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# Partitioning of Octyl Glucoside between Octyl Glucoside/ Phosphatidylcholine Mixed Aggregates and Aqueous Media as Studied by Isothermal Titration Calorimetry

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ABSTRACT Stepwise dilution of lipid-surfactant mixed micelles first results in extraction of surfactant from the mixed micelles into the aqueous medium. Subsequently mixed micelles transform into vesicles, within a range of compositions that corresponds to equilibrium coexistence between these two types of aggregates. Further dilution results in extraction of surfactant from the resultant mixed vesicles. In the present study, we have investigated the heat evolution of these processes, as they occur in mixed systems composed of egg phosphatidylcholine (PC) and the nonionic surfactant octylglucoside (OG). A combined use of isothermal titration calorimetry (ITC) and photon correlation spectroscopy (PCS), capable of monitoring phase transformations, revealed that 1) The sum of all of the studied processes (i.e., extraction of OG from mixed micelles and vesicles and the phase transformation) is isocaloric at ~40°C throughout the whole dilution. At lower temperatures, all of the dilution steps are exothermic, whereas at higher temperatures all of them are endothermic. 2) At all temperatures, the absolute value of the heat associated with each dilution step within the range of coexistence of micelles and vesicles is almost constant and larger than in either the micellar or the vesicular range. We give an interpretation of these calorimetric data in terms of the relationship between the composition of the mixed aggregates $R_e$ and the aqueous concentration of surfactant monomers $D_w$. Assuming that the main contribution to the heat evolution is due to extraction of surfactant from mixed aggregates to the aqueous solution, we deduce the relationship $D_w(R_e)$ characterizing the system over the whole range of compositions. We find that, in accord with thermodynamic expectations, $D_w$ is almost constant throughout the range of coexistence of mixed micelles and vesicles.

## GLOSSARY

| | |
|---|---|
| cmc | critical micellar concentration |
| $D_w^o$ | aqueous concentration of OG monomers in the range of co-existence |
| $D_t$ | total surfactant concentration |
| $D_t^{in}$ | initial total surfactant concentration |
| $D_w$ | concentration of monomeric surfactant |
| $D_w^{in}$ | initial concentration of monomeric surfactant |
| $D_b$ | concentration of surfactant which resides in bilayers |
| $L$ | lipid concentration |
| $L^{in}$ | initial lipid concentration |
| $R_e$ | (effective) ratio between surfactant and lipid in mixed aggregates ($R_e = D_b/L = (D_t - D_w)/L$) |
| $R_e^{sat}$ | the value of $R_e$ at the onset of solubilization |
| $R_e^{sol}$ | the value of $R_e$ required for complete solubilization |
| $\Delta Q$ | amount of heat measured by ITC |
| $\Delta Q^{hydroph}$ | heat of hydrophobic effect |
| $\Delta Q^{head}$ | heat due to the difference between energy of polar head of a surfactant molecule within an aggregate and in pure water |
| $\Delta Q^{trans}$ | heat resulting from transformation of micelles into vesicles |
| $T^*$ | temperature at which the heats of dilution vanish |
| $\mu_D$ | chemical potential of the surfactant |
| $dD_w$ | the change in the concentration of surfactant monomers caused by dilution |
| $dD_t$ | the change in the total surfactant concentration due to dilution |
| $dD_{ext}$ | the change in the aqueous concentration of surfactant monomers due to extraction |
| $\Delta H_D^{a-w}$ | molar heat of extraction of surfactant molecules from aggregates to water |
| $n$ | injection number |
| $dn$ | the change in injection number (in our case $dn = 1$) |

## INTRODUCTION

The state of aggregation of amphiphiles in aqueous solutions is an issue of great interest in many scientific disciplines. Much effort has therefore been devoted to gaining an understanding of the forces involved in the self-assembly of amphiphiles, especially in aqueous solutions (Tanford, 1980), where the structure of the resultant aggregates has been correlated with the molecular structure of the amphiphile (Israelachvili et al., 1980; Gruner et al., 1986).

Of special interest is the state of aggregation in mixtures of bilayer-forming and micelle-forming amphiphiles, such

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as mixtures of phospholipids and surfactants, commonly referred to as detergents. The state of aggregation in these mixtures is determined by the molar ratio of the components within the aggregates (Lichtenberg, 1985, 1993). Thus, mixing biological membranes with an excess of surfactants results in the formation of mixed micelles, namely in solubilization of the membranes. This process is not only essential for isolation and characterization of integral membrane proteins, but is also important for studying the function of such proteins and its dependence on lipid-protein interactions. This requires reconstitution of the proteins into well-defined closed lipidic spheroids, commonly referred to as proteoliposomes (Helenius and Simons, 1975; Klausner et al., 1984; Racker, 1985; Walter, 1990). Reconstitution can be achieved by removal of the surfactant from mixed micellar systems made of surfactants, phospholipids, and proteins, using different techniques such as dialysis, gel permeation chromatography, surfactant-binding "biobeads," or simple dilution of the mixed micellar system, which results in "extraction" of surfactant from the mixed micelles into the aqueous medium.

In view of the importance of these processes, much work has been devoted to structural and kinetic aspects of the solubilization of phospholipid model membranes by various surfactants and their reconstitution upon removal of the surfactant (Schurtenberger et al., 1985; Almog et al., 1986, 1990; Goni et al., 1986; Ollivon et al., 1988; Paternostre et al., 1988; Vinson et al., 1989; Walter et al., 1991). Although the latter investigations yielded some understanding of these processes, several basic issues remained essentially unexplored. The most fundamental issues that have gained little experimental attention thus far are those associated with the thermodynamic aspects of the processes involved in composition-induced alteration of the mixed surfactant-phospholipid systems.

The few calorimetric studies of such systems published thus far yielded interesting findings on the heat associated with micellization, introduction of surfactant molecules into phospholipid bilayers, and solubilization of the bilayers by surfactants at sufficiently high concentrations (Zimmerer and Lindenbaum, 1979; Kresheck et al., 1980; Kresheck and Nimsgern, 1983; Malloy and Binford, 1990; Paula et al., 1995; Heerklotz et al., 1995). However, despite these extensive studies, our knowledge of the interactions between phospholipids and surfactants is quite limited. A systematic investigation of the heat evolution of the various processes that occur in lipid/surfactant mixtures in the whole range of compositions can be used to gain an understanding of basic properties of the system, such as the interactions between amphiphilic molecules of different kinds in different types of amphiphilic assemblies. Gaining this information requires the combined use of a highly sensitive calorimeter and techniques capable of monitoring the state of aggregation in phospholipid-surfactant mixtures of varying compositions. Such systematic studies have not been carried out thus far.

In the present study we have employed a high-sensitivity isothermal titration calorimeter (ITC) in conjunction with a photon correlation spectrometer (PCS), capable of monitoring changes in the size of lipid-surfactant mixed aggregates, to investigate the enthalpy associated with a stepwise dilution of mixed micelles of egg phosphatidylcholine (PC) and the very widely used nonionic surfactant octyl-$\beta$-D-glucopyranoside (OG). Structural and kinetic aspects of the interaction between these two amphiphiles have been previously studied in detail by several investigators, including ourselves (Jackson et al., 1982; Ollivon et al., 1988; Paternostre et al., 1988, 1995; Vinson et al., 1989; Almog et al., 1990). Having a high critical micellar concentration (cmc) (Eidelman et al., 1988), OG can be removed from OG-PC mixed micelles by a relatively small dilution. When sufficient surfactant is removed from these "wormlike" mixed micelles, they transform into mixed vesicles (Vinson et al., 1989). The latter very rapidly undergo "postvesiculation size growth," and further removal of OG from the resultant vesicles has only a slight effect on their size (Almog et al., 1990).

The results of our calorimetric stepwise dilution experiments indicate that the major contributor to the heat evolution throughout the whole range of phases is the heat associated with extraction of OG from mixed aggregates. Under this assumption, the calorimetric results are used to compute the dependence of the composition of OG/PC mixed aggregates on the concentration of OG monomers in the aqueous solution.

## MATERIALS AND METHODS

### Materials

Both egg PC and OG were purchased from Sigma Chemical Co. (St. Louis, MO). Tris buffer was purchased from Fluka (Buchs, Switzerland). NaCl and EDTA were analytical grade (Merck, Darmstadt, Germany).

### Preparation of mixed micelles

A solution of PC in chloroform was dried under a stream of nitrogen and solubilized by an OG solution of the appropriate concentration in buffer A (140 mM NaCl, 0.5 mM EDTA, 0.02% $NaN_3$, and 10 mM Tris, pH 7.4).

### Photon correlation spectrometer measurements

Particle size was measured on a Malvern photon correlation spectrometer (model 4700) equipped with an argon laser (wavelength of 488 nm), at 28°C, as previously described (Almog et al., 1990).

### Calorimetric measurements

All of the calorimetric experiments were carried out using an OMEGA ITC (MicroCal, Northampton, MA). The titrated sample was constantly stirred at 400 rpm. Titrations were carried out at a constant temperature within the range of 9.6–69.5°C.

The design of the calorimeter is such that the volume within the cell ($V_c$ = 1358 $\mu$l) is kept constant throughout the titration; the addition of $V_t$ $\mu$l of the diluting medium is accompanied by simultaneous evacuation of

$V_t$ μl of the solution. Thus, after $n$ steps of dilution, the concentration of the titrated solution with respect to each component is approximately equal to $c_o[(V_c/(V_c + V_t)]^n$, where $c_o$ is the initial concentration of the respective component. In the experiments described in this report, $V_t$ was 25 μl. Hence the concentration of each of the components after $n$ steps of dilution was $c_o \times 0.982^n$.

## RESULTS

Titration of 25 μl of buffer into PC-OG mixed micellar solution (1.358 ml) containing 1.4 mM PC and 28 mM OG at 25°C in the ITC cell resulted in an exotherm of 650 μcal. Subsequent titration steps resulted in slightly smaller exotherms, as depicted in Fig. 1 in terms of the dependence of heat evolution on the "serial number" of the titration step.

To correlate the results of Fig. 1 with structural changes that occur upon dilution, we have carried out PCS studies using the same protocol employed in the ITC studies. Specifically, 1.358 ml of the solution used in the ITC experiment of Fig. 1 was diluted with 25 μl of the same buffer. Subsequently, 25 μl of the mixed solution was removed, and the mean size of the particles in the sample was measured by PCS. The size of the particles obtained after this and the subsequent 29 steps of dilution is depicted in Fig. 2 as a function of the "serial number" of the titration step.

Comparison of the results in Figs. 1 and 2 shows that during the first 16 steps of dilution, the particles present in the solution grew monotonically in size from ~10 nm to 30 nm, whereas the exotherms became smaller. The subsequent six dilution steps, beginning at step number 17 (Fig. 2), resulted in a large increase in particle size, indicating transformation of mixed micelles into vesicles. By comparison, the 17th step of dilution in the calorimetric titration resulted in a sharp increase in the exotherm of the calorimetric titration (Fig. 1). The subsequent six steps of dilution involved very similar exotherms. At dilution step numbers 24–26 the size of the particles remained nearly constant (Fig. 2), whereas the exotherms decreased (Fig. 1) to very small values.

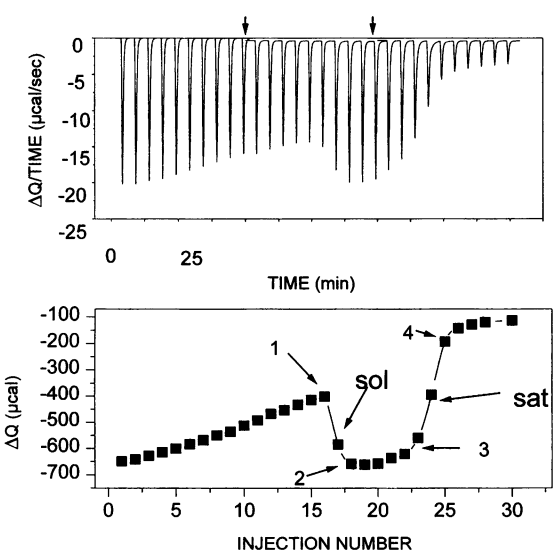


FIGURE 1 Heat evolution as a function of a stepwise dilution of an OG/PC mixed micellar solution. The heat evolution was obtained for repetitive injections of 25 μl of buffer A into a mixed micellar solution containing 1.4 mM PC and 28 mM OG and subsequent removal of 25 μl of the mixed solution. The upper panel presents the titration curves as a function of time (*arrows* depict changes in the titration syringe). The lower panel depicts the heat evolution as a function of the serial number of titration.

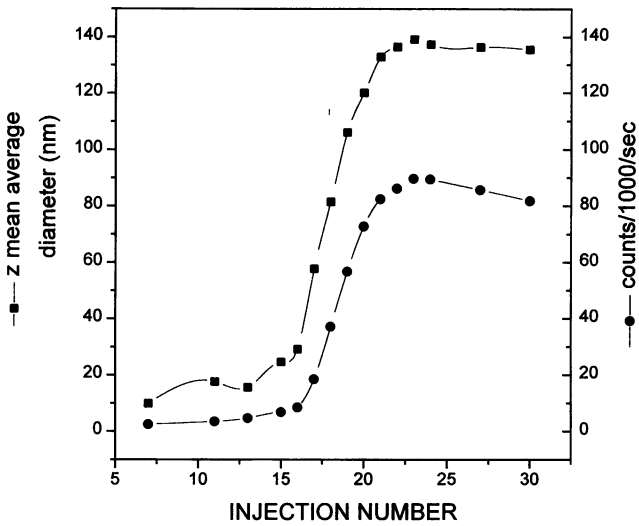


FIGURE 2 Particle size as a function of dilution of a mixed micellar solution. The changes in particle size were measured by PCS, using the same dilution protocol as for Fig. 1. Also given is the count of photons scattered at 90°.

Similar experiments, carried out with PC-OG mixtures of different OG/PC molar ratios at 28°C, yielded qualitatively similar results (Fig. 3). Specifically, in all of these experiments the first several steps of dilution yielded monotonic growth of the particles (not shown), whereas the exotherms became smaller (Fig. 3). Subsequently, a point was reached at which further dilution yielded a large increase in particle size, due to conversion of mixed micelles into vesicles, and the exotherms in the calorimetric titrations increased sharply. The subsequent dilution steps resulted in similar exotherms, until a point was reached at which further dilution yielded decreasing exotherms and subsequent dilution steps resulted in much smaller exotherms (Fig. 3).

Each calorimetric titration can be characterized by the two points at which the change in $\Delta Q$ is a maximum (*sol* and *sat* in Fig. 1). Each of these points corresponds to the heat evolution of diluting a mixture of a specific composition by a factor of 0.982 (see Materials and Methods). Because the process occurring within the range between these two points relates to the formation of vesicles, the compositions that correspond to these two points can be regarded as boundary compositions. Specifically, the surfactant concentration in this system at the point denoted by *sol* is just sufficient for the onset of vesicle formation in this system (i.e., it is $D_t^{sol}$ for the given lipid concentration $L$), whereas *sat* relates to the transformation of residual mixed

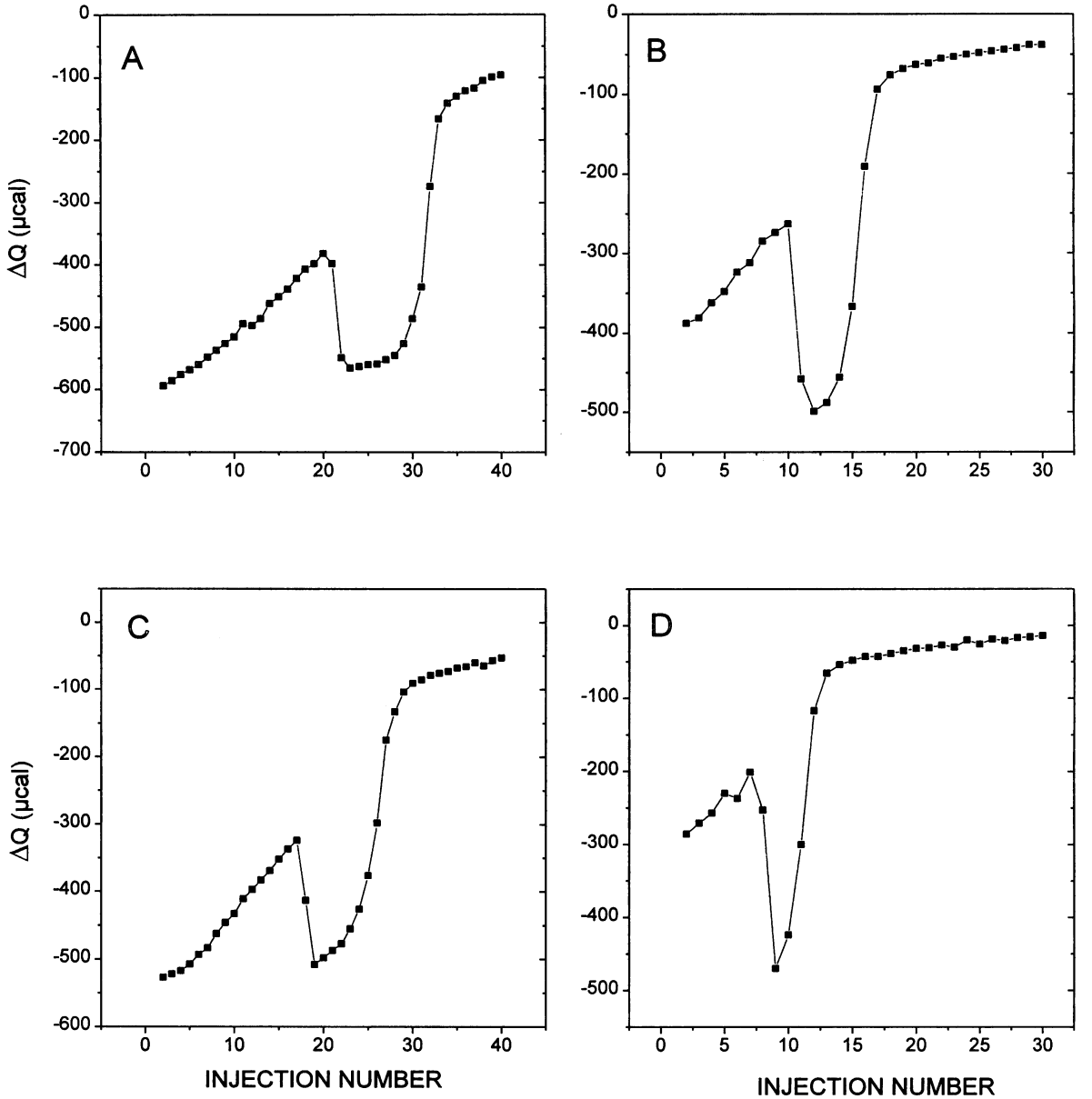


FIGURE 3 Effect of concentration on the heat evolution. Experiments similar to those described in Fig. 1 were carried out, using PC-OG mixtures of four different compositions at 28°C. Initial compositions: (*A*) 2.7 mM PC and 32 mM OG; (*B*) 0.95 mM PC and 22 mM OG; (*C*) 1.71 mM PC and 28 mM OG; (*D*) 0.45 mM PC and 20 mM OG.

micelles into vesicles ($D_t^{sat}$ for the given $L$). The values of both $D_t^{sat}$ and $D_t^{sol}$ were evaluated from the minimum and maximum of the first derivatives of Fig. 3 (not shown), respectively. Fig. 4 depicts these values as functions of the corresponding lipid concentrations. From the slopes of these dependencies, it is possible to evaluate the phase boundaries in terms of the maximum surfactant/phospholipid ratio in vesicles ($R_e^{sat}$) and the minimum effective ratio in mixed micelles ($R_e^{sol}$). The intercept of these dependencies ($D_w^{sat}$ and $D_w^{sol}$) are the monomer concentrations at the onset and completion of the solubilization process, respectively. All of these values are consistent with those obtained for OG/PC systems by other techniques (Almog et al., 1990; Ollivon et al., 1988; Paternostre et al., 1995).

The results of calorimetric titrations obtained with a mixed micellar solution containing 1.4 mM PC and 28 mM OG at five different temperatures (9.6°C, 25°C, 39.8°C, 54.5°C, and 69.5°C) are depicted in Fig. 5. Similar to the dilution of pure OG (Paula et al., 1995), at 39.8°C all of the titration steps resulted in very small exotherms. At higher temperatures, all of the titration steps became endothermic and the endotherms increased upon an increase in temperature, whereas at temperatures lower than 39.8°C the titrations yielded exotherms whose absolute magnitude increased upon a decrease in temperature. Noticeably, the concentration of OG monomers within the range of coexistence appeared to be bell-shaped functions of temperature. This may indicate that, like to the cmc of the pure OG

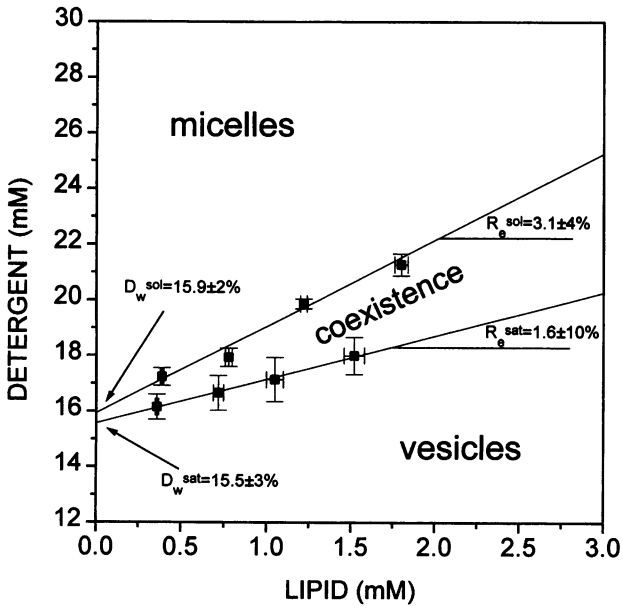


FIGURE 4 Phase diagram describing the phases that occur in PC-OG mixtures. The composition of the mixed aggregates at the onset and completion of solubilization is described in terms of the dependence of surfactant concentration on lipid concentration. Also given are the slopes of the two lines, which represent $R_e^{sol}$ and $R_e^{sat}$, and the intercepts of these lines with the surfactant axis, which represent $D_w^{sol}$ and $D_w^{sat}$ (see text).

(Paula et al., 1995), the values of both $D_w^{sat}$ and $D_w^{sol}$ are minimal at ~40–50°C and become larger at either lower or higher temperatures. This is reminiscent of the temperature dependence of the free energy of transferring surfactant hydrocarbon chains out of water and into the oil-like interior of a micelle, which in turn determines the temperature behavior of the cmc of pure surfactant (Gill and Wadsoe, 1976; Evans and Ninham, 1986).

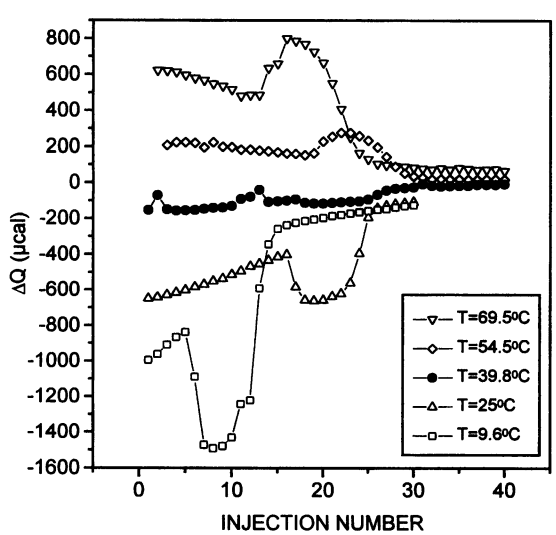


FIGURE 5 Effect of temperature on the heat evolution. Experiments similar to those described in Fig. 1 were carried out using mixed micellar solutions containing 0.45 mM PC and 28 mM OG at five different temperatures (as given in the figure).

## DISCUSSION

Stepwise dilution of surfactant/phospholipid mixed micelles results in extraction of surfactant molecules from the mixed micelles. As a consequence, the surfactant/lipid ratio within the mixed micelles decreases gradually, and the system then passes through the micellar range into a range of coexistence of vesicles and micelles and, subsequently, through the vesicular range, where dilution results in a decrease in the surfactant content of the mixed vesicles.

For interpretation of the results of calorimetric studies, we propose a model based on the following assumptions:

1. After each step of dilution, the system reaches thermodynamic equilibrium. Therefore, within the micellar range, the system consists of mixed surfactant-phospholipid micelles in equilibrium with aqueous solution of surfactant monomers; no pure surfactant micelles are present. Within the range of coexistence, mixed micelles of composition $R_e^{sol}$ are in equilibrium with mixed vesicles of composition $R_e^{sat}$ and with aqueous solution of surfactant monomers. And, finally, within the vesicular range, the mixed phospholipid-surfactant vesicles are in equilibrium with surfactant monomers with no pure phospholipid vesicles in the solution.

2. The major determinant of the heat evolution of the system is the heat of extraction of surfactant monomers from the mixed aggregates (micelles or vesicles) into water. This heat, per mole of extracted surfactant, will be denoted $\Delta H_D^{a-w}$. The sum of all other contributions, such as the heat related to the transformation of surfactant and lipid molecules from micelles into vesicles, can be neglected in comparison to $\Delta H_D^{a-w}$.

3. We can neglect the dependence of the heat $\Delta H_D^{a-w}$ on the composition of the mixed aggregates from which the surfactant molecules are extracted and consider $\Delta H_D^{a-w}$ as a function of temperature only. This assumption implies that the heat of extracting surfactant molecules from mixed aggregates is equal to the heat of demicellization of pure surfactant micelles, $\Delta H_D^{a-w} = \Delta H_{demic}$.

The second and third assumptions are based on the observed temperature dependence of the heat evolution of the stepwise dilution of the OG-PC mixtures. These assumptions were introduced to explain why, according to our measurements, at ~40°C the process became isocaloric ($\Delta Q = 0$) simultaneously in all of the ranges of composition. For temperatures above and below 40°C, the process was, respectively, endothermic and exothermic in all ranges of composition. The reasoning supporting these assumptions is discussed below.

Obviously, the measured heat involves several contributions of different physical nature (Heerklotz et al., 1995, 1996). Dilution within one-phase regions (micellar or vesicular) results in extraction of surfactant monomers from

aggregates into water. The main contribution to the heat of this process $\Delta Q_D^{a-w}$ relates to the hydrophobic effect, $\Delta Q^{hydroph}$, of taking the hydrocarbon chains of the extracted molecules from the hydrophobic core of aggregates and putting them in water. Another contribution, $\Delta Q^{head}$, is due to the difference between the energy of the polar head of a surfactant molecule within an aggregate and its energy in pure water. This value should depend on the composition of the aggregates, $\Delta Q^{head}(R_e)$.

In the range of coexistence, we expect an additional contribution to the heat, resulting from transformation of micelles into vesicles, $\Delta Q^{trans}$. This heat can be seen as being related to the change in curvature of the surfactant and lipid molecules that form the aggregates.

As $\Delta Q^{hydroph}$, $\Delta Q^{head}$, and $\Delta Q^{trans}$ result from different kinds of interactions, their dependencies on temperature are expected to be different. In particular, the temperatures $T^*$ where each of those heats vanishes,

$$\Delta Q^{hydroph}(T^*_{hydroph}) = 0,$$

$$\Delta Q^{head}(T^*_{head}, (R_e)) = 0,$$

$$\Delta Q^{trans}(T^*_{trans}) = 0$$

should differ

$$T^*_{hydroph} \neq T^*_{head}\,(R_e) \neq T^*_{trans}$$

If $\Delta Q^{hydroph}$, $\Delta Q^{head}$, and $\Delta Q^{trans}$ had comparable values, we would not have observed that their sum vanishes at the same temperature $T^* = 40°C$ in the whole range of $R_e$, including the range of coexistence, in contrast to our findings. Therefore, the most straightforward explanation of our experimental results is that the heat of transformation $\Delta Q^{trans}$ is negligible compared to the other contributions (assumption 2) and that the dependence of the heat of polar heads on composition can also be neglected ($\Delta Q^{head}(R_e)$ = constant; assumption 3).

In the following discussion, we use the simple model formulated above in conjunction with our experimental results to determine the relationship between the concentration of surfactant monomers in the aqueous medium, $D_w$, and the composition of the mixed aggregates $R_e$, in all of the phase states of the mixed system. This relationship is a basic characteristics of the physical properties of the system. Agreement between the properties of the function $D_w(R_e)$ and the general thermodynamic expectations will serve as a test for the validity of the assumptions of our model. The resultant relationship can then be used as a basis for future theoretical and experimental studies of the physical properties of the system.

## Determination of the relationship $D_w(R_e)$ from calorimetric measurements

Determination of the function $D_w(R_e)$ will be performed in two steps. First, we will calculate the change in the concentration of surfactant monomers $D_w$ in the course of dilution of the OG-PC mixtures by buffer. For this purpose, it will be convenient to present the results in terms of the dependence of $D_w$ on the total concentration of detergent $D_t$ (i.e., $D_w(D_t)$). Using this function together with the known changes in $D_t$ and in the total lipid concentration $L$ upon dilution, we can compute $D_w(R_e)$.

### *Computation of $D_w(D_t)$*

As discussed under Materials and Methods, the change in total concentration of surfactant $D_t$ from its initial value $D_t^{in}$ is determined by the number of injections $n$:

$$D_t = D_t^{in} \cdot \left(\frac{V_c}{V_c + V_t}\right)^n \tag{1}$$

where $V_t$ is the volume of injection in each step and $V_c$ is the volume of the OG-PC solution.

The concentration of surfactant monomers in the aqueous solution, $D_w$, does not follow such a simple law, as it is determined by, aside from the dilution itself, extraction of surfactant monomers from mixed aggregates. Therefore, we must derive a differential equation for $D_w(D_t)$ and solve it.

The change in $D_w$ for any step of dilution can be presented as

$$dD_w = dD_{w,dil} + dD_{w,ext} \tag{2}$$

where the first term $dD_{w,dil}$ is due to the addition of buffer per se, whereas $dD_{w,ext}$ results from extraction of surfactant monomers from aggregates into the solution.

The first contribution can be expressed as

$$dD_{w,dil} = \frac{D_w}{D_t} \cdot dD_t \tag{3}$$

(Note that in our procedure $dD_t$ is negative and, consequently, $dD_{w,dil}$ is negative). According to Eq. 1,

$$dD_t = D_t \cdot \ln\left(\frac{V_c}{V_c + V_t}\right) \cdot dn \tag{4}$$

According to assumption 2 of our model, the contribution of extraction of monomers to the change in $D_w$ can be related to the heat evolution of the system by

$$dD_{w,ext} = \frac{1}{V_c} \cdot \frac{dQ}{\Delta H_D^{a-w}} \tag{5}$$

where $dQ$ is the heat resulting from one step of dilution.

Inserting Eqs. 3 and 5 into Eq. 2 and dividing all terms by Eq. 4, we obtain the differential equation for the dependence of $D_w$ on $D_t$:

$$\frac{dD_w}{dD_t} - \frac{D_w}{D_t} = \frac{1}{V_c} \cdot \frac{1}{\Delta H_D^{a-w}} \cdot \frac{1}{\ln\left(\frac{V_c}{V_c + V_t}\right)} \cdot \frac{1}{D_t} \cdot \frac{dQ}{dn} \tag{6}$$

The function $dQ/dn$ on the right side of Eq. 6 can be taken directly from the calorimetric experiments (e.g., Figs. 1, 3,

and 5), in which $\Delta Q$, the heat resulting from one injection (i.e., $dn = 1$), is presented as a function of the injection number $n$. According to assumption 3 of our model, $\Delta H_D^{a-w}$ is constant. Hence Eq. 6 can be rewritten as

$$D_t \cdot \frac{dD_w}{dD_t} - D_w = C \cdot \Delta Q \tag{7}$$

where $C = 1/V_c \cdot \ln[V_c/(V_c + V_t)]\Delta H_D^{a-w}$. Numerical solution of Eq. 7 gives the function $D_w(D_t)$. Specifically, we assume $D_w^{in}$ to be the initial monomer concentration and use Eqs. 4 and 7 to compute the change in $D_w$ after the first step of dilution. This can then be used for determination of $D_w$ after this step of dilution, and the latter value can then be used to determine the change in $D_w$ caused by the second step of titration. The same procedure can then be used to evaluate $D_w$ after each of the subsequent titration steps.

### *Computation of $D_w(R_e)$*

Knowing the values of $L$, $D_t$, and $D_w$ (computed as described above) after each step of dilution, we can also compute $R_e$ from its definition:

$$R_e = \frac{D_t - D_w(D_t)}{L(D_t)} \tag{8}$$

where the dependence of the lipid concentration $L$ on the total detergent concentration, $L(D_t)$, is given by

$$L = \frac{L^{in}}{D^{in}} \cdot D_t \tag{9}$$

where $L^{in}$ is the initial concentration of the lipid.

Hence $D_w$ can be presented as a function of $R_e$.

### *Determination of adjustable parameter*

To solve Eqs. 7 and 8, we need to know the initial value of the concentration of surfactant monomers $D_w^{in}$ before the titration. This value cannot be obtained directly from the calorimetric measurements and, therefore, remains an adjustable parameter. To illustrate the dependence of the results on $D_w^{in}$, we show in Fig. 6 the functions $D_w(R_e)$ resulting from the solution of Eqs. 7 and 8 for the experimental $\Delta Q$ values depicted in Fig. 3 ($D_t^{in} = 22$ mM and $L^{in} = 0.95$ mM) at several reasonable values of $D_w^{in}$. The values of the other parameters were taken to be $V_c = 1358$ μl, $V_t = 25$ μl, and $\Delta H_D^{a-w} = -1691$ cal/mol. The latter value is the heat of demicellization of pure OG micelles at 28°C, as measured from a stepwise titration of a solution of OG in buffer A (not shown). Interestingly, this molar enthalpy is similar to that observed in similar experiments conducted in pure water ($\Delta H_{demic} = -1670$ cal/mol; Paula et al., 1995), and to the heat of extraction of OG molecules from palmitoyl oleyl phosphatidylcholine vesicles ($\Delta H_D^{a-w} = -(1300-1900)$ cal/mol; Wenk et al., 1997). We see that the form of the curve is sensitive to the assumed value of $D_w^{in}$. However,

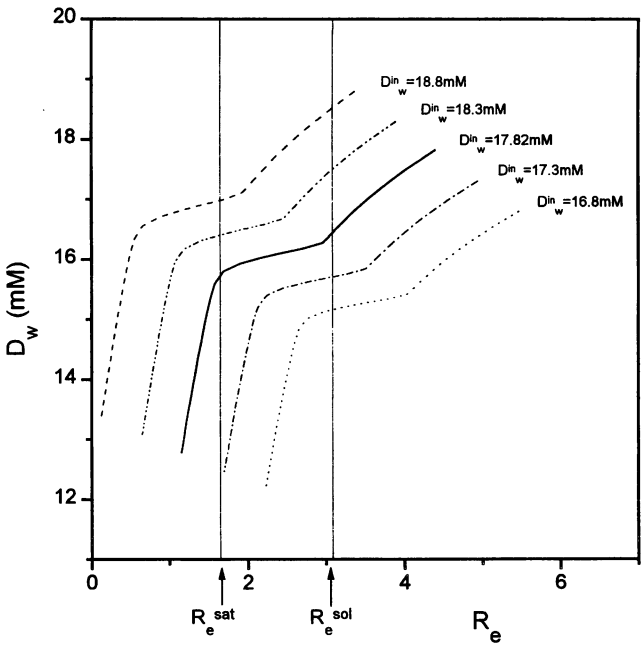


FIGURE 6 Dependence of the monomer concentration of the surfactant on the effective ratio between surfactant and lipid. The different lines were computed from the calorimetric data, assuming different values for the initial monomer concentration ($D_w^{in}$), as given in the figure (see text for details).

on all of the lines of Fig. 6 one can readily recognize a region of smaller slope corresponding to the range of coexistence between the mixed micelles and vesicles. The boundaries of this region depend on the choice of the value of $D_w^{in}$. Because the values of these boundaries, $R_e^{sat}$ and $R_e^{sol}$, were determined independently (Fig. 4), the value of $D_w^{in}$ can be chosen from the fit between the resultant predictions and the experimental boundaries of the region of coexistence (i.e., the values of $R_e^{sat}$ and $R_e^{sol}$ obtained in Fig. 4).

### *Results of computation of $D_w(R_e)$* for different lipid concentrations

Using the method described above, we calculated the dependencies $D_w(R_e)$ for the different titration curves obtained at 28°C (Fig. 3). The results presented in Fig. 7 have the following properties:

1. The function $D_w(R_e)$ is only slightly dependent on the initial composition of the mixture, i.e., within experimental error the function $D_w(R_e)$ appears to be a universal function, independent of the values of the total surfactant and lipid concentrations ($D_t$ and $L$, respectively).

2. In the range of coexistence between mixed micelles and mixed vesicles, the concentration of surfactant monomers in the solution, $D_w$, increases only slightly upon an increase in the OG concentration. The average monomer concentration within this range, denoted $D_w^o$ below, is an important characteristic of the phase transition between micelles and vesicles.

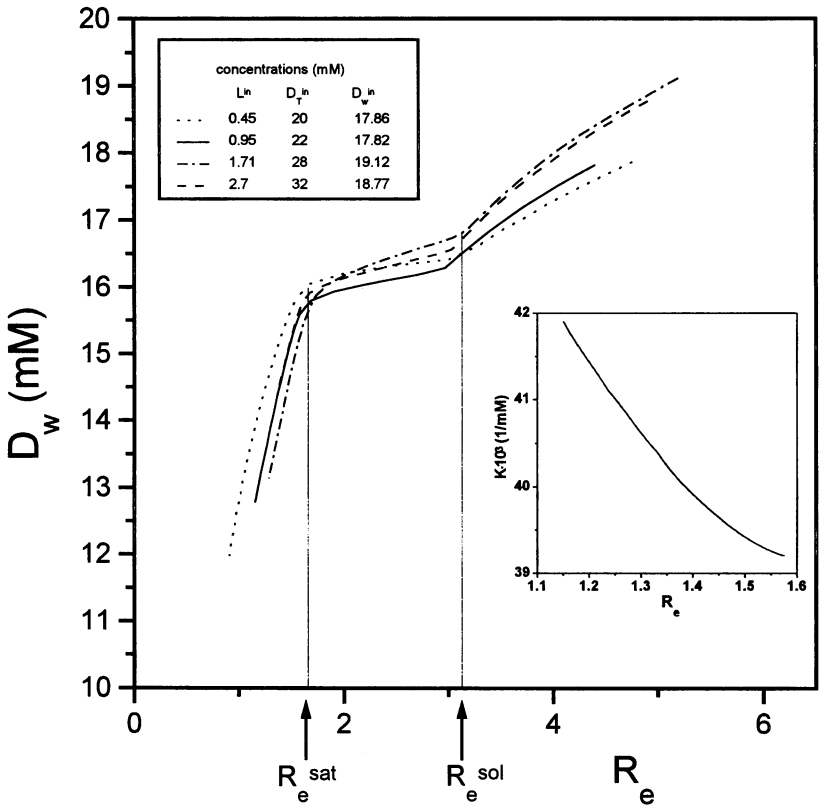


FIGURE 7 Dependence of OG monomer concentration on the effective ratio between OG and PC for different initial concentrations of the two components (as given in the figure). The inset presents the dependence of the empirically defined partition coefficient $K = R_e/D_w(1 + R_e)$ on $R_e$ for the initial concentration, represented by the solid line in the figure.

3. For each PC/OG mixture within the vesicular range, the partitioning of OG between PC vesicles and the aqueous medium can be given in terms of an empirically defined partition coefficient, $K = R_e/D_w(1 + R_e)$. This factor, as computed from the values of $R_e$ and $D_w$ given by the solid line in Fig. 7, is depicted in the inset of Fig. 7 as a function of $R_e$ for PC/OG mixtures of $R_e$ values of 1.1–1.6. Similar dependencies were obtained from the data of the other titrations, although the actual value of $K$ at relatively low $R_e$ values varied by up to ±5%. Similar to the results of Parternostre et al. (1995) and Ueno (1989), $K$ is a decreasing function of $R_e$. Near saturation, $K$ approaches a minimum value of 0.039 $mM^{-1}$ (Fig. 7, *inset*), as compared to literature values of 0.033–0.044 $mM^{-1}$ (Almog et al., 1990; Jackson et al., 1982; Ueno, 1989; Parternostre et al., 1995).

## Validity of the model

The properties of the universal function $D_w(R_e)$, mentioned above, are in agreement with general thermodynamic expectations and, therefore, support the validity of the assumptions of the simple model used to interpret our calorimetric results.

Let us consider this agreement in more detail:

1. According to thermodynamic considerations, equilibrium partitioning of surfactant molecules between the aqueous solution of monomers and the mixed aggregates is determined by equal chemical potentials of surfactant, $\mu_D$, in these two states. The chemical potential of monomers is determined by their aqueous concentration, $\mu_D^w = \mu_D^w(D_w)$, whereas the chemical potential in the aggregates depends only on their composition, $\mu_D^a = \mu_D^a(R_e)$. (The latter consideration is valid under the conditions of all of our experiments, in which, on one hand, each aggregate consists of a large number of molecules and, on the other hand, the dispersion is sufficiently dilute for the contributions to $\mu_D^a$ of both the translational entropy of aggregates and the interactions between them to be neglected). The condition of equilibrium in its most general form, $\mu_D^w(D_w) = \mu_D^a(R_e)$, can be considered a requirement that the concentration of monomers in the solution is a function of composition of aggregates only, $D_w(R_e)$, and not of the total concentrations of surfactant and lipid. This general expectation is reasonably satisfied by the universal concentration-independence of the function $D_w(R_e)$ (Fig. 7).

2. Similar reasoning predicts a constant concentration of monomers in the aqueous medium throughout the region of coexistence of mixed micelles and vesicles. Within this range, micelles of a constant composition $R_e^{sol}$ are in equilibrium with vesicles of a constant composition $R_e^{sat}$ and with monomers in the aqueous solution. The only value that changes upon dilution is the ratio between the amount of material contained in micelles and vesicles. The chemical potentials of surfactant in the micelles and vesicles, determined by $R_e^{sol}$ and $R_e^{sat}$, respectively, do not change. Therefore, the chemical potential of monomers and, consequently, their aqueous concentration have to remain constant. This expectation is only partially consistent with

the second property of the functions $D_w(R_e)$ depicted in Figs. 6 and 7 (see above) and with the experimental data of Fig. 4, which reveal a difference between $D_w^{sat}$ and $D_w^{sol}$. In our estimate of the accuracy of determination of the phase boundaries and intercepts, the difference between $D_w^{sat}$ and $D_w^{sol}$ in Fig. 4 lies within the experimental error. However, more experimentation and theoretical studies are required to determine whether these two values should be considered equal.

In conclusion, the most straightforward interpretation of the temperature dependence of our calorimetric results is that the heat evolution of the dilution experiments is predominantly due to extraction of OG from OG-PC mixed aggregates. This assumption, in turn, was used to determine the dependence of the monomer concentration ($D_w$) in all of the studied mixtures of OG and PC as a concentration-independent function of the OG/PC ratio in the mixed aggregates ($R_e$). Nonetheless, because the contribution of the heat of extraction is much larger than that of the heat evolution due to the phase transformation (in the range of coexistence), the heat of phase transformation cannot be evaluated from experiments based on the dilution protocol used in this study. Alternative protocols, conducted in conjunction with the appropriate theoretical approach, are described in the following paper.

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