz system, through heating from above with an IR lamp. All aqueous electrolyte solutions were checked for organic impurities by surface tension measurements. The surface tension values were always equal to or higher than the value for water.

Aqueous lecithin solutions were prepared in the following manner. An appropriate amount of an alcoholic solution of the lecithin was pipetted into a small pre-weighed glass bottle and taken to dryness with a rotavapor. Then the lecithin was dried at 80°C in vacuum ($10^{-2}$ mm Hg), for 20 hours in the presence of phosphorus pentoxide. After reweighing, solvent was added and concentrations were calculated on a weight basis. Phosphor analysis [38] agreed to within 1% of the calculated value for monohydrate. The lecithin solutions mostly contained a phosphate buffer ($10^{-2}$M, pH = 6.9 ± 0.2) in order to suppress possible influences of traces of charged surface active impurities. We never found any effect of the buffer on the CMC and on micellar weights.

### 2.1. Surface tension measurements

The surface tensions of dihexanoyllecithin solutions were measured with the drop-weight method [39,40]. A stalagmometer was mounted directly above the pan of a Mettler balance. The tip with an effective radius of 0.404 cm, as obtained by calibration with water, was placed in a small erlenmeyer containing the solvent. The dropping time was always greater than one minute, which proved long enough for adsorption to be complete within the experimental accuracy of 0.1 dyne $cm^{-1}$ or better. The measurements on solutions of $diC_7$-, $diC_8$- and $diC_9$-lecithin were performed with the drop-volume method [41], as the dropping times had to be much longer (5 to 30 min) owing to the lower CMCs. A stainless steel tip with a radius of 0.307 cm was used.

## 3. Results

In fig. 1 surface tension values are plotted against the logarithm of the lecithin concentration in $10^{-2}$M phosphate buffer. In some cases especially at low surfactant concentrations of $diC_8$- and $diC_9$-lecithin the amount of solute adsorbed at the air–water interface was not negligible in relation to the amount within the bulk of the drop. We corrected for this by calculating the amount on the surface from the total drop area and the area/molecule, $1/\Gamma_i$, as found from the Gibbs adsorption isotherm:

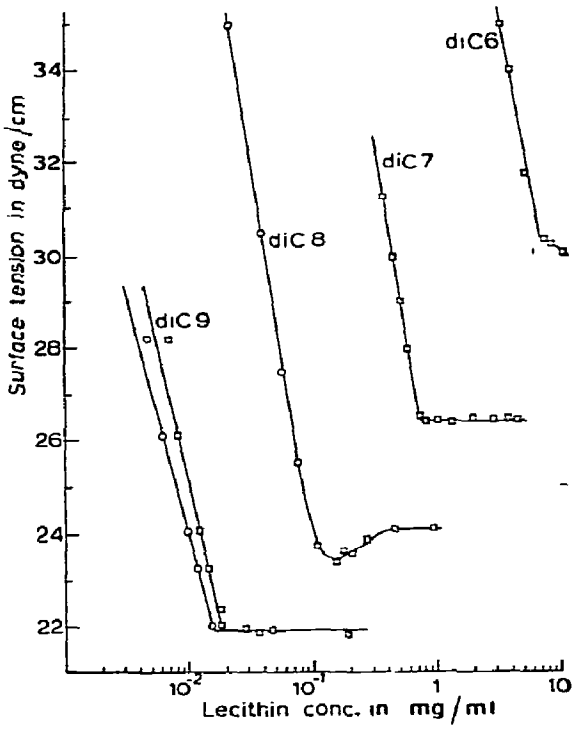


Fig. 1. Surface tension values (in dyne $cm^{-1}$) for several short-chain lecithin homologues at different lecithin concentrations (in mg $ml^{-1}$) in aqueous solutions containing $10^{-2}$mole $\ell^{-1}$ phosphate buffer, pH = 6.9 ± 0.2. In the data indicated with □ the initial bulk concentrations were used, while in the data indicated with ○ the bulk concentrations were corrected for adsorption at the air–water interface (see section3).

$$\partial\gamma/\partial\mu_i = (RT)^{-1}\partial\gamma/\partial \ln c_i = -\Gamma_i, \quad (1)$$

where $\gamma$ is the surface tension, $\mu_i$ is the chemical potential, $c_i$ is the concentration (activity coefficient assumed to be constant) and $\Gamma_i$ is the surface excess of the surfactant (component $i$). Assuming that the diffusion through the capillary hole of the tip was negligible we were able to calculate the decrease in the bulk concentration. It is evident, that this correction method will only be valid if the concentration change in the bulk is not too large. Moreover, at the lowest concentrations the dropping-time dependence of the drop-volume causes an extra inaccuracy in the $\gamma$ measurements and especially in the calculation of the area per molecule. In fig. 1 initial (□) and calculated concentration (○) of $diC_9$-lecithin are both plotted.

From the $\gamma$ versus log $c$ plots the critical micelle concentrations are found by the intersection of straight lines. The $\gamma$ versus log $c$ curve for $diC_8$-lecithin showed a minimum. In this case we assumed the CMC to be within a concentration range around the minimum. This minimum indicates a surface-active impurity [43], which we were not able to remove. It was present in three samples synthetised and purified separately and also in a sample kindly given to us by dr. W.A. Pieterson of the Department of Biochemistry. The values of the CMCs are given in table 1 together with the results obtained from lightscattering (to be published) and the values obtained by de Haas and coworkers [1, 42], and by Roholt and Schlamowitz [21]. Limiting area's per molecule are also included in this table.

Table 1

CMC in mg(monohydrate) $ml^{-1}$ and area per molecule in $A^2$ $molec.^{-1}$ [eq. (1)] for several lecithins in aqueous solutions ($10^{-2}$M phosphate buffer)

| Compound | Surface tension | Light scattering | Literature value [1, 21, 42] | Area/molec.[a] |
|---|---|---|---|---|
| $diC_6$ | 6.9 | 6.5 | 6.5–5.8 | 66 ± 1 |
| $diC_7$ | 0.71 | 0.8 ± 0.04 | 1.0 ± 0.9 | 60 ± 1 |
| $diC_8$ | 0.12–0.16 | 0.13 | 0.10 | 63 ± 3 |
| $diC_9$ | 0.016[b] | | | 85 ± 2[b] |

a) From surface-tension data.

b) These values were obtained by using the corrected concentrations; using initial concentrations a CMC of 0.018 mg $ml^{-1}$ and an area of 77 ± 3 $A^2$ $molec.^{-1}$ was found.

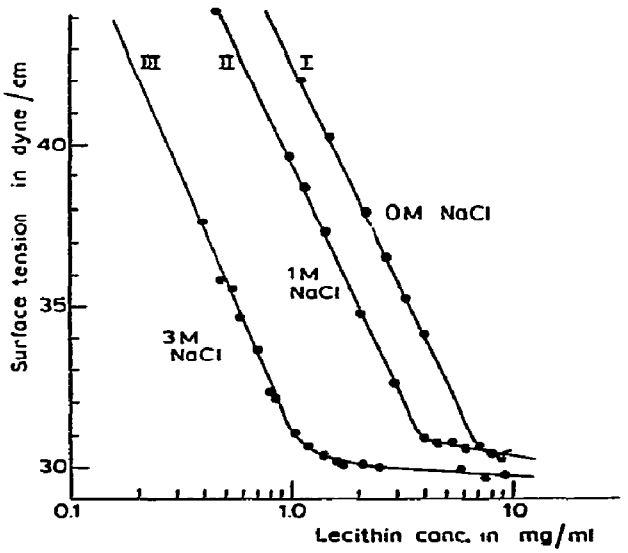


Fig. 2. Surface tension (in dyne $cm^{-1}$) of $diC_6$-lecithin (in mg $ml^{-1}$) in aqueous solutions containing $10^{-2}$ mole $\ell^{-1}$ phosphate buffer pH = 6.9 ± 0.2, and varying concentration of NaCl. Curve I: 0 mole $\ell^{-1}$ NaCl; curve II: 1 mole $\ell^{-1}$ NaCl; curve III: 3 mole $\ell^{-1}$ NaCl.

Some of the $\gamma$ versus log $c$ curves, in aqueous solutions containing high electrolyte concentrations, are plotted in figs. 2 and 3. The influence of the salt concentration on the CMC is also summarized in fig. 4 and table 2.

## 4. Discussion

### *4.1. Effect of acyl chain length on the standard free energy of micellisation*

For the association equilibrium $M_1 \rightleftarrows (1/n)M_n$ between monomers ($M_1$) and monodisperse micelles ($M_n$) with association numbers $n$, the standard free energy per mole for micelle formation $\Delta G^0$ is given by

$$\Delta G^0 = -RT\ln K = -(RT/n)\ln[M_n] + RT\ln[M_1]. \quad (2)$$

In eq. (2) the association constant is called $K$ and symbols in square brackets represent mole fractions. If the micelles have a distribution in aggregation number,

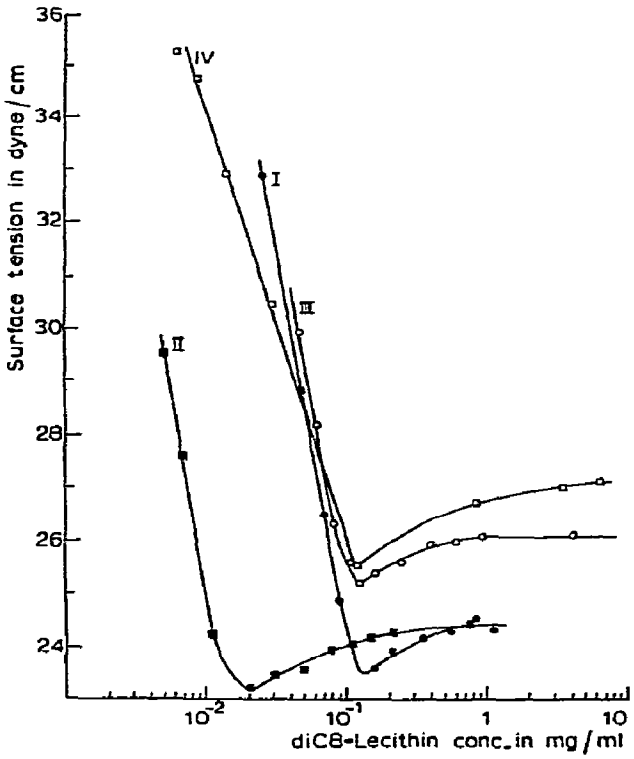


Fig. 3. Surface tension (in dyne $cm^{-1}$) for different concentrations (in mg $ml^{-1}$) of $diC_8$-lecithin in aqueous solutions containing, in addition to $10^{-2}$ mole $\ell^{-1}$ phosphate buffer (pH = 6.9 ± 0.2), variable concentrations of electrolytes. Curve I (•) no extra added electrolyte; II (▪) 1 mole $\ell^{-1}$ NaCl; III (○) 1 mole $\ell^{-1}$ LiI; IV (□) 1 mole $\ell^{-1}$ NaCl + 3 mole $\ell^{-1}$ LiI.

Table 2

Influence of salt concentrations (in mole $\ell^{-1}$) on the CMC (in mg $ml^{-1}$) of $diC_6$-, $diC_7$-, $diC_8$-lecithin

| Lecithin | Salt | Concentration (mole $\ell^{-1}$) | CMC (mg $ml^{-1}$) | Area/molec. ($A^2$/molec.) |
|---|---|---|---|---|
| $diC_6$ | – | – | 6.9 | 66 ± 1 |
| | $Na_2SO_4$ | 0.310 | 3.29 | 67 ± 1.5 |
| | | 0.657 | 1.62 | 65 ± 1.5 |
| | | 0.727 | 1.12 | 61 ± 1 |
| | | 1.715 | 0.29[5] | 70 ± 2 |
| | NaF | 0.595 | 3.01 | 64 ± 3 |
| | NaCl | 1.00 | 3.74 | 62 ± 1 |
| | | 3.00 | 1.10 | 58 ± 2.5 |
| | LiI | 1.18 | 5.91 | 68 ± 1 |
| | | 3.68 | 4.63 | 69 ± 1.5 |
| | | 4.82 | 6.20 | 76 ± 1 |
| | NaCl + LiI | 1.00 1.85 | 3.6 | 73 ± 1 |
| $diC_7$ | – | – | 0.71 | 60 ± 1 |
| | NaCl | 1.00 | 0.43 | 75 ± 1 |
| | | 1.98 | 0.2 | 74 ± 2 |
| $diC_8$ | – | – | 0.12–0.16 | 60 ± 3 |
| | NaCl | 1.00 | 0.02 | 61[5] ± 1 |
| | LiI | 1.00 | 0.11–0.14 | 59 ± 2 |
| | | 3.00 | 0.1 –0.14 | 67 ± 2 |
| | NaCl + LiI | 1.00 3.00 | 0.14–0.16 | 118 ± 2 |

average concentrations and association numbers have to be used and an average free energy will be obtained [20]. For large association numbers the term containing the micellar concentration will vanish and the free energy change per monomer may be approximated by

$$\Delta G^0 = RT \ln [M_1] = RT \ln [CMC]. \quad (3)$$

In cases where the micellar species have to be taken into account we have followed Mukerjee [44]. At a total concentration of $C_t$ = CMC the micelle concentration equals 2% of the monomer concentration. In table 3 the change in standard free energy of the monomers, when associating in micelles, is given for the different lecithins in $10^{-2}$M phosphate buffer. Column I was calculated on the basis of eq. (3), from surface tension data (table 1). The results based on eq. (2) are given in column II and III. In column II Mukerjee's approximation was made, while the values in column III were calculated from light scattering and ultracentrifugation data (to be published). The latter techniques in principle allow for an independent evaluation of $[M_1]$ and $[M_n]$, so that Mukerjee's approximation can be avoided. Comparison of column II and III indicates how well Mukerjee's approximation applies to our systems.

In fig. 5 we have plotted $\Delta G^0/RT$ against the chain length of the lecithin. The slope of this graph gives for the increase in free energy per mole $CH_2$ a value of 1.08 ± 0.02. This magnitude agrees with the hydrocarbon

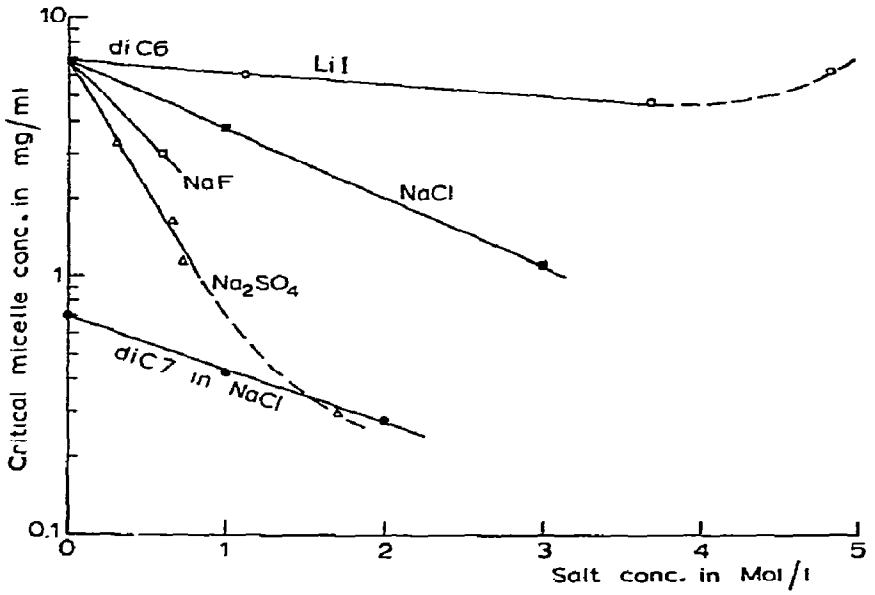


Fig. 4. The influence of varying concentrations in mole $\ell^{-1}$ of several electrolytes on the CMC (in mg $ml^{-1}$) of $diC_6$-lecithin and of NaCl on the CMC of $diC_7$-lecithin.

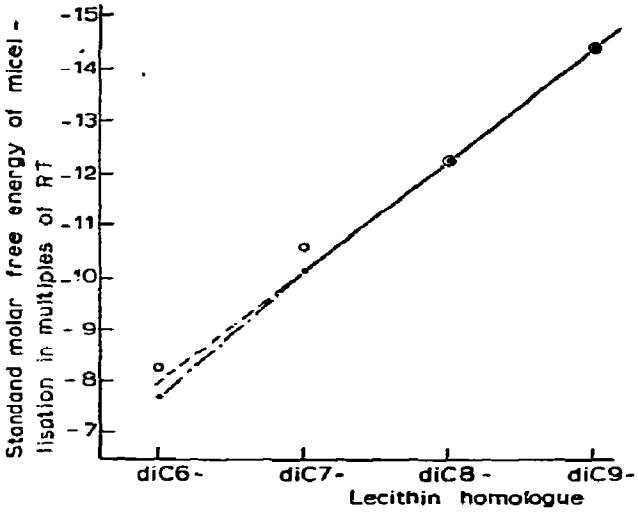


Fig. 5. Standard free energy of micellisation for the four lecithin homologues. The points indicated by an open circle (o) are calculated from eq. (3) (from CMC values only); dots (•) are calculated by eq. (2) (with the help of Mukerjee's approximation). Values from table 3 column I and II respectively.

Table 3

Standard molar free energy of micellization in multiples of $RT$

| Compound | I [a] | II [b] | III [c] |
|---|---|---|---|
| $diC_6$ ($n \approx 30$) | − 8.2[5] | − 7.7 | − 7.8 |
| $diC_7$ ($n \geq 40$) | −10.5[5] | −10.12 | −10.15 (to −10.30) |
| $diC_8$ ($n \geq 470$) | −12.3 ± 0.15 | −12.3±0.15 | −12.3 ± 0.15 |
| $diC_9$ (liquid crystals) | −14.4 ± 0.06 | −14.4±0.06 | – |

a) $\Delta G^0/RT = \ln[CMC]$ ; [CMC] in mole fraction = CMC (in g $ml^{-1}$) × $18/M_1$.
b) $\Delta G^0/RT = \ln[M_1] - (1/n)\ln[M_n]$, where $M_n$ was calculated as 2% of the CMC on a gram basis.
c) $\Delta G^0/RT = \ln[M_1] - (1/n)\ln[M_n]$, calculated from light-scattering or ultracentrifugation (to be published).

contributions found for many other surfactants containing one hydrocarbon chain [45, 46]. From this agreement we may conclude that the two relatively short chains in our molecules are independent of each other in the monomer molecule, i.e., there is no substantial association of the chains in the single molecule.

Similar conclusions for other surfactants can be drawn from the data of Williams et al. [13] and Ralston [14], when taking the CMC values given by Shinoda [46]. On the other hand the extensive data of Evans [12] on alkylsulphates with varying sulphate position have been interpreted by Smith and Tanford [47] by assuming an interaction of the alkylchains in the single molecule. The associated part in the molecule is already shielded from the water and contributes less to the hydrophobic bond than free hydrocarbon chains. This interpretation is based on data concerning compounds with two chains of unequal lengths. An estimate (with the help of eq. (2) and Mukerjee's approximation) of the standard free energy of micellisation using the data of Evans [12] on sulphates with two equal chains gives a value for the micellisation free energy per mole methylene group of 0.6 $RT$. When eq. (3) is used, 0.5 $RT$/mole $CH_2$ is obtained. The value for the soaps with the sulphate in the 1 position is about 0.7 $RT$/mole $CH_2$, which is a normal value for an ionic micellar system [45, 46]. Although these values are expressed per mole $CH_2$ they do in fact also account for changes in the contributions of the polar group, since these values are calculated from the slope of $\Delta G^0$ versus chainlength (as in fig. 5). In spite of the approximations and uncertainties it seems fair to conclude, from the difference between 0.6 and 0.7 $RT$/mole $CH_2$, that at least in this system there is some interaction between the alkyl-groups in the single dissolved molecule.

Smith and Tanford [47] determined the CMC of dipalmitoyllecithin to be $4.6 \times 10^{-10}$M ($\equiv \ln[\text{CMC}] = -25.5$). If we combine this value with ours for the CMC of $diC_9$ ($\ln[\text{CMC}] = -14.4$) by drawing a straight line between these two points as in fig. 5, we arrive at a calculated value of 0.8 $RT$/mole $CH_2$. This might be an indication that with longer carbon chains ($c > 9$) there is some association of the chains on the monomer.

### *4.2. Effects of electrolytes on the critical micelle concentrations*

The change in the critical micelle concentration, on addition of an inert electrolyte to a nonionic or zwitterionic surfactant, can often be expressed by the equation

$$\log \text{CMC} = -k_s C_s + (\log \text{CMC})_{C_s=0}, \qquad (4)$$

where $k_s$ is a constant, which depends on the salt and the soap studied, and $C_s$ is the electrolyte concentration [17, 32, 33]. Mukerjee [32] has given a theoretical explanation for this relation by using the McDevit–Long theory [29, 30] for salting-out effects of electrolytes on nonpolar solutes in water and the mass action equilibrium for micelle formation. He made the following approximations:

(a) The influence of the salt on the polar group of the single dissolved monomer equals the effect on that group in the micellar interface.

(b) The apolar part of the surfactant in the micelle exposed to the water is very small.

(c) The term $(1/n)\ln[M_n]$ from eq. (2) does not change significantly with the salt concentration. With these approximations the following equation was derived,

$$k_s = \overline{V}_i(V_s - \overline{V}_s)/2.3\,RT\beta_0, \qquad (5)$$

where $\overline{V}_i$ is the partial molal volume of the apolar part of the monomer, $(V_s - \overline{V}_s)$ equals the electrostriction of the electrolyte in solution and $\beta_0$ is the compressibility of water at temperature $T$. We could in principle substitute the value for $k_s$ from eq. (5) in eq. (4), but this generally leads to an overestimate of salt effects by about a factor of 3 [29, 30, 33, 48]. However, on comparing different systems the relative values are often found to correlate well with one another. If for example we take the coefficient of NaCl and benzene ($k_{\text{NaCl,benz.}}$) as a reference we can estimate values for the salting-out coefficients in other systems by using a modified form of eq. (5):

$$k_s = (\overline{V}_i/\overline{V}_{\text{benz.}})\,[(V_s - \overline{V}_s)/(V_{\text{NaCl}} - \overline{V}_{\text{NaCl}})]\,k_{\text{NaCl,benz.}} \qquad (6)$$

The influence of different salts on the CMC of $diC_6$-lecithin and of NaCl on $diC_7$-lecithin is shown in fig. 4 and the data are given in table 2. In tables 4 and 5 our results of $k_s$ values for different salts with $diC_6$- and of NaCl with different lecithins are presented, together with values calculated on the basis of eq. 6. We used the same values for the volumes as Ray and Nemethy [33], i.e., $\overline{V}_{CH_3} = 25.5$ cm$^3$/mole [49, 50], $\overline{V}_{CH_2} = 15.9$ cm$^3$/mole [49, 50], $\overline{V}_{\text{benz.}} = 86$ cm$^3$/mole [48], and the $(V_s - \overline{V}_s)$ values from Deno and Spink [48] and Mukerjee [51]. The coefficient for LiI was obtained by assuming additivity of the electrostrictions for ions.

Table 4
Salting-out effects on $diC_6$-lecithin

| Salt | $k_s$(obs.) | $k_s$ [eq. (6)] |
|---|---|---|
| $Na_2SO_4$ | 1.04 ± 0.08 | 1.14 |
| NaF | 0.6 | 0.58 |
| NaCl | 0.26[6] ± 0.01 | 0.414 |
| LiI | 0.05 ± 0.01 | 0.08 |

From table 4 we see that the results for $diC_6$-lecithin are in qualitative agreement with the theory. Table 5, however, indicates that the theory is not satisfactory when comparing the effects of one salt (NaCl) on various surfactants.

Very surprising was the effect of a mixture of NaCl and LiI on $diC_8$-lecithin. The theory assumes additivity of the salt effects and this apparently does not apply since LiI, which by itself has hardly any influence on the CMC of an electrolyte free solution, counteracts the lowering of CMC due to added NaCl (see table 2). This increase in CMC is parallelled by an increase in the area per molecule (table 2). The reason for this increase may be an association of the lecithin at concentrations below the CMC (for instance a nearly complete dimerisation), or an increase in the hydration of the polar group. This latter assumption was proposed by Kurzendörfer [52] as an explanation for the increase in area/molecule of alcohols at the air–water interface when adding high concentrations of urea and sodium benzoate.

In this connection a few remarks about the area per molecule, given in table 2, should be made. The limiting areas for insoluble higher homologues in a monolayer are about 35 to 40 Å$^2$ per molecule [53, 54]. The areas in the L–α lamellar liquid crystalline phase, however, are about 60 Å$^2$ [55]. An area per molecule of around 60 Å$^2$ is also found for lecithin in the rodlike structures H, Q and R, where the paraffin chains are also in the liquid state. These phases occur at high temperatures and lipid concentrations [55, 56]. The monolayers of the soluble short-chain lecithins probably have the same packing as the higher homologues in the L–α phase.

Table 5
Salting-out effects for NaCl on $diC_6$-, $diC_7$-, $diC_8$-lecithin

| Compound | $k_s$(obs.) | $k_s$ [42] | $k_s$ [eq. (6)] |
|---|---|---|---|
| $diC_6$ | 0.26[6] ± 0.01 | 0.26[7] ± 0.01 | 0.414 |
| $diC_7$ | 0.21 ± 0.01 | 0.24 ± 0.04[a)] | 0.488 |
| $diC_8$ | 0.8 | | 0.562 |

[a)] CMC values for 0.1, 1, and 2 M NaCl were used; if only the value for 0.1 and 1 M NaCl are used a $k_s$ of 0.3 is found.

The effect of the salts on the area/molecule is small and barely above the experimental error except in the case of LiI + NaCl. This unexpected non-additivity deserves further experiments.

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