Gel to Liquid-Crystal Transitions in Synthetic Amphiphile Vesicles

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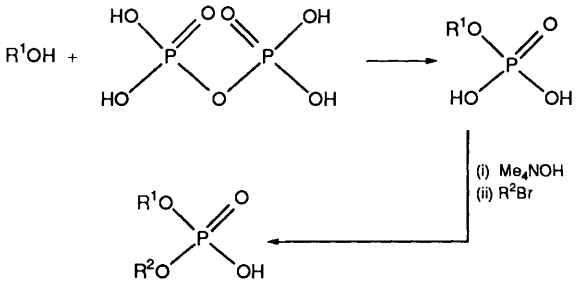
1 Introduction

Throughout Nature the need for compartmentalization is often answered by biomembranes constructed using lipid bilayer structures. The simplest of such structures are closed bilayer vesicles[1] (liposomes) which can be formed in aqueous systems by phospholipid-based molecules. In Nature these bilayers provide the core of cell membranes and involve complex systems often incorporating proteins. Consequently, enormous interest followed the discovery that synthetic dialkyl amphipathic salts (*e.g.* sodium didodecylphosphate) can also form vesicles in aqueous solutions.[2,3] These discoveries presented the opportunity to probe the properties of bilayer systems formed from one chemical substance. A further point of interest emerged from the discovery that these closed bilayer structures can entrap large molecules.[4] This property raises the possibility that these synthetic vesicular systems may form the basis of drug delivery systems.[5,6]

Dialkylphosphates where R^1 and $R^2 \geq C_{12}H_{25}$ form at sufficiently high temperatures spherical vesicles in solution, and the starting monomer can be readily prepared[7] *via* the alkyldihydrogen phosphate in a procedure based on Scheme 1.

The vesicles (*cf.* Schemes 2 and 3) formed from these dialkylphosphates in aqueous solution (see below for comments on preparation) are unilamellar, having diameters of the order 100



Scheme 1

Professor Michael J. Blandamer graduated from the University of Southampton with B.Sc. and Ph.D. degrees in 1961. Following post-doctoral research at NRC in Ottawa (Canada) he joined the staff at the University of Leicester where he was appointed to a Personal Chair in 1990. He is Visiting Professor in the Department of Organic and Molecular Inorganic Chemistry at the University of Groningen, The Netherlands. His research interests concern the thermodynamic and kinetic properties of solutes in aqueous solutions. Otherwise, he is learning to play the piano, the plan being to attain a standard which is likely to be a pale shadow of that demonstrated by the late Thelonious Monk.

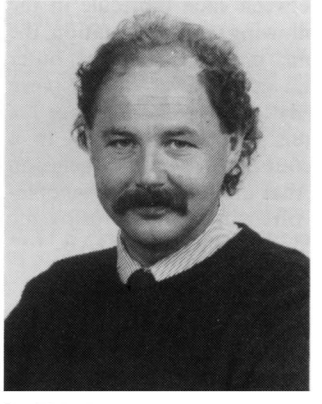
Dr. Barbara Briggs graduated in 1973 from the University of Leicester with a First Class honours degree in Chemistry and then trained as a Junior School teacher. Following reorganization of schools in Leicester, she returned to the University of Leicester to study for the degree of Ph.D. She returned to teaching science to, amongst others, trainee hairdressers at a local college. But Chemistry pulled again and in 1989 she returned to Leicester as a Research Associate in the Department of Chemistry.

Professor Paul M. Cullis graduated from the University of Exeter in 1974 and then studied for the degree of D. Phil. at the University of Oxford. Post-doctoral appointments followed at the University of North Carolina and, again, at the University of Oxford. In 1981 he joined the staff at the University of Leicester being appointed to the Chair of Bioorganic Chemistry in 1990. The latter appointment is not inconsistent with his interest in cooking and good red wine.

Professor Jan B. F. N. Engberts graduated in 1967 with a Ph.D. degree from the University of Groningen. Following a post-doctoral appointment at the University of Amsterdam he returned to the University of Groningen, taking up a full Professorship in 1978. A major part of his research interests currently centre around the synthesis and properties of novel surfactants in aqueous solutions. But these interests do not stop him setting aside every Wednesday evening for riding his horse.


Michael J. Blandamer


Barbara Briggs


Paul M. Cullis


Jan B. F. N. Engberts

nm, although the size depends on the method of preparation and the concentration of added electrolyte. The hydrodynamic diameters[8] of vesicles formed by dioctadecyldimethylammonium bromide and chloride and by sodium dihexadecylphosphate in aqueous solution range from 23 to 300 nm. Vesicles formed by sodium dihexadecylphosphate in aqueous solution[9] have a diameter of 270 nm. Vesicles formed by dioctadecyldimethylammonium bromide contain 48 500 monomer units.[10] An interesting set of vesicles has been prepared[11] from diastereomeric surfactants. In broad terms, synthetic vesicles resemble tennis balls, the bilayer having a thickness of less than 10 nm, aqueous solution being within and surrounding each vesicle. This Review centres on changes in the organization of the alkyl chains in the bilayers following an increase in temperature; Figure 1. At low temperatures the alkyl chains are fully extended, ordered but tilted. At the phase transition temperature, the structure 'melts' to liquid-like arrangements. We illustrate these phenomena by concentrating attention on vesicles formed by dioctadecyldimethylammonium bromide (Scheme 2) and by a series of dialkylphosphates (Scheme 3).

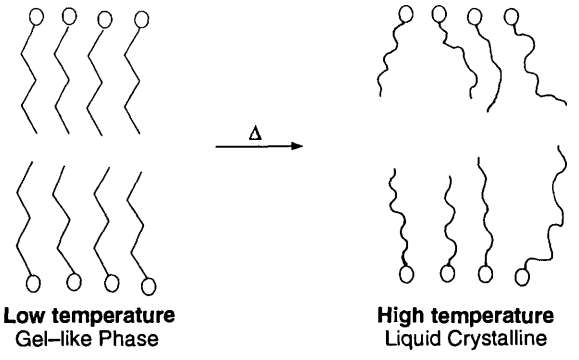
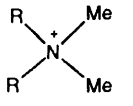


Figure 1 Gel to Liquid-Crystalline Transition.



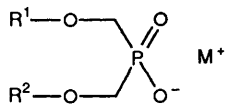
X^-; $R = C_{18}H_{37}$ (i) $X^- = Br^-$ DOAB (ii) $X^- = Cl^-$ DOAC

Scheme 2

A simple calculation[12,13] offers an insight into relevant distances within a solution containing vesicles. For example, the mean hydrodynamic radius of dioctadecyldimethylammonium chloride vesicles[10] is 47 nm, each vesicle containing approximately 5×10^4 monomers. Assuming that vesicles of similar size and composition are formed by DOAB (Scheme 2), then in a solution prepared using (see below) 2×10^{-3} (monomer mol) dm^{-3}, the concentration of vesicles is 4×10^{-8} (vesicle mol) dm^{-3}. Assuming that each DOAB monomer is placed at the centre of a cube, the distance between each molecule in the prepared solution is 9.4 nm. But following vesicle formation, the vesicle–vesicle distance is 346 nm, implying that the outer surfaces of the vesicle are 250 nm apart. Over this distance vesicle–vesicle interactions are likely to be weak and hence the properties of the overall solutions are close to ideal in a thermodynamic sense. This is, of course, a sweeping conclusion when set against the observation that an important phenomenon is vesicle fusion[14] although often induced by fusiogenic agents such as Ca^{2+} for DDP[15–17] and sulfate for DOAB.[18,19]

2 Thermodynamic Background

This Review focuses on the characteristics associated with the gel-to-liquid crystalline transition. The schematic in Figure 1 shows a process characterized by the temperature T_m in which the bilayer remains intact but the ordered alkyl chains gain local



(A) $DDP^- M^+$; $R^1 = R^2 = C_{12}H_{25}$
(i) $M^+ = Na^+$
(ii) $M^+ = K^+$
(iii) $M^+ = Me_4N^+$
$(C_{12}H_{25}O)_2PO_2^-Na^+$ = sodium di-*n*-dodecylphosphate

(B) $DTDP^- M^+$; $R^1 = R^2 = C_{14}H_{29}$
(i) $M^+ = Na^+$
(ii) $M^+ = K^+$
(iii) $M^+ = Me_4N^+$
e.g. $(C_{14}H_{29}O)_2PO_2^-Me_4N^+$ = tetramethylammonium di-*n*-tetradecylphosphate

(C) $DHDP^- M^+$; $R^1 = R^2 = C_{16}H_{33}$
(i) $M^+ = Na^+$
(ii) $M^+ = K^+$
(iii) $M^+ = Me_4N^+$
e.g. $(C_{16}H_{33}O)_2PO_2^-K^+$ = potassium di-*n*-hexadecylphosphate

(D) $DODP^- M^+$; $R^1 = R^2 = C_{18}H_{37}$
(i) $M^+ = Na^+$
(ii) $M^+ = K^+$
e.g. $(C_{18}H_{37}O)_2PO_2^-Na^+$ = sodium di-*n*-octadecylphosphate

Scheme 3

freedom such that long range order is lost. Early studies of this melting were reported in terms of phase diagrams (*cf.* ref. 2) in which T_m was plotted as a function of concentration, the latter extending from 'pure water' to 'pure solute'.

Fortunately, very sensitive differential scanning microcalorimeters are now available which allow measurement of the melting temperature T_m (and associated parameters – see below) for vesicular systems prepared using relatively low concentrations of appropriate monomers; *e.g.* 2×10^{-3} mol dm^{-3} dimethyldioctadecylammonium bromide (DOAB).[12] In our research we use a differential scanning microcalorimeter manufactured by MicroCal Limited (USA). This calorimeter has sufficient sensitivity to allow study of a small sample volume (1.2 cm^3) of dilute aqueous solutions. We comment here on results drawn from a significant body of recently acquired data. A general conclusion that emerges from these studies is that calorimetric methods are able to demonstrate marked differences in thermal behaviour of vesicles formed from a particular surfactant according to the method of vesicle preparation. We have found that preparation of vesicle solutions according to procedures often described in the literature, as will be shown below, gives rise to macromolecular assemblies that show variable thermodynamic properties both between independently prepared vesicular solutions and within a single sample rescanned in our DSC experiments.

Although it is difficult to conclude details of structural information from DSC experiments alone, one is forced to the conclusion that vesicle solutions prepared according to several accepted literature methods are not only heterogeneous in nature but also variable in structure according to the precise conditions used in their preparation. The current sensitivity of our calorimeter has not only revealed this variation of vesicle structure but has also provided the technique for evaluating alternative protocols that can give defined and fully reproducible properties of vesicle solutions.

2.1 Differential Scanning Microcalorimetry

The basis of DSC can be understood in terms of an aqueous solution comprising water and a single solute X. The assumption is made that at low temperatures the solute is in the form X(aq) whereas at high temperature the solute is in the form Y(aq). Consequently, the isobaric heat capacity of such a solution when

plotted against temperature forms a bell-shape if it can be assumed that the isobaric heat capacity of reaction $\Delta_r C_p^\infty(aq)$ [$= C_p^\infty(Y, aq) - C_p^\infty(X, aq)$] is small (zero) Hence, knowing the amount of solute X used to prepare the solution, a plot is obtained of the molar heat capacity of the solute $C_{p\,m}$ as a function of temperature The maximum in this plot corresponds to the temperature where the equilibrium constant is unity, the standard Gibbs energy of reaction $\Delta_r G^\circ$ is zero and equal amounts of X and Y are present in solution The area enclosed by the bell defines the limiting (calorimetric) enthalpy of reaction $\Delta_r H^\infty$(cal) The shape of the bell is described assuming the thermodynamic properties of the solution are ideal, by equation 1

$$C_{p\,m} = [K/(1 + K)^2]\ [\Delta_r H^\infty(\nu H)]^2/RT^2 \qquad (1)$$

By fitting the observed dependence of $C_{p\,m}$ on T to equation 1 an estimate of the van't Hoff enthalpy of reaction $\Delta_r H^\infty(\nu H)$ is obtained In the simple case described $\Delta_r H^\infty$(cal) equals $\Delta_r H^\infty(\nu H)$

The DSC records the differential heat capacity δC_p of an aqueous solution relative to that of the solvent-water For systems such as enzymes[20] and simple surfactants,[21,22] the dependence of δC_p on temperature is compared with scans recorded where both sample cell and reference cell contain water In the cases considered here the signal for transitions in vesicular systems proved so strong that subtraction of a 'water–water baseline' had little impact on the overall pattern Two complications emerge in the context of DSC curves recorded for vesicles in aqueous solutions We consider these in turn

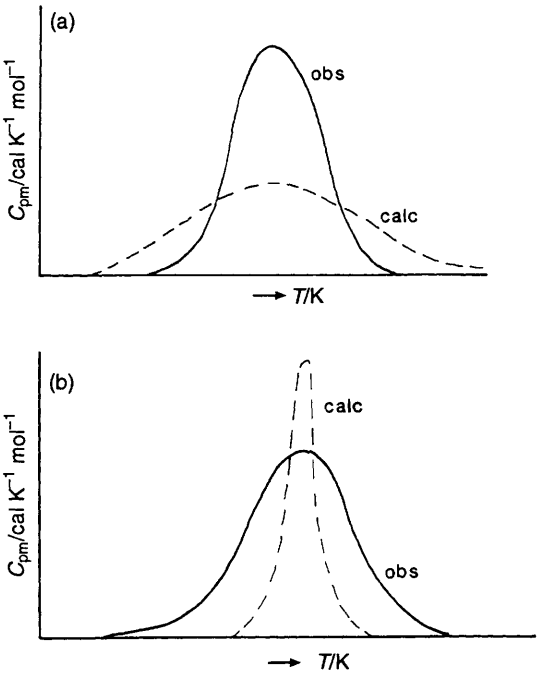


Figure 2 Development of the concept called Patch Number, comparison of the dependence of molar isobaric heat capacity on temperature as observed (——) and calculated (------) using equation 1 where the gel-to-liquid state transition involves (a) a single solute monomer and (b) the total vesicle comprising more than 1000 monomer units

2.2 The Concept of Vesicle Patch Numbers

In most cases, the envelope recorded cannot be accounted for in terms of a simple two-state single equilibrium in the form X(aq) $\rightleftharpoons$ Y(aq) In other words, further complexities have to be considered One possibility is a coupled equilibrium in which three substances are coupled by two equilibria, *e g* X(aq) $\rightleftharpoons$ Y(aq) $\rightleftharpoons$ Z(aq) The resulting equation[23] in $\delta C_{p\,m}$ is slightly more complicated than equation 1 Related schemes involve two independent equilibria involving four states, *e g* X(aq) $\rightleftharpoons$ Y(aq) and W(aq) $\rightleftharpoons$ Z(aq) In fact, the DSC traces for many vesicular systems are satisfactorily accounted for using analyses based on the latter scheme The required curve fitting is conveniently undertaken using ORIGIN software supplied by Micro-Cal Limited

The second problem is slightly more complicated but prompted an important proposal for vesicular systems based on the concept of *patch numbers* To illustrate the point, we imagine that we have recorded a dependence on temperature of an isobaric heat capacity for 1 2 cm^3 of an aqueous solution containing 2×10^{-3} mol dm^{-3} of DOAB(aq) Here the symbol 'mol' refers to the monomer salt, *i e* $R_2N^+Me_2\ Br^-$ Hence by simple proportion we transpose the measured dependence to a dependence of the (monomer) molar isobaric heat capacity on temperature This does not imply that the equilibrium process observed by DSC involves the isolated monomer Indeed, simulation of the DSC trace for a transition involving isolated monomers (Figure 2a) deviates substantially from the experimental data, being very much broader and less intense Similarly, a transition involving the cooperative melting of all of the monomers within a given vesicle (aggregation number for DOAB = 1000) can be modelled Again the fit to the experimental data is poor (Figure 2b) – this time being too sharp and intense Thus an iterative fitting procedure can determine the size of the cooperative unit in an analogous fashion to the well-established methods for determining the oligomeric states of proteins by DSC Analysis of experimental data typically yields co-operative units (*patch number*) involving ~100 to 200 monomers which represents a small fraction of the vesicle surface That vesicles are comprised of a series of well-packed regions rather than one continuous-packed bilayer seems entirely reasonable, and the size of this 'patch' would be expected to depend on the structure of the monomer, the counterion, *etc* , as observed In this way agreement is obtained (see below) between the calculated and observed dependences of molar isobaric heat capacity on temperature Moreover, for many such systems we find that the calorimetric and van't Hoff enthalpies are in agreement, *i e* $\Delta_f H^\infty(\nu H) = \Delta_f H^\infty$(cal) Therefore, the ratio of the molar mass of the aggregate undergoing the transition to the molar mass of monomer yields the patch number This patch number identifies a localized grouping of monomers which undergoes cooperative melting

2.3 Methods of Preparation

In the early stages of this investigation we were surprised by the inability to obtain reproducible DSC plots for an aqueous solution containing, for example, dimethyldioctadecylammonium bromide or sodium di-*n*-dodecylphosphate (DDP) It emerged that widely used methods of solution preparation yielded vesicle solutions with variable thermodynamic properties clearly suggesting heterogeneous structures We show in Figure 3 a comparison of the scans for sodium DDP ($8\ 4 \times 10^{-3}$ mol dm^{-3}) in aqueous solutions prepared in three different ways Curve A (Figure 3) is recorded for a solution prepared using the well-established ethanol-injection method Briefly, the vesicle monomer is dissolved in ethanol and hot aliquots are injected into a buffered aqueous solution kept at a temperature above T_m for the gel-to-liquid transition

Indeed, the solid surfactant dissolves rapidly using this method Nevertheless, the DSC trace differs (Figure 3) from that obtained for vesicle solutions prepared by dissolving sodium DDP directly in hot water (55 Celsius) although both traces show a feature at 35 Celsius, but the solution prepared using simply the hot water method shows a single feature at 35 Celsius When ethanol is subsequently added to this solution the maximum shifts to lower temperature Furthermore, the trace obtained using the hot water method is reversible in that the same pattern emerges through several heat-cool-heat cycles This is not the case for solutions prepared using the ethanol-injection method

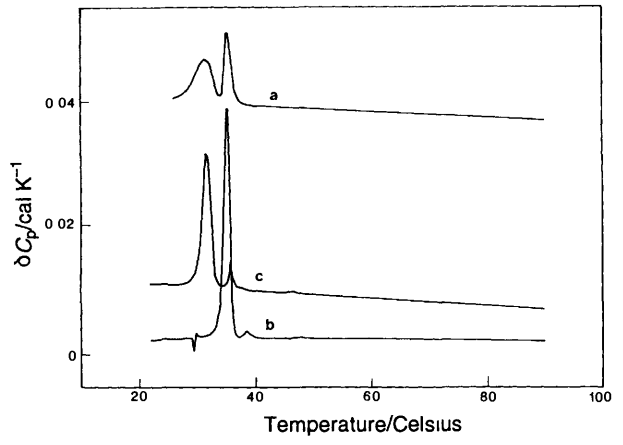


Figure 3 Dependence on temperature of the relative isobaric heat capacity for sodium DDP (8 4 × 10^{-3} mol dm ^{3}), (A) Solution prepared using ethanol injection method (see text), extrema at 31 and 35 Celsius (B) Solution prepared using hot water method (see text), single sharp extremum at 35 Celsius (C) Solution prepared by adding ethanol (mole fraction 0 011) to solution prepared using hot water method All plots have been displaced for clarity on the heat capacity axis

Similar contrasts occur between scans recorded for dimethyldioctadecylammonium bromide (DOAB) vesicles in solutions prepared in three different ways, Figure 4 Moreover, we show in Figure 5 four traces obtained for sodium DDP in freshly prepared solutions The transitions recorded for vesicles prepared using the hot water method are reversible over a number of cycles and confirmed for new freshly prepared solutions The scans shown in Figure 5 confirm the apparent simplicity of the plots when prepared using the simple hot water method [24] We have found that when ethanol was replaced by other organic cosolvents in the injection method, the scans from the DSC were also complex, irreversible, and irreproducible In other words, we express our grave concern over methods which start out by dissolving the dialkyl derivative in an organic solvent Certainly the sensitivity of scanning microcalorimeters is sufficient to signal otherwise unforeseen complexities

2.4 Dimethyldioctadecylammonium Bromide

This salt offers a well-characterized 'onium bromide vesicular[12] system The DSC trace (Figure 4) for DOAB (aq , 2 × 10^{-3} mol

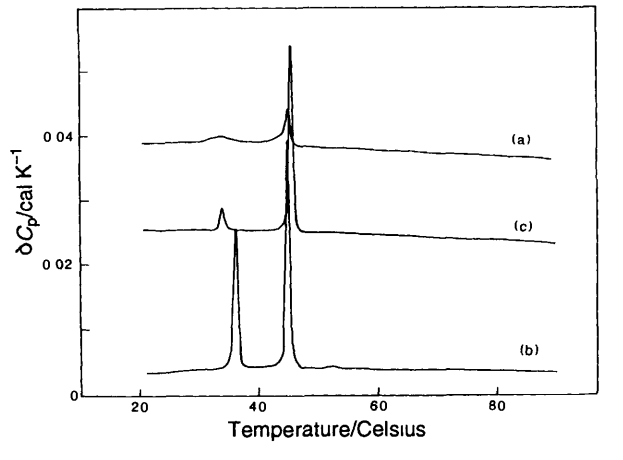


Figure 4 Dependence on temperature of differential isobaric heat capacity for dimethyldioctadecylammonium bromide (2 × 10^{-3} mol dm^{-3}) vesicles prepared using (A) ethanol-injection method, (B) hot water method, and (C) hot water method followed by added ethanol, in plot (B) the main features are at 36 and 45 Celsius The curves have been displaced for clarity on the heat capacity axis

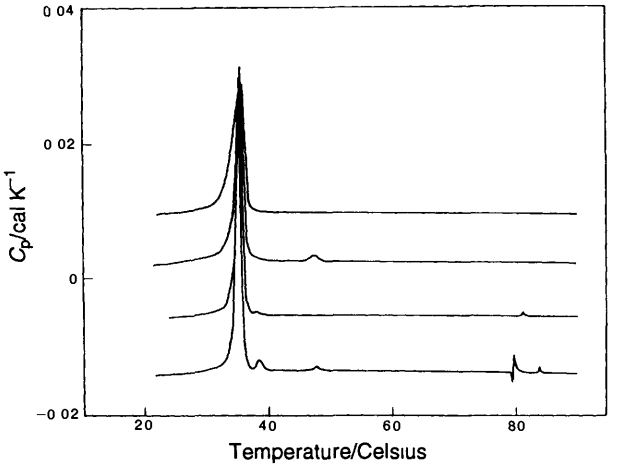


Figure 5 Dependence on temperature of differential isobaric heat capacity for sodium DDP (8 4 × 10^{-3} mol dm ^{3}) for four fresh solutions prepared using the hot water method, the curves have been displaced for clarity on the heat capacity axis The extrema are at 35 Celsius

dm^{-3}) shows an extremum near 45 Celsius and a less intense extremum near 36 Celsius For more dilute solutions (*e g* 1 × 10 ^{4} mol dm^{-3}) only the extremum at 45 Celsius is recorded, the scan being reversible over a period of 15 hours We attribute the extremum to a gel-to-liquid crystal transition in DOAB vesicles which at this monomer concentration are widely separated in solution This conclusion follows from the observation that if the vesicles comprise 1000 monomer units, the concentration of vesicles is of the order 10^{-8} (mol vesicle) dm^{-3} At such low concentrations, the vesicles are separated by domains comprising solely water Analysis of the scan pattern using the concept of patch number reveals that for DOAB the patch number is approximately 130, the enthalpy change accompanying the melting being approximately 8 kJ (monomer mol)$^{-1}$ The good agreement between calculated and observed dependences of isobaric heat capacities (Figure 6) is reassuring

The concept of the patch number is intuitively attractive It overcomes the difficult alternative explanation of the DSC pattern which would require that the melting process requires the co-operative reorganization of the alkyl chains across the whole vesicle The patch concept might also account in part for the leakage/porosity of the bilayers in which channels are formed between patches However, we do not see the patches as long-lived structures Rather we see the clustering of monomers into patches and declustering of patches as a dynamic process A model of a vesicle at any single instance would show channels between the patches

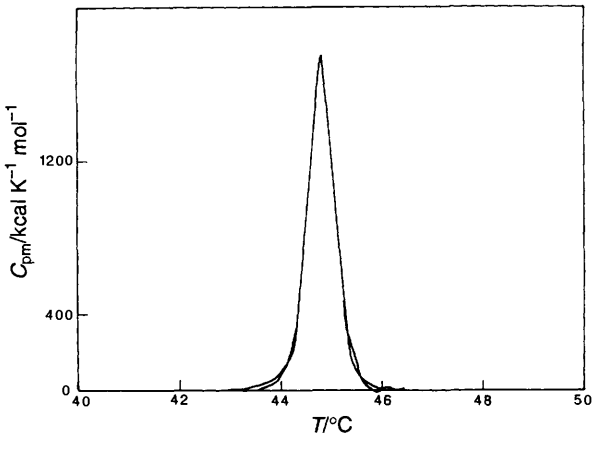


Figure 6 Comparison of observed and calculated dependence on temperature of the molar heat capacity for DOAB (aq , 2 × 10 ^{3} mol dm ^{3})

2.5 Dialkylphosphate Vesicles

Dialkylphosphate vesicles offer an interesting contrast to the DOAB system in the way the charge on the head group has changed from positive to negative The DSC scan recorded[24] for a DDP solution prepared using the hot-water method shows a single extremum near 35 Celsius No other feature is recorded over the range 15 to 90 Celsius The intensity of the extremum assigned to the gel-to-liquid crystal transition decreases steadily with decrease in concentration of DDP (sodium) monomer, Figure 7 Again the sharpness of the melting for a solution containing only 8 42 × 10^{-3} (monomer mol) dm^{-3} is remarkable An analysis based on equation 1 using the concept of the patch number with reference to a solution containing 8 42 × 10^{-3} (monomer mol) dm^{-3} proved satisfactory yielding approximately 170 for the patch number and an enthalpy of fusion equal to approximately 4 0 kJ (monomer mol) [1] With decrease in concentration the patch number increased, a trend consistent with the idea that for small vesicles the co-operativity of the melting increases

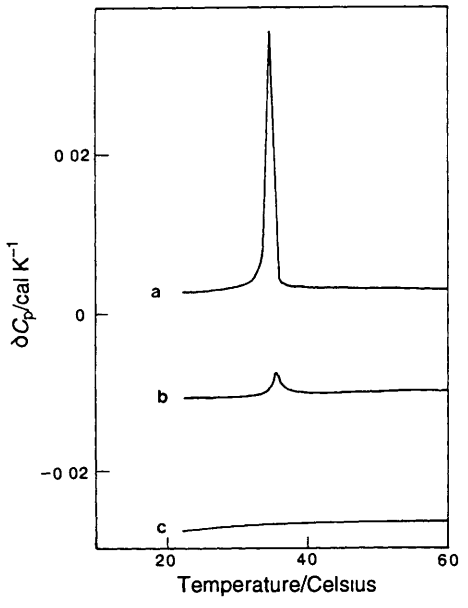


Figure 7 Dependence on temperature of the differential heat capacity of DDP (sodium) in aqueous solution where concentration of DDP is (a) 8 4 × 10 $^{-3}$, (b) 1 × 10^{-3}, and (c) 1 × 10^{-4} mol dm^{-3}

With increase in chain length but with constant counter cation, the melting temperature T_m increases[25] reaching 77 1 Celsius for $DODP^-Na^+$ (Scheme 3) This trend agrees with that deduced on the basis of fluorescence polarization of the hydrophobic fluorescent probe, *trans-trans-trans*-1,6-diphenylhexa-1,3,5-triene [26] In addition, the DSC measurements show that on going from DDP^-Na^+ to $DODP^-Na^+$ the patch number falls from 170 to 50 A similar trend is observed[25] for the corresponding K^+ derivatives although for the same dialkyl phosphate the patch number is slightly smaller The melting temperature is also sensitive to the counter cation [25] Over the range from C_{12} to C_{16} derivatives, T_m for the K^+ vesicles are approximately 1 Celsius lower than the Na^+ derivatives but at least 20 Celsius lower for the Me_4N^+ derivatives, Figure 8 The destabilization of the gel state by the latter cation was a little surprising but must indicate that the neutralizing effect of the counter cation in reducing head group repulsion is important

Recently we studied the impact on the DSC patterns when vesicular solutions are prepared by mixing two dialkylphosphates (sodium) which differ in chain length [27] For equimolar mixtures of dialkylphosphates, $(R^1O)_2PO_2^-Na^+$, where R^1 and R^2 differ by only two methylene groups, the trace shows clear evidence of two distinct types of patches, presumably containing high proportions of each of the dialkylphosphates A striking

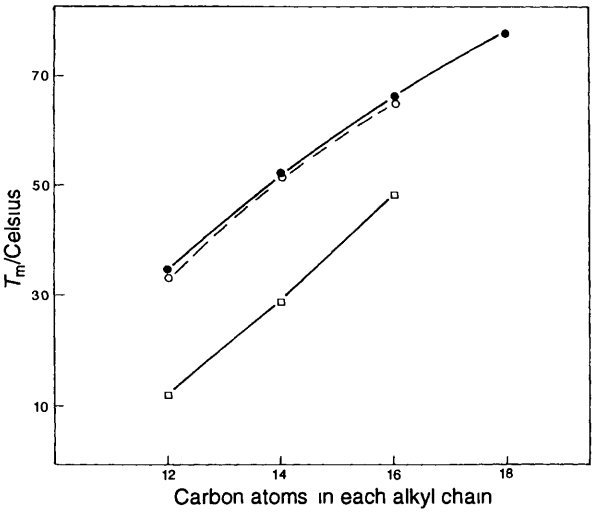


Figure 8 Dependence of melting temperature T_m on alkyl chain length in $(RO)_2PO_2^-M^+$ where the counter cation is (a) Na^+ (●), (b) K^+ (○) and (c) Me_4N^+ (□)

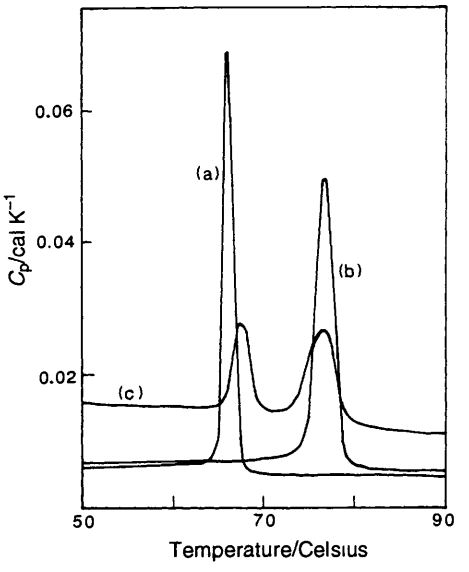


Figure 9 Dependence on temperature of differential heat capacities for aqueous solutions containing sodium dialkylphosphates (8 4 × 10 $^{-3}$ mol dm^{-3}) or a mixture of sodium dialkylphosphates (4 2 × 10 $^{-3}$ mol dm^{-3}), $(R^1O)_2PO_2^-Na^+$, and $(R^2O)_2PO_2^-Na^+$ (a) $R^1 = C_{16}H_{33}$, T_m = 66 3 Celsius (b) $R^2 = C_{18}H_{37}$, T_m = 77 1 Celsius (c) $R^1 + R_2$, T_m = 67 6 and 75 7 Celsius

example is shown in Figure 9 where the two well-resolved transitions are detected When the differences between R^1 and R^2 are more than two-CH_2 groups, the recorded trace is very complicated, indicating that many types of patches containing mixtures of monomers are formed

At the next level of complexity the DSC plots have been recorded[28] for mixtures of sodium dialkylphosphates (aq) where the alkyl chains within the monomer differ in length [29] The scans are complex and reflect the extent of hydrophobic mismatch between lengths of alkyl chains [28 30] One example is shown in Figure 10 which shows that for the equimolar mixture the melting occurs over an extended temperature range consistent with melting in localized domains of varying compositions

2.6 Effect of Added Cosolutes

The comments in this section again emerge from a recognition of the importance of methods of vesicle preparation on the DSC

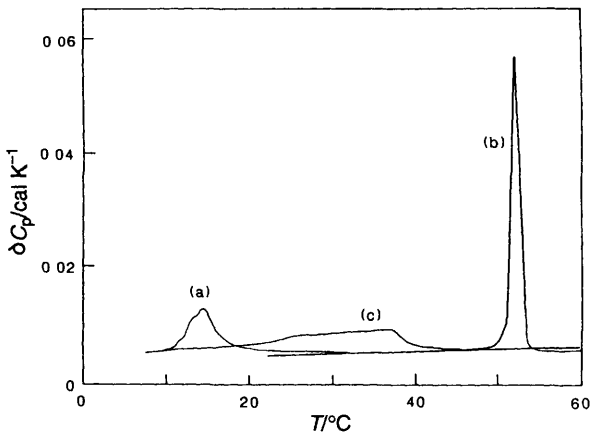


Figure 10 Dependence on temperature of differential heat capacities (reference = water) for aqueous solutions containing vesicles prepared from sodium dialkylphosphates Scans recorded for (a) surfactant $(C_{10}H_{21}O)(C_{14}H_{29}O)PO_2^-Na^+$ (aq , 8 4 × 10 $^{-3}$ mol dm $^{-3}$) where T_m = 14 4 ± 0 1 °C, (b) surfactant $(C_{14}H_{29}O)_2PO_2^-Na^+$ (aq , 8 4 × 10 $^{-3}$ mol dm $^{-3}$) where T_m = 52 2 ± 0 1 °C, and (c) equimolar mixtures (4 2 × 10^{-3} mol dm $^{-3}$) of $(C_{14}H_{29}O)_2PO_2Na^+$ (aq) and $(C_{10}H_{21}O)(C_{14}H_{29}O)PO_2^-Na^+$ (aq)

scans Therefore, we have studied the effects of various added solutes (*e g* organic cosolvents and salts) on T_m and patch number When ethanol, *n*-propanol, and THF are added to sodium DDP vesicular solutions, T_m shifts to lower temperatures but the complexity of the scans[31] points towards domains (patches) which differ in the amount of alcohol incorporated into the bilayers An additional complexity is signalled by an ageing process for vesicle solutions containing alcohol which are stored for more than 12 hours The key consideration centres around the extent to which the added solute is incorporated into the bilayer At the other extreme, the melting temperature for DOAB vesicles is relatively insensitive to added urea, T_m = 43 6 Celsius in the presence of urea (2 5 × 10^{-3} mol dm^{-3}) compared to 44 8 Celsius in the absence of added solute Perhaps the most interesting comparison centres on those studies where we have added another surfactant When CTAB (aq , 1 5 × 10^{-3} mol dm^{-3}) is added to DOAB (aq , 2 × 10^{-3} mol dm^{-3}) the two extrema at approximately 35 and 45 Celsius are replaced by a single broad extrema near 40 Celsius [33] The shift of the most intense transition from 45 to 40 Celsius (Figure 11) is attributed to penetration of the bilayer by the mono-alkyl surfactant and

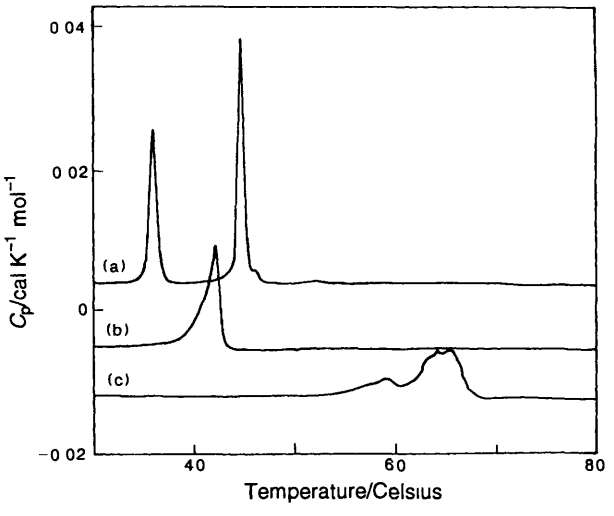


Figure 11 Dependence on temperature of the relative isobaric heat capacity for DOAB (aq , 2 × 10^{-3} mol dm^{-3}), (a) no added surfactant, and in the presence of (b) added CTAB (1 5 × 10 $^{-3}$ mol dm^{-3}), and (c) SDS (1 5 × 10 $^{-3}$ mol dm^{-3})

destabilization of the gel state The latter is accounted for in terms of poor packing within the bilayer and an increased repulsion between $-N^+Me_3$ and $=N^+Me_2$ head groups When SDS was added, a complex trace emerged at much higher temperature The stability of the gel has been raised by incorporation of the anionic surfactant, as a result of decreasing head group $\{=N^+Me_2\}$–$\{N^+Me_2=\}$ repulsion However, this penetration is not "homogeneous" in that the melting now takes place over a wider range of temperatures When CTAB and SDS are added to DDP a similar pattern is followed Addition of CTAB (aq , 1 5 × 10^{-3} mol dm^{-3}) to sodium DDP (aq , 2 × 10^{-3} mol dm^{-3}) raises T_m but broadens the trace These patterns form an interesting starting point for a discussion of the interaction between surfactants and biomembranes [34]

Another interesting contrast is observed[35] between the effects of added NaCl and added $CaCl_2$ on sodium DDP vesicles Thus addition of NaCl (aq , 0 145 mol dm^{-3}) simply shifts the melting temperature to 51 Celsius Addition of $CaCl_2$ has a more profound effect producing a complex scan with a sharp transition near 87 Celsius, attributed to melting in patches having most head group binding sites in the double layers occupied by calcium ions

3 Conclusions

This has been a necessarily brief survey into the calorimetric studies being undertaken in this collaborative research involving groups at the University of Leicester and the University of Groningen Our aim has been to survey some key results but also to demonstrate how careful calorimetric work can be enormously informative

Acknowledgements We thank SERC for their support under the Molecular Recognition Initiative (MRI) and the Royal Society for their support This Review was written in part during the appointment of MJB to a Visiting Professorship at the University of Groningen (The Netherlands)

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