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HEAT CONDUCTION MICROCALORIMETER FOR THERMOKINETICS AND TITRATION EXPERIMENTS

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Abstract

A heat conduction microcalorimeter with a sensitivity of 103 μV mW^{-1} and a vessel of 3 cm^3 has been designed. The device is equipped with an automatic dosimeter, an original system for injection in titration experiments, and a PC for control, data recording and data analysis. The software allows the determination of (i) overall heat effects, (ii) thermokinetics, and (iii) changes in enthalpy and equilibrium constants in titration experiments.

Keywords: microcalorimeter, thermokinetics, titration

Introduction

Recent decades have seen increasing interest in the application of calorimetry in studies of biological and biochemical processes [1–3]. The characteristic feature of these processes is in general very small heat effects. High-precision calorimetry is therefore necessary for their study. For various types of binding reactions, e.g. enzyme reactions or macromolecule complexations, the equilibrium constant (K) is very important. The application of titration inside the microcalorimeter also allows the determination of all binding parameters (K, ΔH, and ΔS) in a single experiment, i.e. without reloading the measuring vessel.

The up-to-date technological possibilities were utilized to design a new microcalorimeter. It can also be used as part of a combined device KR [4], elaborated previously in the Institute of Physical Chemistry of the Polish Academy of Sciences.

Design of the microcalorimeter

The calorimeter system consists of several blocks, besides the microcalorimeter itself. They serve for thermostating, recording of the signal, stirring, calibration and injection during titration (Fig. 1).

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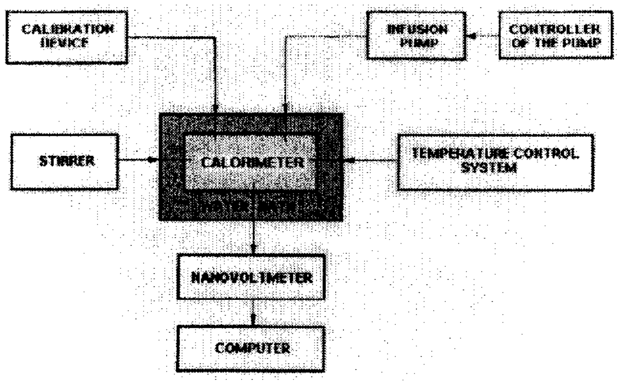

Fig. 1 Block diagram of calorimetric system

The elaborated microcalorimeter is of the heat conduction type, in which the heat evolved in the vessel is quickly conducted to the isothermal heat sink. The device consists of four identical measuring units (Fig. 2), which can function as a system of individual calorimeters or in a differential way. These units are installed in a common thermostat. This consists of an aluminium block (2) placed in a double-walled metal box and then immersed in a water-bath (1). Thermal stability of the calorimetric vessel (11) is achieved by temperature control of the

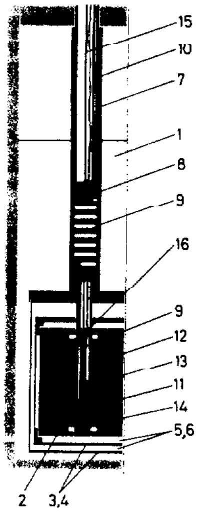

Fig. 2 Cross-section of a single unit of the microcalorimeters: (1) water-bath; (2) aluminium block; (3), (4) metal shields; (5), (6) insulation (air); (7) plastic tube; (8) thermostat of injection capillary; (9) injection capillary, (10) brass tube (sleeve for introduction of vessel); (11) calorimetric vessel; (12) calibration heater; (13) stirrer; (14) Peltier thermopile; (15) rod to stirrer motor; (16) lid of vessel

water-bath with a precision of $5 \cdot 10^{-4}$ K (temperature controller type 650, UNIPAN), the damping effect of the successive metal and insulating shields (3–6), and the main heat sink (2). Changes in the temperature of the calorimetric vessel (11) are measured by means of the semiconductor Peltier element battery localized between the isothermal block (2) and the jacket of the vessel. Intensive heat exchange between these elements and the block (2) ensures quasi-isothermal conditions for the studied process.

The calorimetric signal, θ (named the output function, the response to the heat evolved), from the Peltier thermopile (14) is recorded by means of a Keithley 181 nanovoltmeter and stored in the computer memory. The signal runs can also be observed on the monitor. The calorimetric vessel has a volume of 3 cm^3. The vessel is screwed onto a plastic tube (7), where the vessel lid (16) is mounted. Tightness of the vessel-lid seal is attained by means of a Teflon ring. In the lid, there are three holes. One is for the stirrer rod. Through the others, the injection tube and the calibration heater are tightly introduced into the vessel. The vessel, the lid, the stirrer (12) and the heater jacket are made from tantalum. In the middle part of the tube (7), a copper block (8) is installed. This is in good thermal contact with the thermostated metal tube (10) and plays the role of a thermostat for the injection capillary (9) (Teflon or stainless steel) which passes through it in the form of a spiral. At the lower end of the capillary, a needle (inner diameter 0.2 mm) is mounted. The effusion end of the needle is situated in the central part of the vessel, which is the sample compartment. By means of this injection set, the desired amount of liquid reagent can be introduced into the vessel, in one portion or in a periodic mode. The latter is used in stepwise titration experiments. In this case, an automated dosimetric device is used. The device consists of an infusion pump (type 340A, UNIPAN), a digital controller of the pump (home-made) and a 2 cm^3 glass or plastic syringe. By this means, a set of injections of the reagent in the required doses (precision 0.01 mm^3) and at the required time intervals can be realized.

Experimental procedures and methods

Calorimetric experiments were carried out at 298.15 K (25°C). The vessel was loaded with 2–2.5 cm^3 liquid sample (2.5 cm^3 of water in the case of calibration) outside the calorimeter and then inserted into the measurement position. Stirring was applied during each experiment (rate 60 rpm). After 40–60 min of temperature equilibration, it was possible to begin the signal recording. The stability of the baseline was ±0.05 μV on a short time scale (ca. 1 h) and 0.5 μV during one day.

For testing of the device, electrical calibration and two complexation experiments were performed.

Calibration

For calibration, the electric heater installed inside the vessel was used. Heat effects were produced by means of the calibrator (home-made), which allows the generation of a known current during required periods. Three types of experiments were performed:

1) Steady-state calorimetric signals, θ, were attained for constant heat effects of various power P_i. The values of the heat loss coefficient, α, were evaluated: $\alpha = P/\theta$.

2) Cooling curves were recorded, and the main time constant τ_1 of the calorimeter was determined.

3) Five square impulses of various constant thermal power, each of 6 s duration, were generated in 3 min time invervals. Determination of the total heat, Q, the thermokinetics, $P(t)$, ($P(t) \equiv \mathrm{d}Q/\mathrm{d}t$), and verification of α on the basis of the recorded $\theta(t)$ were carried out. For the thermokinetics determination, an algorithm based on a 1st-order differential equation [5] was applied. This equation is

$$P(t) = \alpha\left[\tau_1 \frac{\mathrm{d}\theta(t)}{\mathrm{d}t} + \theta(t)\right] \quad (1)$$

Complexation

Two types of experiments were performed. The aim of the first type was the determination of total heat and thermokinetics. The aim of the second one was the determination of binding constant K and enthalpy change ΔH.

1) *D*-mannonaphtho-crown 6 ether was complexed with *L*-phenylalanine. The vessel was charged with 2 cm^3 of a 0.01 M aqueous solution of ether. To this solution, 0.2 cm^3 of a 0.1 M solution of *L*-phenylalanine was injected by capillary. The total heat effect of the reaction and thermokinetics were determined by using Eq. (1).

2) Titration experiments were performed for the binding reaction of Ba^{2+} to 18 crown 6 ether. This reaction is recommended for the testing of calorimeters when the equilibrium constant and the enthalpy change are to be determined [6]. The vessel contained 2 cm^3 of a 0.01 M aqueous solution of 18 crown 6 ether (Sigma product). After thermal equilibration, stepwise titration with a 0.1 M solution of $BaCl_2$ (Roth product) was carried out. About 26.7 mm^3 of Ba^{2+} solution was added to the vessel in each injection. Fifteen injections at 20 min intervals were made. A separate titration experiment was carried out for determination of the heat of dilution of Ba^{2+} solution in water.

K and ΔH were computed by means of an iterative procedure [7], where the conjugate gradient [8] of minimization of the function $\chi^2 = \sum (Q_{n,\mathrm{calc.}} - Q_{n,\mathrm{exp.}})^2$ was applied [8, 9]. The Q_n values are the sums of the heat portions evolved from the 1st to the *n*-th injection.

Results

It was observed that the values of the heat loss coefficient α for the designed device are nearly independent of the thermal power evolved in the vessel (Table 1). This indicates that the calorimeter can be treated as a linear dynamic object (of constant parameters) in a sufficiently large range of the measured signal. Such a property simplifies the mathematical treatment of the results. The mean value of α, 9.7 W V^{-1}, corresponds to a sensitivity of 103 μV mW^{-1}.

Table 1 Heat loss coefficient α for various thermal powers (verification of linearity of the system)

Thermal power, P/W	Signal, θ/V	α/W V^{-1}
$9.70\cdot10^{-6}$	$1.0\cdot10^{-6}$	9.70
$9.46\cdot10^{-5}$	$9.8\cdot10^{-6}$	9.65
$3.813\cdot10^{-4}$	$3.94\cdot10^{-5}$	9.68
$1.287\cdot10^{-3}$	$1.32\cdot10^{-4}$	9.74
$2.424\cdot10^{-3}$	$2.50\cdot10^{-4}$	9.69

Besides the heat loss coefficient, dynamic properties of the device were also determined. The main time constant, τ_1, of the vessel (loaded with 2.5 ml of water) was 151 s. In the case of relatively slow heat effects (in comparison with the inertial response of the microcalorimeter), the 1st-order equation (Eq. 1) is sufficient for a correct determination of the thermokinetics. For faster effects, a differential equation of higher order [5, 10] or other methods of signal deconvolution are needed [10–12].

In consequence of the inertia of the microcalorimeter, overlapping of the responses to the successive five impulses generated in the vessel was observed (Fig. 3a). Application of the 1st-order differential equation, with only one time constant, deconvolutes this output signal to the course shown in Fig. 3b. Separation of the impulses and their correct localization in the time scale is achieved.

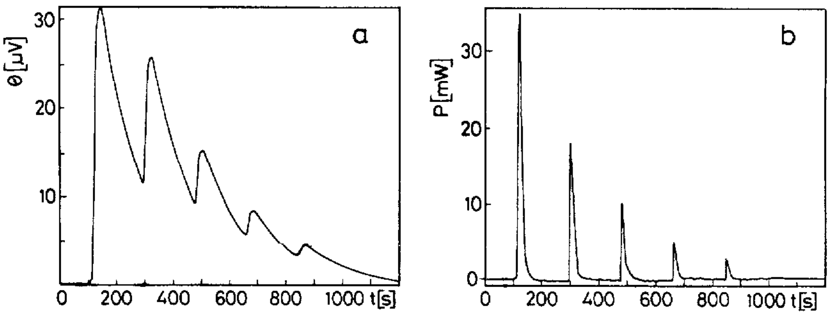

Fig. 3 Calibration series of five impulses (squares); a) calorimetric response; b) thermokinetics, i.e. reconstructed thermal power

The total heat effect calculated on the basis of θ *vs.* t, using α determined from steady-state measurements, is in good agreement (0.2%) with the sum of the energy generated in the vessel during the successive impulses. This confirms the linearity of the system. The characteristics of the microcalorimeter are given in Table 2.

Table 2 Characteristics of the microcalorimeter

Total volume of vessel	3 cm^3
Time constant, τ_1 (2.5 cm^3 of water)	151 s
Sensitivity	0.103 V W^{-1}
Lowest detectable continuous heat effect	$6\cdot10^{-7}$ W
Baseline drift	$\sim0.1\cdot10^{-6}$ V h^{-1}
	$\sim0.5\cdot10^{-6}$ V day^{-1}

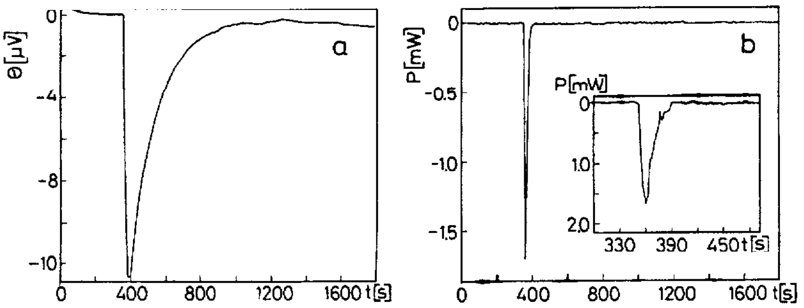

Fig. 4 Complexation of *D*-mannonaphtho-crown 6 with *L*-phenylalanine (1:1 stoichiometry); a) calorimetric response; b) thermokinetics, i.e. reconstructed thermal power

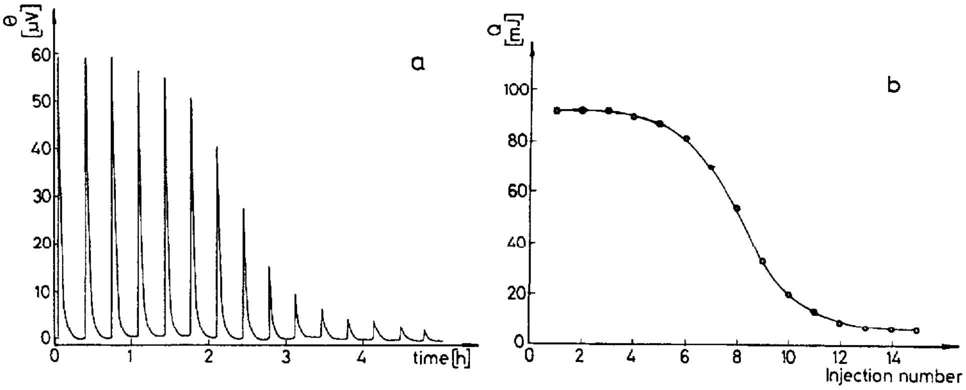

Fig. 5 Titrationof 18 crown 6 with Ba^{2+}; a) calorimetric response, i.e. signal recorded for fifteen injections; b) heats evolved in successive injections

The signal θ obtained for the complexation of *D*-mannonaphtho-crown 6 ether with *L*-phenylalanine (Fig. 4a) indicates that this reaction is accompanied by an endothermic effect. The deconvolution of θ using the 1st-order differential equation revealed a fast heat effect (Fig. 4b), where the duration of the reaction is comparable with that of the reagent injection into the vessel. In a separate measurement, the heat of dilution of *L*-phenylalanine in water was determined. The total heat of the studied complexation was measured: ΔQ=–0.025 J (–1.25 kJ mol^{-1}).

Results of microcalorimeter testing in titration experiments are shown in Fig. 5. The series of peaks of θ obtained for successive injections are presented in Fig. 5a. On this basis, values of heats corresponding to the successive injections were evaluated (Fig. 5b). These were then used in the iterative procedure of K and ΔH calculations. The computed values are K=6098±1128 mol dm^{-3} and ΔH=–32.2+–0.25 kJ mol^{-1}, while those reported by Wadsö [6] are K= 5900±200 mol dm^{-3} and ΔH=–31.42±20 kJ mol^{-1}.

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