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DIFFERENTIAL SCANNING CALORIMETRY AND HYDRODYNAMIC STUDY OF BACTERIAL VIRUSES
About possible heat effects in hermetically closed calorimetric vessels with free volume above the liquid

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Abstract

The phase transitions occurring in aqueous solutions of macromolecules and their complex structures (like order–disorder transitions in proteins or nucleic acids solutions) are usually accompanied by small changes in their specific partial volumes. If the quantity of these substances in the closed calorimetric vessels is relatively large (few mg) and if the phase transition is accompanied by a high change of specific partial volume (like in solution of bacteriophages), it is possible to detect some *imaginary* heat effects in the DSC calorimeters which have closed (sealed) vessels with free volume above the liquid.

Keywords: DSC, heat effects in phage suspensions, partial volume effects, sealed calorimetric vessels

Introduction

One of the most important problems in differential microcalorimetry is filling of both vessels with equal quantities of the standard and investigated liquids and also the existence of free volume above the liquid. It is easily seen that if we measure the difference heat capacity with the precision of 10^{-5} J K^{-1}, the precision of filling the vessels must be not less than 10^{-5} g.

Calorimeters with a construction in which vessels are removed, are characterized by bad reproducibility of the results: after each procedure of refilling, the slope of the registered imaginary line of difference heat capacity is changed.

The accuracy of the measurement of the heat capacity of a liquid also depends on the existence of free volume above the liquid. It is clear that it is impossible to fill the sealed vessels because the heat coefficients of expansion of liquid and vessels are

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different. If there is free volume in the vessels, additional vaporization occurs in the process of heating, with additional energy cost. The vapour pressures above the solvent and the solution of macromolecules are practically identical, but the difference on the heat effect of vaporization is very high because the specific heat of vaporization is very high (~2000 J g^{-1} for water). This effect increases with the increase of free volume, but a decrease of free volume produces other effects related with the expansion of the liquid by heating (see next paragraph). This problem can be solved by using a DSC with measurements of definite volume of substance. This type of microcalorimeters have a non-removable block and the heat capacity is measured under an excess constant pressure (Figs 1 and 2) [1–4]. It is important to notice that the main advantage of the capillary calorimetric vessel (Fig. 1a and b) as compared with the cylindrical one (Fig. 2) is the smaller temperature gradient inside the vessels [1, 2, 4]. This gives the possibility of measuring the heat capacity of macromolecule solutions with high (and not constant during the time of scanning) viscosity [1, 2, 4].

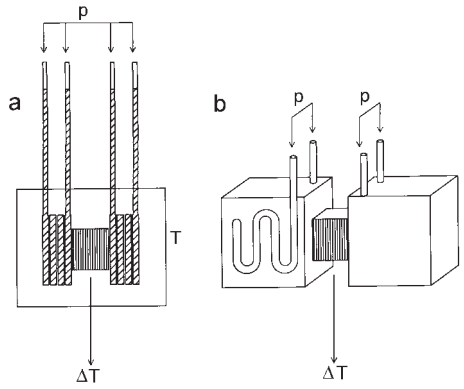

Fig. 1 Platinum capillary calorimetric vessels [1] (a); Capillary calorimetric vessels: solid blocks of gold with platinum inlet and outlet tubes [4] (b), ΔT – thermosensors; T – thermal shield; p – excess constant pressure

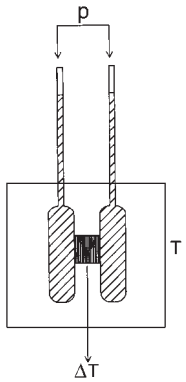

Fig. 2 Calorimetric vessels of gold with inlet capillary tubes for filling and cleaning [3], ΔT – thermosensors; T – thermal shield; p – excess constant pressure

In differential calorimetry, we measure the difference between the heat capacities of two vessels containing the solution of macromolecules (phages) and the solvent. The difference in heat capacity between a solution containing biological materials (proteins, nucleic acids, membranes, phages) and the pure solvent is negative because the heat capacity of the solution is smaller than the heat capacity of the same volume of solvent. For the observed difference in heat capacity between the solution and the solvent, we have:

$$-\Delta C_p(T)^{ap}_{pr,sol/solv} = C_p(T)_{pr}\, m(T)_{pr} - C_p(T)_{solv}\, \Delta m(T)_{solv} \quad (1)$$

where, $C_p(T)_{pr}$ is the partial specific heat capacity of the protein at temperature T, $m(T)_{pr}$ is the mass of protein which is in the calorimetric vessel at temperature T and $\Delta m(T)_{solv}$ is the mass of solvent displaced by proteins in solution. The latter is:

$$\Delta m(T)_{solv} = m(T)_{pr}\,[v(T)_{prot}/v(T)_{solv}] \quad (2)$$

where, $v(T)_{pr}$ and $v(T)_{solv}$ are, respectively, the partial specific volume of proteins and the partial specific volume of the solvent, at temperature T.

Therefore, the value of ΔC_p depends on the balance between all the terms in the above relations.

From Eqs (1) and (2) we obtain the partial specific heat capacity of the protein:

$$C_p(T)_{pr} = C_p(T)_{solv}\,[v(T)_{prot}/v(T)_{solv}] - \Delta C_p(T)^{ap}_{pr,sol/solv}/m(T)_{pr} \quad (3)$$

The specific heat capacity of the solvent can be determined over the desired temperature range by the same method, using as reference pure water, the specific volume and heat capacity of which are well known. The operational volume of the calorimetric vessel and the mass of protein in the vessel at temperature T can be calculated from their values at room temperature and known coefficients of thermal expansion of the solution and vessel material (for details [4]).

It should be noted that the protein (phage) heat capacity determined by the above equation is not strictly the partial heat capacity since the latter is the value that is obtained by extrapolation to an infinite dilute solution. However, if we take into account that the protein concentration in the solution used for the scanning calorimetric experiment is usually less than 10^{-4} M, and this solution does not show any concentration dependence of the heat capacity, it becomes evident that the value determined by Eq. (3) can be considered as a partial heat capacity of the protein in solution (for details [4] and references therein).

Results and discussion

Heat effects in hermetically closed calorimetric vessels

The phase transitions occurring in aqueous solutions of macromolecules and their complex structures (like order–disorder transitions in proteins or nucleic acids solutions) are usually accompanied by small changes in their specific partial volumes. If

the quantity of these substances in the closed calorimetric vessels is relatively large (few mg) and if the phase transition is accompanied by a high change of specific partial volume, it is possible to detect some imaginary heat effects in DSC calorimeters which have closed vessels (sealed vessels) with free volume above the liquid (Fig. 3). This kind of DSC instruments (like SETARAM micro DSC III) have very high sensitivity but without the possibility of measuring the heat capacity at constant pressure, in comparison with capillary type vessels used in DSC DASM–4 (Fig. 1a) [1] or in the new generation of *nanocalorimeters* (Figs 1b and 2) [3, 4].

If we admit that in the aqueous solutions of macromolecules the partial volume of macromolecules increases significantly (in the temperature interval where phase transition occurs) this can produce two kinds of heat effects:

I) Heat effect related with *production of work* by the solution in the hermetically closed (sealed) vessel, where partial volume is increasing. This *work* can be calculated using the well-known relation:

$$W = Q = p\Delta V \tag{4}$$

where p is the pressure inside the hermetically closed vessel (equal to the pressure inside the vessel in the phase transition region); ΔV is the increment of the volume of solution (this is a result of changing of partial volumes of macromolecules in the process of phase transition):

$$\Delta V = (v_2 - v_1)m \tag{5}$$

where v_1 and v_2 are the specific partial volumes of macromolecules, before and after the phase transition and m is the mass of protein (phages).

The equation

$$W = -nRT \ln(V_{\text{final}}/V_{\text{initial}}) \tag{6}$$

could be used to calculate the work of an isothermal compression of the liquid. However, the expansion of the liquid is not really an isothermal process – in the phage solution, the volume change is the result of a phase transition that takes place in a temperature interval (40–78°C).

II) Heat effect resulting from the condensation of saturated water vapour, associated with volume change.

Heat effects in solutions of bacteriophages in the temperature interval of ejection of ds-DNA

The heat effects for solutions of bacteriophages in the temperature interval where DNA releases from the head of phages (see previous paper [6] in this volume) can be calculated. In order to do that, two states must be considered: the phages in native and disrupted states. In the native state the specific partial volume of phages is $[v_1]=1/\rho_1=0.65$ mL g^{-1} and in the disordered state of the phages the specific partial volume is $[v_2]=1/\rho_2=1.91$ mL g^{-1} [6, 7].

If we have in the calorimetric vessel 1 mL of suspension of phages with a concentration of 1 mg mL^{-1}, at the temperature of disruption of phages (T~70°C) the volume of solution in the closed vessel (Fig. 3) increases:

$$\Delta V = (v_2 - v_1)\ \text{mL g}^{-1} \cdot 10^{-3}\ \text{g} = 1.26 \cdot 10^{-3}\ \text{mL} = 1.26 \cdot 10^{-9}\ \text{m}^3 \quad (5a)$$

So the disruption of 1 mg of phages produces an increase of volume $\Delta V = 1.26 \cdot 10^{-3}$ mL$=1.26 \cdot 10^{-9}$ m^3. Really the percentage increase volume is ΔV=194%.

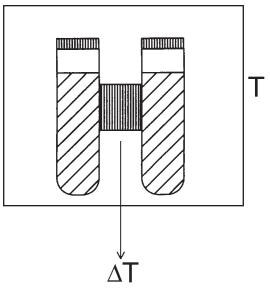

Fig. 3 Hastelloy C 276 sealed twin vessels [5], ΔT – thermosensors; T – thermal shield

Calculation of the heat effects

I) The heat effect originated by the increment of volume is:

$$Q_1 = p_1 \Delta V \quad (7)$$

where p_1 is the pressure in the hermetically sealed vessel at a given temperature (in our case it is the temperature of release of DNA from the phage head – T~70°C).

At room temperature (25°C) this pressure is $p_0=10^5$ Pa. This is the pressure in the vessel when it is sealed. If temperature increases the pressure also increases and $p_1 > p_0$. In our conditions $p_1=1.2\ p_0$. This result is based on Charles law: $p_1/p_0 = T_1/T_0$ where T_1=343 K and T_o=298 K.

$$Q_1 = 1.2 \cdot 10^5\ \text{Pa}\ (-1.26 \cdot 10^{-9}\ \text{m}^3) = -1.5 \cdot 10^{-4}\ \text{J} \quad (7a)$$

II) The heat effect resulting from condensation of saturated water vapour contained in volume $\Delta V = 1.26 \cdot 10^{-9}$, at temperature T=70°C, is:

$$Q_2 = Lm \quad (8)$$

where L is the heat of condensation of saturated water vapour ($L = -2.3$ K J g^{-1}) and m is the mass of condensed vapour.

The value of m at 70°C can be calculated using the equation:

$$pV = mRT/M \quad (9)$$

or

$$m = pVM/RT \quad (10)$$

where p is the pressure of saturated water vapour at 70°C, $p=3.1\cdot10^4$ Pa (value from the literature); $V=1.26\cdot10^{-9}$ m^3, M is the molar mass of water, $M=18.0$ g mol^{-1}; $R=8.314$ J K^{-1} mol^{-1}; T is the phase transition temperature, $T=343$ K, and we obtain:

$$m=3.1\cdot10^4 \text{ Pa}\cdot1.26\cdot10^{-9} \text{ m}^3\cdot18.0 \text{ g mol}^{-1}/(8.314 \text{ J K}^{-1} \text{ mol}^{-1}\cdot343 \text{ K})=2.4\cdot10^{-7} \text{ g} \quad (10a)$$

and
$$Q_2=Lm=-2.3\cdot10^3 \text{ J g}^{-1}\cdot2.4\cdot10^{-7} \text{ g}=-5.5\cdot10^{-4} \text{ J} \quad (8a)$$

Therefore, the total heat effect is:

$$Q=Q_1+Q_2=(-1.5\cdot10^{-4}-5.5\cdot10^{-4}) \text{ J}=-7.0\cdot10^{-4} \text{ J} \quad (11)$$

Heat effects of this magnitude can be detected by using such sensitive instruments as SETARAM micro DSC.

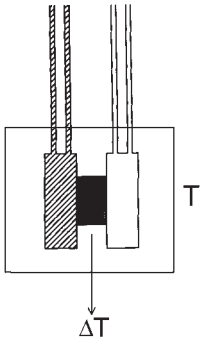

Fig. 4 Hastelloy C 276 liquid heat capacity vessels with tubes [5], ΔT – thermosensors; T – thermal shield

Thus we suggest that the accuracy of measurement of the heat effect of a biological liquid using closed calorimetric vessels depends on a correction term due to the vapour phase above the liquid. Fortunately, in order to overcome this difficulty, a special calorimetric vessel has been designed by SETARAM (Fig. 4). The main feature of this vessel is a tube welded to the experimental vessel, which is filled through the tube by means of a syringe, until the liquid comes out through a second tube. When the liquid is heated it expands freely in the tubes but the volume of liquid in the vessel, located in the detection zone of the calorimeter, remains constant. The determination of the heat capacity of this corresponding volume is achieved using the step-heating mode [5].

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