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# Sodium di-*n*-dodecylphosphate vesicles in aqueous solution: effects of added ethanol, and $Ca^{2+}$ and $Na^{+}$ ions on the gel–liquid phase transition

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**Abstract**

For aqueous solutions containing vesicles formed by sodium di-*n*-dodecylphosphate, the gel–liquid-crystal transition occurs near 35°C at the temperature $T_m$. When ethanol is added, $T_m$ decreases. When sodium chloride is added, $T_m$ shifts to higher temperatures whereas very complex scans with several transitions are recorded when calcium chloride is added. The complexities are attributed to the binding of $Ca^{2+}$ ions by phosphate groups at the vesicle surface.

*Keywords:* DDP; Ethanol; Phase transition; Sodium compound

## 1. Introduction

In aqueous solutions, synthetic double-chain amphilphiles [1] aggregate to form vesicles [2–4]. These interesting systems resemble natural membrane systems [5]. The amphilphile, di-*n*-dodecylphosphate (DDP) as its sodium salt, forms vesicles as

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confirmed by electron microscopy [6]. Each vesicle of DDP has a diameter [1] of less than 100 nm.

Apart from the intrinsically interesting properties of vesicle solutions, the exciting possibility emerges that vesicles can be used as drug carriers, the active drug being transported within the bilayer systems. Alternatively, hydrophobic drugs can be contained in the hydrophobic part of the bilayers. Linked into these applications is the phenomenon by which vesicles fuse, thereby merging the solutions entrapped within the bilayer systems.

Recently, Engberts and coworkers [5,7] showed that artificial and natural vesicular systems would fuse in the presence of the fusogenic agent $Ca^{2+}$. In the reported study, the vesicle system was an aqueous solution containing sodium di-*n*-dodecylphosphate (DDP). We have therefore examined the effect of added $Ca^{2+}$ ions on the DSC for DDP, contrasting the changes with the effect of added sodium chloride. We report that a new extremum in the differential scan is produced by added $Ca^{2+}$ and that the shape of the trace at the extremum near 35°C is significantly changed.

## 2. Experimental

### 2.1. Materials

The surfactants and other materials were prepared as previously described [1].

### 2.2. Calorimetry

The differential scanning microcalorimeter (MicroCal Ltd., USA) recorded [8] the heat capacities of DDP solutions relative to that of a corresponding solution which contained no DDP. The volume of the cell was 1.2 $cm^3$. The scan rate with rising temperature was approximately 60 K $h^{-1}$. As previously described [8], a water–water baseline was subtracted from each scan using ORIGIN software (MicroCal Ltd.). Therefore, in the figures described below we report the dependence of the differential isobaric heat capacity $\delta C_p(\text{sln}; T)$ on temperature.

A known weight of DDP(s) was added to 2.2 $cm^3$ of water, heated to 55°C and held at this temperature for approximately 30 min with stirring. The solution was allowed to cool to room temperature and the required volume of ethanol(l) was added. The solutions were placed in the sample cell and cooled to 15°C. The differential scans were recorded between 15 and 90°C. In all cases, the solutions were cooled from the higher to the lower temperature cited above. The differential heat capacity was then re-measured, in some cases almost immediately and, in other cases, after equilibration at the lower temperature for a specified time.

In the examination of the effects of added $Ca^{2+}$ ions and $Na^+$ ions, Hepes buffer solution ($5 \times 10^{-3}$ mol per $dm^{-3}$ $CH_3COONa$ and $5 \times 10^{-3}$ mol per $dm^3$ Hepes) containing the appropriate concentration of $CaCl_2$(aq) and NaCl(aq) was added to DDP(s). The solution was heated to 55°C and held there for 1 h. The reference cell

contained the identical solution except that no DDP was present. A fresh DDP solution at 55°C, prepared as described above, was placed in the sample cell and scanned over the range 55–90°C. The solution was cooled to 15°C and re-scanned to 90°C. This cool-scan cycle was repeated several times.

## 3. Results

In the scan recorded for DDP (aq; $8.4 \times 10^{-3}$ monomer mol $dm^{-3}$), the single dominant feature is an extremum at 35°C. The shape and position of the scan are the same when the aqueous solutions are prepared using Hepes buffer ($5 \times 10^{-3}$ mol per $dm^3$ $CH_3COONa$ + Hepes), Fig. 1. The temperature extremum at 35°C for DDP(aq) moved to lower temperatures with an increase in vol% ethanol (Fig. 1).

When $Ca^{2+}$ ions (as the chloride) were added to DDP(aq) in Hepes buffer, the original extremum at 35°C was lost and a broader extremum obtained at slightly higher temperature. For direct comparison, we show in Fig. 2 the recorded traces for the third scans (following two heat–cool cycles) for a series of DDP solutions containing added $Ca^{2+}$ ions. For solutions containing $Ca^{2+}$ ($2.5 \times 10^{-3}$ mol $dm^{-3}$), the extremum was at 40°C. With increase in [$Ca^{2+}$], a new extremum was also recorded near 87°C.

The extent of reproducibility of these traces is illustrated in Fig. 3. After 10 h, the essential features of the differential scan remained unchanged but a new extremum emerged. The origin of these new extrema obtained after storage of the solutions for more than 7–11 h is obscure. Certainly they are not reproducible and do not, we suggest, impinge on the overall pattern and explanation discussed here.

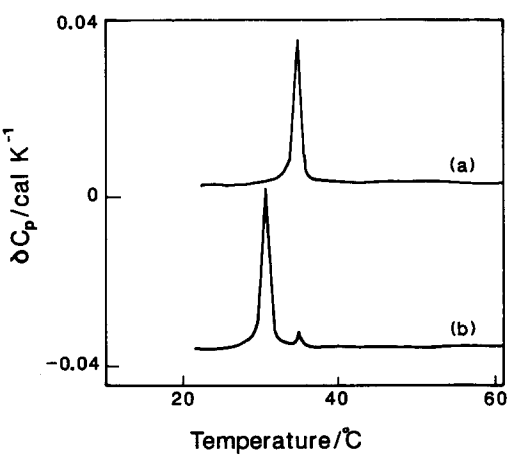


Fig. 1. Dependence on temperature of the differential heat capacity $\delta C_p$ for DDP ($8.4 \times 10^{-3}$ monomer mol $dm^{-3}$) in (a) aqueous solution, $T_m = 3.5$°C and (b) 3.9 vol% ethanol, $T_m = 31$°C. One joule is 4.184 calories.

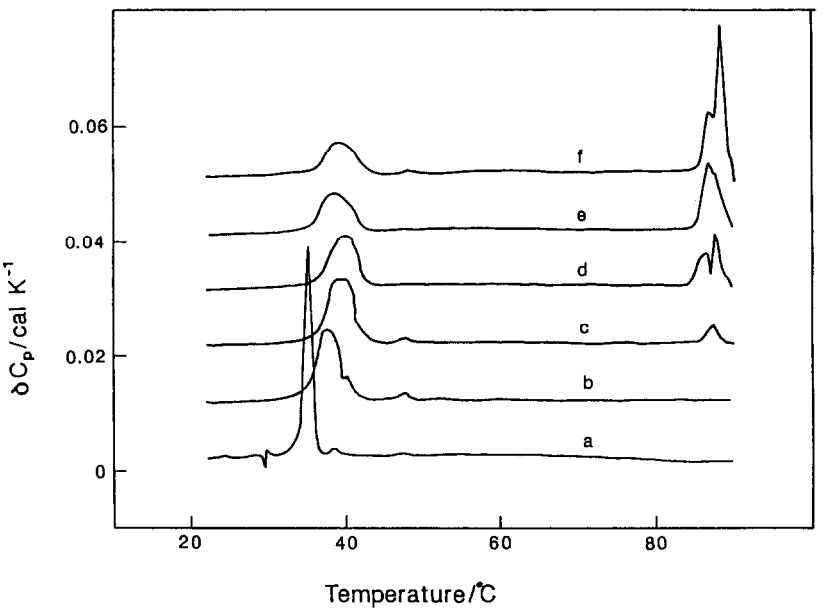


Fig. 2. Dependence of differential heat capacity on temperature for DDP (aq; $8.42 \times 10^{-3}$ mol $dm^{-3}$) in Hepes buffer solution containing (a) 0, (b) $0.5 \times 10^{-3}$, (c) $1.0 \times 10^{-3}$, (d) $1.5 \times 10^{-3}$, (e) $2.0 \times 10^{-3}$ and (f) $2.5 \times 10^{-3}$ mol per $dm^3$ $CaCl_2$. (The traces have been displaced for clarity on the heat capacity axis.) One joule is 4.184 calories.

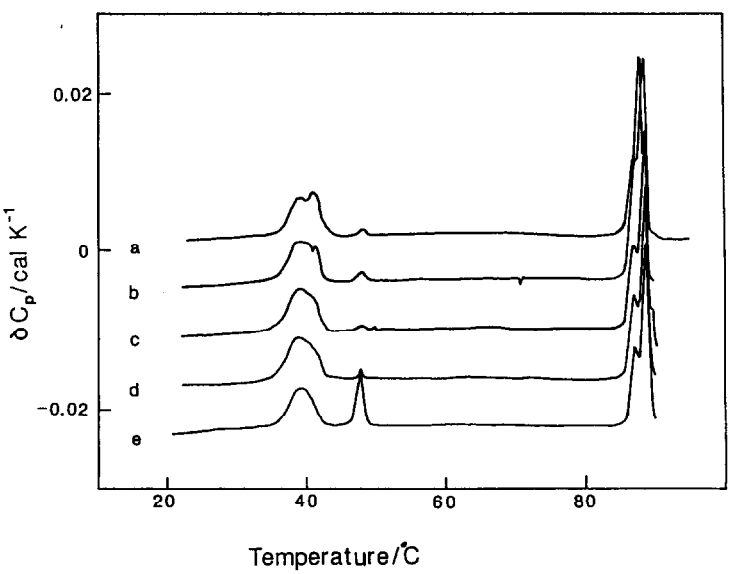


Fig. 3. Dependence on temperature of differential heat capacity on temperature for DDP (aq; $8.4 \times 10^{-3}$ mol $dm^{-3}$) and $CaCl_2$ ($2.5 \times 10^{-3}$ mol $dm^{-3}$) in Hepes buffer recorded in the (a) first, (b) second, (c) third and (d) fourth scans following a heat–cool–heat cycle, and (e) after 10 h. One joule is 4.184 calories.

By comparison with the effects of added $Ca^{2+}$ salt, the impact on the recorded scans of added NaCl was much less dramatic. For a solution containing NaCl (aq; 0.145 mol $dm^{-3}$), reasonably concentrated in the present context, the extremum was shifted to 51°C but this was the only recorded maximum. No high temperature extremum comparable to that produced by added $CaCl_2$(aq) was observed. The extremum near 51°C remained sharp and again did not broaden in the manner observed when $CaCl_2$ was added.

## 4. Discussion

The transition at 35°C for DDP vesicles is attributed to the gel–liquid-crystal transition. When ethanol is added, the transition temperature shifts to lower temperatures. The overall pattern reflects the adsorption of alcohol molecules into the DDP vesicles. The gel phase of the vesicles with added NaCl (aq; 0.145 mol $dm^{-3}$) has a much greater thermal stability, i.e. $T_m = 51$°C in the latter solution. Presumably the effect on water structure of the presence of NaCl(aq) results in a stabilising effect on the gel phase of the vesicles. Addition of $Ca^{2+}$ ions has a profound effect on the nature and extent of the structural transition in the DDP. The dramatic effect of relatively low concentrations of $Ca^{2+}$ ions means that these ions interact directly with the phosphate head groups of the vesicles. The patterns can be understood in terms of displacement of $Na^{2+}$ ions by the $Ca^{2+}$ ions in the double layers associated with the bilayers. Moreover, the $Ca^{2+}$ ions bind strongly across two phosphate head groups. So we attribute the low-temperature extremum to a gel–liquid-crystal transition in the bilayer involving patches of DDP monomers with $Na^{+}$ ions close to the phosphate head groups but perturbed by local $Ca^{2+}$ ions. With increase in [$Ca^{2+}$], there emerge patches on the surface of the bilayers where many sites are occupied by $Ca^{2+}$ ions. These ions introduce greater structural rigidity into the associated patches and hence the gel–liquid transition moves to a higher temperature, e.g. 87°C (Fig. 3).

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## References

[1] A. Wagenaar, L.A.M. Rupert, J.B.F.N. Engberts and D. Hoekstra, J. Org. Chem., 54 (1989) 2638.
[2] J.H. Fendler, Acc. Chem. Res., 13 (1980) 7.
[3] T. Kunitake, Angew. Chem. Int. Ed. Engl., 31 (1992) 709.
[4] A.M. Carmona-Ribeiro, Chem. Soc. Rev., 21 (1992) 209.
[5] T.A.A. Fonteyn, J.B.F.N. Engberts and D. Hoekstra, in S. Ohki (Ed.), Cell and Model Membrane Interactions, Plenum Press, New York, 1991, p. 215.
[6] T.A.A. Fonteyn, D. Hoekstra and J.B.F.N. Engberts, J. Am. Chem. Soc., 27 (1988) 8870.
[7] L.A.M. Rupert, J.B.F.N. Engberts and D. Hoekstra, Biochemistry, 27 (1988) 8232.
[8] M.J. Blandamer, B. Briggs, P.M. Cullis and G. Eaton, J. Chem. Soc. Faraday Trans., 87 (1991) 1169.