A Titration Microcalorimetric Study of the Effects of Halide Counterions on Vesicle-Forming Aggregation in Aqueous Solution of Branched-Chain Alkylpyridinium Surfactants

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Titration microcalorimetry is used to study the influences of iodide, bromide, and chloride counterions on the aggregation of vesicle-forming 1-methyl-4-(2-pentylheptyl)pyridinium halide surfactants. Formation of vesicles by these surfactants was characterised using transmission electron microscopy. When the counterion is changed at 303 K through the series iodide, bromide, to chloride, the critical vesicular concentration (cvc) increases and the enthalpy of vesicle formation changes from exo- to endothermic. With increase in temperature to 333 K, vesicle formation becomes strongly exothermic. Increasing the temperature leads to a decrease in enthalpy and entropy of vesicle formation for all three surfactants. However the standard Gibbs energy for vesicle formation is, perhaps surprisingly, largely unaffected by an increase in temperature, as a consequence of a compensating change in both standard entropy and standard enthalpy of vesicle formation. Interestingly, standard isobaric heat capacities of vesicle formation are negative, large in magnitude but not strikingly dependent on the counterion. We conclude that the driving force for vesicle formation can be understood in terms of overlap of the thermally labile hydrophobic hydration shells of the alkyl chains.   © 2000 Academic Press

Key Words: titration microcalorimetry; vesicles; aggregation; alkylpyridinium surfactants.

INTRODUCTION

When surfactant monomers are dissolved in aqueous systems, a fascinating range of aggregate structures are formed. These structures (1) include (a) spherical micelles, (b) worm-like micelles, and (c) bilayers, which may close to form vesicles. The actual aggregate morphology formed by a given surfactant depends on the molecular architecture of the surfactant, temperature, surfactant concentration and both nature and concentration of added salts. In aqueous systems, single-chained surfactants usually form micelles whereas double-chained surfactants usually form bilayers. The driving force for aggregation results primarily from hydrophobic interactions between the alkyl chains of a surfactant (2).

In this paper we focus attention on the aggregation of vesicle-forming surfactants using titration microcalorimetry. To our knowledge, this technique has not been previously used to examine vesicle formation from either natural phospholipids or synthetic amphiphiles, because vesicles are usually formed at very low concentrations. These systems exhibit critical vesicle concentrations (cvcs) of the order of 10^{-6} mol dm^{-3}. Consequently the task of measuring enthalpies of vesicle deaggregation seemed unrealistic. Vesicle formation from branched single-chain amphiphiles has been documented previously (3). Fortunately, and perhaps significantly, the surfactants used in the study reported here form vesicles at relatively high concentrations, of the order of 10^{-3} mol dm^{-3}. Hence the possibility emerged of using a microcalorimeter to characterise the thermodynamics of vesicle formation by surfactants in aqueous solution. This study builds on previously reported studies which concerned the use of titration microcalorimetry (4–6) to understand the thermodynamics of micelle formation by surfactants in aqueous solution. In those studies we showed that the deaggregation of micelles formed by alkylpyridinium and alkyltrimethylammonium salts in aqueous solution to monomers could be monitored and both standard enthalpies of deaggregation and critical micellar concentrations (cmcs) could be estimated. We showed that the recorded enthalpogram could also reflect the extent to which the thermodynamic properties of the micellar and surfactant solutions are not ideal (6–7). In such cases the recorded enthalpograms are often complicated, but, for a given surfactant system, can be classified on the basis of the overall pattern of the enthalpogram. A similar diversity of enthalpograms is recorded for deaggregation of vesicles in aqueous solution. Nevertheless, estimates of the thermodynamic parameters characterising vesicle deaggregation can be obtained from the recorded enthalpograms. The outcome is an estimate of the cvc for a given vesicle system which leads to the standard Gibbs energy for the formation of vesicles from one mol of surfactant monomer. Combination with the corresponding enthalpy of deaggregation obtained from the recorded enthalpogram

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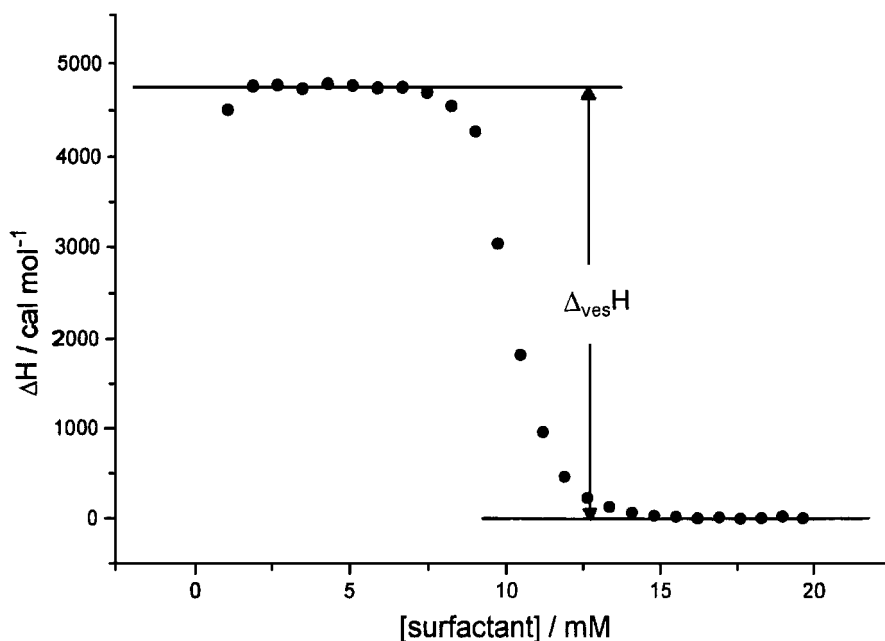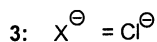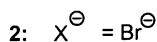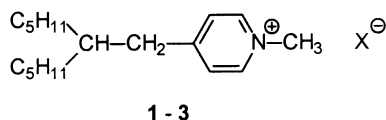[2] To whom correspondence should be addressed.

FIG. 1. Dependence on concentration of surfactant **1** of the enthalpy change accompanying injection of aliquots from the syringe into the sample cell using a titration microcalorimeter.

provides an estimate of the corresponding standard entropy of vesicle formation. Further by recording the enthalpograms over a range of temperatures the corresponding standard isobaric heat capacity for vesicle formation for one mol of surfactant is obtained. These heat capacities are strongly negative and almost independent of counterion although the individual enthalpies of vesicle formation depend on the counterion.

RESULTS AND DISCUSSION

The formation of vesicles by surfactants **1**, **2**, and **3** (Scheme 1) was confirmed using transmission electron microscopy. The diameters of vesicles formed by surfactant **1** were between 100 and 120 nm. Vesicles formed by surfactant **2** in a solution having a concentration of 3×10^{-2} mol dm^{-3} had diameters between 40 and 100 nm. For surfactant **3**, (vesicles formed in a solution having a concentration of 5×10^{-2} mol dm^{-3}), the corresponding diameters were between 40 and 200 nm. The micrographs exhibit no unusual features and therefore are not reported here.

C$_5$H$_{11}$
$\quad$CH–CH$_2$–⟨pyridinium⟩N$^{\oplus}$–CH$_3$ $\quad$ X$^{\ominus}$
C$_5$H$_{11}$

1 - 3

1: X$^{\ominus}$ = I$^{\ominus}$

2: X$^{\ominus}$ = Br$^{\ominus}$

3: X$^{\ominus}$ = Cl$^{\ominus}$

SCHEME 1

A typical enthalpogram recorded by the titration calorimeter for the deaggregation of vesicles formed by surfactant **1** is shown in Fig. 1. The plot yields directly the standard enthalpy of vesicle deaggregation. Further, using the procedure developed by van Os and co-workers (6) the cvc is readily obtained (Fig. 2). Using the phase equilibrium model [2] now carried over to describe the formation of vesicles from monomeric surfactants, the standard Gibbs energy of vesicle formation per mol of surfactant (where

TABLE 1
Thermodynamic Parameters Describing Vesicle Formation by Surfactants 1–3[a]

Surfactant	T (K)	cvc (10^{-3} mol dm^{-3})[b]	$\Delta_{\text{ves}} H^{\circ}$ (kJ mol^{-1})[c]	$\Delta_{\text{ves}} G^{\circ}$ (kJ mol^{-1})[d]	$\Delta_{\text{ves}} S^{\circ}$ (J mol^{-1} K^{-1})[e]
1	303	8.7	−14.5	−23.9	31.0
1	313	10.6	−19.9	−23.7	12.1
1	323	11.7	−21.9	−23.9	6.2
1	333	12.0	−24.1	−24.1	0.0
2	303	14.8	−0.4	−21.2	68.6
2	313	18.6	−4.8	−20.7	50.8
2	323	18.5	−8.6	−21.4	39.6
2	333	19.5	−11.7	−21.8	30.3
3	303	20.7	3.1	−19.5	74.6
3	313	—	−2.1	—	—
3	323	25 ± 1	−4.9	−19.8	46.1
3	333	25 ± 1	−8.0	−20.4	37.2

[a] Isobaric heat capacities for vesicle formation $\Delta_{\text{ves}} C_p^{\circ}$/J mol^{-1} K^{-1}: surfactant 1, -308 ± 57; 2, -377 ± 21; and 3, -361 ± 38.
[b] Estimated error: $\pm(0.2–0.3) \times 10^{-3}$ mol dm^{-3} or if stated otherwise.
[c] Estimated error: $\pm(0.2–0.3)$ kJ mol^{-1}.
[d] Estimated error: $\pm(0.1–0.2)$ kJ mol^{-1}.
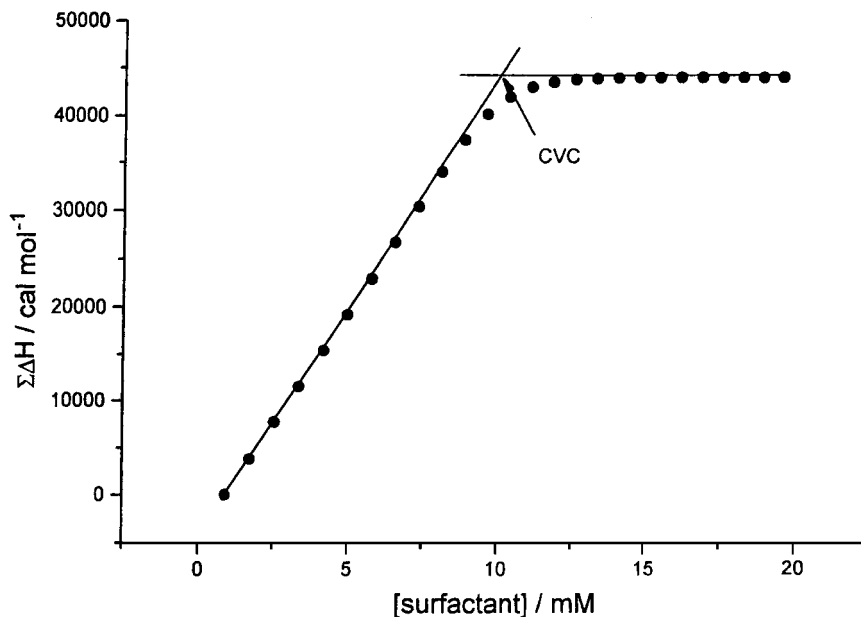[e] Estimated error: ±1.5 J mol^{-1} K^{-1}.

FIG. 2. Cumulative enthalpies of dilution against surfactant concentration in the sample cell using the enthalpogram shown in Fig. 1; a van Os plot.

the latter is a 1 : 1 salt) is then calculated together with the standard entropy of vesicle formation, Eqs. [1] and [2], where R is the gas constant, T is temperature, and c_r is the reference concentration, 1 mol dm^{-3} (Table 1).

$$\Delta_{ves}G^\circ = 2RT \ln(cvc/c_r). \qquad [1]$$

The two standard states are (i) 1 mol of surfactant in the pure vesicular phase, and (ii) 1 mol of surfactant in an ideal aqueous solution, concentration 1 mol dm^{-3}.

Further,

$$\Delta_{ves}S^\circ = (\Delta_{ves}H^\circ - \Delta_{ves}G^\circ)/T. \qquad [2]$$

Moreover, the standard isobaric heat capacity of vesicle formation is related to the dependence of the enthalpy on temperature using Eq. [3].

$$\Delta_{ves}C_p^\circ = (\delta\Delta_{ves}H^\circ/\delta T)_p. \qquad [3]$$

With increase in temperature to 313 K, the enthalpogram for surfactant **1** retained the same shape, the enthalpies of dilution above the cvc being close to zero (Fig. 3). The enthalpogram indicates that the enthalpy of vesicle formation is more exothermic at 313 K than at 303 K (Table 1). Hence, the isobaric heat capacity for vesicle formation is negative in the case of surfactant **1**.
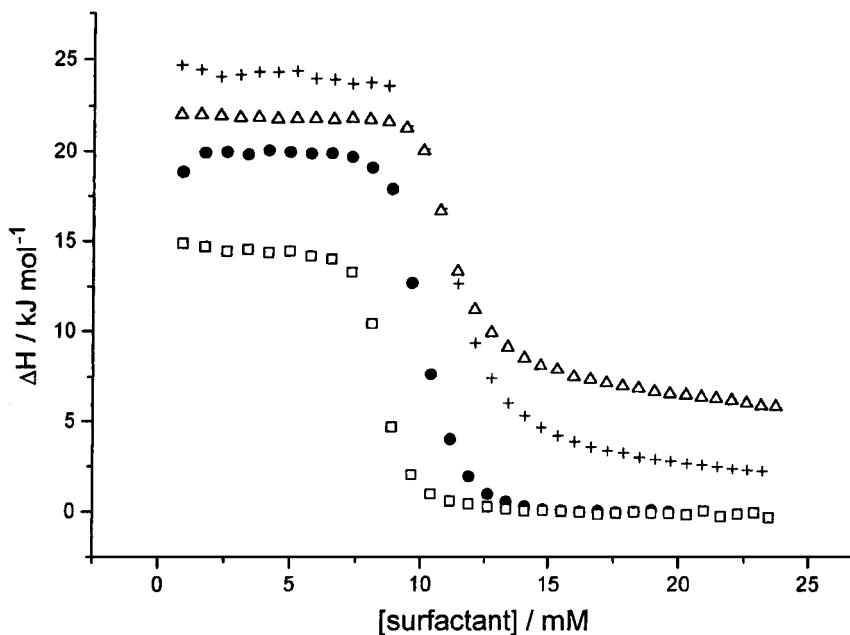

FIG. 3. Enthalpograms for surfactant **1** at four temperatures: 303 (□), 313 (●), 323 (△), and 333 K (+).

With further increase in temperature, the pattern broadly continues, and the recorded enthalpies of dilution at low injection numbers increase with increase in temperature. However, when the concentration of surfactant in the sample cell is above the cvc, the enthalpies of dilution do not fall to essentially zero. Thus below the cvc the vesicles deaggregate to form surfactant monomers. However, with reference to the enthalpograms for the higher temperatures when the concentration in the sample cell exceeds the cvc, we attribute the non-zero enthapy of dilution to aggregation of the surfactant monomers to a range of different structures by the hydrophobic solutes. Fortunately, the

recorded enthalpies of dilution, when the concentration of surfactant is below the cvc, can be confidently used to comment on the enthalpies and isobaric heat capacities of vesicle formation.

The shapes of the enthalpograms recorded for surfactant **1** at 303 and 313 K (Fig. 3) form a benchmark against which to compare the enthalpograms for surfactants **2** and **3** (Fig. 4). The enthalpograms in the latter figure show that below the cvc, the enthalpies of dilution for a given surfactant and temperature, depend on the concentration of surfactant in the sample cell. A similar pattern was observed in the case of several surfactants which form micelles (7). We noted that the thermodynamic properties
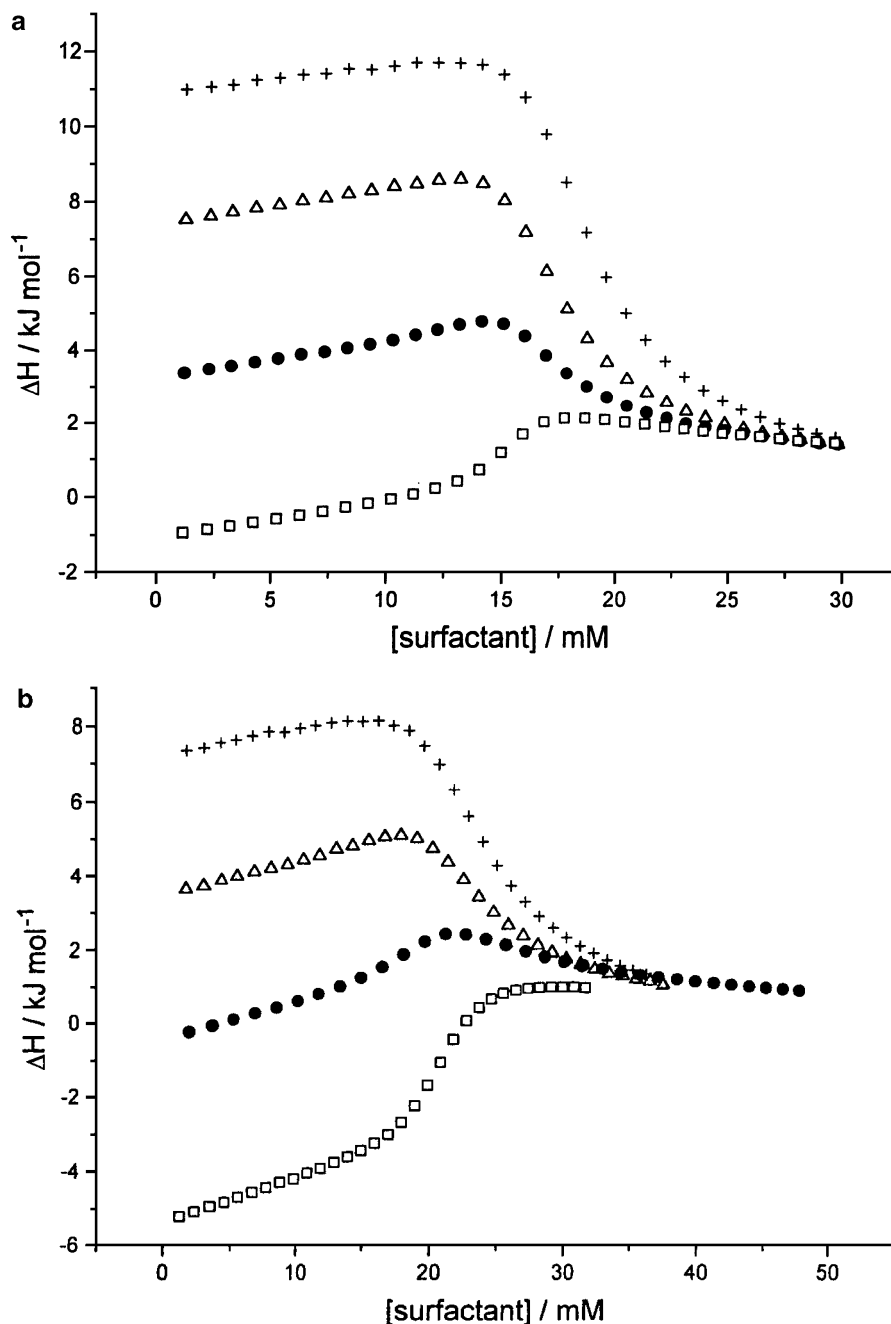

FIG. 4. Enthalpograms for (a) surfactant **2** and (b) surfactant **3** at four temperatures: 303 (□), 313 (●), 323 (△), and 333 K (+).

of the solutions in the sample cell depend on composition to an extent which increases with increase in cmc. This pattern is a consequence of the need to use more concentrated solutions in order that the enthalpograms cover the concentration range from below to above the cmc. A similar explanation accounts for the patterns recorded in Fig. 4 in that through the series I^-, Br^-, to Cl^-, the cvc increases (Table 1). A significant feature of Fig. 4 is the change in sign of the enthalpy of dilution below the cvc with increase in temperature. Mehrian *et al.* (8) found a similar change in sign with respect to enthalpies of micelle formation. The cvcs for surfactants **2** and **3** are more difficult to determine than that for surfactant **1** as a result of the change in sign in the enthalpies of dilution. For surfactant **2**, the cvc at 303 K was estimated using the change in slope in the enthalpogram. For surfactant **3** at 313 K enthalpies of dilution are small below and above the cvc, so both the cvc and enthalpy of vesicle formation are difficult to estimate. In general terms for the three surfactants, the cvcs increase with increase in temperature. We attribute this pattern to the fact that at higher temperatures, the monomers are less strongly hydrated and therefore the driving force for aggregation is smaller. A second feature is that the enthalpy of vesicle formation decreases with increasing temperature (i.e., vesicle formation becomes more exothermic). Furthermore, the entropy decreases with increasing temperature. These findings lead to a Gibbs energy which is either hardly or only slightly temperature dependent due to the enthalpy–entropy compensation (9–11).

Heat capacities of micelle formation (8, 9, 11) are understandable in terms of hydrophobic hydration of the hydrophobic parts of the monomers. The heat capacities for vesicle formation are a measure of the changes in the hydrated hydrophobic surface area and with increasing length of the hydrophobic alkyl chains the partial molar heat capacities become more negative. The effect of counterions is not completely clear. While Johnson and Olofsson (12) found a trend for aromatic counterions between the position of the counterions with respect to the headgroup area and the heat capacities, no such trend was observed by Bijma *et al.* (13). The large negative isobaric heat capacities for vesicle formation are a consequence of the fact that the aggregates are formed through overlap of the thermally labile hydrophobic hydration shells (2).

CONCLUSIONS

The formation of vesicles of 1-methyl-4-(2-pentylheptyl) pyridinium surfactants at relatively high surfactant concentrations in aqueous solutions has been confirmed by transmission electron microscopy. The remarkably high cvcs for the surfactants **1**–**3** makes possible the application of titration microcalorimetry to determine thermodynamic parameters describing vesicle formation. For this group of surfactants the cvc and enthalpy of vesicle formation increases with increase in size of hydrated counterions. Further for each surfactant the cvc increases with increasing temperature.

EXPERIMENTAL PROCEDURES

Materials

The water was demineralized and distilled twice. All commercially available chemicals were purchased from Merck, Aldrich, Janssen, or Fluka and were used as received.

Elemental analyses were performed in the analytical department of our laboratories by Mr. H. Draayer, Mr. J. Ebels, and Mr. J. Hommes. The analysis of surfactant **3** was hampered by the hygroscopic nature of this compound. No impurities were detected using NMR.

NMR Measurements

^{1}H-NMR spectra were recorded for surfactants dissolved in deuterated chloroform using a 200 MHz Varian machine. The ^{1}H chemical shifts are reported in δ units (ppm) relative to the solvent as an internal standard and are converted to the TMS scale using $\delta(CHCl_3) = 7.24$ ppm. The splitting patterns are designated as follows: s (singlet); d (doublet); t (triplet); and m (multiplet). ^{13}C-NMR spectra were recorded in deuterated chloroform using a 300 MHz Varian machine. The chemical shifts are reported in δ units (ppm) relative to the solvent as an internal standard and are converted to the TMS scale using $\delta(CDCl_3) = 76.9$ ppm.

Synthesis

Synthesis of surfactant **1** has been described previously by Nusselder *et al.* (14, 15). 6-Bromoundecane was synthesized from 6-undecanone in two steps. Surfactant **1** was prepared using the general method described by Sudhölter *et al.* (16). For surfactant **2** the same procedure as used for surfactant **1** was used, but methyl bromide was used instead of methyl iodide. Surfactant **3** was prepared using ion-exchange methods, starting from surfactant **1**.

An ion-exchange column was made with Dowex 1×8 200–400 mesh (Merck). The column was saturated with the desired anionic counterion by treating the column with an aqueous solution of the corresponding sodium salt (0.2 mol). After the regeneration, the column was washed with water (1.5 L) and methanol (0.5 L). Compound **1** was dissolved in 3 mL of methanol and introduced on the column. The product was fractionally collected and the fractions which contained product (using a UV detection test) were combined. Subsequently, the solvent was evaporated using a rotary evaporator. The product was crystallized from either ethyl acetate or ether. Finally, the compound was freeze-dried for 1 or 2 days.

1-Methyl-4-(2-pentylheptyl)pyridinium iodide (**1**). Mp = 96–99°C. ^{1}H-NMR: $\delta = 0.86$ (t, 6H, CH_3), 1.26 (m, 16H, alkyl chain), 1.75 (m, 1H, CH), 2.78 (d, 2H, CH_2), 4.67 (s, 3H, N^+-CH_3), 7.76 (d, 2H, pyridinium ring), 9.26 (d, 2H, pyridinium ring) ppm. ^{13}C-NMR: $\delta = 14.00$ (CH_3, alkyl chain), 22.56–32.98 (CH_2, alkyl chain), 39.32 (CH, alkyl chain), 40.77 (CH_2, attached to pyridinium ring), 48.71 (CH_3, N^+-CH_3), 128.35

(CH, pyridinium ring), 144.74 (CH, pyridinium ring), 162.92 (C, pyridinium ring) ppm.

1-Methyl-4-(2-pentylheptyl)pyridinium bromide (**2**). To a solution of 2-pentylheptylpyridine (9.93 g, 40.13 mmol) in acetone (65 mL), methyl bromide (30 g, 0.32 mol) was added at $-20°C$ via a gas inlet. The solution was stirred for 5 h at this temperature followed by one night of stirring at room temperature. The product precipitated and subsequently the solvent was evaporated. The crude material was crystallised from acetone and dried under vacuum. An off-white solid was obtained (6.51 g, 48%). Mp = 99–101°C. ^{1}H-NMR: $\delta = 0.86$ (t, 6H, CH$_3$, alkyl chain), 1.24 (m, 16 H, alkyl chain), 1.72 (m, 1H, CH), 2.77 (d, 2H, CH$_2$), 4.72 (s, 3H, N$^+$-CH$_3$), 7.73 (d, 2H, pyridinium ring) 9.37 (d, 2H, pyridinium ring) ppm. ^{13}C-NMR: $\delta = 13.76$ (CH$_3$, alkyl chain), 22.33–32.78 (CH$_2$, alkyl chain), 39.13 (CH, alkyl chain), 40.53 (CH$_2$, attached to pyridinium ring), 48.04 (CH$_3$, N$^+$-CH$_3$), 128.15 (CH, pyridinium ring), 144.83 (CH, pyridinium ring), 162.60 (C, pyridinium ring) ppm. Anal. calcd. for C$_{18}$H$_{32}$NBr: C, 63.15; H, 9.42; N, 4.09; Br, 23.34. Found: C, 62.91; H, 9.16; N, 4.15; Br, 23.59.

1-Methyl-4-(2-pentylheptyl)pyridinium chloride (**3**). Mp = 50–52°C. ^{1}H-NMR: $\delta = 0.87$ (t, 6H, CH$_3$, alkyl chain), 1.24 (m, 16H, alkyl chain), 1.77 (m, 1H, CH), 2.77 (d, 2H, CH$_2$), 4.74 (s, 3H, N$^+$-CH$_3$), 7.72 (d, 2H, pyridinium ring), 9.44 (d, 2H, pyridinium ring) ppm. ^{13}C-NMR: $\delta = 13.76$ (CH$_3$, alkyl chain), 22. 34–32.77 (CH$_2$, alkyl chain), 39.15 (CH, alkyl chain), 40.49 (CH$_2$, attached to pyridinium ring), 47.79 (CH$_3$, N$^+$-CH$_3$), 128.17 (CH, pyridinium ring), 144.94 (CH, pyridinium ring) ppm. Anal. Calcd. for C$_{18}$H$_{32}$NCl (1.16% crystal water): C, 67.81; H, 10.85; N, 4.39; Cl, 11.12; Found: C, 67.48; H, 10.70; N, 4.47; Cl, 11.34.

Vesicle Preparation

Vesicle solutions were prepared by dispersion of the surfactants with concentrations between 0.05–0.15 M in double–distilled water at 50°C by means of a Branson B15 sonication immersion tip (5 min).

Transmission Electron Microscopy

Samples were examined using a Philips EM 300 electron microscope operating at 60 kV. Samples were prepared using the techniques of negative staining or freeze-fracture. In case of the negative staining technique, the samples were stained with a solution of either uranyl acetate (UA) or phosphotungstic acid (PTA). Freeze-fracture samples were prepared as described previously (17). For surfactant **1**, the formation of vesicles was previously observed using negative staining and freeze fracture techniques (14). Vesicles formed by surfactant **2**, were stained with PTA whereas vesicles formed by surfactant **3** were identified using the freeze-fracture technique.

Titration Microcalorimetry

Enthalpograms were recorded using a titration micro-calorimeter (Microcal, Northampton, MA) (18, 19). All solu-tions were degassed before use. In a typical experiment the sample cell (1.3 cm^3) and reference cell were filled with doubly distilled water. The syringe contained a vesicle solution having a concentration of approximately 15 times the cvc. The sample cell was stirred (350 rpm) to ensure complete mixing. It had been shown previously (13) that the stirring speed has no effect on the recorded enthalpograms for the deaggregation of micelles. We confirmed this observation by repeating a number of experiments using a stirring speed of 800 rpm. No change in the recorded enthalpogram was recorded. In a typical microcalorimetric experiment, aliquots (10×10^{-6} dm^3 in 20 s) of a concentrated vesicle solution (concentration approximately 10 times larger than the cvc) were injected into the sample cell which contained, initially, doubly distilled water. Deaggregation of vesicles injected from the syringe was monitored by the accompanying heat which in the case of the example quoted in Fig. 1 is endothermic. A period of 210 s between two injections ensured that the system in the sample cell had attained thermodynamic equilibrium before a new aliquot was injected into the sample cell. This procedure was repeated until the desired concentration range was covered. With increase in concentration of surfactant in the sample cell, a stage is reached where the cvc is exceeded. Hence the extent of the heat either liberated or absorbed decreases. The calorimetric data were analysed using Omega software (Origin 2.9). Titration microcalorimetric experiments were performed in duplicate at discrete temperatures in the range 303–333 K.

A given enthalpogram displayed two distinct patterns, below and above the cvc: cf. Fig. 1. The two parts were used to calculate the standard enthalpy of vesicle deaggregation, $\Delta_{ves}H°$. The cvc was calculated using a plot of the cumulative enthalpy change versus the concentration. In an extension of the procedure developed by van Os and co-workers (6), for the estimation of a cmc, the intersection of the two lines was used in the study reported here to identify the cvc for a given surfactant: e.g., Fig. 2.

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