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AN ALTERNATIVE EXPLANATION FOR PHASE TRANSITIONS OBSERVED IN QUICK FROZEN CALCIUM CARDIOLIPIN SOLUTION

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It is demonstrated that the phase changes reported for a quick frozen calcium cardiolipin solution containing $CaCl_2$ are virtually identical to those seen in pure $CaCl_2$. This introduces uncertainty as to whether the data in fact reflect the behavior of cardiolipin or of the associated $CaCl_2$.

In a recent publication Melchior et al. [1] presented data in their Fig. 5 indicating that the calcium salt of cardiolipin forms glasses upon cooling at rates of 5 K/min.

The cardiolipin was purportedly prepared with 0.2 M $CaCl_2$ in the cardiolipin solution.

Noting that $CaCl_2$ is a notorious glass former [2], I present differential calorimetric analysis traces of 0.2 M $CaCl_2$ in distilled water, 2.0 M $CaCl_2$ in distilled water and 2.0 M $CaCl_2$ cardiolipin solution. The 20 mg aliquot of 0.2 M $CaCl_2$ was frozen at 200 K/min and heated at 5 K/min in a Perkin-Elmer DSC 4 Scanning Calorimeter in a sealed aluminum sample pan. In Fig. 1 the whole trace is displayed, and in Fig. 2 the power scale is expanded 7.5-times to show the glass transition more clearly. Clearly the glass transition at peak A, the devitrification at peak B, and the eutectic melt at peak C are virtually identical to Melchior's. The completed melt, peak D, is far different, and consistent with a $CaCl_2$ concentration of 0.2 M [3].

Fig. 3 illustrates 4.4 mg 2.0 M $CaCl_2$ solution frozen at 200 K/min and measured calorimetrically as in the 0.2 M aliquot. Note that the peaks A, B, and C have not changed, whereas the temperature of the completion of the melt at D is consistent with a solution composition of 2.0 M $CaCl_2$ [3]. Thus it is the 2.0 M $CaCl_2$ solution that gives a curve virtually identical to Melchior's.

Fig. 4 illustrates 2.0 M $CaCl_2$ with 2.5 mg associated cardiolipin (U.S. Biochemical Corp 10% chloroform solution). The sample was prepared by evaporation to constant weight of the chloroform cardiolipin solution in an aluminum sample pan at room temperature and then the addition (and mixing in the sample pan) of 5.6 mg of 2.0 M $CaCl_2$ solution. The resulting 10 K/min heating curve is identical to Fig. 3 except for the two events above 0°C. The +5°C event did not repeat after the sample was run to 50°C, refrozen at 200 K/min, the rewarmed at 10 K/min. The +17°C peak was highly repeatable in several samples and is assumed to be the actual calcium cardiolipin melting peak.

Thus my $CaCl_2$ curve raises two questions: (1) do the authors' data actually demonstrate phase transitions in calcium cardiolipin or are their peaks those of the associated $CaCl_2$; and (2) if their measurement is dominated by $CaCl_2$, why is the melt complete at about −20°C corresponding to 2.0 M $CaCl_2$, rather than about −1.0°C as would

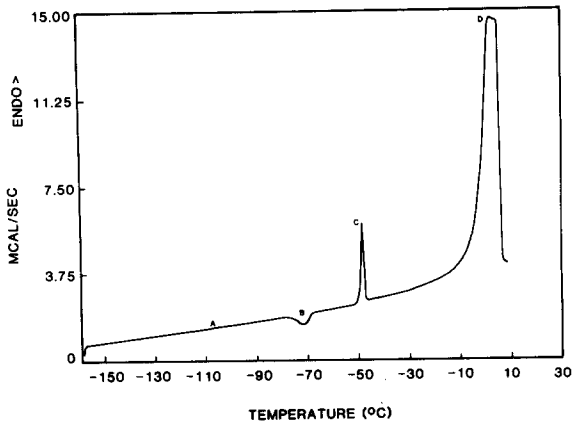

Fig. 1. 20 mg 0.2 M $CaCl_2$ solution cooled at 200 K/min, warmed at 5 K/min on a Perkin-Elmer DSC 4. Peak A, glass transition; Peak B, devitrification of supercooled eutectic; Peak C, eutectic melt; Peak D, complete melt of all remaining ice.

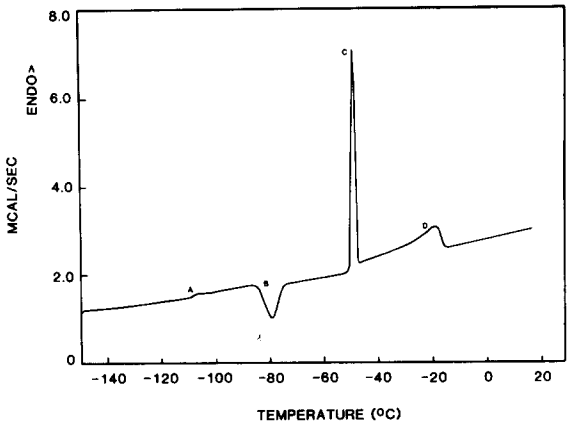

Fig. 3. 4.4 mg of 2.0 M $CaCl_2$ cooled at 200 K/min then warmed at 5 K/min in DSC 4. Peaks A, B, C as in Fig. 1. Peak D is complete melt of 2.0 M salt solution.

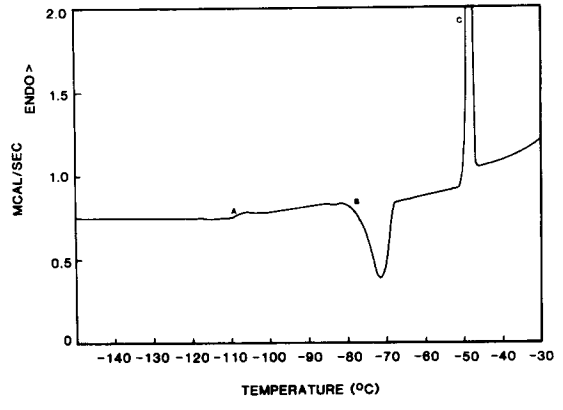

Fig. 2. As Fig. 1 but power scale expanded 7.5-fold to illustrate peak structure more clearly.

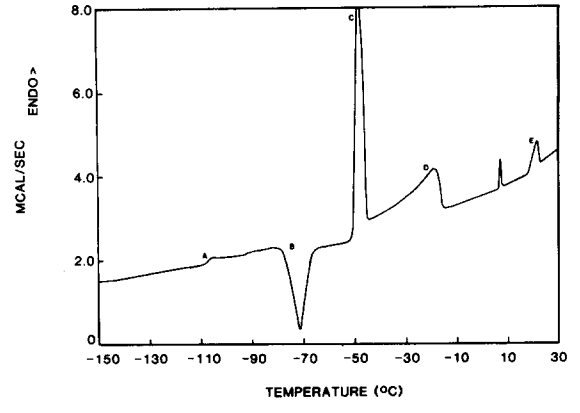

Fig. 4. 5.6 mg 2.0 M $CaCl_2$ solution mixed with 2.5 mg cardiolipin; cooled at 200 K/min then warmed at 10 K/min. Peaks A–D as in Fig. 3. Peak E apparently is a calcium cardiolipin event.

be expected from a solution of 0.2 M $CaCl_2$? The latter observation may be related to events occurring during their precipitation of the calcium cardiolipin. According to the authors this was achieved by dropping a water-cardiolipin emulsion into a concentrated $CaCl_2$ solution until a final $CaCl_2$ concentration of 0.2 M was reached, then sedimenting the precipitated lipid. $CaCl_2$ brine is dense and, if care was not taken to mix the suspension vigorously, the cardiolipin may in fact have been pelleted into a concentrated $CaCl_2$ solution. The likelihood that the calcium cardiolipin itself suppressed the freezing point to $-20°C$ seems remote, especially in view of the author's contention that it was not in water solution, but a suspension.

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