

Thermodynamic Characterization of Dilute Aqueous Lipid/Detergent Mixtures of POPC and C₁₂EO₈ by Means of Isothermal Titration Calorimetry

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The solubilization of POPC lipid bilayers by the nonionic detergent C₁₂EO₈ was studied by isothermal titration calorimetry. The characteristic transfer enthalpies for the detergent and the lipid between bilayers and micelles were determined by titration of detergent micelles to lipid membranes and vice versa. For purpose of comparison, the enthalpy and Gibbs free-energy changes for the aggregation of aqueous detergent monomers to micelles as well as for the partitioning into lipid bilayers were analyzed. The phase boundaries between pure bilayers and pure micelles, i.e., the detergent mole fraction, when the bilayers become saturated with detergent and first mixed micelles appear and the mole fraction when the bilayers are completely solubilized and only mixed micelles are present could be easily determined from the titration experiments. The detergent binding to membranes does not follow the mass action law because there are no specific binding sites. An equation using partitioning of detergent between water and bilayers gave good fits to the experimental data. The experiments lead to a consistent set of transfer enthalpies and entropies for the system of monomers, micelles, and bilayers. Suggestions are made about the thermodynamic nature of solubilization and partitioning. Finally, besides the limiting compositions of bilayers and micelles, another composition-driven transition was detected within the mixed micellar range. This can be imagined to correspond to a variation of the micellar shape and size and/or intermicellar interactions.

Introduction

The interaction of detergents with lipid membranes is a field of continued interest because several scientific questions are still unclear but have to be solved because they are related to technical applications, particularly the problem of biomembrane reconstitution,^{1–4} or to the understanding of modifications of model membrane systems for studying basic membrane properties like the hydration force.⁵ The aim of this work is a thermodynamic characterization of the ternary system consisting of the lipid POPC, the nonionic detergent C₁₂EO₈ and water by means of isothermal titration calorimetry (ITC). This detergent is well studied, and numerous data on its physical properties in water have been published using other methods.^{6–8}

The transition of lipid bilayers to micelles upon addition of a micelle-forming detergent has been called solubilization. Whereas only little information is available on the complete ternary phase diagram of such lipid/detergent/water systems,⁹ much more data were published about highly diluted systems close to the “water corner” of the ternary phase diagram. These dilute lipid/detergent mixtures can be treated as pseudobinary systems, and the liquid-crystalline lamellar/micellar phase transition can be well described in terms of the Lichtenberg model.^{10–14} According to this model, detergent molecules are incorporated into lipid membranes up to a limiting detergent mole fraction X_{sat} . Then, lipid-saturated micelles with composition X_{sol} start to be formed. In the composition range between X_{sat} and X_{sol} , membranes and micelles with the definite compositions X_{sat} and X_{sol} coexist with varying proportions. Increasing the total detergent content to values above X_{sol} destroys all bilayers, and only micelles exist.

Various extensions to this model have been made based on morphological observations. It has been stated, for example, that just below X_{sat} , the bilayer vesicles are broken up, forming stacks of large lamellar fragments.^{13,15,16} For the thermodynamic properties, this has little effect as long as edge effects can be neglected.

Composition-driven second-order transitions are known to occur in mixed micelles beyond a critical lipid content. It is known that addition of lipid to detergent micelles can reduce the clouding temperature considerably.^{17,18} Ollivon et al.¹³ found macroscopic phase separation effects for EYPC/octyl glycoside mixtures ([EYPC] > 2 mM) around X_{sol} (“breakpoint C”), where the viscous lower phase had a composition close to X_{sol} , i.e., close to that of the mixed micelles. Other authors observed similar macroscopic phase separation effects for other systems.^{12,15,16} Furthermore, a sphere-to-rod transition of the micellar shape and subsequent size growth were reported to happen beyond a critical lipid content of the micelles.^{6,19,20} Brown et al.²¹ showed that such growth effects as well as critical fluctuations due to intermicellar interactions²² occur when the system approaches the cloud point. We are going to summarize these effects as “preclouding” phenomena, because the calorimetric measurements cannot differentiate between these different effects. For a review see ref 23.

Recently, we observed that the critical compositions of solubilization can be easily obtained using ITC.²⁴ Now, we present a detailed quantitative ITC analysis of this solubilization experiment for the POPC/C₁₂EO₈/water system. We performed four different types of ITC experiments with the ternary system consisting of water, detergent, and phospholipid.

In the two ITC solubilization experiments, aqueous detergent micelles were added to lipid vesicles or vice versa. From the heat effects observed in these experiments, we could calculate

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on the basis of models described below the enthalpy changes for the detergent and lipid during these processes as well as the Gibbs energy and the entropy changes for the transfer of these molecules between micelles and bilayers. Additionally, another transition is detected within the micellar range, which can be related to the clouding or preclouding effects discussed above.

Additional information was obtained from the detergent partitioning experiment, i.e., the addition of pure lipid vesicles to detergent monomers, as well as from the detergent demicellization experiment where detergent micelles are diluted to concentrations below their cmc.

For the partitioning of detergents into lipid bilayers, the mass action model used to explain the binding of molecules to specific binding sites on membranes or membrane proteins^{25–27} fails because a detergent does not have a restricted number of binding sites on the membrane but dissolves in the bilayer.⁸ Zhang and Rowe²⁸ solved the problem of determining the partition coefficient of butanol between water and DPPC by a series of about 20 ITC experiments. Our alternative approach requires only one single ITC experiment. Lipid vesicles are titrated to an aqueous detergent solution of very low concentration. The more easily the detergent partitions into the lipid bilayers (i.e., the higher the partition coefficient), the faster (i.e., at the less lipid injected) the aqueous detergent concentration decreases after injection of lipid vesicles. The nonlinear dependence of the observed reaction heat on lipid concentration in the sample yields the partition coefficient, the enthalpy of transfer for detergent between water and bilayer, and the heat of dilution within one single experiment. For a control, the heat of dilution was also measured in a separate experiment by injecting lipid vesicles into pure water.

By means of the demicellization experiment, the demicellization enthalpy, the critical micelle concentration (cmc), and an estimate of the aggregation number can be obtained.^{29–35} For consistency with our data, we repeated the demicellization experiment of Olofsson²⁹ with C₁₂EO₈.

Experimental Procedures

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipids, and octa(ethylene oxide) dodecyl ether (C₁₂H₂₅(OCH₂CH₂)₈OH [C₁₂EO₈]) from Nikko Chemicals. All substances were used without further purification. Appropriate amounts of the substances were mixed with purified water (0.06 μ S/cm) to obtain aqueous solutions or dispersions of suitable concentration. The lipid vesicles were extruded using Costar/nuclepore polycarbonate membranes, yielding unilamellar vesicles of 100-nm diameter. Only the concentrated (40 or 100 mM) lipid dispersions were prepared by sonication (Branson sonifier, power 2, 20%, 5 \times 2 min). This procedure was sufficient to avoid sedimentation processes of the lipid within the injection syringe. Reference experiments using vortexed lipid dispersions yielded no systematic deviations but only slightly increased the random errors for the heats observed after injection.

All titration calorimetry experiments were performed at 25 °C using a MCS ITC calorimeter (Microcal, Northampton, MA). Before the experiments, all solutions were degassed by stirring for 10 min at 3 kPa to prevent air bubbles.

The experiments consisted of a series of injections from a 50-, 100-, or 250- μ L syringe to a 1.34-mL sample in the cell. Actual available syringe volumes are up to about 60, 130, and 300 μ L, respectively. The differential heating power needed to maintain zero temperature difference between reference and sample cell after injection of the titrant is recorded vs time. The experimental data were evaluated using the MicroCal

Observer and MicroCal Origin software. The concentrations of titrant and the sample in the cell are calculated automatically by the instrument software, correcting for the small sample replacement effects due to the injection.

A 1- μ L preliminary injection was used to correct for volume errors of the first injection, which is caused by inserting the filled syringe into the cell. This preliminary injection was considered in the titrant concentration scale, but the corresponding heat was excluded from the evaluation.

As mentioned above the following types of ITC experiments were performed:

Demicellization Experiment. This experiment has been described by various authors.^{29–35} A micellar detergent solution of 2.5 mM C₁₂EO₈ was titrated into pure water until the detergent concentration in the cell was above the cmc. After 39 injections of 3 μ L each (118 μ L in total, including an initial addition of 1 μ L), a final titrant concentration of 0.2 mM was reached within the sample cell.

Partitioning Experiment. This is similar to the common binding experiment.^{25–27} Lipid vesicles were injected (15.1 mM) into a C₁₂EO₈ monomer solution (0.05 mM) using the 50- μ L syringe. Thus, a final lipid concentration of 0.65 mM was reached in the sample cell. The data were evaluated according to the partition model with only two free parameters, the average partition coefficient and the average transfer enthalpy. One has to take care that only bilayers of variable composition but no mixed micelles are formed during the experiment. The concentrations used in the experiment gave optimum results without reaching the saturation concentration for the detergent in the bilayer.

Solubilization Experiment. In this experiment, either lipid vesicles are titrated to detergent micelles or vice versa. The concentrations of the reactants are high enough to ensure that almost all detergent and lipid molecules are incorporated into bilayers or micelles. Thus, the concentration of detergent and lipid monomers in water can be neglected in the analysis. We either used an initial concentration of detergent of 2.5 mM in the cell and injected a 40 mM lipid suspension (reaching 7 mM in the cell) or filled the cell with a 4.9 mM lipid vesicle suspension and titrated a 95 mM detergent solution into the vesicle suspension (final detergent concentration in cell: 16.5 mM). In both cases, the 250- μ L syringe was used. Because for this type of experiment, a mole fraction scale is more appropriate; the injection volumes were varied, e.g., initially from 0.7 μ L up to 40 μ L (cf. Figures 4B and 5B).

Results

Figure 1 shows a schematic diagram of the types of titration experiments we performed. In region I, bilayers and, in region III, micelles are the only stable aggregation form. These two regions are separated by a two-phase region (II) where micelles and bilayers are in equilibrium. The two straight lines should intercept the ordinate at a particular detergent concentration, which is generally lower than the cmc of the pure detergent and constitutes the hypothetical cmc of a mixed lipid/detergent system.¹⁰

The demicellization experiment is done by diluting an aqueous micellar detergent solution into water. This corresponds to a vertical line on the ordinate at zero lipid concentration (arrow 1). In the partition experiment, lipid vesicles are added to detergent monomers (arrow 2). These experiments studying monomers have to be performed at very low concentrations (cf. the different concentration scales in parts A and B of Figure 1). The two types of solubilization experiments

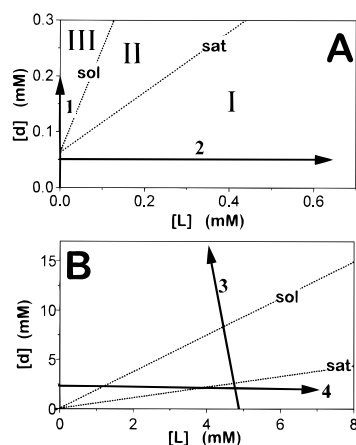


Figure 1. Phase diagram of aqueous POPC/C₁₂EO₈ mixtures at 25 °C. Ranges I, II, and III correspond to the lamellar, coexistence, and micellar ranges, respectively. The sample compositions reached during the various ITC experiments are indicated as arrows. The experiments investigating detergent monomers have to be carried out at very low concentrations (A): Demicellization experiment (arrow 1), partitioning experiment (arrow 2). The solubilization experiments are performed at high concentrations, to keep monomers negligible (B): detergent titration to lipid (arrow 3) and lipid titration to detergent (arrow 4). Upon titration, small volumes are added to the cell, replacing the cell content. The corresponding decrease of the concentration is significant for the 250- μ L syringe (arrows 3 and 4) but low for the 50- μ L syringe (arrow 2).

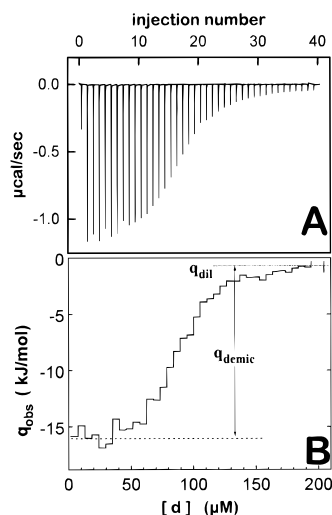


Figure 2. ITC demicellization experiment of C₁₂EO₈ at 25 °C. A 2.5 mM micellar C₁₂EO₈ dispersion was titrated into pure water. (A) Measured heat power vs injection number. (B) Observed titration heat q_{obs} vs C₁₂EO₈ cell concentration. Up to a concentration of 70 μ M, one observes a constant heat due to total dissolution of the injected micelles. Between 70 and 110 μ M, demicellization vanishes, indicating incomplete dissolution of the micelles injected. The demicellization heat is -16 kJ/mol.

(Figure 1B) are indicated by arrows 3 and 4. In both cases, the coexistence region of mixed micelles and bilayers is crossed.

Demicellization Experiment. The demicellization experiment yields the enthalpy and Gibbs free-energy differences upon detergent transfer from the aqueous monomer solution into a micelle, $h_D^{\text{m}}(\infty) - h_D^{\text{w}} = -q_{\text{demic}}$ and $\mu_D^{\text{m}}(\infty) - \mu_D^{\text{w}} = -RT \ln(w/\text{cmc})$. Figure 2A shows the heat peaks detected upon gradual injection of a 2.5 mM micellar C₁₂EO₈ solution into (initially) pure water vs the injection number. Every injection of 3 μ L increases the detergent concentration $[d]$ within the sample cell by about 5.6 μ M. Integration and normalization of the peaks yields the heat per mole of injectant, q_{obs} , as a function of concentration ($[d]$) of C₁₂EO₈ in the sample cell (Figure 2B).

For low $[d]$, the injected micelles are completely dissolved into monomers. This is accompanied by a heat of $-(16 \pm 0.5)$ kJ/mol. Injection of C₁₂EO₈ micelles into the cell with C₁₂EO₈ concentrations higher than the cmc results in no demicellization. Only the heat of dilution of the injectant is observed, which is found to be $-(0.8 \pm 0.5)$ kJ/mol. This heat of dilution of micelles is assumed to be constant for the complete titration experiment, since the micellar concentration within the sample is lower than that of the injectant by at least 1 order of magnitude. Consequently, we obtain for the demicellization enthalpy $h_D^{\text{w}} - h_D^{\text{m}} = \Delta h_{\text{demic}} = -(15.2 \pm 1)$ kJ/mol as indicated by the double arrow in Figure 2B. Because we know the cmc of the detergent, we can calculate that the injections include about 4% detergent in the monomeric form which does not contribute to the experimentally observed heat. Therefore, Δh_{demic} has to be corrected, giving 15.6 kJ/mol.

The heats of demicellization and dilution reported here agree well with the values published by Olofsson, who determined -15.3 and -0.9 kJ/mol, respectively, using isothermal titration calorimetry.²⁹ Rosen et al.³⁶ calculated $\Delta h_{\text{demic}} = -13.2$ kJ/mol from the temperature dependence of the cmc, which is also consistent with our data.

The cmc has been defined to be the concentration where the first micellar aggregates appear³⁴ or as the point of inflection of the micellization transition (i.e., the minimum of the first derivative of the experimental titration curve).^{31,37} In the phase separation model, the cmc is also the monomer concentration in a micellar solution of any concentration above the cmc; i.e., it is independent of the total concentration. In reality, we observe not a sharp micellization but a micellization range. In the case of C₁₂EO₈, the first aggregates are formed at about 70 μ M, the point of inflection is at about 90 μ M, and the micellization seems to be completed at about 110 μ M C₁₂EO₈. Experiments detecting the existence of hydrophobic aggregates by the fluorophore solubilization and by the surface tension yielded cmc values of about 70 μ M.^{38,39} Olofsson reports a value for the cmc of 90 μ M from his ITC studies.²⁹ We determined the aqueous monomer concentration of C₁₂EO₈ coexisting with C₁₂EO₈/POPC mixed micelles in a study of the partitioning of C₁₂EO₈ into lipid bilayers.⁸ Extrapolating these results to vanishing lipid yields a limiting detergent monomer concentration of 110 μ M. All cmc values reported using different methods are obviously inside the micellization range observed in our ITC experiments.

The important results of the demicellization experiment are the enthalpy and free energy of micellization, $h_D^{\text{m}}(1) - h_D^{\text{w}} = -\Delta h_{\text{demic}} = +(16 \pm 1)$ kJ/mol and $\mu_D^{\text{m}}(1) - \mu_D^{\text{w}} = -(33.0 \pm 0.3)$ kJ/mol, respectively.

Partitioning Experiment. The partitioning experiment allows the determination of the molar enthalpy and Gibbs free-energy change of transfer of aqueous detergent monomers into a mixed lipid/detergent bilayer.

Figure 3 shows the experimental ITC data for the titration of 15 mM aqueous POPC dispersion to 0.05 mM C₁₂EO₈ and the plot of the normalized heats q_{obs} vs the lipid concentration $[L]$.

The solid line is a fit using a partition model which is described below in the Comparison with experiments section.⁸

Solubilization Experiments. Figure 4 shows the results of a titration of a micellar solution of C₁₂EO₈ (95 mM) to an initially pure POPC unilamellar vesicle dispersion (5 mM). The reverse experiment, injecting a POPC vesicle suspension (40 mM) to an initially pure dispersion of detergent micelles (2.5 mM), is shown in Figure 5.

The top graph shows the experimental heat peaks observed after addition of the volumes ΔV from the syringe as shown in

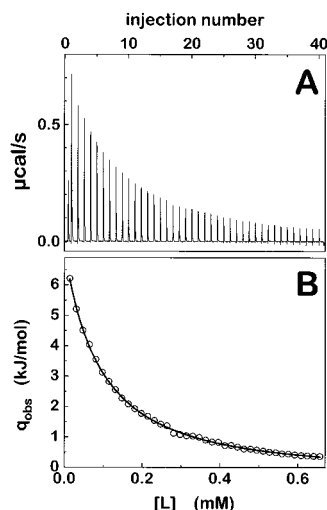


Figure 3. ITC partitioning experiment at 25 °C. Measured heat power upon titration of 15 mM POPC to 0.05 mM C₁₂EO₈ vs injection number (A). B shows the corresponding integrated titration heats per mole of lipid injected (○) vs average molar lipid concentration in the sample corresponding to a given injection. The model curve according to eqs 3 and 4 (solid line) was fitted with $q_D^{b/m} = +31.5$ kJ/mol and $P = 2.1 \times 10^5$ in the range of $[L]$ up to 650 μ M corresponding to an effective mole fraction of detergent in bilayers $X_e \approx 0.33 - 0.07$ (range I).

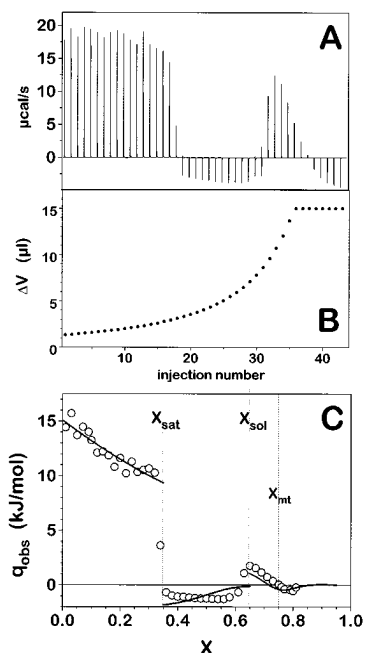


Figure 4. ITC solubilization experiment at 25 °C. Observed heat power upon titration of 95 mM C₁₂EO₈ to 4.9 mM POPC vesicles using the 250- μ L syringe vs injection number (A). Injection volumes have been increased gradually up to 15 μ L with injection number (B). The integrated heats of A were normalized by injection mole number (proportional to B) and corrected for injectant dilution heat of -0.2 kJ/mol, measured separately (not shown) (○) (C). The lines drawn correspond to the parameters given in Figure 6B, and the contributions corresponding to the phenomenological micellar transition heat shown in Figure 8.

the middle of the figures. We used an injection schedule with increasing ΔV to get better signal-to-noise ratios at the end of the titration experiments. In Figure 4, the mole fraction X of the detergent increases from left to right when detergent is added to lipid vesicles. The reverse is observed when lipid vesicles are added to a detergent dispersion as indicated at the top of Figure 5. Both experimental approaches cover a range of mole fractions X where saturation and finally solubilization of lipid

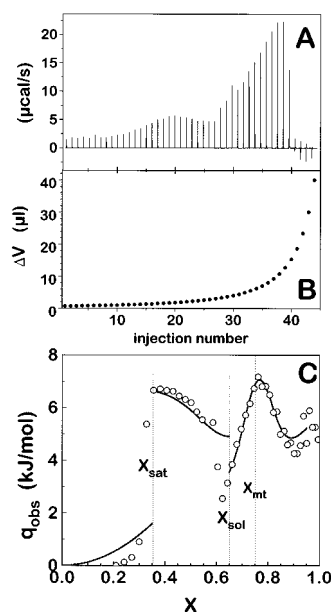


Figure 5. ITC solubilization experiment at 25 °C. Observed heat power upon titration of 40 mM POPC vesicles to 2.5 mM C₁₂EO₈ (using the 250- μ L syringe) vs injection number (A). Injection volumes have been increased gradually with injection number (B). The integrated heats of A were normalized by injection mole number (proportional to B) and corrected for injectant dilution heat of -0.05 kJ/mol, measured separately (not shown) (○) (C). In this type of experiment, the titration heat data (○) are measured by decreasing X during the titration. The lines drawn correspond to the parameters given in Figure 6B and the contributions corresponding to the phenomenological micellar transition heat shown in Figure 8.

bilayers occurs. The raw data in these figures were integrated, corrected for injectant dilution heats (see legends), and normalized using the ΔV values of the injection schedule to give the graphs shown at the bottom of Figures 4 and 5.

In Figure 4, three different regimes of X can easily be seen. There is first a constant decrease of q_{obs} from approximately 15 to 10 kJ/mol in the mole fraction range up to $X = 0.35$. Then a sudden drop is seen, and q_{obs} is small and negative (-1 kJ/mol) between $X = 0.35$ and 0.65 . Above $X = 0.65$, q_{obs} is again slightly positive but decreases with increasing X and becomes negative again. The two breakpoints at $X = 0.35$ and 0.65 agree with the published values^{6,8} for the mole fractions X_{sat} and X_{sol} , i.e., the mole fractions where the bilayers become saturated with detergent and the first mixed micelles appear and the mole fraction where all bilayers have disappeared and only micelles are present. At the mole fraction X_{mt} , another breakpoint is detected, which corresponds to a micellar transition. The micellar samples with X up to $X_{mt} = 0.76$ exhibit a substantial turbidity, but we did not see a total macroscopic phase separation (checked for $X = 0.6$ and 0.7 at 25 °C). Thus, one can relate the observed micellar transition to clouding, or better, preclouding phenomena, which are to be expected for these systems (see Introduction). The procedure to derive the transition enthalpy will be explained below.

The three different concentration ranges are also seen in Figure 5 for the reversed experiment. However, in this experiment, the preclouding effect is much more pronounced.

It should be noted that the heat power peaks measured particularly within range II show a superposition of exothermic and endothermic contributions of different kinetics (cf. Figures 4A and 5A). Within this work, we only refer to the enthalpy differences between the equilibrium states before and after

injection. These are given by the time integral over all the injection heat powers due to the various elementary processes occurring.

Theory

In this section, we will derive equations which will enable us to extract thermodynamic data for the transfer of lipid and detergent monomers from bilayers to micelles and from water to micelles or bilayers, respectively. We calculated the experimental heats of titration for the three concentration ranges, which we want to call range I, $0 < X < X_{\text{sat}}$, i.e., the range where only bilayers exist and the detergent partitions into the bilayers; range II, $X_{\text{sat}} < X < X_{\text{sol}}$, i.e., the region where mixed bilayers and mixed micelles coexist, and range III, $X > X_{\text{sol}}$, i.e., the range where only mixed micelles are stable.

Partitioning Experiment. The partition coefficient P is defined as the ratio of the mole fractions of the detergent in the bilayer and in the aqueous subphases.⁴⁰ With the number of moles of the detergent incorporated in bilayers d_b and in the aqueous subphase d_w , we obtain for P

$$P = \frac{d_b(d_w + w)}{(d_b + L)d_w} \quad (1)$$

where L and w are the number of moles of lipid and water, respectively. Because of the extremely low cmc of the lipid, lipid monomers can be neglected. With $d_w = d - d_b$ and the approximation $d_w + w \approx w$, one finds

$$d_b = \frac{1}{2P} [P(d - L) - w + \sqrt{P^2(d + L)^2 - 2Pw(d - L) + w^2}] \quad (2)$$

To determine the change of d_b occurring upon injection of ΔL moles of lipid to detergent micelles, we have to differentiate eq 2 with respect to L :

$$\frac{\Delta d_b}{\Delta L} = -\frac{1}{2} + \frac{P(d + L) + w}{2\sqrt{P^2(d + L)^2 - 2Pw(d - L) + w^2}} \quad (3)$$

The observed heat per mole of lipid injected is due to the incorporation of detergent monomers into the lipid bilayers. This incorporation leads to a change of the detergent's molar enthalpy from that of an aqueous surrounding h_D^w to that within the bilayer h_D^b :

$$q_{\text{obs}} = \frac{\Delta d_b}{\Delta L} [h_D^b(X) - h_D^w] + q_{\text{dil}} \quad (4)$$

Combination of eq 3 with eq 4 enables a two-parameter fit of the experimental values with P and $h_D^b(X) - h_D^w$ as parameters.

Solubilization Experiment. The total sample composition is given by the mole fraction of detergent X , with the number of moles of lipid denoted as L and detergent as d :

$$X = \frac{d}{d + L} \quad (5)$$

In the two solubilization experiments, X increases in one case when detergent is injected into the sample cell, because d increases whereas the mole number of lipid L remains constant. For the reverse experiment, d is constant and L increases; thus, X is decreased.

Calculations assuming Ideal Mixing of Detergent and Lipid. In the first calculation, we assume that the molar enthalpy of components in the bilayers or micelles is inde-

pendent of composition X of the aggregate, i.e., that the mixing is ideal. Injecting detergent micelles (increase in X) to lipid/detergent mixed bilayers (i.e., $X < X_{\text{sat}}$, range I), the micellar detergent is transferred to bilayers, which is accompanied by the observed molar heat $q_D^{b/m}$. For the assumption of X -independent enthalpies, we find that $q_D^{b/m}$ is just the difference of the molar enthalpies of the detergent in bilayers h_D^b and in micelles h_D^m , respectively:

$$q_D^{b/m} = h_D^b - h_D^m \quad (6)$$

Injecting lipid bilayers (decrease in X) to mixed bilayers (range I), no heat effect will occur ($q_L^{b/b} = 0$). Analogously, $q_D^{m/m} = 0$. Titration of lipid bilayers to pure or mixed micelles (range III) yields the heat of transfer of lipid molecules from bilayers to mixed micelles $q_L^{m/b}$ as the difference of the molar enthalpies of the lipid between micelle h_L^m and bilayers h_L^b :

$$q_L^{m/b} = h_L^m - h_L^b \quad (7)$$

Within range II, lipid-saturated micelles of composition X_{sol} are in equilibrium with detergent-saturated bilayers of composition X_{sat} , and only the relative amounts of both types of aggregates vary. Here the total enthalpy of the sample H changes not only due to the incorporation of the injectant but also due to internal transfer of molecules between the coexisting bilayers and micelles, which are caused by a variation of the total mole fraction of detergent X .

The system consists of the sample in the cell and the injectant in the syringe. The system enthalpy H includes contributions of the components in the various aggregation states. We obtain

$$H = L_b h_L^b + L_m h_L^m + d_b h_D^b + d_m h_D^m + \text{inj} h_{\text{INJ}} \quad (8)$$

with L_b and L_m being the mole number of lipid in bilayers and micelles and d_b and d_m being the number of moles of detergent in bilayers and micelles, respectively. The last term reflects the contribution of the injectant (detergent or lipid) given by its mole number, inj, and the corresponding molar enthalpy h_{INJ} . Because the concentration of detergent and lipid in monomeric form is very low, it can be neglected in all further calculations.

For the solubilization experiment where detergent micelles are titrated to lipid bilayers, we have to find the derivative of H with respect to d at constant L . We obtain

$$\frac{\Delta H}{\Delta d} = \frac{\Delta L_b}{\Delta d} h_L^b + \frac{\Delta L_m}{\Delta d} h_L^m + \frac{\Delta d_b}{\Delta d} h_D^b + \frac{\Delta d_m}{\Delta d} h_D^m + \frac{\Delta d_{\text{inj}}}{\Delta d} h_{\text{INJ}}^m \quad (9)$$

To calculate this expression, we have to find expressions for the five derivatives. An injection of detergent Δd to the cell diminishes the mole number of injectant in the syringe, d_{inj} , just by Δd so that $\Delta d_{\text{inj}}/\Delta d = -1$. The derivatives in eq 9 can be calculated from the mass balance equations. The total number of moles of detergent and lipid in the cell are d and L , respectively; those fractions of molecules residing in bilayers and micelles are d_b , L_b and d_m , L_m , respectively. As mentioned above, the total concentrations used in the solubilization experiments are sufficiently high (compared to the corresponding critical micelle concentrations) that aqueous monomers (d_w , L_w) can be neglected. With the balance equations

$$L = L_b + L_m \quad (10)$$

$$d = d_b + d_m \quad (11)$$

we obtain for the mole fractions X_{sat} and X_{sol} in range II where bilayers and micelles coexist the two mole fractions

$$X_{\text{sat}} = \frac{d_b}{L_b + d_b} \quad (12)$$

$$X_{\text{sol}} = \frac{d_m}{L_m + d_m} \quad (13)$$

This system of four equations (10), (11), (12), and (13) has four unknown variables L_b , L_m , d_b , and d_m . It can be solved, yielding

$$L_m = L - L_b = \frac{(1 - X_{\text{sol}})(1 - X_{\text{sat}})}{X_{\text{sol}} - X_{\text{sat}}}d - \frac{X_{\text{sat}}(1 - X_{\text{sol}})}{X_{\text{sol}} - X_{\text{sat}}}L \quad (14)$$

$$d_b = d - d_m = -\frac{X_{\text{sat}}(1 - X_{\text{sol}})}{X_{\text{sol}} - X_{\text{sat}}}d + \frac{X_{\text{sol}}X_{\text{sat}}}{X_{\text{sol}} - X_{\text{sat}}}L \quad (15)$$

We now need the derivatives with respect to d for constant L :

$$\frac{\Delta L_m}{\Delta d} = -\frac{\Delta L_b}{\Delta d} = \frac{(1 - X_{\text{sol}})(1 - X_{\text{sat}})}{X_{\text{sol}} - X_{\text{sat}}} \quad (16)$$

$$\frac{\Delta d_b}{\Delta d} = 1 - \frac{\Delta d_m}{\Delta d} = -\frac{X_{\text{sat}}(1 - X_{\text{sol}})}{X_{\text{sol}} - X_{\text{sat}}} \quad (17)$$

Inserting these expressions into eq 9 using eqs 6 and 7 gives the titration heat of detergent titration to a sample within the coexistence range, q_D^{sol} :

$$q_D^{\text{sol}} = -\frac{X_{\text{sat}}(1 - X_{\text{sol}})}{X_{\text{sol}} - X_{\text{sat}}}[q_D^{\text{b/m}}] + \frac{(1 - X_{\text{sol}})(1 - X_{\text{sat}})}{X_{\text{sol}} - X_{\text{sat}}}[q_L^{\text{m/b}}] + [q_D^{\text{m/m}}] \quad (18)$$

The last term $q_D^{\text{m/m}} = h_D^{\text{m}}(X) - h_D^{\text{m}}(1)$ vanishes for ideal mixing.

If $q_D^{\text{b/m}}$ and $q_L^{\text{m/b}}$ are both endothermic, we see from eq 18 that the transfer of the detergent from micelles to bilayers makes a negative contribution and the lipid transfer from bilayers to micelles a positive contribution to the observed heat q_D^{sol} , the heat observed in range II.

For the solubilization experiment where lipid vesicles are titrated into a detergent micellar dispersion, a similar procedure yields q_L^{sol} , the observed titration heat upon lipid addition to the bilayer/micelle equilibrium. In this case, d is constant and L increases by the injection of lipid vesicles. Consequently, we have to differentiate eq 8 with respect to L holding d constant:

$$\frac{\Delta H}{\Delta L} = \frac{\Delta L_b}{\Delta L}h_L^{\text{b}} + \frac{\Delta L_m}{\Delta L}h_L^{\text{m}} + \frac{\Delta d_b}{\Delta L}h_D^{\text{b}} + \frac{\Delta d_m}{\Delta L}h_D^{\text{m}} + \frac{\Delta L_{\text{inj}}}{\Delta L}h_L^{\text{b}} \quad (19)$$

Again, $\Delta L_{\text{inj}}/\Delta L = -1$, and we have to find expressions for the remaining four derivatives on the right-hand side of this equation. These are given by

$$\frac{\Delta L_m}{\Delta L} = 1 - \frac{\Delta L_b}{\Delta L} = -\frac{X_{\text{sat}}(1 - X_{\text{sol}})}{X_{\text{sol}} - X_{\text{sat}}} \quad (20)$$

$$\frac{\Delta d_b}{\Delta L} = -\frac{\Delta d_m}{\Delta L} = \frac{X_{\text{sat}}X_{\text{sol}}}{X_{\text{sol}} - X_{\text{sat}}} \quad (21)$$

We note here that $\Delta L_m/\Delta L < 0$, which means that the mole number of lipids situated in micelles is diminished ($\Delta L_m < 0$) upon injection of lipid ($\Delta L > 0$) and that the number of moles

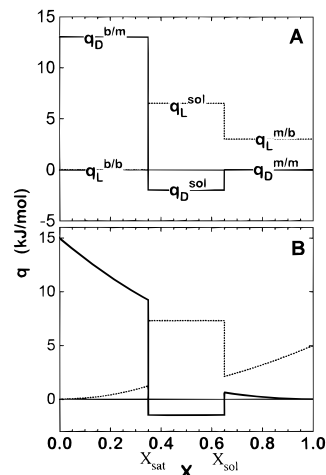


Figure 6. Calculated curves of q vs X for ideal mixing (A) and nonideal mixing (B). The simulation is based on (A) eqs 6, 7, 18, and 22 with $q_D^{\text{b/m}} = 13$ kJ/mol and $q_L^{\text{m/b}} = 3$ kJ/mol and (B) eqs 30, 32, 33, 34, 35, and 36 with $\rho_0^{\text{b}} = +10$ kJ/mol, $h_D^{\text{b}}(1) - h_D^{\text{m}}(1) = 5$ kJ/mol, $\rho_0^{\text{m}} = 5.5$ kJ/mol, and $h_L^{\text{m}}(0) - h_L^{\text{b}}(0) = 0$. The fit curves in Figures 4C and 5C are based on these parameters and additional precluding contributions.

d_b and L_b increase; i.e., we obtain transfer of detergent and lipid from micelles to bilayers.

For the observed heat q_L^{sol} in this type of experiment, we obtain from eq 19 with eqs 6 and 7

$$q_L^{\text{sol}} = \frac{X_{\text{sat}}X_{\text{sol}}}{X_{\text{sol}} - X_{\text{sat}}}q_D^{\text{b/m}} - \frac{X_{\text{sat}}(1 - X_{\text{sol}})}{X_{\text{sol}} - X_{\text{sat}}}q_L^{\text{m/b}} + q_L^{\text{b/b}} \quad (22)$$

The curves calculated according to eq 18 and eq 22 are shown in Figure 6A using values of 13 and 3 kJ/mol for $q_D^{\text{b/m}}$ and $q_L^{\text{m/b}}$, respectively. As is evident from the curves, the general features of the experimental data are reproduced but a good fit cannot be obtained.

Calculations assuming Nonideal Mixing of Detergent and Lipid. We refined our model by the assumption that the mixing of detergent and lipid in bilayers and in micelles might be nonideal. Accordingly, we applied a regular solution model with nonideality parameters ρ_0^{b} and ρ_0^{m} to describe the deviations from ideality.

Range I ($X < X_{\text{sat}}$). By using the assumption of nonideal mixing of detergent and lipid in bilayers, one has to consider that the sample enthalpy H depends on the mole fraction X . For region I, we assume for H

$$H = dh_D^{\text{b}}(X) + Lh_L^{\text{b}}(X) \quad (23)$$

Following the approach of Johnson et al.,^{32,33} we describe the composition dependence of the enthalpies with an excess term $h_{\text{EXC}}^{\text{b}}(X)$ which describes the mixing effect. The constant reference enthalpies are the molar enthalpy of the lipid in pure lipid bilayers, $h_L^{\text{b}}(0)$, and the molar enthalpy of the detergent molar in a hypothetical pure detergent bilayer, $h_D^{\text{b}}(1)$. Thus, eq 23 changes to

$$H = dh_D^{\text{b}}(1) + Lh_L^{\text{b}}(0) + (d + L)h_{\text{EXC}}^{\text{b}}(X) \quad (24)$$

Using the regular solution model,^{33,8,41} we obtain

$$h_{\text{EXC}}^{\text{b}}(X) = \rho_0^{\text{b}}X(1 - X) \quad (25)$$

The nonideality parameter ρ_0^{b} is a constant and characterizes the nonideal lipid/detergent interaction.

To the heat of titration $q_D^{b/m}$ observed upon injection of Δd moles of detergent to a constant lipid quantity (increase in X), we now have to add an extra heat effect due to the enthalpy variation of the sample caused by the excess enthalpy $h_{\text{EXC}}^b(X)$. Therefore, we need the derivative of eq 24 with respect to d :

$$\frac{\Delta H}{\Delta d} = h_D^b(1) + h_{\text{EXC}}^b(X) + (d + L) \frac{\Delta h_{\text{EXC}}^b}{\Delta X} \frac{\Delta X}{\Delta d} \quad (26)$$

With the first derivative of eq 25

$$\frac{\Delta h_{\text{EXC}}^b}{\Delta X} = \rho_0^b(1 - 2X) \quad (27)$$

and

$$(d + L) \frac{\Delta X}{\Delta d} = 1 - X \quad (28)$$

We obtain then from eq 26 the common regular solution term:^{41,8}

$$\frac{\Delta H}{\Delta d} = h_D^b(1) + \rho_0^b(1 - X)^2 \quad (29)$$

The observed heat per mole of detergent injected into the cell containing lipid vesicles is now the molar heat of transfer of the detergent $q_D^{b/m}(X)$ from pure detergent ($X = 1$) micelles to mixed bilayers of composition X . Therefore, eq 6, valid for ideal mixing, has to be corrected by the expression for change in sample enthalpy $\Delta H/\Delta d$ (eq 29), yielding

$$q_D^{b/m}(X) = [h_D^b(1) - h_D^m(1)] + \rho_0^b(1 - X)^2 \quad (30)$$

The expression in the first bracket of eq 30 can be imagined as the transfer enthalpy of the detergent from micelles to speculative pure detergent bilayers. The following excess term describes the deviation from ideal mixing.

For the experiment where lipid vesicles are titrated into a micellar solution, a similar approach can be used. The number of moles of detergent d is now constant, and L changes by ΔL upon injection of pure lipid bilayers. With

$$(d + L) \frac{\Delta X}{\Delta L} = -X \quad (31)$$

we therefore obtain for range I

$$q_L^{b/b}(X) = \rho_0^b X^2 \quad (32)$$

Range III ($X > X_{\text{sol}}$). For a sample composition within range III, i.e., $X > X_{\text{sol}}$, the observed heat is now $q_D^{m/m}(X)$ or $q_L^{m/b}(X)$ for detergent and lipid injections, respectively. We use the same regular solution model for nonideal mixing in micelles with a nonideality parameter ρ_0^m and obtain analogously to eqs 30 and 32

$$q_D^{m/m}(X) = \rho_0^m(1 - X)^2 \quad (33)$$

and

$$q_L^{m/b}(X) = [h_L^m(0) - h_L^b(0)] + \rho_0^m X^2 \quad (34)$$

The first term of eq 34 corresponds to the transfer of the injected lipid from bilayer vesicles to hypothetical pure lipid micelles.

Range II ($X_{\text{sat}} < X < X_{\text{sol}}$). For concentration range II, the compositions of the bilayers (X_{sat}) and the micelles (X_{sol}) remain constant. Therefore, q_D^{sol} and q_L^{sol} are not dependent on X ;

only the values of $q_D^{b/m}$ and $q_L^{m/b}$ calculated for ranges I and III depend on X (see eqs 30 and 34). The transfer of lipid and detergent molecules in range II occurs not between pure micelles and pure bilayers but between aggregates of compositions X_{sol} and X_{sat} , respectively. Thus, the actual heats of transfer have to be calculated by correcting, for example, $q_D^{b/m}(X_{\text{sat}})$ with the heat of transfer $q_D^{m/m}(X_{\text{sol}})$ from pure to lipid-saturated micelles. With these corrections for detergent and lipid, we get eqs 35 and 36, which correspond to eqs 18 and 22 of the model using ideal mixing:

$$q_D^{\text{sol}} = - \frac{X_{\text{sat}}(1 - X_{\text{sol}})}{X_{\text{sol}} - X_{\text{sat}}} [q_D^{b/m}(X_{\text{sat}}) - q_D^{m/m}(X_{\text{sol}})] + \frac{(1 - X_{\text{sol}})(1 - X_{\text{sat}})}{X_{\text{sol}} - X_{\text{sat}}} [q_L^{m/b}(X_{\text{sol}}) - q_L^{b/b}(X_{\text{sat}})] + q_D^{m/m}(X_{\text{sol}}) \quad (35)$$

$$q_L^{\text{sol}} = \frac{X_{\text{sat}}X_{\text{sol}}}{X_{\text{sol}} - X_{\text{sat}}} [q_D^{b/m}(X_{\text{sat}}) - q_D^{m/m}(X_{\text{sol}})] - \frac{X_{\text{sat}}(1 - X_{\text{sol}})}{X_{\text{sol}} - X_{\text{sat}}} [q_L^{m/b}(X_{\text{sol}}) - q_L^{b/b}(X_{\text{sat}})] + q_L^{b/b}(X_{\text{sat}}) \quad (36)$$

Curves calculated according to the model for nonideal mixing of lipid and detergent in bilayers and in micelles are shown in Figure 6B. For that purpose, eqs 30 and 32–36 were used with the parameters indicated in the legend. As can be seen, the theoretical curves describe the experimental data in ranges I and II much better than those obtained under the assumption of ideal mixing (Figure 6A). However, the result in range III is still unsatisfactory. In this concentration range, an additional micellar transition contributes to the experimental heat effects.

Contribution from a Micellar Transition. The experimental results indicate that an additional enthalpic transition within the micellar range must be considered, which is accompanied by a decrease of the system enthalpy within a range centered at a critical detergent mole fraction X_{mt} . We characterize the micellar state up to X_{mt} by an endothermic enthalpy contribution $h_{\text{mt}}(X)$ (given per mole of detergent and lipid), which amounts to $h_{\text{mt}}^{\text{max}}$ below the transition range and then vanishes gradually around X_{mt} . In fact, Olofsson⁴² suggested micellar growth to be accompanied by an endothermic enthalpy contribution.

The simplest assumption for the coexistence range (range II) is that the micelles, having a fixed composition $X_{\text{sol}} < X_{\text{mt}}$, are characterized by $h_{\text{mt}}^{\text{max}}$, whereas the vesicles are not involved in the micellar transition effect. Because $h_{\text{mt}}^{\text{max}}$ refers to micelles and $h_{\text{mt}}(X)$ refers to all aggregates, $h_{\text{mt}}(X)$ must be rescaled with the fraction of the micellar detergent and lipid:

$$h_{\text{mt}}(X) = h_{\text{mt}}^{\text{max}} \frac{d_m + L_m}{d + L} \quad (37)$$

With eqs 14 and 15, one finds a linear behavior of $h_{\text{mt}}(X)$ within range II (cf. Figure 7):

$$h_{\text{mt}}(X) = \frac{h_{\text{mt}}^{\text{max}}}{X_{\text{sol}} - X_{\text{sat}}} (X - X_{\text{sat}}) \quad (38)$$

The contributions of the micellar transition enthalpy $h_{\text{mt}}(X)$ to the titration heats for both solubilization experiments, namely, $q_D^{\text{mt}}(X)$ for detergent titration and $q_L^{\text{mt}}(X)$ for lipid titration, respectively, can be derived analogously to the procedure applied to $h_{\text{EXC}}(X)$ (cf. eq 26):

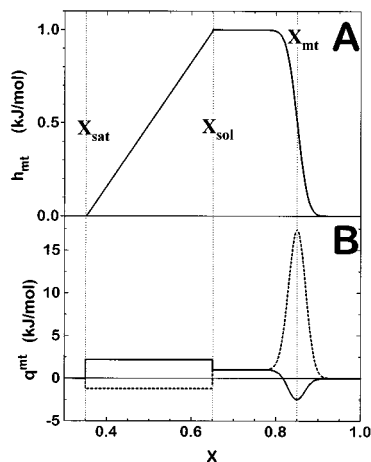


Figure 7. Schematic enthalpy contribution due to a different state of the lipid-rich micelles vs total detergent mole fraction X in ranges II and III (A) and the corresponding contribution to the heats upon lipid (···) and detergent (—) titration observed in the ITC solubilization experiment. The simulation was done with $X_{mt} = 0.85$ (width 0.02) and $h_{mt}(X_{sol}) = 1$ kJ/mol.

$$q_L^{mt}(X) = h_{mt}(X) - X \frac{\Delta h_{mt}(X)}{\Delta X} \quad (39)$$

$$q_D^{mt}(X) = h_{mt}(X) + (1 - X) \frac{\Delta h_{mt}(X)}{\Delta X} \quad (40)$$

The behavior of $q_L^{mt}(X)$ and $q_D^{mt}(X)$ obtained for the $h_{mt}(X)$ curve in Figure 7A is shown in Figure 7B.

Depending on the type of experiment, the contributions arising from the micellar transition around X_{mt} differ not only in sign but also in magnitude. Its influence on the experimental heats, when lipid vesicles are titrated to micelles, is substantially larger than when detergent is titrated to lipid vesicles. With the assumptions done with respect to the coexistence range, one obtains constant values of q_L^{mt} and q_D^{mt} for $X = X_{sat} - X_{sol}$ (with eqs 38–40), which are implicitly considered in the eqs 35 and 36 by the terms $q_D^{m/m}(X_{sol})$ and $q_L^{m/b}(X_{sol})$. That means that in this simplest case, these general equations for the coexistence range must not be extended by an additional contribution. However, eqs 33 and 34 for the micellar range have to be updated by adding eqs 39 or 40, respectively.

Comparison with Experiments

Partitioning Experiment. From a fit of our experimental data using eqs 3 and 4 (cf. Figure 3B, solid line), we obtained for the enthalpy of transfer of the detergent from water into bilayers ($h_D^b - h_D^w$) a value of $+31.5 \pm 1$ kJ/mol, for the partition coefficient P the value $(2.1 \pm 0.1) \times 10^5$, and for the heat of dilution of vesicles q_{dil} the value -0.13 kJ/mol. The errors include the contributions of the variation of $[d]$ (minimized by use of the 50- μ L syringe) and $h_D^b - h_D^w$. The average partition coefficient is somewhat lower than the data obtained by fluorescence spectroscopy⁸ $((4.4-3.0) \times 10^5$ for range I) and light scattering⁶ $(3.0 \times 10^5$ for X_{sat}). The heat of dilution obtained by the fitting procedure was essentially the same as the one determined by injecting lipid vesicles into water (not shown).

The enthalpy of incorporation of detergents into bilayers was found to vary significantly with bilayer composition X (solubilization experiment; see below). A similar variation for the transfer enthalpy should also occur in the partitioning experiment. Indeed, it was recently found that the partition coefficient depends also on composition.⁸ However, the model assuming

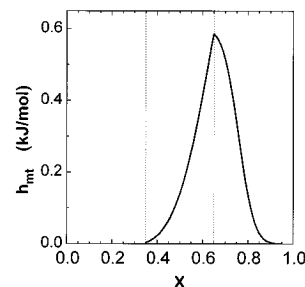


Figure 8. Hypothetical concentration dependence of h_{mt} vs X as used in the simulated curves in Figures 4 and 5.

constant and thus average values was found to fit the data quite well. Therefore, a more elaborate model seemed not to be necessary.

Solubilization Experiment. Range I. From the experimental data, we obtained (cf. Figure 4C and eq 30) that the detergent transfer heat from pure micelles to mixed bilayers of composition X , $q_D^{b/m}(X)$, decreases from $+15$ kJ/mol for detergent addition to almost pure lipid bilayers ($X \approx 0$) to about 10 kJ/mol at the limiting detergent content X_{sat} . This decrease of $q_D^{b/m}(X)$ was modeled by eq 30 (see solid lines in Figures 4C and 5C) using the transfer enthalpy of the detergent from a pure detergent micelle to a hypothetical detergent bilayer, $h_D^b(1) - h_D^m(1) \approx +5$ kJ/mol and the nonideality parameter $\rho_0^b = +10$ kJ/mol.

Range III. Within range III, the enthalpic contributions of a micellar transition must be considered additionally, (cf. eqs 39 and 40). These are small but not negligible. We varied the maximum precluding enthalpy, the composition at the transition, and the transition widths of our arbitrary $h_{mt}(X)$ function as shown in Figure 8 in order to obtain an empirical approximation of $h_{mt}(X)$, which would be consistent with the behavior observed for $q_L^{m/b}(X)$ (cf. Figure 5). Using eqs 34 and 39 with a $h_{mt}^{max} = +0.6$ kJ/mol, $X_{mt} = 0.76$ (width 0.05), a nonideality parameter $\rho_0^m = 5.5$ kJ/mol, and $h_L^m(0) - h_L^b(0) = 0$, we obtained a reasonable fit of the experimental data in Figure 5. With the same parameters, also $q_D^{m/m}$ in Figure 4 was simulated (solid line) using eqs 33 and 40. These parameters therefore give a satisfactory fit of both of our experimental data sets.

Range II. If our model were to be correct, the experimental data in solubilization range II should also be described. We simulated q_D^{sol} and q_L^{sol} using eqs 35, 36, 39, and 40 and the parameters obtained from the simulation of the experimental data in ranges I and III. In contrast to our theoretical approach (cf. Figures 6 and 7), the titration heats within range II were found to vary with X (cf. Figure 5). This fact could be explained with a nonlinear increase of $h_{mt}(X)$; i.e., the coexistence of mixed vesicles seems to partially prevent the micelles from contributing to $h_{mt}(X)$. Consequently, at low X , the incomplete participation of the micelles in the transition enthalpy $h_{mt}(X)$ causes lower contributions to the titration heats than have been considered implicitly by eqs 39 and 40. Namely, the parameters $q_D^{m/m}(X_{sol})$ and $q_L^{m/b}(X_{sol})$ obtained from range III refer to micelles with h_{mt}^{max} . Consequently, they apply only to micelles with h_{mt}^{max} within range II, which occur close to X_{sol} but possibly not for lower X . Thus, using these parameters, eqs 39 and 40 yield $q_D^{sol}(X_{sol})$ and $q_L^{sol}(X_{sol})$. Then, the actual values of q_D^{sol} and q_L^{sol} for lower X are given by the X dependence of q^{mt} . For demonstration, we used the curves for q_D^{mt} and q_L^{mt} derived from the arbitrarily chosen $h_{mt}(X)$ by eqs 39 and 40. The corresponding curves for q_D^{sol} and q_L^{sol} are shown in Figures 4C and 5C. The calculated curves fit the experimental results quite well. This shows that the assumption of nonideal mixing in bilayers and micelles (eqs 21 and 22) is reasonable and that

TABLE 1: Enthalpy and Standard Chemical Potential Differences Δh and $\Delta\mu^0$, Respectively, for the Transfer of Detergent and Lipid between Various Phases of the Ternary System Water/POPC/C₁₂EO₈ at 25 °C^a

kJ/mol	Δh		$\Delta\mu^0$		$-T\Delta s$	
	det	lipid	det	lipid	det	lipid
bilayer–water (partition expt)	31.5 ± 1 ^b		−30.4 ± 0.2 ^b		−62 ± 2 ^b	
micelle–water (demic. expt)	16 ± 1 ¹		−33.1 ± 0.3 ¹		−49 ± 2 ¹	
→ bilayer–micelle	15.5 ± 2.5 ^b		+2.7 ± 0.5 ^b			
bilayer–micelle (solubzn expts)	15 ± 1 ⁰ , 10.0 ± 0.5 ^{sat}	−5 ± 1 ^m , −2.5 ± 0.5 ^{sol}	+1.5 ± 0.5 ^{sat/sol}	−1.5 ± 0.3 ^{sat/sol}	≈ −8	≈ +1

^a All data are presented in kJ/mol. The phase compositions the values refer to are given as superscripts: 0 = pure lipid bilayer ($X = 0$); b = mixed bilayer, $X \approx 0.1 - X_{\text{sat}}$ (average values); sat = detergent-saturated bilayer, $X = X_{\text{sat}}$; sol = lipid-saturated micelles, $X = X_{\text{sol}}$; m = mixed micelles, average value for $X \approx X_{\text{sol}} - 50$; 1 = pure detergent micelles, $X = 1$. The first row corresponds to the partition experiment, the second to the demicellization experiment. The third gives the estimation for the bilayer → micelle transfer values using the data of the demicellization and partitioning experiment. This is for comparison with the values determined directly by the solubilization experiment (fourth row).

the experimental data in all three concentration ranges can be successfully fitted.

The chemical potential differences for the detergent and the lipid between bilayers and micelles in the coexistence range were determined from $X_{\text{sat}} = 0.35 \pm 0.01$ and $X_{\text{sol}} = 0.65 \pm 0.02$.⁸

$$\mu_{\text{D}}^{\text{b}}(X_{\text{sat}}) - \mu_{\text{D}}^{\text{m}}(X_{\text{sol}}) = 0 = \Delta\mu_{\text{D}}^0(\text{b/m}) + RT \ln(X_{\text{sat}}/X_{\text{sol}}) \quad (41)$$

$$\mu_{\text{L}}^{\text{b}}(X_{\text{sat}}) - \mu_{\text{L}}^{\text{m}}(X_{\text{sol}}) = 0 = \Delta\mu_{\text{L}}^0(\text{b/m}) + RT \ln[(1 - X_{\text{sat}})/(1 - X_{\text{sol}})] \quad (42)$$

For the standard chemical potential differences, we obtain $\Delta\mu_{\text{D}}^0(\text{b/m}) = -\Delta\mu_{\text{L}}^0(\text{b/m}) = +1.55 \pm 0.15$ kJ/mol. As expected, the transfer of lipid from micelles to bilayers and the transfer of detergent to micelles are processes which would occur spontaneously ($\Delta\mu^0 < 0$).

Experimental Errors. The experimental errors of the solubilization experiment are related to the approximations that the concentration of monomeric aqueous detergent can be neglected and that the sample replacement by the injected volume is not considered. If one takes into account the amount of aqueous monomers, the effective aggregate composition X_{e} is found to differ from the total, X , maximally by 0.004. This is far below the resolution of our experiment.

The transfer of detergent molecules to the water phase upon detergent titration is maximum at low X . This transfer is accompanied by the heat of demicellization. The experimental data can be corrected by subtraction of this heat of demicellization and renormalization. The maximum correction amounts to +0.8 kJ/mol for our experiments presented here. Upon titration of lipid vesicles to detergent micelles, part of the aqueous detergent is transferred into mixed micelles. The maximum correction here is −0.7 kJ/mol, occurring for $X \rightarrow 1$. This error can be controlled by the concentrations applied or compensated afterwards.

The sample replacement by the injections plays no role as an error source in the solubilization experiments. Figures 4C and 5C are based on automatically corrected D and L values. In theory, the cell content was assumed to be constant solving eqs 28 and 31. The corresponding errors of $\Delta X/\Delta d$ and $\Delta X/\Delta L$ are lower than 2%.

Discussion

Table 1 shows the enthalpies of transfer for detergent and lipid and chemical potential differences obtained by three types of ITC experiments in comparison with published data and calculated values (see below). The transfer entropies were obtained using $\Delta\mu = \Delta h - T\Delta s$ and given as $-T\Delta s$ in order to

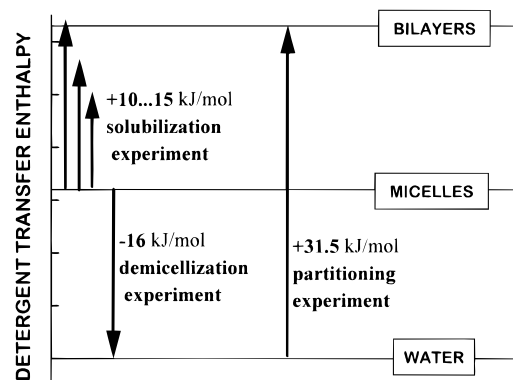


Figure 9. Schematic illustration of a Born–Haber cycle of the results of the different ITC experiments. The numbers given are the enthalpies of transfer in kJ/mol of detergent (cf. Table 1). The total heat over the closed loop should vanish, indicating good consistency of the data.

be directly comparable to the transfer enthalpy and chemical potential differences.

A Born–Haber cycle for the enthalpies of transfer is illustrated for the case of detergent transfer in Figure 9. The enthalpy of transfer from micelles to bilayers observed in the solubilization experiment (+10–15 kJ/mol) agrees with a hypothetical two-step transfer where detergent in micelles is first transferred to water (−16 kJ/mol) and then the monomers are incorporated into the bilayers (+31.5 kJ/mol), leading to an overall transfer enthalpy of +15.5 kJ/mol. The transfer enthalpies of both separate steps were determined by the demicellization and the partition experiment, respectively. The latter two-step route corresponds to row 3 of Table 1, which has to be compared to row 4.

Analogously, the various chemical potential differences are in accord with each other (cf. Table 1).

We note a substantial increase of the enthalpy for the detergent when it is transferred from micelles to bilayers (15–10 kJ/mol), which decreases with increasing detergent content of the bilayer.

The molecular interpretation of this behavior should be possible in terms of headgroup hydration and conformation, because the enthalpic differences of the hydrocarbon chains caused by different chain conformations in bilayers and micelles probably have only a minor contribution to the total enthalpy.^{29,31} Olofsson²⁹ discussed the micellization heat of C₁₂EO₈ exclusively in terms of headgroup dehydration. If one uses the same assumption for our mixed system, a dehydration of the ethylene oxide headgroup upon transfer from micelles to bilayers has to be suggested. This explanation would be compatible with the fact that the space available for a detergent headgroup is considerably reduced within the lamellar structure. Dehydration results in a volume decrease of the headgroup, reducing the molecular asymmetry of the truncated, cone-shaped C₁₂EO₈.^{43–45}

Otten et al.⁴³ considered the dehydration-driven decrease of C₁₂EO₈ headgroup repulsion to be one origin of the thermotropic micellar/lamellar transition, which is observed even for C₁₂EO₈. This detergent forms lamellar structures within a narrow range of low hydration (25 wt % water) at room temperature.⁴⁶

One has to take into account the possible fluidization of the bilayer upon incorporation of detergent.^{8,43–45,47,48} As a consequence, the lateral pressure in the headgroup region decreases, which is the origin of the endothermic dehydration processes. Thus, the fluidization due to detergent/detergent contacts should cause a positive contribution to the enthalpic nonideality parameter.

The regular solution model assumes a random mixing of the molecules within the mixed membrane, yielding composition dependencies of the enthalpy according to eqs 32 and 33. Further neglecting all entropic effects besides the lipid–detergent mixing entropy, the entropy should be composition independent and the deviations of the enthalpy as well as of the chemical potential from ideal behavior should be characterized by one and the same nonideality parameter ρ_0 . This is in contradiction to our observation of the enthalpic nonideality parameter $\rho_0 = +10$ kJ/mol and the $\rho_0 = -1.7$ kJ/mol for standard chemical potential differences determined recently.⁸ That means, there must be a considerable entropic gain additionally to the random mixing entropy considered by the regular solution model. This is fairly consistent with the idea that the enthalpic effects observed are mainly due to dehydration processes, which change the order of water molecules. The fact that EO–detergents were found to shield the hydrophobic core of lipid membranes from water⁴⁵ should yield a further water entropy gain due to the hydrophobic effect.

For lipid incorporation into mixed micelles, we estimated values of $\rho_0^m \approx +5.5$ kJ/mol and $h_L^m(0) - h_L^b(0) \approx 0$. Obviously, a transfer of a lipid molecule from a bilayer to a micelle does not change its enthalpy. This can be understood by the fact that the lipid is almost fully hydrated even in the bilayer and more headgroup space in the micelle does not increase its hydration. The nonideality parameter for the mixing in micelles is considerably lower in micelles than in bilayers. In fact, the lateral pressure in the headgroup region should be much lower in the highly curved system. However, for micelles also a nonrandom arrangement of the molecules should be considered to be possible. Namely, the cone-shaped detergent molecules were assumed to be located preferentially at the highly curved edges of disk-shaped micelles, whereas the lipid prefers the less curved regions.^{13–16}

Conclusions

Isothermal titration calorimetry (ITC) is a suitable method to investigate the thermodynamics of the composition-driven bilayer-to-micelles transition (solubilization). All thermodynamic parameters and transfer enthalpies as well as changes in Gibbs free energy and in entropy can be determined from one and the same type of experiment.

In the case of the detergent C₁₂EO₈, the limiting compositions of solubilization X_{sat} and X_{sol} can be easily determined using ITC (cf. ref 24). Furthermore, it was shown also that preclouding phenomena (i.e., micellar growth and/or intermicellar interactions) can be detected by ITC. Such effects occur for C₁₂EO₈/POPC micelles up to $X_{\text{mt}} = 0.76 \pm 0.05$ (transition width) and are accompanied by an endothermic enthalpy contribution of up to 0.6 kJ/mol.

For C₁₂EO₈, a demicellization enthalpy of -16 kJ/mol and an average cmc of $90 \mu\text{M}$ (70 – $110 \mu\text{M}$) have been observed.

The partition coefficients and water-to-membrane transfer enthalpies of detergents between mixed membranes and water can be conveniently obtained by a single ITC experiment using a partition fitting function (eqs 3 and 4 instead of the mass action law). The composition dependence of both values is not accessible by this type of experiment; instead, average values are determined.

The enthalpy and standard chemical potential differences upon detergent transfers water \rightarrow bilayer, water \rightarrow micelle, and bilayer \rightarrow micelle have been determined, yielding a consistent thermodynamic Born–Haber cycle. Obviously, the system can be well described by our model using characteristic enthalpies of the detergent as well as of the lipid in water, micellar, and bilayer states. Assuming nonideal mixing of detergent and lipid in bilayers as well as in micelles, it was possible to quantitatively describe the heat effects observed in the calorimetric experiments upon solubilization of bilayers by the detergent.

The thermodynamic data obtained can be discussed in terms of headgroup hydration (dominating enthalpy changes) and the hydrophobic effect (dominating entropy changes). Partial headgroup dehydration is suggested upon incorporation of detergent monomers into a micelle and, even more, upon incorporation into a lipid bilayer.

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