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Plenary lectures

DIFFERENTIAL SCANNING AND TITRATION CALORIMETRIC STUDIES OF MACROMOLECULES IN AQUEOUS SOLUTION

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Abstract

An enormous amount of detailed information can be obtained concerning macromolecules in aqueous solution using data obtained from differential scanning and titration microcalorimetry. This claim is supported by reference to examples taken from recent work concerned with micelles (e.g. CTAB), vesicles (e.g. DOAB and DDP) and enzymes (e.g. CAT and DNA gyrase).

Keywords: enzymes, microcalorimetry, micelles, vesicles

Introduction

The last decade witnessed dramatic technological developments in calorimetry which opened up new challenges for chemists. In particular, these developments allow chemists to probe quite subtle intramolecular and intermolecular interactions involving macromolecules in aqueous solutions. At the University of Leicester we have exploited the sensitivity of two microcalorimeters to probe the chemistry of micelles, vesicles and enzymes in aqueous solutions. Here we survey this research. We start our account with relatively simple systems and then, with increasing complexity, survey recent studies involving two enzymes – chloramphenicol acetyltransferase and DNA gyrase.

Calorimeters

The group at the University of Leicester uses two microcalorimeters (Micro-Cal Ltd., USA); a differential scanning microcalorimeter and a titration micro-

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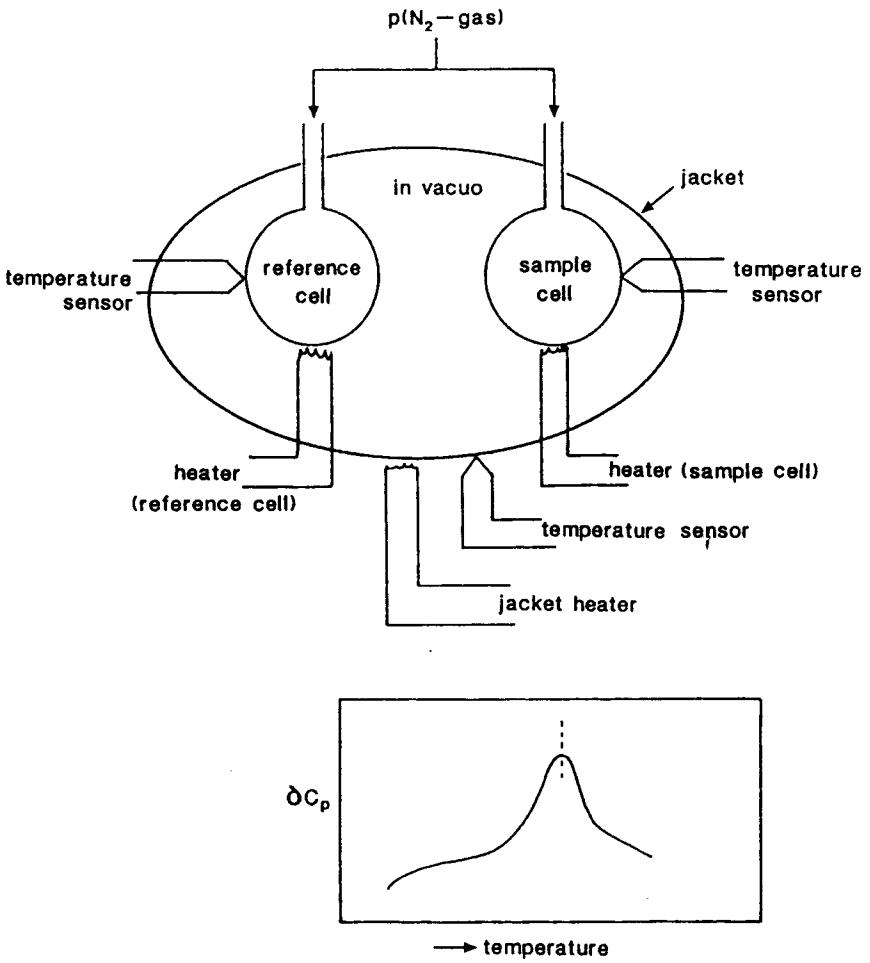

Fig. 1 Diagrammatic presentation of a differential scanning microcalorimeter [inset shows recorded dependence of relative isobaric heat capacity on temperature]

calorimeter; Figs 1 and 2. The essential features of the two calorimeters are common. Two small cells (volume = approx. 1.4 cm^3) are held in an evacuated "adiabatic" jacket. Control units monitor the temperatures of the cells and jacket. In the DSC, the control unit heats and gradually increases the temperatures (e.g. 60 $deg \cdot h^{-1}$) of the two cells. The system records the relative isobaric heat capacity of the solution in the sample relative to the liquid (solvent) in the reference cell. If the solution in the sample cell which contains solute X (at low temperature) which forms solute Y at high temperature; the relative isobaric heat capacity δC_p passes through a maximum (Fig. 1). The dependence of δC_p

on temperature is analysed [1, 2] to yield a characteristic melting temperature T_m (*cf.* maximum in δC_p), and the enthalpy of reaction for the process

$$X(aq) \rightarrow Y(aq),\ \Delta_r H^\circ(aq).$$

In the titration microcalorimeter (Fig. 2), small aliquots of one solution are injected into the sample cell containing another solution. Interaction/reaction between solutes produces a pattern of pulses (see inset to Fig. 2) which are analysed to yield enthalpies of interaction/reaction. Considerable skill is required of the operator in deciding the concentrations of solutions in syringe and sample cell. We should stress that the titration calorimeter probes a series of equilib-

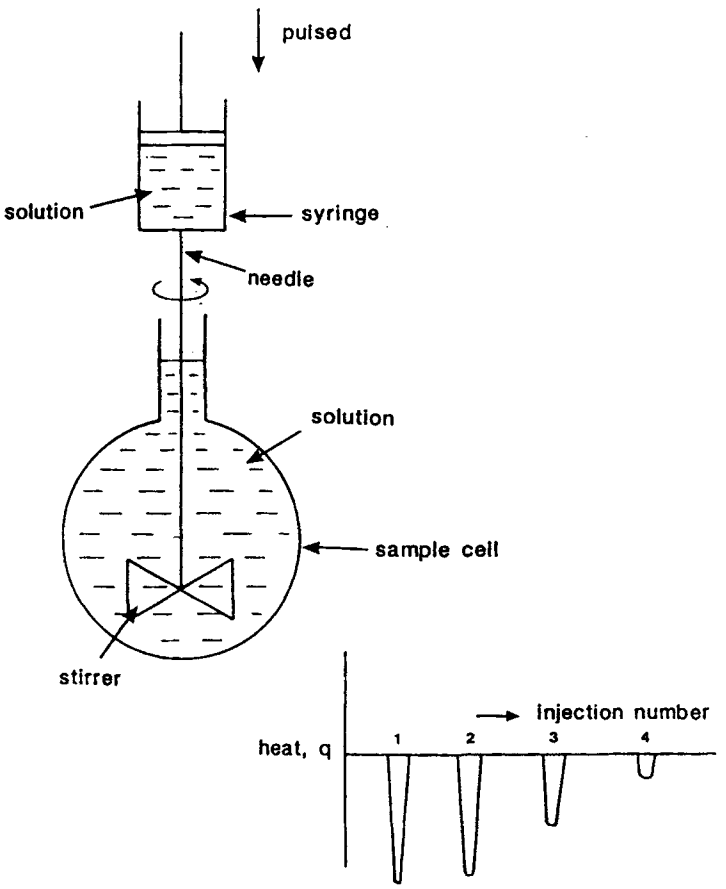

Fig. 2 Diagrammatic presentation of a titration microcalorimeter showing sample cell (*cf.* Fig. 1) with syringe (plus stirrer) which injects aliquots of solution into the sample cell [inset shows a formal plot of exothermic pulses accompanying several injections from the syringe]

rium states. Originally, this calorimeter was designed to probe the extremely small enthalpies of binding of substrates to enzymes [3] but clearly the calorimeter has wider application. In general terms, the heat q recorded following the k^{th} injection of solution from the syringe into the sample cell is given by equation [1].

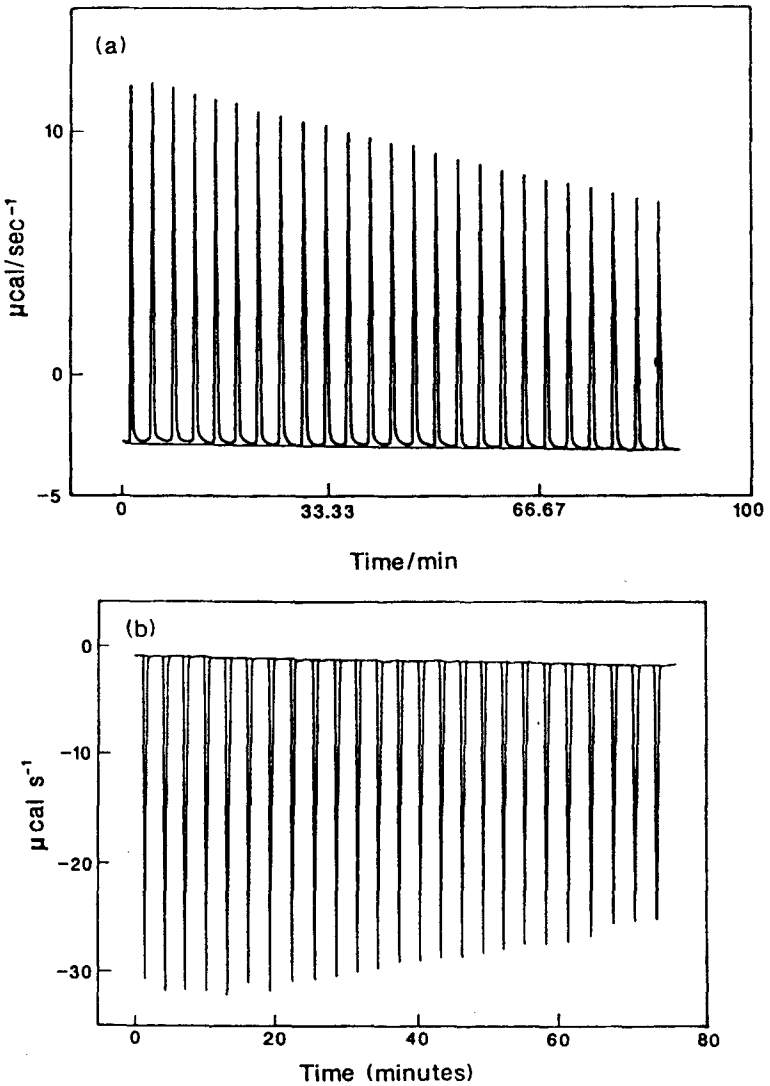

Fig. 3 Titration calorimetry; injection plots showing dependence of rate of heating on time of injection when aqueous solutions are injected into the reservoir, initially containing only water; (a) urea (0.65 mol·dm^{-3}) and (b) HMT (0.53 mol·dm^{-3}) are solutions in the syringe

$$q = H[k; \text{aq}] - H[(k-1); \text{aq}] - H[I; \text{aq}] \quad (1)$$

where $H[(k-1); \text{aq}]$ is the enthalpy of the solution in the sample cell (Fig. 2) prior to injection k whereas $H[I; \text{aq}]$ is the enthalpy of the solution in the aliquot injected into the sample cell at the k^{th}. In each type of application the key task concerns setting down explicit equations for the three enthalpies in equation (1). Nevertheless, this task is relatively straightforward allowing a gallimaufry of systems to be investigated as indicated below.

Enthalpies of dilution

In a formally simple but quintessential experiment the titration calorimeter (Fig. 2) is used to probe enthalpy changes accompanying dilution of an aqueous solution. For neutral solutes and using the concept of the Gurney cosphere [4], dilution of an aqueous solution simply involves an increase in mean separation of {solute molecules + cospheres} in solution. In Fig. 3 we contrast the plots obtained [5] for dilutions of aqueous solutions containing the hydrophilic solute, urea and the hydrophobic solute hexamethylene tetramine (HMT). Dilution of HMT(aq) is exothermic (Fig. 3). In other words, mutual approach of HMT(aq) molecules in aqueous solution is endothermic [5], a trend generally regarded as characteristic of hydrophobic interactions. The titration microcalorimetric data are analysed to yield pairwise enthalpic interaction parameters [5, 6], h(HMT) = 1119 ±57 J·kg^{-1} whereas h(urea) = −324 ±24 J·kg^{-1}. The contrast in sign is the clearest signal of the contrast in character of these two solutes.

Micelle deaggregation

A more complicated situation arises in titration microcalorimetry when another process in addition to simple dilution occurs. If the sample cell contains initially water and the syringe contains a solution of a surfactant above its critical micellar concentration [7–9] (*cmc*), the heat recorded by the titration calorimeter includes a contribution from deaggregation of micelles. Enthalpies of formation of micelles from monomers with long alkyl chains tend to be exothermic and so dilution (deaggregation) is endothermic [10].

A typical example concerns the surfactant, hexadecyltrimethylammonium bromide (CTAB) where for aqueous solutions at 298.2 K and ambient pressure the *cmc* [10, 11] is 0.95 mol·m^{-3}. The corresponding titration plot is shown in Fig. 4(a). Each peak is integrated to yield the dependence on injection number of the enthalpy of dilution of the solution in the syringe. With increase in injection number so the concentration of surfactant in the sample cell (Fig. 2) increases, equals and then exceeds the *cmc*. Over the first series of injections the calorimeter records the impact of micelle deaggregation but eventually the only change is micelle dilution. The change in the pattern of dilution enthalpies oc-

curs at the *cmc* (Fig. 4). The titration curve can be accounted for [12] in terms of the enthalpy of micelle formation and the dependence on ionic strength of the relative apparent molar enthalpies of both micelles and the simple deaggregated salt. For these calculations we used Pitzer's equations to express the dependence on ionic strength of relative apparent molar enthalpies [13, 14].

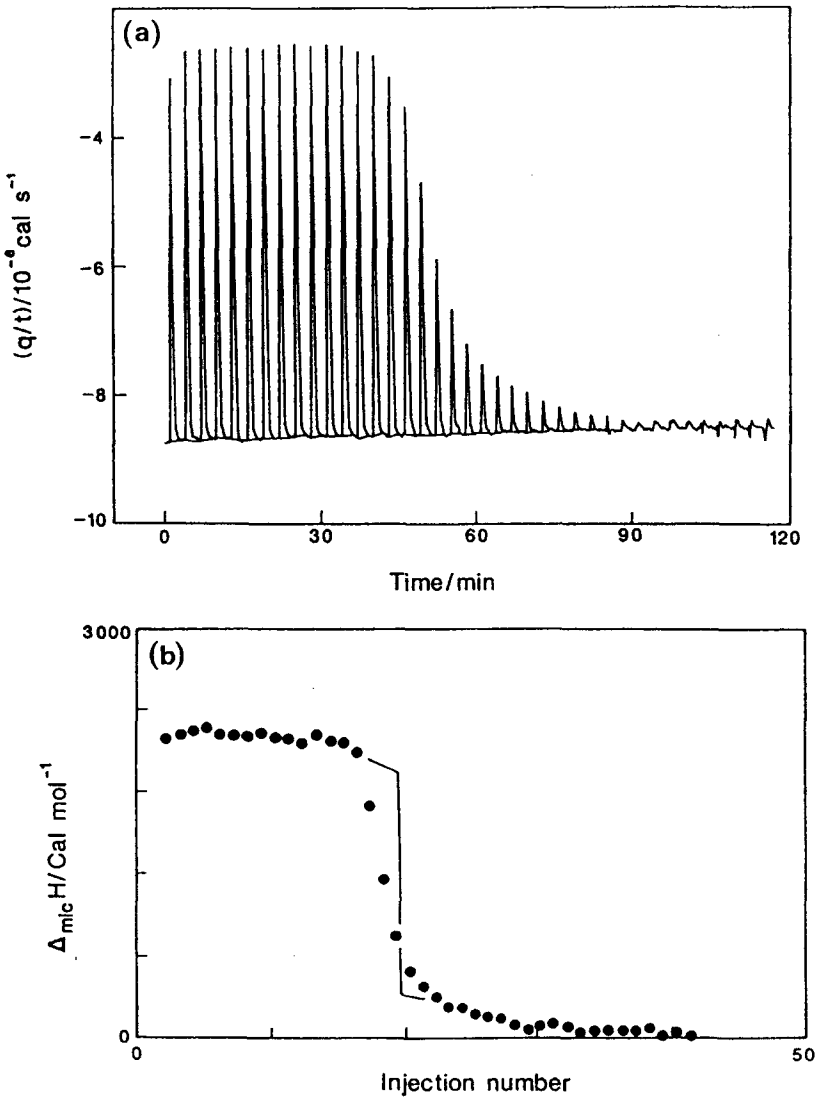

Fig. 4 Titration plots for CTAB(aq) where [CTAB] in the syringe is $14.8 \cdot 10^{-3}$ mol·dm^{-3}; (a) recorded titration plot and (b) integrated plot. [In plot (b) the points are the experimental enthalpies of dilution and the full line is the calculated dependence]

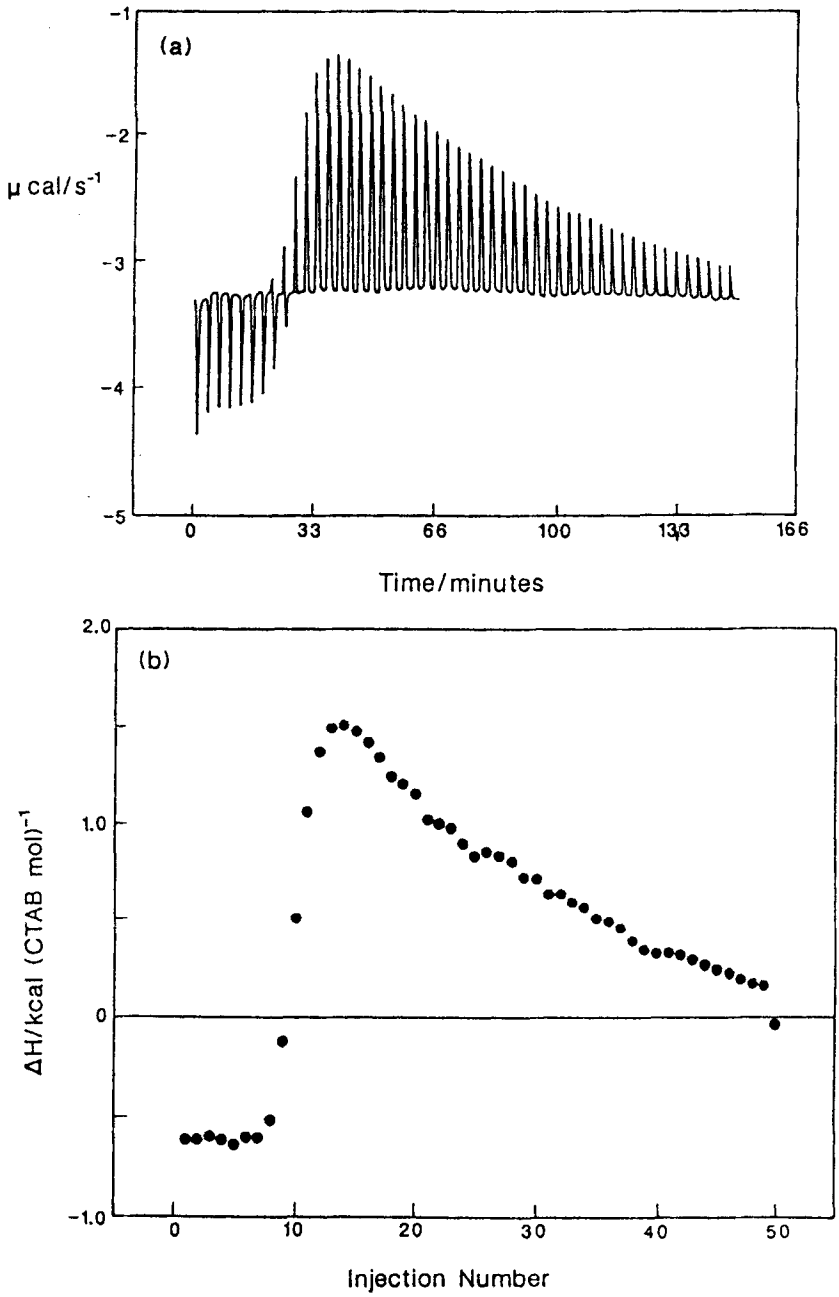

Fig. 5 Titration calorimetry; injection of aliquots containing CTAB (aq; $4 \cdot 10^{-3}$ mol·dm^{-3}) and pentanol (aq; 0.11 mol·dm^{-3}) into pentanol (aq; 0.11 mol·dm^{-3}); (a) recorded titration plot and (b) integrated plot

The full line in Fig. 4(b) shows the calculated dependence based on the following estimates; cmc = 0.95 mol·m^{-3}, aggregation number N = 92, residual charge = 0.21 and $\Delta_{mic}H^{\circ}$ = −2.45 kcal·(monomer mol)$^{-1}$. We have extended these studies to probe the impact on micellar thermodynamics of adding other solutes prompted in part by the current interest in solubilization [15]. The impact of adding, for example, pentanol is dramatic as shown in Fig. 5 where the solvent in both sample cell and syringe contains equal concentrations of pentanol. We draw particular attention to the change from exo- to endothermic heats accompanying deaggregation. These data offer convincing evidence of the dramatic impact of incorporating into the CTAB micelles a solute such as pentanol. We have linked these calorimetric studies with a complementary examination of the effects of added PeOH on micellar catalysis by CTAB.

With increase in CTAB concentration the properties of the aqueous solutions become more complicated. Many models have been proposed for these 'post-micellar' phenomena. Strong evidence for such processes involving, for example, changes in micellar shape and in counterion binding emerge from the DSC traces [2, 17] (Fig. 1) for these solutions (Fig. 6). Analysis of these traces is unfortunately complicated when two or more processes are coupled [18].

CTAB provides a useful marker for examining the properties of micellar solutions. Certainly the titration calorimetric plots (Fig. 4) are readily accounted for, at least qualitatively. With decrease in alkyl chain length (and hydrophobicity), the titration calorimetric plots become more complicated. An example is shown is Fig. 7 for decyltrimethylammonium bromide. In this case the enthalpies associated with deaggregation and solute-solute interactions are comparable, leading to complicated dependence of recorded heat on injection number.

Vesicles

In aqueous solution surfactants having two long alkyl chains can associate to form vesicles [19]. The latter comprise a shell of hydrocarbon chains, presenting polar head groups to aqueous solutions inside and outside the vesicle. Interest in these systems stems from the similarity of these structures to natural lipid bilayer systems. A characteristic of these synthetic bilayer systems is the gel-to-liquid crystal transition at a characteristic melting temperature T_m. For example, T_m for vesicles formed from dimethyldioctadecylammonium bromide (DOAB) is 44.8°C and in this melting a patch of approx. 130 monomers undergo this cooperative transition [20]. The transitions are clearly seen in the DSC traces for quite dilute solutions. In fact, the solutions are very dilute in vesicles bearing in mind that the aggregation numbers are 70 000. Moreover, the scans are reproducible over several hours showing that the melting transitions are fully and thermodynamically reversible (Fig. 8).

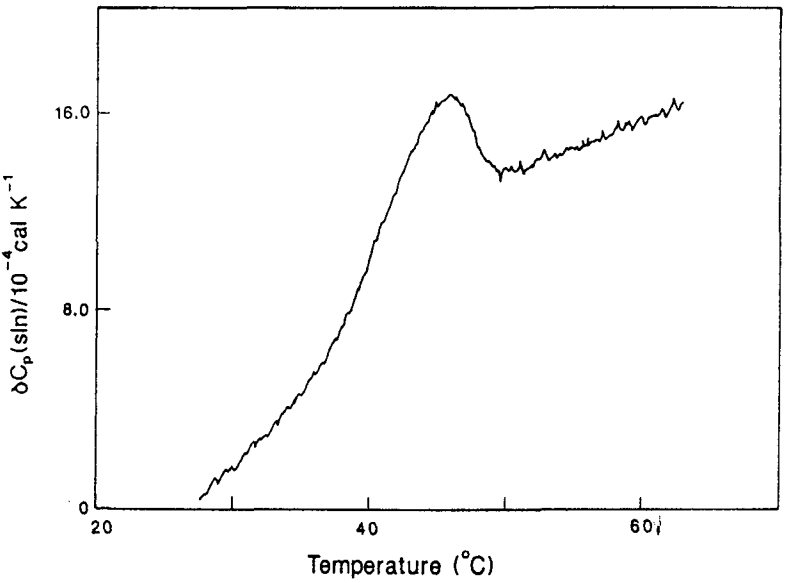

Fig. 6 Dependence of differential isobaric heat capacity for CTAB (aq; 0.02 mol·dm^{-3}) on temperature

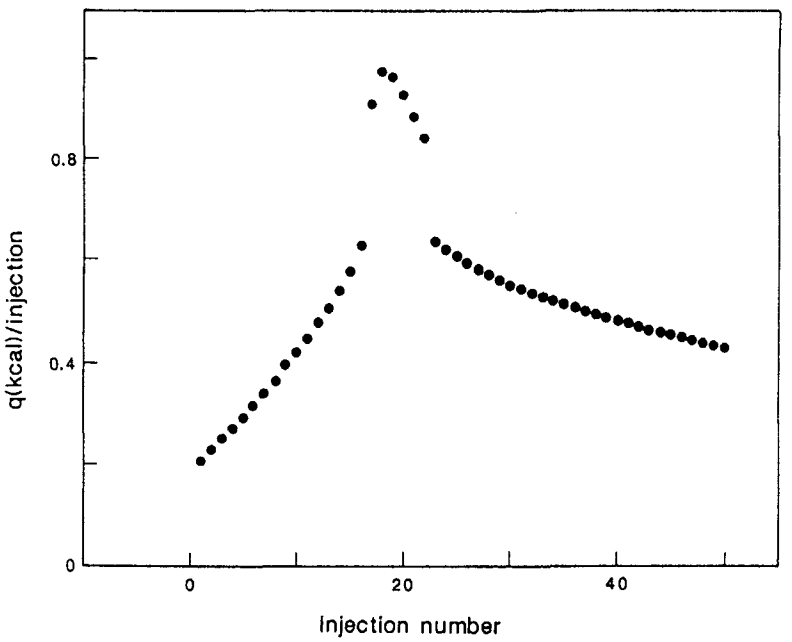

Fig. 7 Titration calorimetry; integrated plot showing the dependence of recorded heat on injection number for dilution of decyltrimethylammonium bromide (aq; 1.10 mol·dm^{-3})

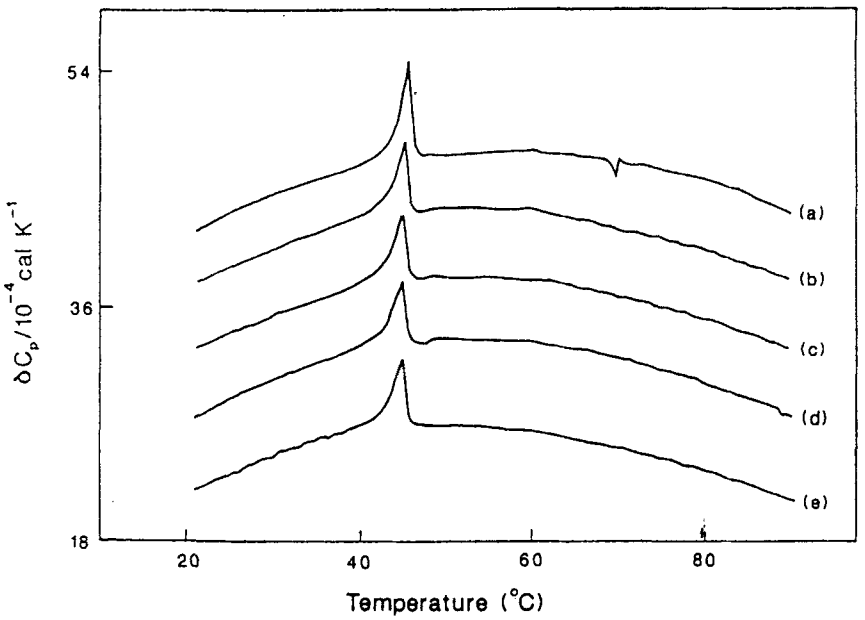

Fig. 8 DSC traces recorded for DOAB (aq; $1.0 \cdot 10^{-4}$ mol·dm^{-3}). The five traces were recorded after a series of heat-cool-heat ···· cycles over a period of eleven hours

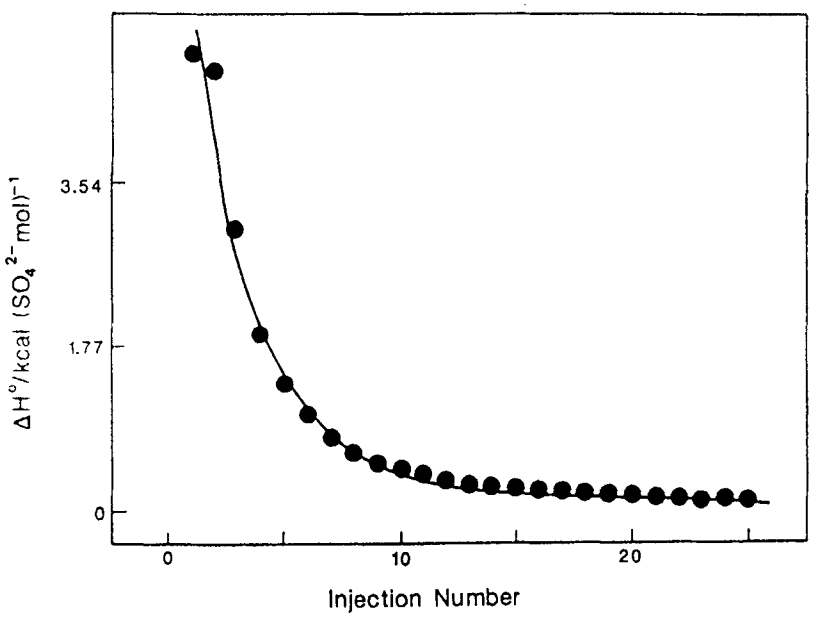

Fig. 9 Titration calorimeter; integrated plot for the titration of aliquots ($5.0 \cdot 10^{-6}$ dm^3) containing SO_4^{2-} (aq; $22 \cdot 10^{-3}$ mol·dm^{-3}) into 1.4115 cm^3 of DOAB (aq; $1 \cdot 10^{-3}$ mol·dm^{-3})

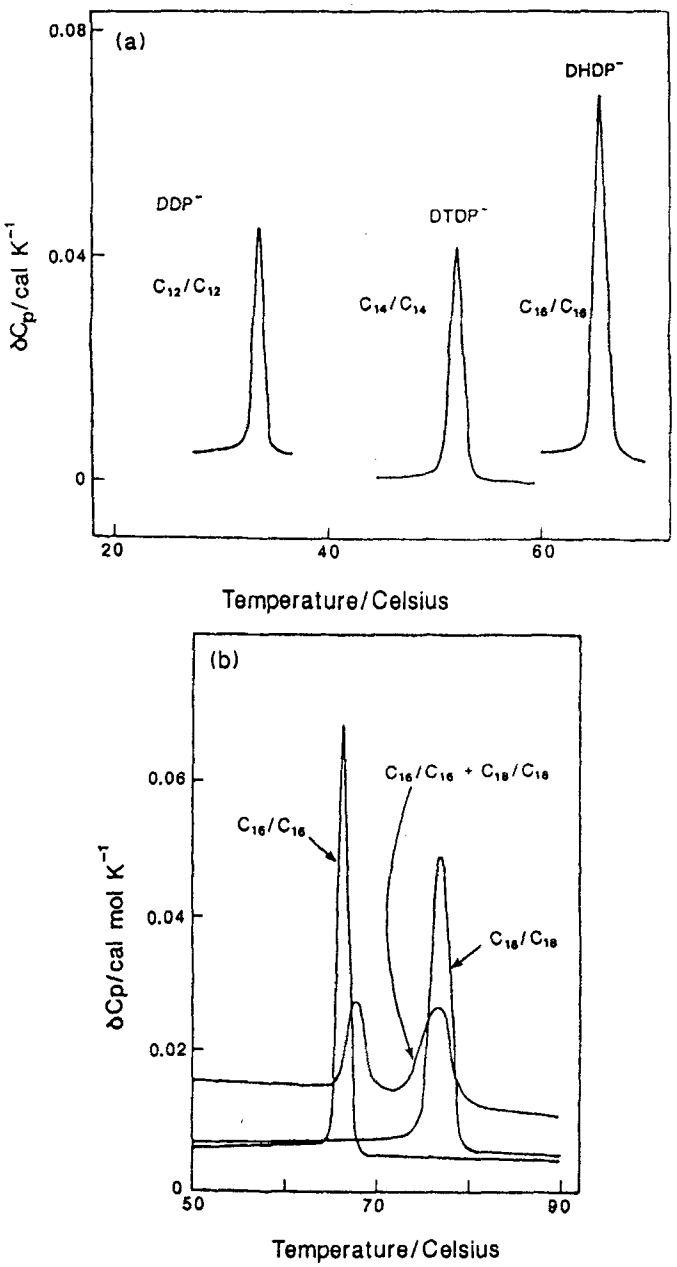

Fig. 10 DSC; gel-to-liquid crystal melting temperatures for dialkylphosphate vesicles ($8.4 \cdot 10^{-4}$ mol·dm^{-3}); (a) dependence of T_m on chain length for potassium salts, (b) dependence of relative heat capacities on temperature for mixture of two sodium dialkylphosphates

An important biological process is the fusion of natural lipid bilayer membranes. In analogous fashion vesicles formed from simple synthetic surfactants also fuse, a process initiated by fusogenic agents. This process can be probed using a titration microcalorimeter [21]. To our surprise, the fusogenic process initiated by adding sulphate (sodium) to DOAB(aq) is endothermic (Fig. 9). However, detailed consideration shows that the processes recorded by the calorimeter are linked with processes involving anions in the double layer around the DOAB head groups rather than the actual coalescence of the bilayer structures.

Recently we have examined in considerable detail the characteristics of vesicles formed by (sodium) didodecylphosphate (DDP). For vesicles formed by this surfactant we record a melting temperature T_m at 35°C; the co-operative melting involving a patch of around 170 monomers in a solution prepared using $8.42 \cdot 10^{-3}$ mol·dm^{-3}. The melting temperature depends on alkyl chain length, T_m, increasing with increase in chain length (Fig. 10). The melting temperature also depends on the counter cation, decreasing on going from Na^+ to Me_4N^+. We have also investigated the DSC traces recorded for mixtures of dialkylphosphate vesicular solutions. Where the chain lengths differ by one or two methylene groups, the traces show evidence of separate domains within the vesicles. However the traces for mixtures of systems having quite different chain lengths are very complicated and no simple pattern seems to emerge. Overall the patterns point to the key role played by the stereochemical properties of the alkyl chains in determining cohesion within the bilayers.

Enzymes

As noted in the Introduction, we have in this Review tracked systems of increasing complexity. The last group to consider here centres on solutions containing enzymes. Chloramphenicol acetyltransferase is an enzyme responsible for induced bacterial resistance to the antibiotic, chloramphenicol [22, 23]. CAT is a trimer in which each subunit has molecular weight 25 000. The integrity of the trimer is critical to the catalytic activity. Enzymes undergo a transition at a characteristic temperature as the structure changes reversibly or irreversibly from catalytically active to inactive forms. CAT denaturation is irreversible in the DSC experiment and shows a high thermal stability. The transition for CAT (aq; TRIS buffer *pH* 7.5; 0.44 mg·cm^{-3}) occurs at 84°C with an enthalpy of denaturation equal to 290 kcal·mol^{-1} (Fig. 11). In the catalytic reactions, the key step involves binding of chloramphenicol to CAT. We have used the titration calorimeter to show that the standard enthalpy of binding is -43.1 kJ·mol^{-1}.

In our last example, we have used DSC to probe the domain structure of DNA gyrase [24] from *Escherichia coli*. The enzyme introduces negative su-

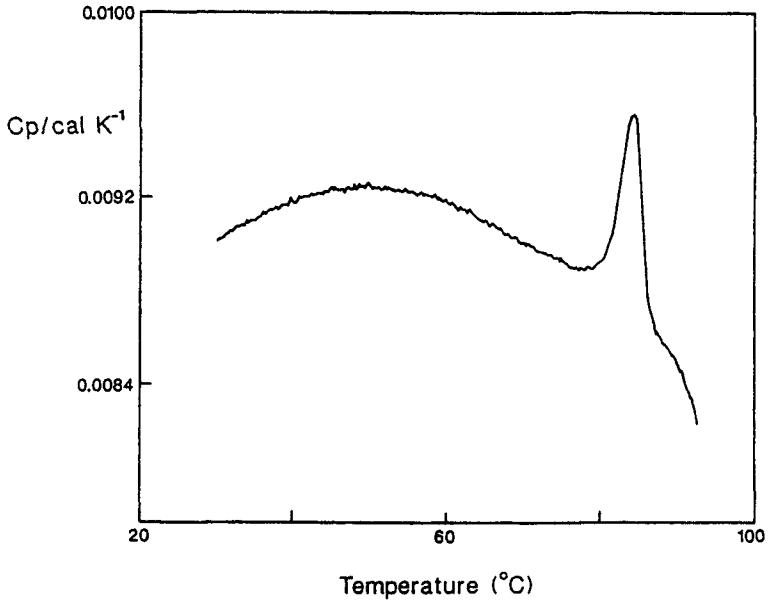

Fig. 11 DSC curve for CAT showing thermal denaturation at 84°C

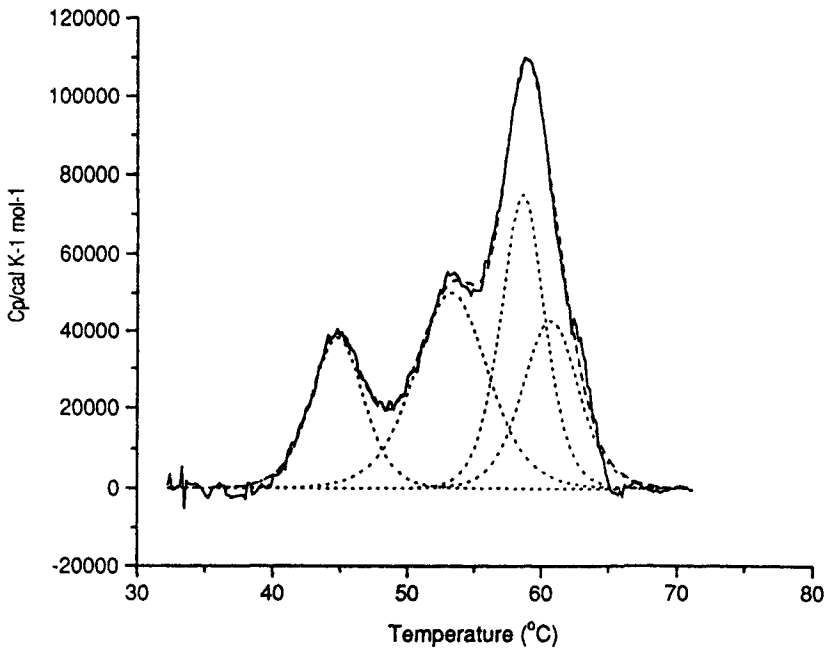

Fig. 12 DSC scans; *E. coli* DNA gyrase (A_2B_2; aq; 1.4 mg·cm^{-3}); the data are shown with reference to four independent transitions

percoils into closed-circular DNA using energy coupled with ATP hydrolysis. Remarkably, the DSC scans show at least four transitions with T_m's near 44.8, 53.3, 58.6 and 60.7°C. Further evidence leading to identification of the domains responsible for these transitions are obtained from the traces for the subunits of the A_2B_2 enzyme and for genetically engineered fragments.

* * *

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Zusammenfassung — Durch Einsatz von Angaben aus DS- und Titrationsmikrokalorimetrie kann ein erheblicher Umfang an Informationen über Makromoleküle in wäßrigen Lösungen erhalten werden. Diese Behauptung wird durch Bezugnahme auf Beispiele aus jüngsten Arbeiten über Mizellen, Bläschen und Enzyme bestätigt.