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CONTINUOUS GRADIENT METHOD IN FLOW MICROCALORIMETRY. PART I. RELATION BETWEEN ELECTRICAL AND CHEMICAL CALIBRATION

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ABSTRACT

The application of the continuous gradient techniques to flow microcalorimetry, requires the introduction of corrections for the thermal lag due to the high instrumental time constant of heat leakage calorimeters. To this purpose, the system must be properly modeled, with careful consideration of dynamic corrections. Therefore, the calorimetric response was analysed both for electrical and chemical input and Tian's equation parameters were evaluated, when applied to flow microcalorimetry. It is demonstrated how it is possible to calculate the power emitted by a given process, in a steady-state experiment, independently from the electrical calibration, taking into account the residual heat flowing out with the liquid. Application to the protonation enthalpies of D-cycloserine is shown.

INTRODUCTION

Almost every chemical, physical and biological process takes place with heat consumption or evolution. Calorimetric techniques are more and more widely used, not only to obtain thermodynamic information, but also as analytical tools to measure, in a non-destructive way, the entity of a given event. In biological investigations, it is important to use microcalorimeters the response of which is proportional to the thermal power (heat-leakage calorimeters), because the power developed characterizes, more or less unequivocally, a complex biological process [1,2]. In the field of solution equilibria, in order to evaluate the enthalpies of the metal–ligand equilibrium reactions with good accuracy, a large number of experimental points is needed, particularly when several species are present. The same argument is valid when kinetic experiments are carried out. Even if the flow microcalorimeter [2–4], one of the several types of heat-leakage instruments commercially available, is a speedy and manageable apparatus, the time required to acquire a single point in a steady-state experiment is considerably long. This calorimeter can operate: (a) in flow-through mode, suitable

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for studying biological systems or sufficiently slow kinetics, in which the reaction is started in an external vessel and the reacting solution flows at a constant flow rate through the calorimetric cell; (b) in mixing mode, suited to fast reactions, in which two different solutions are pumped continuously through the measuring cell where they are mixed and react. In the latter mode, one of the feeding pumps can be connected to a continuous gradient generating device, so as to realize a sort of "continuous titration" and to collect a large number of points by a single experimental run [5–7]. In such a way, it is necessary to extract from the instrumental signal the actual instantaneous thermal output and to correlate it with the concentration of the eluteol reactant. In order to solve this problem, we looked for an accurate evaluation of the parameters affecting the calorimetric response and first of all we have tried to verify if there is any discrepancy between different electrical or chemical calibration procedures. As a matter of fact, according to our experience, the thermal data obtained by flow microcalorimetry are greater than those obtained by other calorimetric techniques. This fact suggests the hypothesis of a systematic error, probably due to the kind of instrumental calibration adopted.

EXPERIMENTAL

A flow microcalorimeter (LKB 2107), kept in a thermostated room at 25 ± 0.5 °C, was employed. The solutions were delivered into the mixing cell by LKB Perspex 10200 peristaltic pumps. In addition to the standard equipment, the calorimeter was connected to a Hewlett-Packard 3555A digital voltmeter and 5150A thermal printer with timer, in order to facilitate collection of the calorimetric data and to study cooling kinetics.

The experiments concerning the electrical calibration were performed as described by Poore and Beezer [8], varying the flow rate from zero to 0.01 $cm^3\ sec^{-1}$.

The chemical calibration data were obtained by a strong acid–strong base neutralization reaction with solutions of the following concentrations: HCl 1×10^{-3} mol dm^{-3} and KOH 2×10^{-3} mol dm^{-3}. The heats so obtained were corrected for the dilution.

The instrumental deflections were measured referring to a base line obtained from a background solution flowing at the same rate as the reaction liquid. The reactant solutions were introduced by switching on three-way HPLC stopcocks.

DATA PROCESSING

All experimental data were processed by programs working on an IBM XT personal computer. A set of calculation programs in BASIC were assembled in order to evaluate the calibration parameters and to determine the enthalpies for proton–ligand and metal–ligand equilibrium reactions directly from flow microcalorimetric data.

The program REDA [9] stores the experimental parameters: reacting solutions composition, flow rates, density, thermal data, number and kind of species present in solution with their relative equilibrium constants. The program GORIZIA [9] computes: (i) the solution composition before and after reaction, solving the mass balance equations by the iterative Newton–Raphson method; (ii) the thermal output from the electrical calibration or from Tian's equation parameters; (iii) the reaction enthalpies by a least-squares regression.

MODEL FOR THE MICROCALORIMETRIC RESPONSE

The relation between power developed by a given process, W, and the calorimetric response, Δ, depends on the system used and is described by Tian's equation [1,8]. When applied to flow microcalorimetry, the equation becomes:

$$W = \alpha_0 \Delta + \eta_1 \frac{\mathrm{d}\Delta}{\mathrm{d}t} + \eta_2 \frac{\mathrm{d}^2\Delta}{\mathrm{d}t^2} + \ldots + \eta_n \frac{\mathrm{d}^n\Delta}{\mathrm{d}t^n} + \beta \tag{1}$$

where α_0 is the calibration constant in static condition or exchange coefficient. The parameters $\eta_1, \eta_2, \ldots, \eta_n$ depend on the calorimetric time constants $\tau_1, \tau_2, \ldots, \tau_n$ and on the exchange coefficient α_0; β is the correction term for the power flowing out of the cell with the liquid.

The α_0 parameter can be evaluated by electrical calibration at steady-state conditions with zero flow rate, in which the derivatives and the β term in eqn. (1) vanish. The signal so obtained is indicated, according to Poore and Beezer [8] as Δ^0_∞. Then,

$$\alpha_0 = W/\Delta^0_\infty \tag{2}$$

The β term is assumed to be linearly proportional to the power supplied, W, and to the flow rate R:

$$\beta = \gamma W R \tag{3}$$

and it can be derived from the difference between the signal at thermal equilibrium, Δ^0_∞, and the steady-state signal, Δ_∞, at R flow rate:

$$\beta = \alpha_0 (\Delta^0_\infty - \Delta_\infty) \tag{4}$$

For the chemical calibration, the value of W is known and the theoretical signal at the thermal equilibrium is obtained simply by dividing W by α_0.

It can be demonstrated, by limiting eqn. (1) to the first derivative, that

$$\eta_1 = \alpha_0 \tau \tag{5}$$

where τ is the calorimetric time constant depending on the flow rate [8]. The electrical calibration is very useful to determine τ_0, the intrinsic time constant at the thermal equilibrium ($R = 0$). It can be estimated by switching off the electrical heating and collecting the couples signal-time of the calorimetric cooling curves. If we assume an exponential decay, at zero flow rate, we obtain $\tau_0 = 71.1(4)$ s, which is in accordance with the literature data [8,10].

RESULTS AND DISCUSSION

In a steady-state experiment, it is assumed that an accurate measurement of the heat evolved is obtained by carrying out an electrical calibration, provided that the electric thermal effect is comparable with that of a chemical reaction taking place in the same experimental conditions. Nevertheless, even in steady-state conditions, a flow microcalorimeter is not in thermal equilibrium, because only a portion of the whole heat generated in the cell is detected. Monk and Wadsö [2] have shown that the electrical calibration constant is a function of the flow rate; Poore and Beezer [8] have been the first to underline the systematic errors deriving from non-equilibrium conditions.

When the instrumental gain is set to the minimum value of 10^3 (sensitivity $\times$ 1) and the instrumental deflection is measured in mV, the intrinsic calibration constant, α_0, from eqn. (2), results 0.1475(1) μW $(\mathrm{mV})^{-1}$. It can be obtained in thermal equilibrium conditions by neglecting the very small fraction of heat, lost in the gap between the cell and the thermopiles.

The β term is the most sensitive to the differences between electrical and chemical calibration. Referring to the electrical calibration and indicating γ of eqn. (3) by γ_e, our results confirm a linear dependence between power and flow rate: from the slopes of the straight lines in Fig. 1 we obtain $\gamma_e = 6.15(5) \times 10^{-3}$ s ml^{-1}.

Figure 2 shows the percentage of the heat loss as a function of R for the chemical reaction: the power loss is linearly correlated with R, the proportionality coefficient γ_c being smaller ($\gamma_c = 5.72(7) \times 10^{-3}$ s ml^{-1}) than γ_e. The difference between γ_c and γ_e, even when small, is statistically significant. This means that the power supplied by a chemical reaction and distributed over the whole cell is revealed more efficiently than the power generated by the Joule effect in the small calibration heater. Even for the chemical reaction, however, there is not a finite flow rate under which either the system can be considered at thermal equilibrium or the electrical calibration is perfectly comparable with a chemical reaction. The discrepancy between electrical and chemical calibration becomes more and more evident with increasing flow rate.

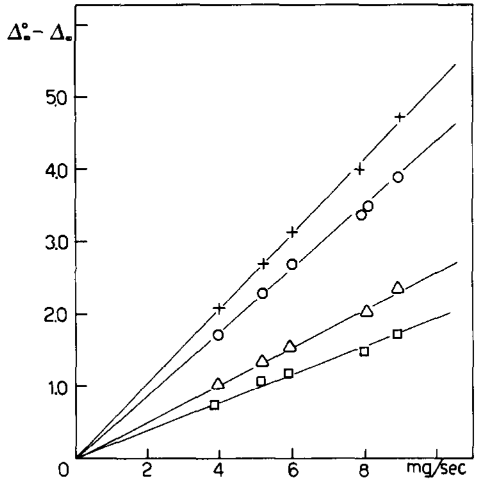

Fig. 1. Electrical calibration: $\Delta^0_\infty - \Delta_\infty$ as a function of the flow rate R for different power input, W: (□) 451 μW; (△) 614 μW; (○) 1014 μW; (+) 1252 μW.

For a fast chemical reaction at the steady state, Tian's equation becomes:

$$W = \alpha_0 \Delta_\infty / (1 - \gamma_c R) \tag{6}$$

When the parameters α_0 and γ_c are known, it is possible to calculate W from the Δ_∞ value, without electrical calibration. By this method, the systematic overestimation of heats connected with the electrical calibration is avoided and much experimental time is saved.

As a test reaction we chose the protonation equilibria of D-cycloserine (D-4-aminoisoxazolidin-3-one, HL) in aqueous solution at 25 °C and $I = 0.1$ mol dm^{-3} KCl. The protonation constants had been evaluated previously

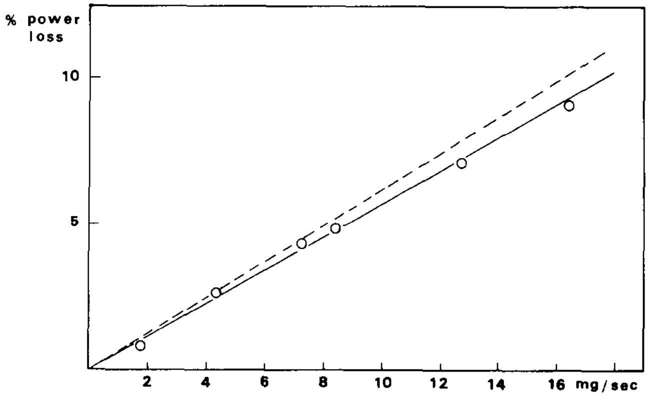

Fig. 2. Percentage power loss for the chemical calibration (———) in comparison with the electrical calibration (- - - -).

TABLE 1

Protonation enthalpies of D-cycloserine (at 25°C and $I = 0.1$ mol dm^{-3} KCl) and comparison of values obtained by electrical and chemical calibration

n	Reaction	$\log K_n$	ΔH^0 (kJ mol^{-1})	
			Electrical calib.	Chemical calib.
1	$L^- + H^+ = HL$	7.346(5)	−33.30(22)	−33.23(22)
2	$HL + H^+ = H_2L^+$	4.388(6)	−15.32(25)	−15.19(25)

[11]. Fifteen steady-state experiments were performed with the relative electrical calibration. The total flow rate was varied between 6 and 10 mg s^{-1}. Data were processed by computer programs [9] starting both from the electrical calibration and from chemical calibration, via Tian's equation. The results are reported in Table 1.

CONCLUSIONS

The agreement between the enthalpy values, obtained with the two types of calibration for the test reaction, is very high, with those from Tian's equation being, as predicted, a little lower; the accuracy of enthalpies is not affected by the method chosen to calculate the power developed, thus demonstrating the perfect reliability also of the chemical calibration parameters. The low values of total flow rate, used in accordance with the manufacturer's recommendations, justify the fact that the difference between the values evaluated by electrical and chemical calibration is within the experimental error: in this range, the systematic error due to the electrical calibration, even if present, is not so high as to become significant.

The correction term, obtained from the chemical calibration as a continuous function of W and R, makes it possible to work with flow rates higher than the recommended ones, without loss of precision or accuracy, and to correct the data directly by a computer program.

When a continuous gradient method is applied, the importance must be stressed of correctly evaluating the thermal output independent from the electrical calibration: because of the high value of the time constant, the dynamic corrections are absolutely necessary and Tian's equation parameters for the chemical reaction must be evaluated.

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