The use of high-sensitivity differential scanning calorimetry to characterise dilute aqueous dispersions of surfactants

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Summary

Four synthetic surfactants, consisting of alkyl chains of either 16 or 18 carbon units, with a polar head of either three or seven ethylene oxide units (termed $C_{16}(EO)_3$, $C_{18}(EO)_3$, $C_{16}(EO)_7$ and $C_{18}(EO)_7$) have been studied using thermal analysis in both their hydrated and non-hydrated states. The non-hydrated samples were investigated using differential thermal analysis (DTA), and dilute aqueous dispersions were studied using high-sensitivity differential scanning calorimetry (HSDSC). The non-hydrated $(EO)_3$ surfactants exhibited a single melting transition, whilst the equivalent experiment for the $(EO)_7$ systems revealed a pre-melting transition, which could be a change in crystal structure. Multiple transitions were observed with all of the hydrated samples following HSDSC investigation. These transitions are probably related to different levels of hydration of a non-homogeneous dispersion of the surfactant in the water. The following general conclusions were reached: the surfactants exhibit a phase transition at a temperature which is predominantly determined by the alkyl chain length, but the increasing chain length of the head group (polyoxyethylene) causes a decrease in transition temperature. It has proved possible to estimate the probable contribution to the enthalpy of transition that is associated with a methylene group and an ethylene oxide unit. This allows predictions to be made regarding the behaviour of similar surfactants. It was concluded that HSDSC is potentially of great value for studies of dilute solutions/dispersions of surfactants.

Introduction

The solution-phase properties of surfactants are of great importance, since changes in conformation, association and hydration phenomena, as a function of temperature, are not just of fundamental interest, but will affect the behaviour of products in which such molecules are contained. Changes in conformation/association/hydration may influence product stability (e.g., in creams, emulsions and suspensions), as well as the wetting and solubility of associated components in the product. It is also possible that such thermally induced changes may alter the toxicity of surfactant molecules. Thus, there is considerable justifi-

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cation for pharmaceutical scientists to have an interest in studies on the physico-chemical properties of such systems.

A group of surfactants which is of particular interest is the polyoxyethylene alkyl ethers. In high concentrations in water, these surfactants can form several (liquid) crystalline structures e.g., the cubic, viscous isotropic, hexagonal, and lamellar phases (Mitchell et al., 1983). These phases may form the basis of cream or ointment formulations for transdermal drug delivery. Recently, Hofland et al. (1989) demonstrated that, at low concentrations, these surfactants can (when mixed with cholesterol) form vesicles, which might be considered as potential drug delivery systems for several routes of delivery. With the use of low concentrations of surfactants in drug delivery systems, it becomes of increasing importance to assess their behaviour thermodynamically, in dilute aqueous solution/dispersions.

Differential scanning calorimetry (DSC) has been used to investigate the thermal properties of polymers. Normally, investigations relate to studies of melting, fusion, crystallisation (and degree of crystallinity), and glass transitions (see Ford and Timmins (1989) for examples). Such studies are usually undertaken in either the solid state (using sample sizes in excess of 10 mg) or in reasonably concentrated solutions or gels (i.e., $\gg 10\%$ w/w).

There are almost no data on surfactant polymer transitions in dilute aqueous solutions or dispersions, due to a lack of sensitive instrumentation. It is clear that the data obtained at high concentrations cannot be assumed to be applicable to predict transitions in (the often more realistic) dilute systems. In a recent publication (Mitchard et al., 1990), what is believed to be the first example of a phase transition of a synthetic organic block copolymer (pluronic F87) in dilute aqueous solution was recorded. The use of high-sensitivity differential scanning calorimetry (HSDSC) opens up a new and exciting opportunity to investigate pharmaceutical surfactants (and other synthetic polymers). The data presented here demonstrate phase transitions for dilute aqueous dispersions (1.5% w/w) of four synthetic surfactants, which have been investigated as potential drug delivery systems (e.g., Bouwstra et al., 1990; Hofland et al., 1990). The phase transitions obtained using HSDSC have been compared to the more usual measurements, which are often carried out on the non-hydrated surfactants.

This paper constitutes a preliminary study of synthetic polymers in dilute aqueous dispersion (which would not give rise to responses by conventional DSC experiments, due to limitations in sensitivity) by use of HSDSC, and aims to demonstrate the type of information that can be obtained, and the potential for application of this technique.

Materials and Methods

Materials

The four polyoxyethylene-alkyl ether surfactants consisted of alkyl chains of either 16 or 18 carbon units, with a polar head, which consisted of either three or seven ethylene oxide units. Thus, the surfactants were designated $C_{16}(EO)_3$, $C_{16}(EO)_7$, $C_{18}(EO)_3$ and $C_{18}(EO)_7$ (Servo, Delden, The Netherlands). The purity of the surfactants was assessed by use of a gel chromatography method (THF/antioxidant, flow rate 1 ml min^{-1}, mixed gel, ambient temperature, refractive index detector), and in each case the purity was slightly in excess of 90%. In each case, the major peak had a polydispersity ratio in the range of 1.125–1.226, and each surfactant exhibited two distinct peaks, the smaller one being of slightly lower molecular weight.

Methods

Dispersions of 30 mmol surfactant in phosphate-buffered saline (30 mM phosphate, 15 mM NaCl, pH 7.4) were prepared for each of the four samples. The calorimetric experiments were conducted in a Microcal MC-2 microcalorimeter (Microcal Amherst, MA, U.S.A.), interfaced with an IBM PC 32 personal computer. Each calorimetric run was obtained under excess nitrogen pressure (2 atm). A baseline, obtained by running buffer against buffer was subtracted from the data files before analysis. The HSDSC experiments were

conducted at a scan rate of 60 K h^{-1}, over the temperature range of 5–70°C. The transitions were defined by a calorimetric enthalpy (ΔH_{cal}), which reflects the amount of structure in the melting process; the Van 't Hoff enthalpy (ΔH_{VH}), which reflects the width of the transition; ΔC_p, the heat capacity change for the process, and a melting temperature (T_m), which indicates the stability.

The ΔC_p of an endotherm was determined from the difference in the ΔC_p values between the linearly extrapolated pretransition baseline, and the post-transition baseline. The effect of ΔC_p was removed by drawing a line from the onset to the end-point of the endotherm; the area between the HSDSC curve and this line measures the enthalpy of the total transition.

The ratio between the Van 't Hoff enthalpy and the calorimetric enthalpy gives the cooperative ratio (n). A value for n of below unity suggests the presence of domains and/or intermediate steps in the melting process; a value higher than unity indicates an increased cooperativity of the structure and/or a quantification of aggregation. Equilibrium thermodynamics has been used in the analysis with the sole purpose of defining the width of the transition, i.e., to obtain ΔH_{VH}, and calculate the cooperative ratio (n).

The surfactants were also investigated in the non-hydrated state using differential thermal analysis (Mettler, TM 3000). The surfactants were hermetically sealed into a sample pan (with a sample size of approx. 25 mg), and then heated at a rate of 2.5 K/min.

Results and Discussion

Visual inspection of the surfactants in water revealed that they formed inhomogeneous dispersions. The thermal analysis results would be expected to reveal differences between surfactant molecules that are hydrated to different extents; these aspects will be discussed below.

The phase transitions exhibited by the non-hydrated surfactants are shown in Fig. 1 (DTA results), and those obtained for the dilute aque-
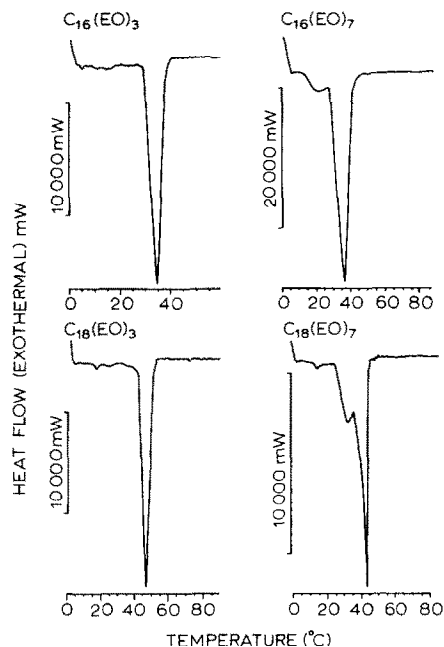

Fig. 1. Plots of exothermal heat flow as a function of temperature obtained for the non-hydrated surfactants by use of DTA.

ous dispersions (using HSDSC) are shown in Figs 2–5.

The DTA results, for the non-hydrated samples, reveal single transitions for the two samples with three oxyethylene units, but two distinct peaks for those samples with seven oxyethylene units. This could imply that the (EO)$_3$ surfactants are comparatively pure, but that the (EO)$_7$ surfactants contain more than one molecular species in sizeable quantities, however, the gel permeation chromatography results demonstrate that each sample has a major component and a much smaller quantity of a lower molecular weight impurity, thus this is not a justifiable conclusion. A further observation from the DTA results for the (EO)$_3$ surfactants (Fig. 1) is that there is a higher baseline before the melting point than is observed above: this was not expected as the ΔC_p of the crystalline state is lower than that of the liquid state. The higher ΔC_p observed at lower temperatures could be explained by the existence of a slow thermal event (such as a phase change) taking place before the melting of the alkyl chains.

Therefore, it is possible that the first major DTA peak that is observed for the $(EO)_7$ surfactants is a phase change between two crystal forms, just prior to the melt. This type of behaviour is often encountered in long-chain alkyl surfactants, e.g., methyl ethers of long-chain alkyl esters (e.g., Van Brommel (1986)). Van Brommel (1986) used an adiabatic calorimeter to demonstrate a very slow transition in long-chain alkyl surfactants (which can take a few days to complete) at a few degrees below the melting point; they further noted that the effects of impurities were difficult to distinguish from crystalline structure modifications, as both resulted in a lowering of the transition temperature or an increase in baseline just prior to the melting point.

The non-hydrated surfactants with C_{18} chains have phase transitions at higher temperatures than those with C_{16} chains (43–47 compared to 34–37°C respectively, for the major peaks) (Fig. 1). The ethylene oxide head only has a minor influence on these peak transition temperatures as for the C_{16} chains the $(EO)_3$ has the slightly lower transition temperature, but for the C_{18} chains the ranking is reversed.

The HSDSC data for the dilute aqueous dispersions all reveal multiple peaks (Figs 2–5). The shape of the diagrams of heat capacity as a function of temperature for the two surfactants with three ethylene oxide units is very similar, i.e., a
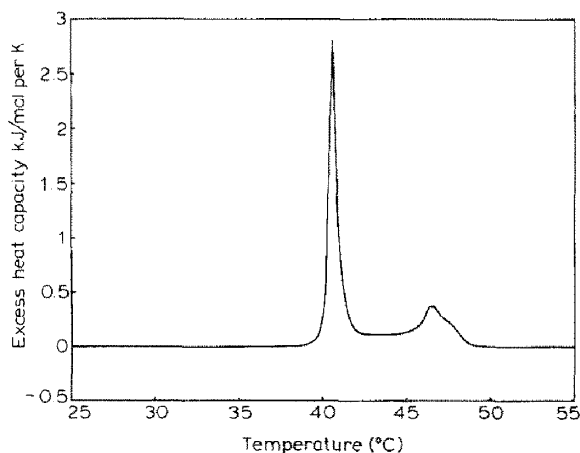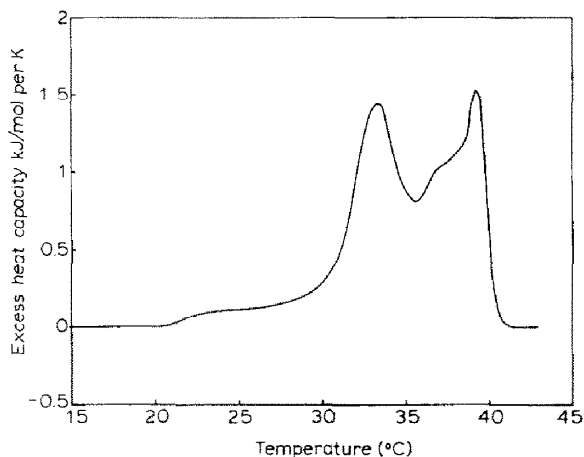
Fig. 3. Excess heat capacity as a function of temperature for a dilute aqueous dispersion of $C_{16}(EO)_7$, obtained by HSDSC.

sharp (near isothermal) peak followed by a region of slightly increased heat capacity and then 4 degrees higher (this temperature difference is the same for both of these surfactants) there is a second (much smaller) peak. This type of transition is similar to, but curiously inverted from, the response seen with 1,2-dipalmitoyl-sn-glycero-3-phosphorylcholine (DPPC) in water (which exhibits a small pre-transition and a subsequent major near-isothermal peak (Chowdhry and Dalziel, 1985)). It is clear that DPPC and these surfactants behave in different manners in water. Further HSDSC investigations of these systems

Fig. 2. Excess heat capacity as a function of temperature for a dilute aqueous dispersion of $C_{16}(EO)_3$, obtained by HSDSC.

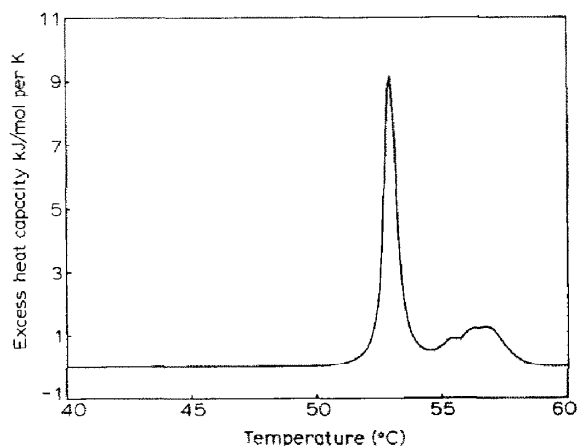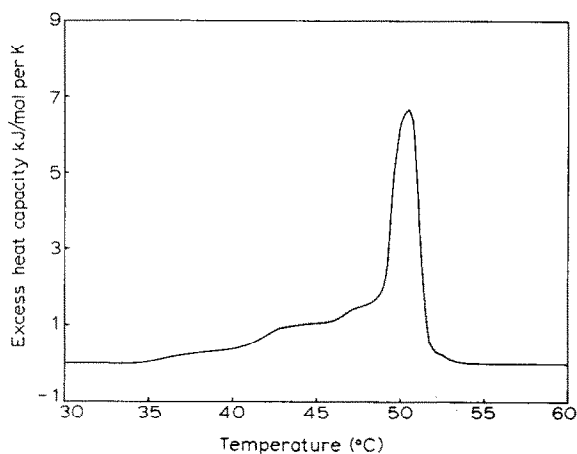Fig. 4. Excess heat capacity as a function of temperature for a dilute aqueous dispersion of $C_{18}(EO)_3$, obtained by HSDSC.

Fig. 5. Excess heat capacity as a function of temperature for a dilute aqueous dispersion of $C_{18}(EO)_7$, obtained by HSDSC.

that are exhibited for the $(EO)_3$ surfactants have been obtained from computer deconvolution of Figs 2 and 4, and the data are presented in Table 1.

The HSDSC data for the $(EO)_7$ surfactants exhibit extremely complicated diagrams of heat capacity as a function of temperature, and in each case there are at least four peaks (Figs 3 and 5). Although it is notionally possible to deconvolute all four peaks, at present only the main peak and the total area under the curve have been studied, which has allowed the calculation of a limited amount of thermodynamic data; this is presented in Table 1. As the level of purity of the $(EO)_7$ surfactants is not significantly different from that of the $(EO)_3$ surfactants, it is unlikely that the multiple peaks observed for the $(EO)_7$ systems are due to impurities.

If the DTA results (Fig. 1) are compared with those obtained by HSDSC (Figs 2–5), a number of interesting differences emerge. Firstly, in most cases the transitions are displaced by a significant amount, to higher temperatures following hydration (i.e., for each surfactant, the HSDSC transitions are mostly at higher temperatures than the equivalent DTA transition). Thus, in dilute aque-

may allow the development of explanations for the differences in behaviour. To this end, characterisation of these transitions, and more importantly their physical significance, will form the basis of further work; it is possible, for example, that the sharp peak is the melt for the alkane and the second peak is related to the poly(oxyethylene). The thermodynamic data for the two peaks

TABLE 1

Thermodynamic parameters for the aqueous dispersions of surfactants scanned at 1 K min^{-1} by HSDSC

Sample	T_m (°C)	ΔH_{cal} (kJ/mol)	ΔH_{VH} (kJ/mol)	n	$\Delta C_p(max)$ (kJ/mol per K)	$\Delta C_p^d(inc)$ (kJ/mol per K)	ΔS_{cal} (J/mol per K)
$C_{16}(EO)_3$							
Overall	40.6	3.88					12.36
Peak 1	40.6	2.16	4660	2157	3.08	0.15	6.88
Peak 2	44.6	0.73	1688	2312	0.02	−0.15	2.30
$C_{16}(EO)_7$							
Overall	39.2	16.08					
Peak 1	33.3						
Peak 2	39.2						
$C_{18}(EO)_3$							
Overall	52.9	12.15					37.26
Peak 1	52.9	7.22	5065	702	10.36	0.61	22.15
Peak 2	56.7	2.16	1827	850	1.1	−0.62	6.55
$C_{18}(EO)_7$							
Overall	49.0	27.89					86.56

Data obtained from computer deconvolution, where possible. The complex nature of the $(EO)_7$ multiple peaks prevents routine deconvolution.

ous dispersion, the phase transition step is hindered to a greater degree than in the non-hydrated state. Secondly, the hydrated samples exhibit multiple transitions, even for surfactants which had single transitions in the non-hydrated state. A lack of purity is not necessarily implicated in these results, as (particularly for the $(EO)_3$ surfactants) the major HSDSC peaks are nearly isothermal, consequently, it is more likely that the multiple peaks are indicative of molecular species with different levels of hydration in an inhomogeneous dispersion. The $C_{16}(EO)_7$ shows multiple non-isothermal transitions, with a level of complexity that reflects a heterogenous dispersion of a heterogenous sample. Thirdly, the overall shape of the HSDSC transitions indicates that the ethylene oxide (comparatively hydrophilic) region controls the type of transitions, but the alkyl chain (hydrophobic) controls the temperature range at which these occur (as with the DTA results, the C_{18} transitions are at a higher temperature than those for the C_{16} chains). The shapes of the HSDSC traces are characteristic of the hydrophilic heads; the $(EO)_3$ surfactants exhibit an initial near-isothermal transition (which is the major peak), with multiple small peaks at higher temperatures over a range of some 7–8°C The $(EO)_7$ surfactants, however, both have multiple small peaks over a temperature range of some 20°C below the major (more isothermal) transition.

The overall calorimetric enthalpy changes (Table 1) are lower for the $(EO)_3$ surfactants than for the $(EO)_7$, indicating the preferred hydration of the lower molecular weight species: equally the C_{16} species have a lower overall calorimetric enthalpy change than the corresponding C_{18} surfactants. By calculating the differences between the calorimetric enthalpies (Table 1) for the overall transition (i.e., from the area under the curve), then from the difference between the $(EO)_3$ and the $(EO)_7$ data the contribution to the enthalpy of transition due to a single ethylene oxide unit can be estimated (i.e., the difference between $C_{16}(EO)_3$ and $C_{16}(EO)_7$ is approx. 12 kJ mol^{-1}, and the difference between $C_{18}(EO)_3$ and $C_{18}(EO)_7$ is approx. 16 kJ mol^{-1}, thus an ethylene oxide unit contributes approx. 3.5 kJ mol^{-1}).

By similar arithmetic, the contribution of a methylene unit is approx. 5 kJ mol^{-1}. These figures are reasonable, as the lower molecular weight, but more hydrophobic methylene unit would be expected to have a higher enthalpy contribution. There is a cause for concern over interpreting the thermodynamic data, having commented that the surfactant dispersions are inhomogeneous. A reasonable test for the validity of the enthalpy values is to reflect on the internal consistency of the data, and as the contribution per methylene group is similar for the $(EO)_3$ and the $(EO)_7$ surfactants, and the contribution per ethylene oxide group is similar for the C_{16} and C_{18} surfactants it is reasonable to assert internal consistency. Thus, despite the inhomogeneous nature of the dispersions and multiple transitions observed, the data appear to be reliable. These calculations assume that there is independence in the contributions of the ethylene oxide and the alkyl group to the overall enthalpy change. We acknowledge that this may not be true; further members of the series will be investigated to confirm or refute these observations.

Assuming the contribution to the enthalpy of transition for the methylene and ethylene oxide groups to be accurate, then the possibility of being able to predict the transition of other members of this series becomes interesting. If one estimates the probable response for lower members of the series, considering changes in the ethylene oxide content, a reduction to $(EO)_2$ would approach thermoneutrality, whilst a reduction to $(EO)_1$ would be predicted to elicit an exothermic transition. It is unlikely that an exotherm would occur, suggesting that $(EO)_1$ would have a distinctly different transition (and/or conformation) to the other members of the series (this is not unexpected, as the non-polymeric head group may well behave in a different manner to others in the series). Considering the alkyl chain, chains of C_{12} and less would be predicted to be exothermic in transition, suggesting that a change in conformation can be expected at this chain length. Further experimental work is in progress to investigate these aspects, however, this preliminary study is encouraging and indicates the potential for application

of HSDSC to studies of dilute macromolecular systems of pharmaceutical interest.

The HSDSC results can also be used to quantify the polymer/water interaction. The cooperativity (n) values (Table 1) for the $(EO)_3$ surfactants indicate extensive aggregation: essentially the numerical value of n is directly related to the number of molecules involved, thus the aggregates of the $C_{16}(EO)_3$ surfactant contain more molecules than those of the $C_{18}(EO)_7$. Difficulties in deconvolution of the $(EO)_3$ traces prevent such comparisons for these surfactants. This technique is, therefore, of value in investigating micellar structures.

Conclusions

Thermal analysis does not, in its own right, provide conclusive proof of, or identification of, any particular process. However, the collated data above allow the formulation of a hypothesis concerning the transitions that occur in these surfactants. The surfactants exhibit a phase transition at a temperature that is determined (apparently almost exclusively) by the alkyl chain length. When the polymers are hydrated, then the transition is inhibited (i.e., the transitions from HSDSC are at higher temperatures than those from DTA), but still occurs at a temperature that is controlled by the alkyl chain length (the major near-isothermal HSDSC peaks being at approx. 40 and 50°C for C_{16} and C_{18} chains, respectively). Even the surfactants which exhibit single transitions when non-hydrated have multiple peaks when hydrated; as purity is not likely to be responsible for this behaviour, it is probable that it is related to the degree of interaction with the water. The shape of the HSDSC thermograms suggests that the interaction with water varies in a manner that is dependent largely upon the length of the oxyethylene region, this is supported by the thermodynamic data.

The thermodynamic data calculated for the transitions are internally consistent and open up the possibility of modelling/predicting the behaviour of dilute macromolecular dispersions. The inhomogeneity of the dispersions resulted in multiple phase transitions, but did not appear to adversely affect the calculated thermodynamic functions.

When considering the behaviour of samples in dilute solution and/or dispersions, it is likely that HSDSC will provide more information and be more reliable than conventional DSC and DTA data. We are only aware of a few other examples (e.g., Mitchard et al., 1990; Schid and Tirrell, 1990) in which HSDSC has been used to investigate such dilute systems of synthetic polymers. The existence of a practicable, direct, accurate method by which thermodynamic transitions can be calculated for such polymers, in dilute solution or dispersion, provides numerous and exciting opportunities for fundamental investigations relating to the behaviour of such molecules when employed as drug delivery systems.

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