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Characterization of the solubilization of lipid bilayers by surfactants

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This communication addresses the state of aggregation of lipid-detergent mixed dispersions. Analysis of recently published data suggest that for any given detergent-lipid mixture the most important factor in determining the type of aggregates (mixed vesicles or mixed micelles) and the size of the aggregate is the detergent to lipid molar ratio in these aggregates, herein denoted the effective ratio, R_e. For mixed bilayers this effective ratio has been previously shown to be a function of the lipid and detergent concentrations and of an equilibrium partition coefficient, K, which describes the distribution of the detergent between the bilayers and the aqueous phase. We show that, similar to mixed bilayers, the size of mixed micelles is also a function of the effective ratio, but for these dispersions the distribution of detergent between the mixed micelles and the aqueous medium obeys a much higher partition coefficient. In practical terms, the detergent concentration in the mixed micelles is equal to the difference between the total detergent concentration and the critical micelle concentration (cmc). Thus, the effective ratio is equal to this difference divided by the lipid concentration. Transformation of mixed bilayers to mixed micelles, commonly denoted solubilization, occurs when the surfactant to lipid effective ratio reaches a critical value. Experimental evaluation of this critical ratio can be based on the linear dependence of detergent concentration, required for solubilization, on the lipid concentration. According to the 'equilibrium partition model', the dependence of the 'solubilizing detergent concentration' on the lipid concentration intersects with the lipid axis at $-1/K$, while the slope of this dependence is the critical effective ratio. On the other hand, assuming that when solubilization occurs the detergent concentration in the aqueous phase is approximately equal to the critical micelle concentration, implies that the above dependence intersects with the detergent axis at the critical micelle concentration, while its slope, again, is equal to the critical effective ratio. Analysis of existing data suggests that within experimental error both these distinctively different approaches are valid, indicating that the critical effective ratio at which solubilization occurs is approximately equal to the product of the critical micelle concentration and the distribution coefficient K. Since the nature of detergent affects K and the critical micelle concentration in opposite directions, the critical ('solubilizing') effective ratio depends upon the nature of detergent less than any of these two factors.

Abbreviations and symbols: PC, phosphatidylcholine; L, total lipid concentration; D_T, total detergent concentration; D_b, detergent concentration in bilayer; D_w, detergent concentration in aqueous medium; cmc, critical micelle concentration; $K = D_b/LD_w$; $R_e = D_b/L$; D_T^{cmc} and D_b^{cmc}, the detergent concentrations (total and in the bilayer, respectively) when D_w = cmc; D_T^c and D_b^c, the detergent concentrations (total and in the bilayer, respectively) when solubilization occurs; $R_e^{cmc} = D_b^{cmc}/L$; $R_e^c = D_b^c/L$; ΔD_T, ΔD_w and ΔD_b, the detergent concentrations (total and in the water and bilayer, respectively) that should be added to dispersions in which D_w = cmc to solubilize the lipids; $K' = \Delta D_b/L\Delta D_w$; $\bar{R}_H$, mean hydrodynamic radius of vesicles or micelles.

Introduction

Biological membranes are complex lamellar assemblies of insoluble amphiphiles, including phospholipids, neutral lipids and membrane proteins Surfactants (commonly denoted detergents) are soluble amphiphiles which above a critical concentration (cmc) form micelles of various sizes and shapes Much of our present knowledge on the composition, structure and function of biological membranes is due to the formation of thermodynamically stable isotropic (mixed micellar) solutions of the various components of biological membranes in the presence of detergents at sufficiently high concentrations [1] Nevertheless, in spite of the extensive use of this procedure for separation and characterization of the various water-insoluble membrane components, its detailed mechanism is not clear Moreover, even the mechanism of solubilization of lipid bilayers is not understood [2]

Previously it has been suggested that for the phase transformation (micellization) to occur, the concentration of the free detergent (D_w) should exceed the critical micellar concentration (cmc) of the surfactant [3] This does not necessarily mean that pure micelles of the surfactant have to be formed, which can then form mixed micelles with the lipids Alternatively, the added surfactant can distribute between the bilayers and the aqueous medium and bring about spontaneous phase transformation when the ratio of detergent to lipid in the bilayer ($R_e = D_b/L$) exceeds a critical ratio R_e^c [1–8] While there is no a priori reason why this ratio cannot be attained for $D_w <$ cmc, there are presently no sufficient data to support this possibility (see below)

Transformation of lamellar structures into mixed micelles is commonly denoted 'solubilization' Explicit definition of the latter term is not trivial because the disruption of the lamellar structures by detergents may involve various stages However, for most membrane biochemists and biophysicists the term 'solubilization' is merely operational As such, it means that 'complete solubilization' of a membrane preparation is defined as that point at which all the membranes were transformed into mixed micelles, yielding a transparent solution Accordingly, turbidity measurements of a membrane preparation as a function of added detergent has been analyzed in terms of 'percent solubilization' [8] In such turbidity versus detergent curves, the turbidity is initially affected only slightly by additional detergent Further detergent addition results in a large decrease of turbidity until complete solubilization' is obtained, at which point additional detergent has only slight effect on the turbidity of the dispersion, probably due to changes in the size and shape of the detergent-lipid (-protein) mixed micelles

In terms of 'percent solubilization', the initial detergent added, up to a point at which the turbidity starts decreasing markedly, is denoted 'subsolubilizing detergent concentration' Throughout the range of detergent addition which causes large decrease of turbidity, it may be assumed that lamellar and micellar structures co-exist [4–13] This view is strongly supported by the agreement between turbidity measurements and NMR studies [8–13] since the latter are especially sensitive to micelles while turbidity is mostly due to lamellar aggregates Analysis of 'solubilization curves', based on the dependence of either the turbidity or various NMR parameters upon the added detergent concentration, demonstrates that this curve can be regarded as an integral of a normal distribution curve [14], indicating either a Gaussian distribution of the added detergent between lipid vesicles or a normal distribution of the vesicle sensitivity to the detergent In any event, the solubilization can be characterized by the ratio at which 50% of the lipid is solubilized (R_e^c) and the width of the Gaussian distribution curve representing the solubilization

The crucial question which remains rather open is that of the value of the critical solubilizing molar ratio in the bilayers, R_e^c This communication addresses this problem

Approaches for derivation of R_e^c

Two approaches can be offered for the derivation of the critical effective ratio, at which the lamellar to micellar transformation occurs Both approaches are based on the linear dependence of D_T^c, the critical detergent concentration at which phase transformation occurs, on the lipid concentration, L Such a linear dependency (sche-

matically presented by the solid line in the inset to Fig 1) can either be described by

$$D_T^c = b + \alpha L \tag{1}$$

or

$$D_T^c = \alpha(a + L) \tag{2}$$

Both approaches for derivation of R_e^c share the following assumptions [4–8]

(1) The lipid monomer concentration is negligible

(2) When phase transformation occurs, the detergent is either monomeric or else included in lipid-detergent mixed aggregates (i e mixed vesicles and/or micelles) At this point, pure detergent micelles are not present

(3) It is the molar ratio of detergent to lipid in the mixed aggregates (R_e) which determines the aggregational state of the lipid (lamellar or mixed micellar) More specifically, if the value of R_e in any given lipid detergent mixed aggregate is higher than the critical ratio R_e^c, solubilization will occur

However, the two approaches differ significantly in terms of one further assumption

(1) $D_w^c = \text{cmc}$

This approach assumes that when the bilayers are transformed into mixed micelles, the detergent concentration in the aqueous medium is about equal to the critical micelle concentration, hence the detergent concentration in the bilayers at this point (D_b^c) is given by $D_b^c \approx D_T^c - \text{cmc}$
The effective detergent to lipid ratio at this point (R_e^c) is therefore given by $R_e^c = (D_T^c - \text{cmc})/L$ Hence

$$D_T^c = \text{cmc} + R_e^c\, L \tag{3}$$

In other words, the value of the slope (α) in the inset of Fig 1 is that of R_e^c while the extrapolated intercept (b) equals the cmc

(2) $D_b/L - D_w = K$ (4)

This approach, recently offered by Schurtenberger et al [4–7], assumes an equilibrium partition of detergent between the bilayers and the aqueous medium From the definition of R_e ($R_e \equiv D_b/L$) it follows that $D_b = R_e\ L$ In addition, $K = R_e/D_w$, hence $D_w = R_e/K$

Material balance requires that $D_T = D_w + D_b$ and the equilibrium partition therefore requires that

$$D_T = R_e\left(L + \frac{1}{K}\right) \tag{5}$$

This means that if a single distribution coefficient can describe the distribution of detergent between the lipid aggregates and the aqueous medium, then the detergent concentration reaches a critical value D_T^c when the molar ratio reaches a critical value R_e^c given by

$$R_e^c = D_T^c/\left(L + \frac{1}{K}\right) \tag{6}$$

In other words, the value of the slope (α) in the inset of Fig 1 is that of R_e^c while the extrapolated intercept (a) equals $1/K$

This 'equilibrium partition model' [4–7] is not very sound thermodynamically because of the following reasons

(1) It ignores the kinetic barriers to thermodynamic equilibration imposed on the system by the slow distribution of many detergents into the inner monolayers, due to their slow rate of trans-membrane 'flip-flop' [15,16]

(2) It ignores specific interactions between lipid and detergents, which has been demonstrated for a variety of amphiphiles [17,18] This indicates that the mixing of lipids and detergents is not ideal A single distribution coefficient can still describe the partition of detergent between the bilayer and the aqueous medium for a certain range of detergent to lipid ratio, but rigorous thermodynamic analysis of such a constant is complex

(3) For ideal mixing of lipid and detergent, equilibrium can be expected when $\mu_b = \mu_w$ where μ_b and μ_w are the chemical potentials of the detergent-in-bilayer and detergent-in-water mixtures, respectively Hence,

$$\mu_L^0 + kT \ln x_b = \mu_w^0 + kT \ln x_w \tag{7}$$

where x_b and x_w are the mole fractions of detergent in the bilayer and in the aqueous medium, respectively Thus, the ratio of mole fractions (x_b/x_w) is constant

$$x_b/x_w = e^{-[(\mu_b^0 - \mu_w^0)/kT]} \tag{8}$$

or

$$[D_b/(D_b + L)]/[D_w/(D_w + w)] = K \quad (9)$$

D_w is always much lower than the water concentration (w) Thus

$$D_b/(L + D_b)D_w] = K \quad (10)$$

Therefore D_b/LD_w is constant only when $D_b \ll L$, that is only for low values of R_e

Under these conditions, the mole fraction of detergent in the bilayers ($D_b/(L + D_b)$) is about equal to R_e and R_e should depend linearly on D_w Otherwise x_b (and not R_e) is a linear function of D_w Hence

$$L/D_b = (1/K'D_w) - 1 \quad (11)$$

which implies that a linear correlation should exist between the reciprocals of D_b and D_w rather than between D_b and D_w

In spite of the shortcomings of the 'equilibrium partition model' of Schurtenberger and his colleagues [4–7], their approach appears to be consistent with experimental data over wide ranges of R_e values (see 'Comparison with available data' below) More specifically, addition of 'sub-solubilizing' concentrations of various detergents results in detergent partition between the bilayer and water such that the detergent concentration in the bilayer (D_b) is a linear function of the detergent concentration in the aqueous medium (D_w) [4–8]

However, when the lipid is solubilized the concentration of monomers in the solution (D_w) might be larger than the cmc of the pure detergent [19]

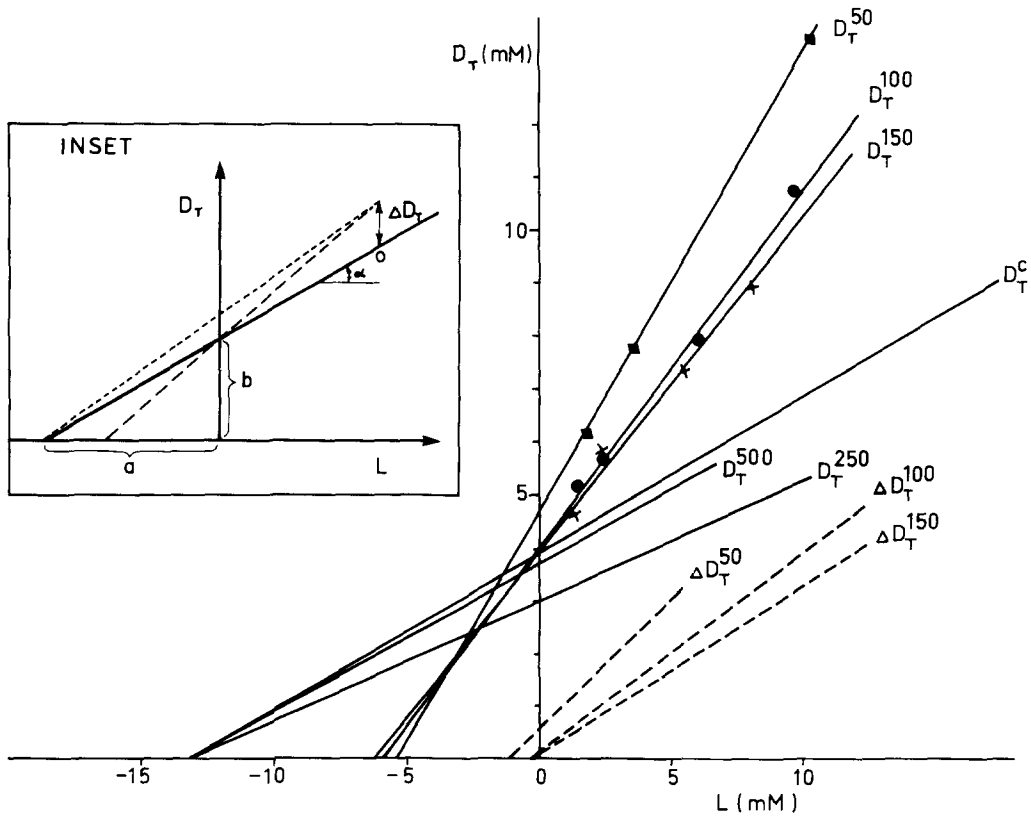

Fig 1 The total detergent concentration is plotted as a function of the lipid concentration for various conditions (data from Schurtenberger et al [4–7]) D_T^c is the phase boundary D_T^{250} and D_T^{500} represent vesicles of hydrodynamic radii ($\bar{R}_H$) of 250 A and 500 A, respectively, D_T^{50} (■) D_T^{100} (●) and D_T^{150} (×) present the composition of mixed micelles of $\bar{R}_H$ of 50 100 and 150 A respectively ΔD_T^{50}, ΔD_T^{100} and ΔD_T^{150} are the differences in dependences ($D_T^{50} - D_T^c$) ($D_T^{100} - D_T^c$) and ($D_T^{150} - D_T^c$) respectively The inset is a schematic representation of a phase diagram of lipid-detergent systems (with L mM lipid and D_T mM detergent), presented for the clarity of discussion in the text, where the various factors are also defined

In this case, the assumption of distribution according to a constant partition coefficient [4–8] implies that even after the concentration of detergent in the aqueous medium exceeds the critical micelle concentration of the pure detergent, the distribution of the detergent obeys the same K I find this implication to be very unlikely

When the limit $D_w = \text{cmc}$ is reached, Eqn 4 requires that the detergent concentration in the bilayer (D_b^{cmc}) will be equal to

$$D_b^{cmc} = \text{cmc}\ K\ L \tag{12}$$

which means that

$$R_c^{cmc} = \text{cmc}\ K \tag{13}$$

The total detergent concentration required to reach the critical micelle concentration in the presence of lipids is therefore given by

$$D_T^{cmc} = \text{cmc}(1 + K\ L) \tag{14}$$

and since $\text{cmc} = R_c^{cmc}/K$ (from Eqn 13) then

$$D_T^{cmc} = R_c^{cmc}\left(\frac{1}{K} + L\right) \tag{15}$$

It can therefore be concluded that when $a = -1/K$ and $b = \text{cmc}$, the solid line in the inset of Fig 1 represents the dependence of D_T^{cmc} on the lipid concentration Thus, only if solubilization occurs when the total detergent concentration is just sufficient to result in a monomer concentration which is equal to the critical micelle concentration, the dependence of D_T^c on L intersects with the lipid axis at $1/K$ and with the detergent axis at the detergent's critical micelle concentration In all other cases $a \neq 1/K$ and/or $b \neq \text{cmc}$

If solubilization occurs when $D_w < \text{cmc}$, the value of (b) in Eqn 1 is lower than the critical micelle concentration and the critical ratio R_e^c (which may then be appropriately described by Eqn 6) is higher than would have been predicted on the basis of the assumption that when solubilization occurs $D_w \approx \text{cmc}$ On the other hand, if the total detergent concentration required for solubilization is higher than that required for reaching a monomer concentration equal to the critical micelle concentration, that is if $D_T^c > \text{cmc}\ (1 + KL)$, then for a system in which $D_w = \text{cmc}$ but $R_e < R_c^c$ (that is $D_b < D_b^c$), additional detergent is likely to distribute between the bilayer and the aqueous medium according to a different distribution coefficient K' ($K' \gg K$, see below) and (a) is likely to be larger than $1/K$ More specifically, if at point O in the inset of Fig 1 $D_w = \text{cmc}$ (that is $a = 1/K$ and $b = \text{cmc}$) and yet at this point the critical ratio for solubilization (R_c^c) has not been reached, then the detergent concentration, which has to be added for solubilization to occur (ΔD_T), depends upon the distribution of the additional detergent The minimal requirement for added detergent is expected if all the additional detergent is incorporated into the membranes ($K' \to \infty$), in which case $\Delta D_b \simeq \Delta D_T$ and D_T^c is described by the broken line in the inset to Fig 1, and by Eqn 16

$$D_T^c = \text{cmc} + R_c^c\ L \tag{16}$$

whereas the maximal required detergent is expected if the distribution of the added detergent is described by K, $K' = K$ In this case, more detergent has to be added and if ΔD_T is needed at point O, D_T^c is described by the dotted line in the inset of Fig 1, and by Eqn 17

$$D_T^c = R_c^c\left(L + \frac{1}{K}\right) \tag{17}$$

Therefore the dependence of D_T^c on L should intersect with the lipid axis at $-1/K$ only if $D_T^c < D_T^{cmc}$ or else, for a system in which $D_T^c > D_T^{cmc}$, when $K' = K$ In all other cases, the value of (a) is not equal to $-1/K$ As a matter of fact, for the two extremes described above (i e $\Delta D_T = \Delta D_b$ and $\Delta D_b/(\Delta D_T - \Delta T_b) = KL$), α is equal to the critical ratio R_e^c But if the new distribution coefficient K' is larger than K but not infinite, then $a \neq \frac{1}{K}$, $b \neq \text{cmc}$ and, in fact, $\alpha \neq R_c^c$

Comparison with available data

As stated above, 'sub-solubilizing' detergent concentration appeared in several studies to be distributed between bilayers and aqueous media according to Eqn 4 Thus, a partition coefficient of 0 074 mM^{-1} described the partition of glycocholate between egg PC vesicles and the aqueous media [7] while octyl glucoside obeyed a partition

coefficient of 0 044 [8] * Analysis of the data points of both these studies in terms of (the more thermodynamically sound) Eqn 11 yield similar correlation coefficients to those obtained for the linear dependence formulated by Eqn 4 and the corresponding values of K were only slightly different from those based on the latter equation Furthermore, in both these studies there is no evidence for a difference between the accessibility of the inner and outer leaflets of the PC bilayer to the added detergent This may be due to detergent-induced increase in the rate of 'flip-flop' of detergent molecules, which may occur at total detergent concentrations at which the ratio of detergent to lipid in the bilayer is lower than the ratio which existed in the distribution studies of references [7] and [8] even for the lowest detergent concentration employed

This was not the case for solubilization of PC bilayers by the non-ionic detergent $C_{12}E_8$ In the study describing the solubilization and reconstitution of PC vesicles by this detergent [9], the ratio of detergent to phospholipid in vesicles (R_e) was described as a function of the detergent concentration in the aqueous medium (D_w) For $R_e < 0\ 15$, D_w depended very strongly upon the method of preparation of the detergent-containing vesicles Thus addition of detergent to preformed vesicles to a point where $D_w = 0\ 012$ mM was accompanied by incorporation of detergent into the vesicle membranes yielding $R_e \approx 0\ 05$, whereas upon formation of vesicles through detergent-removal an R_e value of approx 0 10 was observed for $D_w <$ 0 012 mM Furthermore, the permeability of detergent-containing vesicles to Na^+ and Cl^- was quite different at any level of $R_e < 0\ 15$ but becomes essentially equal at high R_e values However, for $R_e > 0\ 15$, the dependence of R_e on D_w appears to be independent of the method of preparation of the detergent-containing vesicles Solubilization appears to occur at $R_e^c \approx 0\ 30$ since as long as some of the vesicles remained non-solubilized (probably over the range of co-existence of vesicles and mixed micelles) the ratio of detergent to PC in the vesicles phase is approx 0 30 The number of data points [15] in the range of $0\ 15 < R_e < 0\ 30$ is insufficient to conclude whether over this range R_e (thus D_b) is a linear function of D_w However, it is quite possible that this indeed is the case Furthermore, the data points obtained upon addition of detergent to the preformed vesicles to R_e values lower than 0 15 also appear to represent a linear function of R_e on D_w but with a slope half of that obtained for the higher R_e values Moreover, the dependence of x_b on D_w may also be analyzed in terms of two distribution coefficients Initially, for $R_e < 0\ 15$, the distribution of detergent between the outer monolayer and the aqueous medium is described by one partition coefficient At that point where the overall $R_e = 0\ 15$, the molar ratio in the outer monolayer may in fact be twice as high, some lipid may be solubilized and the rate of transmembrane 'flip-flop' of detergent may become sufficiently high to make the inner monolayer accessible for the detergent At higher R_e values, the apparent partition coefficient will therefore represent the partition of detergent between the whole phospholipid bilayer and the aqueous medium

For other detergents [4,5], this facilitated rate of 'flip-flop' may occur at much lower R_e values, so as to obscure the 'biphasic nature' of detergent partition In these (and other) cases, solubilization experiments resulted in a value of (a) which is about equal to $1/K$, and a value of (b) which is about equal to the critical micelle concentration, suggesting that at the phase boundary D_w = cmc As an example, the micellar $\rightleftharpoons$ lamellar phase boundary in mixtures of phosphatidylcholine (PC) and glycocholate (GC), which has been recently studied in detail by Schurtenberger et al [7], intersect with the bile salt concentration axis at a concentration close to the critical micelle concentration of this surfactant [20], while intersecting with the PC axis at a value close to the (independently determined) $-1/K$ (D_T^c in Fig 1) Further-

* In this paper [8], the distribution was analyzed in terms of a concentration-independent distribution coefficient $\bar{K}$ such that $\bar{K} = (D_b/V_b)/(D_w/V_w)$ where V_b and V_w are the volumes of the lipid bilayer and the aqueous medium, respectively For a bilayer of a specific volume $\bar{V}$ made of lipids of an average molecular weight $\bar{M}$ and a total concentration L (expressed in mM), the ratio of volumes is given by $V_b/V_w = \bar{K}\bar{V}L\ 10^{-6}$ $\bar{K}$ is therefore given by $\bar{K} = (D_b/LD_w)\bar{M}^{-1}\bar{V}^{-1}\ 10^6$ And in terms of $\bar{K}$, the value of K is given by $K = \bar{K}\bar{M}\bar{V}\ 10^{-6}$ For the distribution of octyl glucoside between PC vesicles and the aqueous medium we found $\bar{K} \approx 60$ that is $1/K \approx 22\ 5$ mM

more, these authors demonstrated that the size of vesicles obtained upon dilution of glycocholate-PC mixed micelles is a function of R_e, as defined by Eqn 5 Thus, for any given size of vesicles, the bile salt is a linear function of the lipid concentration as exemplified in Fig 1 for PC-glycocholate vesicles of hydrodynamic radii $\bar{R}_H$ of 250 Å (D_T^{250}) and 500 Å (D_T^{500}) These lines, again, intersect with the lipid concentration axis at $-1/K$ but their intersections with the bile salt axis is at values below the critical micelle concentration

The data on glycocholate-PC mixed micelles, given by these authors, are compatible with the assumption that, similar to the PC-glycocholate mixed bilayers, the size of the mixed micelles is also a function of R_e Thus the total concentration of glycocholate in glycocholate-PC mixed dispersions containing micelles of any given size is a linear function of their PC concentration

$D_T^{50} = 0\ 87\ L + 4\ 7$, for micelles of $\bar{R}_H = 50$ A

$D_T^{100} = 0\ 67\ L + 4\ 0$, for micelles of $\bar{R}_H = 100$ A

$D_T^{150} = 0\ 61\ L + 3\ 9$, for micelles of $\bar{R}_H = 150$ A

All these linear dependencies (with correlation coefficients > 0 996) intersect with the lipid axis at about -5 mM This may be interpreted in terms of a distribution coefficient (K_m) between mixed micelles and aqueous media, which has a value of 0 2 mM^{-1} Alternatively, it may be analyzed on the basis of two distribution coefficients that are K for systems in which $D_w <$ cmc and K', for detergent concentrations in excess of D_T^{cmc} (ΔD_T) The latter distribution coefficient $K' = \Delta D_b / L \Delta D_w$) implies that $\Delta D_T = (\Delta D_b/L)(L + 1/K)$ D_T is of course a sum of D_T^{cmc} and ΔD_T, and since both these factors are a function of L, the difference between the dependencies of D_T and D_T^{cmc} on L should describe the dependence of ΔD_T on L Assuming that for glycocholate-PC mixed micellar systems $D_T^{cmc} = D_T^c$, results in the dependencies described by the broken lines in Fig 1, denoted $\Delta D_{0T}^{>}$, ΔD_T^{100} and ΔD_T^{150}, for micelles of $\bar{R}_H$ of 50, 100 and 150 Å, respectively All these lines intersect with the lipid concentration axis at $-0\ 8$ to $-0\ 2$ mM, indicating that $K' = 1\ 25$–$5\ 0$ mM^{-1}, that is $K' \gg K$ Thus, while for bilayers $R_e = D_T/(L + 1/K)$, in the micellar range $R_e \approx (D_T -$ cmc$)/L$ It is therefore not surprising that phase transformation occurs when $(D_T -$ cmc$)/L \simeq K$ cmc, which for $D_w =$ cmc means that $R_e \simeq K$ cmc This argument again supports the hypothesis that phase transformation occurs when $D_w =$ cmc

This conclusion is also supported by our previously study [8] of the solubilization of PC vesicles by the non-ionic detergent octyl glucoside In this study we have shown that over the whole range of coexistence of micelles and vesicles, the ratio of octyl glucoside to PC in the vesicles (R_e^c) is constant and equals 1 29 ± 0 21 The critical micelle concentration of octyl glucoside is about 22 mM whereas its distribution between the bilayer and the aqueous phase is characterized by a distribution coefficient * of $K = 1/22\ 5$ mM^{-1} Accordingly, $R_e^{cmc} =$ cmc $K = 22/22\ 5 = 0\ 98$ The experimental errors in the determination of both K and cmc are quite large Therefore the value of $R_e^{cmc} = 0\ 98$ can be considered to be close enough to the experimental value of R_e^c to support the conclusion that solubilization occurs when $D_w =$ cmc, that is when $R_e \simeq K$ cmc

The non-ionic detergent $C_{12}E_8$ has a very low critical micelle concentration (approx 0 09 mM [9]) On the other hand, its partition between egg PC vesicles and aqueous media follows a very high partition coefficient (according to Eqn 11 $K \approx 5\ 7$ mM^{-1})

The product of these two parameters is equal to about 0 5 This value is higher than the experimentally observed R_e^c of approx 0 3 but in view of the probable experimental errors in determination of this detergent's low critical micelle concentration, the approximation $R_e^c \simeq K$ cmc can be considered to be quite satisfied This latter approximation should only be regarded as a rough estimate of the effective ratio at which solubilization is likely to occur However, as such, it may serve as a valuable guide in designing solubilization experiments

Implications

In practical terms, the above conclusion is likely to be quite general Hydrophobic detergents with low hydrophilic/liphophylic balance can be ex-

* See footnote on p 475

pected to have low critical micelle concentration but high K values Therefore, when D_w = cmc, much of the detergent already resides in the bilayer, if it still has not been solubilized at this point On the other hand, detergents with high critical micelle concentration values are likely to have low K values Thus, when D_w = cmc, K' can be expected to be so much higher than K that even if R_e^c is much higher than R_e^{cmc}, the concentration of detergent that has to be added to the bilayer to cause its solubilization (ΔD_T) is much smaller than D_T^{cmc} and in practical terms, again $D_T^c \simeq D_T^{cmc}$

Altogether, in spite of the lack of a sound theoretical basis for the hypothesis of Dennis and Owens [3], that the concentration of free detergent has to rise to the critical micelle concentration for solubilization to occur, their conclusion appears to be valid Accordingly, although the two approaches to analyze solubilization of lipid bilayers by detergents are based on different grounds, in practice they might be quite similar To distinguish between the two approaches would require a high degree of accuracy in the independent determination of K and the critical micelle concentration The variability of data available to date on the critical micelle concentration is certainly not sufficient to make such a distinction We suggest that since the critical micelle concentration values of most detergents are known, the solubilization in general will be described by Eqn 16 (D_t^c = cmc + R_e^c L) As suggested above, empirically D_T^c obeys this equation as well as Eqn 17 Therefore, the dependence of D_T^c on L results not only in the values of the detergent's critical micelle concentration and of R_e^c but also in a close approximation for K ($K = R_e^c$/cmc)

This conclusion has several other interesting implications First, the dependence of D_T^c on L is such that the 'solubilizing' critical ratio of detergent to lipid in the bilayer (R_e^c) is approximately equal to the product of K and the critical micelle concentration As the hydrophobicity of a detergent is expected to alter these two factors in opposite directions, R_e^c should be affected by the detergent's hydrophobicity less than either K or the critical micelle concentration Second, for a given detergent, a membrane will be solubilized when R_e^c reaches a value directly proportional to K, that is as the affinity of a detergent to the membrane increases (higher K), more detergent can be accommodated in the membrane before solubilization occurs Furthermore, since $K = \bar{K}\bar{M}\bar{V}\ 10^{-6}$ (see footnote on p 475) it also follows that an increase in the membranes specific volume ($\bar{V}$) would result in a requirement for higher detergent concentrations for solubilization, provided that $\bar{K}$ and $\bar{M}$ are constant And last, but not least, while the disruption of the bilayer by a detergent of course depends on the exact nature of detergent and bilayer lipid, as it is a function of the various interactions between these two compounds, a measurement of the distribution coefficient of a sub-solubilizing concentration of a detergent of a known critical micelle concentration between a given preparation of membranes and the aqueous medium is sufficient for a rough approximation of the detergent's concentration needed for solubilization

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