

The use of high sensitivity differential scanning calorimetry to characterise dilute aqueous dispersions of surfactants: 2. Further studies on polyoxyethylene alkyl ethers

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Abstract

Polyoxyethylene alkyl ether surfactants have been investigated by use of differential scanning calorimetry (DSC) in the solid state, and high-sensitivity DSC (HSDSC) for dispersions in phosphate-buffered saline. The surfactants had either 16 or 18 carbons in their alkyl chain, and either 2, 3, 4 or 7 oxyethylene groups in their polar head groups. Two regions of transition were observed for each surfactant (pre-transition and main transition). The pre-transition was identical in both the solid and dispersed state and was seen to be due to many cooperating molecules. It was argued that the pre-transition was related to a solid-state structure. The main transition was significantly different in the aqueous environment than in the dry state. Relationships existed between the structure of the surfactants (both the length of the hydrocarbon chain and the hydrophilic head group) and the nature of the transitions. For the main transition the structure of the dispersion was stabilised as the nature of the surfactant became more hydrophobic. There are clear analogies between the nature of these surfactants and the behaviour of lipid bilayers (e.g., both systems have two transition regions). The fact that the behaviour is similar may well allow the toxicity of these surfactants to be understood, if they form cooperative structures between the surfactant and the lipids.

Key words: Surfactant; Calorimetry; Pre-transition

1. Introduction

In a recent publication (Buckton et al., 1992) we introduced the possibility of the application of high sensitivity differential scanning calorimetry (HSDSC) studies in characterising the properties

of dilute aqueous dispersions of surfactants. It is acknowledged that changes in conformation and hydration of surfactants will affect the properties of the drug delivery systems in which they are incorporated. We have also reported (Hofland et al., 1992) that changes in the physical properties of surfactants affect their toxicity, and on conformational changes in dilute solutions of block copolymer surfactants (e.g., Mitchard et al., 1990, 1992).

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The use of conventional DSC is commonplace in pharmaceutical research, however, the sensitivity is such that it is usual to study solid materials, or solutions of high concentration. HSDSC has the advantage of significantly greater sensitivity, but requires a liquid sample of about 2 ml volume. There are obvious advantages in being able to investigate systems in concentrations which relate to those that are commonly used, as molecules such as surfactants will undoubtedly have altered physical properties as a function of concentration. To this end, it is advantageous to be able to study dilute solutions or dispersions of surfactants, rather than to have to consider concentrated systems or solid-state properties.

Our previous publication (Buckton et al., 1992) constituted a preliminary study of synthetic surfactants in dilute dispersion. The polyoxyethylene alkyl ethers were investigated, and studies were limited to alkyl chains of 16 or 18 methylene units, and hydrophilic head groups of either three or seven oxyethylene units. These surfactants were designated as $C_{16}EO_3$, $C_{16}EO_7$, $C_{18}EO_3$ and $C_{18}EO_7$. In the current study, other surfactants of this general type are considered, and are described using similar nomenclature. The aim here is to consider the effect of method of preparation of the dispersion, and to compare the current data with the previously published work, in order to attempt to draw a clearer understanding of the derived thermodynamic parameters.

2. Materials and methods

2.1. Materials

Three surfactants were studied; these were polyoxyethylene alkyl ethers, and consist of an alkyl chain of a certain carbon chain length, joined to the hydrophilic head group of a certain number of oxyethylene units, by an ether linkage. The nomenclature has been simplified to C_aEO_b , where a is the number of carbons in the alkyl chain, and b the number of ethylene oxide units. The three surfactants examined were $C_{16}EO_2$, $C_{18}EO_2$ and $C_{18}EO_4$. The surfactants from Nikkol were a gift from Chesham Chemicals. All

the surfactants were investigated by gel permeation chromatography, and were found to have single peaks, with relatively narrow molecular weight dispersions.

2.2. Methods

Two methods of dispersion were considered; the first involved heating the sample to 80°C, followed by continuous vigorous shaking during cooling (method 1). The second method was the same as the first, except that samples were placed in an ultrasonic bath for 1 h. The bath was cooled to prevent the temperature exceeding 30°C (method 2). Dispersions were prepared of either 3 mg ml⁻¹ (for the 60 K h⁻¹, main transition investigations, using method 2) or 15 mg ml⁻¹ (all other runs) surfactant in 30 mM phosphate buffered saline (pH 7.2). A fixed mass concentration was used, rather than a molar concentration, to produce liquid systems of similar physical properties. Fixed molar concentrations can yield drastically different systems when considering materials with significantly different molecular weights.

The high sensitivity differential scanning calorimetry (HSDSC) experiments were conducted using 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 nitrogen pressure (2 atm). A baseline obtained by running PBS against PBS was subtracted from the data files before analysis. The HSDSC experiments were conducted at a scan rate of 1 K min⁻¹, unless otherwise stated in the discussion. The transitions were defined by:

- (1) a calorimetric enthalpy (ΔH_{cal}). This reflects the amount of structure in the process, as it is linked to the entropy term. Due to the design of the experiment, no reference is made to the molar dependency of the calorimetric enthalpy;
- (2) the van't Hoff enthalpy (ΔH_{vH}) which reflects the shape of the transition;
- (3) the maximum heat capacity change for the process (ΔC_p^{max}); and
- (4) a melting temperature (T_m) which indicates the stability of the system.

The cooperativity ratio (n) was also calculated ($\Delta H_{\text{VH}}/\Delta H_{\text{cal}}$), which can be taken to reflect a quantification of aggregation, in that it reflects the domains participating in the process. Whilst these domains may reflect the number of molecules involved, it is also possible that there can be more than one participating domain per molecule. These terms were calculated as defined previously (Buckton et al., 1992).

Solid-state surfactants (approx. 13 mg, accurately weighed) were also investigated using a DSC7 (Perkin Elmer), scanning at 2.5 K min^{-1} , in the range $0\text{--}60^\circ\text{C}$.

3. Results and discussion

3.1. Method of dispersion preparation

The HSDSC scan for C_{18}EO_4 is presented in Fig. 1, for the sample prepared using method 1. A reasonably sharp single transition is observed, however, if the sample is re-run after cooling it is seen to be significantly diminished in size. This is because phase separation occurs on heating, and the surfactant is only re-dispersed if it is agitated vigorously on cooling. Samples prepared using method 1 did not give reproducible dispersions. This fact was demonstrated by the observation that scans on different samples prepared by method 1 showed considerable variability.

The second method of preparation (utilising sonication) resulted in the production of more reproducible samples. For sonicated C_{18}EO_4 , not only was a main transition observed (T_m 323.6, Fig. 2), but also a distinct pre-transition occurred (T_m 283.6 K, Fig. 3). The pre-transition also occurs on the downscan. The differences between the upscan and downscan are seen by considering the data in Table 1, where ΔH_{cal} is smaller, ΔH_{VH} is larger, and correspondingly, n is larger for the downscans.

3.2. Pre-transitions

As discussed above, a pre-transition is observed for C_{18}EO_4 almost exactly 40 K below the main transition. From the data in Table 1, it can

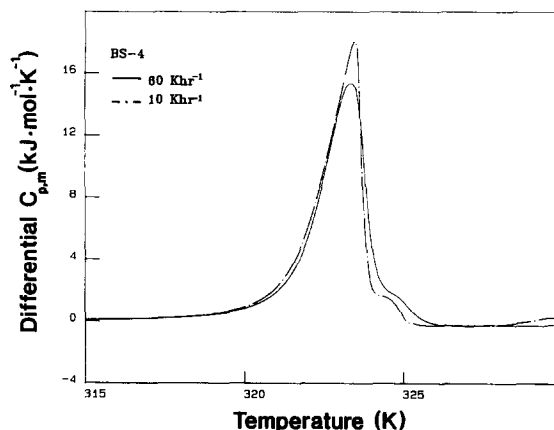


Fig. 1. Repeated HSDSC upscans of the sample of C_{18}EO_4 in PBS, at pH 7.2, 15 mg ml^{-1} at a scan speed of 1 K min^{-1} .

be concluded that large numbers of molecules are cooperating in the thermal event that causes the pre-transition (very high values for n). The value for n represents the number of cooperating domains, and not simply the number of molecules involved in the process. It is possible that there are significantly more than one cooperating domain on each molecule of the surfactants, however, the n values are so large that they do indicate many molecules are involved. Such a large number of cooperating molecules must imply that the pre-transition relates to a large struc-

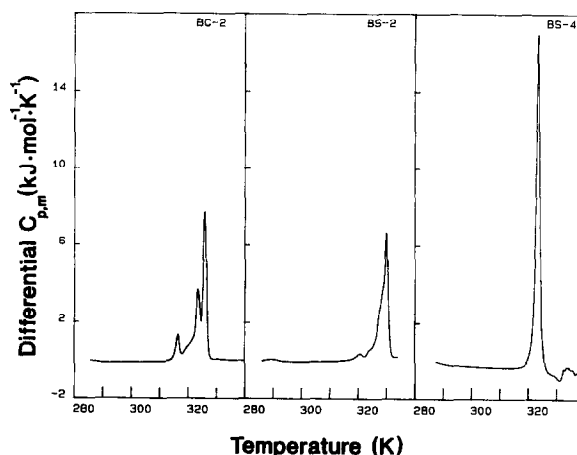


Fig. 2. HSDSC data for the main transitions of the three different surfactants studied as 3 mg ml^{-1} dispersions in water. (BC-2) C_{16}EO_2 ; (BS-2) C_{18}EO_2 ; (BS-4) C_{18}EO_4 .

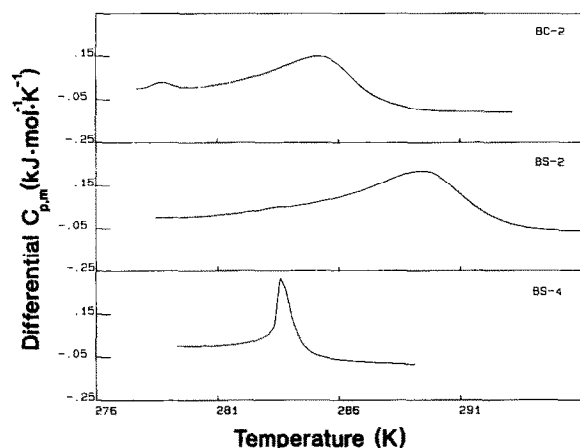


Fig. 3. HSDSC data for the pre-transitions of the three different surfactants studied as dispersions in water (notation as in Fig. 2).

ture which could be either a very large micelle, or a crystalline or a liquid crystalline region. It is interesting to note that the value of n for the downscan of the pre-transition (Table 1) is approximately twice that of the upscan, indicating a further aggregation, which may relate to a joining of two of the original structures.

The data obtained for solid-state thermal analysis also show a pre-transition for each of the surfactants examined (Table 2). The T_m values for the pre-transitions are comparable for the aqueous dispersions prepared by method 2, and the solid-state investigations, for each surfactant; this indicates that the pre-transitions may relate to a solid-state property which endures in the liquid dispersions. Such a solid-state transition is totally in keeping with the high n values (large numbers of cooperating molecules). Mitchell et

Table 1

Thermodynamic parameters for the HSDSC scans of $C_{18}EO_4$, prepared either manually, or by sonication, and after centrifugation to reduce solid matter present

	T_m (K)	ΔH_{cal} (kJ mol ⁻¹)	ΔH_{VH} (kJ mol ⁻¹)	n	ΔC_p^{max} (kJ mol ⁻¹ K ⁻¹)	ΔS (J mol ⁻¹ K ⁻¹)
Manually prepared ^a	323.50	18.60	1 927	104	10.33	57.3
Ultrasound ^a	323.60	29.8	2 016	68	17.23	92.0
Pre-transition ^a	283.62	0.29	3 025	10 370	0.33	1.0
Pre-trans. upscan ^b	283.67	0.27	8 367	31 162	0.84	1.0
Pre-trans. down ^b	283.82	-0.21	13 992	65 599	-1.09	-0.7
Main upscan ^b	323.47	29.3	2 145	73	18.08	90.8
Main downscan ^b	323.52	-3.1	2 959	951	-2.65	-9.6

^a Transitions measured at 60 K h⁻¹.

^b Upscan and downscan experiments measured at 10 K h⁻¹. All samples prepared by sonication, unless otherwise indicated.

Table 2

Solid-state DSC T_m values for the pre-transitions and the main transitions

	T_m pre-trans. (K)	T_m main trans. (K)	Comments
$C_{16}EO_2$	287.0	308.1	main transition single peak on first upscan, multiple peaks on downscan and second upscan
$C_{18}EO_2$	290.8	315.6	main transition single peak on first upscan, multiple peaks on downscan and second upscan
$C_{18}EO_4$	283.3	310.9	reproducible single peak for main transition (first upscan, downscan and second upscan)

NB: thermodynamics are not reported, but the pre-transition values are very similar to the HSDSC data, e.g., $C_{18}EO_4 = 0.8$ kJ mol⁻¹, compared to 0.7 by HSDSC, whilst all enthalpies for the main transition are much larger in the solid state, e.g., 36.3 kJ mol⁻¹ compared to 18.6 for $C_{18}EO_4$.

al. (1983) have reported phase diagrams for other members of the oxyethylene alkyl ethers, although at much higher surfactant concentrations in water than reported here. They describe numerous phase transitions, including the possibility of a transition from water plus solid, to water plus a lamellar phase and then to water plus micellar phases (with increase in temperature). It is most likely that at temperatures below the pre-transition, the surfactants exist as a mixture of solid surfactant and water, which will contain free or aggregated surfactant. The pre-transition may well mark the transformation of the solid material into a gel like phase.

The HSDSC data for the pre-transitions of the surfactants in the series ($C_{18}EO_4$, $C_{16}EO_2$ and $C_{18}EO_2$) are presented in Table 3 and Fig. 3. For both of the surfactants not already discussed ($C_{16}EO_2$ and $C_{18}EO_2$) the pre-transition existed, and in each case was almost exactly 40 K below the main transition (which was taken as the major peak, peak 5, for $C_{16}EO_2$).

The surfactants which were examined in our previous study (Buckton et al., 1992) have been reinvestigated to probe the low temperature pre-transition, which in combination with the HSDSC data reported here allow investigations of how structure affects the pre-transition. These data are also presented in Table 3.

The contribution of a methylene group in the alkyl chain of the surfactant (to T_m of the pre-transition) can be estimated from the data in Tables 2 and 3, using the difference between the C_{16} and C_{18} data. In the solid state, the T_m

increases by 1.9 K per methylene group (EO_2 data, Table 2). In the dispersed state T_m increases by 2 K and 1.5 K per methylene group for the EO_2 and EO_3 surfactants, respectively. The contribution of the ethylene oxide groups to T_m in the dispersed state is opposite to that of the effect of increasing the alkyl chain length. This indicates that the ethylene oxide groups have the effect of causing the surfactant structures to be less 'stable', i.e., more associated with the bulk water. From Table 2, the contribution per ethylene oxide group is 3.7 K, which is similar to the contributions seen in the dispersed state, which range from 2.3 to 3.2 K per group, with the exception of the $C_{18}EO_7$ data which seem to have an idiosyncratically high T_m , when compared with the other data in Table 3. These consistent effects in the solid state and in the dispersions support the discussion above, relating to a solid-state structure enduring after dispersion. A significant difference between the solid-state and dispersion data is that the dispersions showed reproducibility on repeat upscans, but this was not the case for the solid state, where the pre-transitions generally were shallower, and had a peak at a higher T_m on the second upscan.

The calculated volumes of the head groups and the alkyl chains (using GEPOL 87, see reference list) where the surface is as defined by Richards (1977) are presented in Table 4.

Plots of C_p^{\max} , and the ΔH_{VH} as a function of C/EO ratio, C/EO volume ratio and the head group volume resulted in the relationships between the C_{18} and C_{16} surfactants being uncorre-

Table 3

The thermodynamic parameters describing the pre-transition obtained from HSDSC scans on 15 mg ml⁻¹ dispersions of each of the surfactants, all scanned at 60 K h⁻¹, after preparation with ultrasonic treatment before scanning

C	EO	T_m (K)	ΔH_{cal} (kJ mol ⁻¹)	ΔH_{VH} (kJ mol ⁻¹)	n	ΔC_p^{\max} (kJ mol ⁻¹ K ⁻¹)	ΔS (J mol ⁻¹ K ⁻¹)
16	2	285.44	0.76	700	929	0.20	2.6
16	3	283.17	0.24	611	2567	0.06	0.3
18	2	289.39	1.45	487	335	0.25	5.0
18	3	286.22	0.69	2350	3430	0.59	2.4
18	4	283.62	0.70	2199	3160	0.58	2.5
18	7	281.52	0.55	2829	5188	0.59	2.0

Table 4

Calculated C/EO ratios, and molecular volumes (from GEPOL 87)

C	EO	C/EO ratio	Molecular volume C (\AA^3)	Molecular volume EO (\AA^3)	C/EO volume ratio
16	2	8.0	413	141	2.9
16	3	5.3	413	2	2.0
18	2	9.0	463	141	3.3
18	3	6.0	463	204	2.3
18	4	4.5	463	267	1.7
18	7	2.6	463	454	1.0

lated (plots not shown); however, plots of n , T_m (as discussed above), and ΔH_{cal} all produced reasonable correlations (the volume ratio plots are shown in Fig. 4a–c). From Fig. 4a–c, it can be seen that the thermodynamic properties are related to the molecular volume of the EO head group in a non-linear manner. When the ratio of the chain to head groups is considered (either by simple proportion, or by molecular volume), the relationships are linearised for T_m . The linear relationship between the C/EO ratios and n is surprising, as it is derived from non-linear relationships between C/EO and the ΔH_{cal} and H_{VH} .

3.3. The main transition

Comparison of the HSDSC data with the solid-state results reveals that in each case the main transition is significantly lower for the solid material. The transition in the solid state undoubtedly reflects the melting of the remaining crystalline structure of the surfactant. The transition in the dispersed system (Fig. 2) demonstrates that the water has interacted with, and stabilised, the surfactant structures (higher T_m). In line with the phase diagrams of Mitchell et al. (1983) for related surfactants, it can be postulated that the major HSDSC transition reflects a move from a

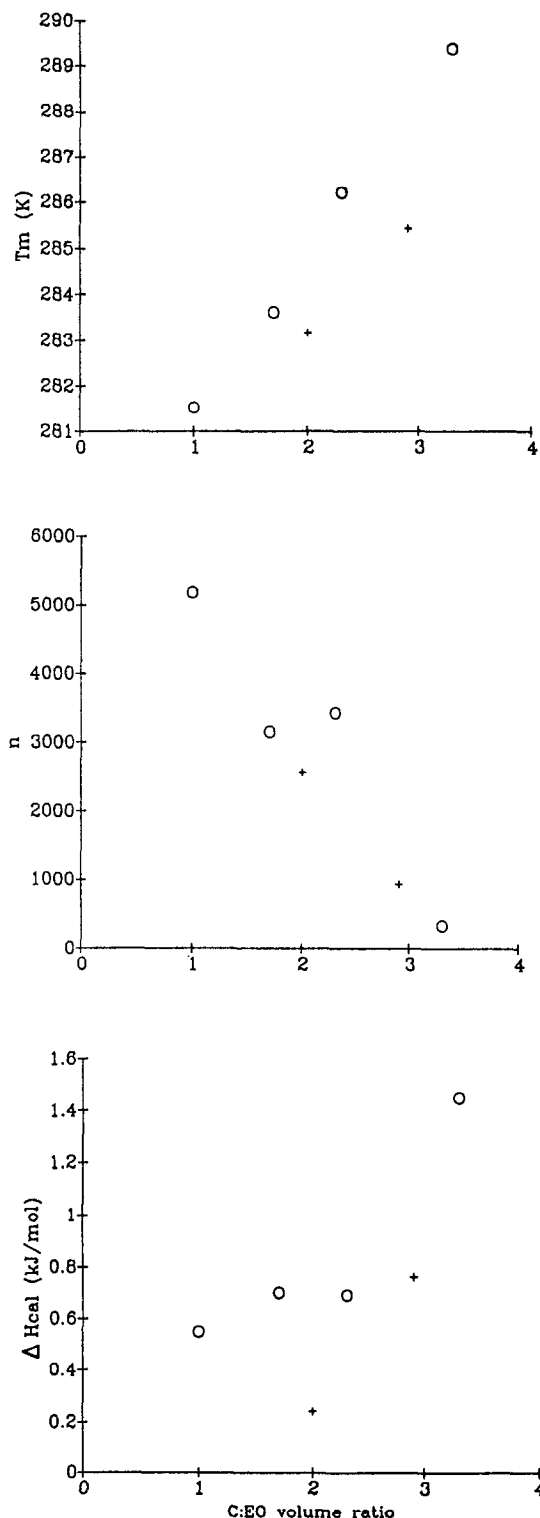


Fig. 4. Relationship between the C/EO volume ratio and (a) the T_m , (b) n and (c) ΔH_{cal} , for the pre-transitions for the dispersions ((○) C₁₈; (+) C₁₆ surfactants).

gel phase to micellar structures. It is interesting to note that the HSDSC main transitions were consistently 40 K above the pre-transitions, whilst for the solid state the difference was much more variable, 16–28 K, again indicating the influence of water in the dispersion system.

3.4. Relationships within the surfactant series for the main transition

By considering the data published previously, in combination with those data presented here (Table 5), it is possible to re-examine the relationships between structure and properties within the polyoxyethylene alkyl ether series.

It was noted before (Buckton et al., 1992) that there appeared to be a contribution to the enthalpy of the main transition which could possibly be ascribed to the polyoxyethylene head group, and another which could be related to the methylene groups on the alkyl chain. As has been noted above, the enthalpy term will indeed be related to the length of the polyoxyethylene head, and to

the alkyl chain, but it is also influenced by the method of preparation. However, the complexity of multiple peaks makes meaningful analysis of the enthalpy changes rather difficult, notwithstanding this, it is unlikely that such a simple relationship exists.

A characteristic which does allow comparison of the results of the previous study with those of the current work is the T_m . There is, of course, still some uncertainty about the results obtained for this parameter, as multiple transitions are observed for many of the surfactants. In each case we have allocated T_m to the major peak. There is a clear relationship between T_m and the number of ethylene oxide groups in the surfactant (Fig. 5). Equally, the alkyl chain length is seen to exert an effect, with two distinct curves being formed (C_{16} surfactants having consistently lower T_m values than C_{18}).

The T_m value is related to the stability of the dispersion, with higher stability being observed with a lowering of the number of polyoxyethylene groups, and with an increasing number of methy-

Table 5
Summary of previously published data (taken from Buckton et al., 1992), and current HSDSC studies of the main transitions

		T_m (K)	ΔH_{cal} (kJ mol ⁻¹)	ΔH_{VH} (kJ mol ⁻¹)	n	ΔC_p^{max} (kJ mol ⁻¹ K ⁻¹)	ΔS (J mol ⁻¹ K ⁻¹)
$C_{16}EO_2$	peak 1	316.5	2.9	2324	801		
	peak 2	321.6	5.0	715	143		
	peak 3	323.3	3.8	3005	791		
	peak 4	324.4	5.4	2216	410		
	peak 5	325.8	11.3	2822	250		
$C_{16}O_3$	overall	313.6	3.88				12.4
	peak 1	313.6	2.16	4660	2157	3.08	6.9
	peak 2	317.6	0.73	1688	2312	0.02	2.3
$C_{16}EO_7$	overall	312.2	16.08				
	peak 1	306.3					
	peak 2	312.2					
$C_{18}EO_2$		329.7	24.5	1363	56	9.23	74.1
$C_{18}EO_3$	overall	325.9	12.15				37.3
	peak 1	325.9	7.22	5065	702	10.36	22.2
	peak 2	329.7	2.16	1827	850	1.1	6.6
$C_{18}EO_4$		323.5	18.6	1927	104	10.33	57.3
$C_{18}EO_7$	overall	322.0	27.89				86.56

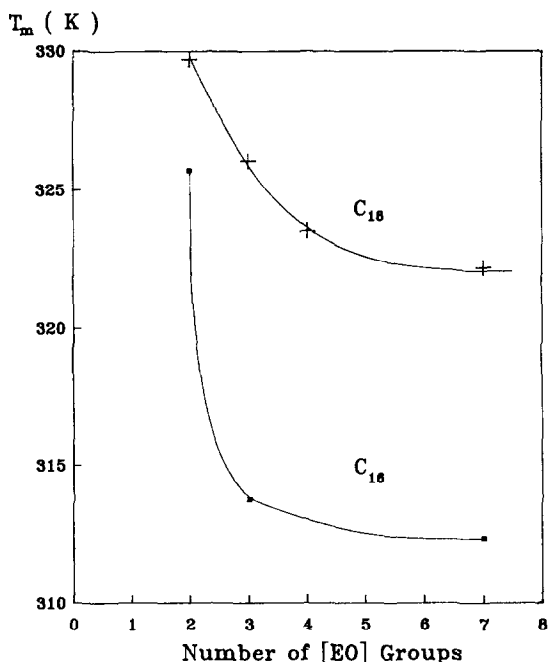


Fig. 5. Relationship between T_m (K) main transition of the dispersions and the number of ethylene oxide groups ((+) C_{18} ; (■) C_{16} surfactants).

lene groups in the alkyl chain. This indicates that the surfactant is stabilised as structures which produce a hydrophobic regime; any effect which makes the molecule more hydrophilic will ease molecular dispersion, and thus reduce the stability of the aggregate.

The T_m values for the surfactants in the solid state are not so well correlated with EO proportion of C/EO ratio as are those obtained on the dispersions. However, the broad classification of the T_m values for the solid state does divide the surfactants into two discrete groups, the C_{16} members have values in the range 307–309 K, whilst those of the C_{18} surfactants studied are in the range 315–319 K. These data are much more tightly grouped than those for the dispersions in water, demonstrating that water has a significant effect on the surfactant structures. It is not surprising that there is no ranking within these tight bands in the solid state, because, as noted in Table 2, there is a tendency for the transitions for certain of the surfactants to alter on repeat scans,

indicating that different packing arrangements are possible (thus increasing the variability in T_m slightly).

3.5. Comparison of results obtained with data on lipid bilayers

The physical and chemical nature of lipid bilayers has been thoroughly investigated, and a large body of data exists relating to the bilayer phase transition. It is interesting to compare the data obtained here for synthetic surfactants to those which exist in the literature for the lipid bilayer systems.

Lipid bilayers are known to have a specific temperature below which X-ray diffraction data indicate a molecular spacing of about 4.2 Å and above which the spacings are significantly larger (e.g., Nagle, 1980). This behaviour is very similar to that observed for long-chain alkanes, and has been described as a 'melting', or disordering, of the hydrocarbon tails of the bilayer. For a series of lecithins, T_m increases in a linear manner, when plotted as a function of $(1/(n - 3))$, where n is the number of carbon atoms in the alkyl chain (see Nagle, 1980). As the carbon chain length in the current study only varied between C_{16} and C_{18} , such a plot cannot be considered, but the rise in T_m with increasing alkyl chain length is a comparable feature between these surfactant structure, and the lipid bilayers.

The major difference between the melt of lipid bilayers and high molecular weight alkanes comes as a consequence of the fact that with the lipid molecules, the alkyl chains are anchored at one end to a hydrophilic moiety, which will be in contact with the aqueous phase. The fact that the lipid molecules have two alkyl chains on each polar head is not considered as being too important for the melt, as the two chains on the same backbone can separate by as much as two neighbouring chains on different molecules (Nagle, 1980). It has also been shown (as reviewed by Nagle, 1980) that hydrated multilayer lipid dispersions and single bilayers have identical phase transition properties. It follows that the similarities between the lipids and the synthetic surfactants considered here could be significant.

4. Conclusion

The low sensitivity DSC studies reveal that the surfactants have two transitions, one low enthalpy endotherm at lower temperatures, followed by the main transition. The HSDSC investigation shows that dilute dispersions also have two transitions, the low temperature one being identical to that noted in the solid state (in terms of T_m and enthalpy), thus indicating that this is the same process both in the solid state and in dispersion. The main transitions are, however, totally different when the solid state is compared with the dispersions. These melts relate to different processes, one a transition of an aggregate which incorporates water, and the other a crystalline melt.

The HSDSC data are valuable in demonstrating the changes in behaviour when the surfactants are dispersed in such low concentrations (such that the transitions cannot easily be detected by other means). The relationships between molecular structure and the main transitions are more significant for the dilute dispersions, and are unrelated to the solid-state data, thus HSDSC is a valuable approach to such investigations. From our previous studies (e.g., Hofland et al., 1992) it is probable that the toxicity of these surfactants when used in certain formulations, will correlate with these phase transitions.

References

- Buckton, G., Chowdhry, B.Z., Armstrong, J.K., Leharne, S.A., Bouwstra, J.A. and Hofland, H.E.J., The use of high sensitivity differential scanning calorimetry to characterise dilute aqueous dispersions of surfactants. *Int. J. Pharm.*, 83 (1992) 115–121.
- GEPOL 87 (QCPE Program No. 554), Pascual-Ahuir, J.L., Silla, E., Tomasi, J. and Bonaccorsi, R., Quantum Chemistry Program Exchange, Department of Chemistry, Indiana University.
- Hofland, H.E.J., Bouwstra, J.A., Verhoef, J., Buckton, G., Chowdhry, B.Z., Ponc, M. and Junginger, H.E., Safety aspects of non-ionic surfactant vesicles: a toxicity study related to the physicochemical characteristics of non-ionic surfactants. *J. Pharm. Pharmacol.*, 44 (1992) 287–294.
- Mitchard, N.M., Beezer, A.E., Mitchell, J.C., Armstrong, J.K., Chowdhry, B.Z., Leharne, S. and Buckton, G., Thermodynamic analysis of scanning calorimetric transitions observed for dilute aqueous solutions of ABA block copolymers. *J. Phys. Chem.*, 96 (1992) 9507–9512.
- Mitchard, N., Beezer, A., Rees, N., Mitchell, J., Leharne, S., Chowdhry, B. and Buckton, G., Polyoxyethylene-polyoxypropylene block copolymers: a novel phase transition in aqueous solution of Pluronic F87 (poloxamer 237). *J. Chem. Soc. Chem. Commun.*, (1990) 900–901.
- Mitchell, D.J., Tiddy, G.J.T., Waring, L., Bostock, T. and McDonald, M.P., Phase behaviour of polyoxyethylene surfactants with water. *J. Chem. Soc. Faraday Trans. 1*, 79 (1983) 975–1000.
- Nagle, J.F., Theory of the main lipid bilayer phase transition. *Annu. Rev. Phys. Chem.*, 31 (1980) 157–195.
- Richards, F.M., Areas, volumes, packing and protein structure. *Annu. Rev. Biophys. Bioeng.*, 6 (1977) 151–176.