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CONTINUOUS GRADIENT METHOD IN FLOW MICROCALORIMETRY. PART II: APPLICATION TO SOLUTION EQUILIBRIA*

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SUMMARY

A flow microcalorimeter operating in mixing mode generates a type of "continuous titration" when one of the feeding pumps is connected to a continuous gradient generating device. The proper correction of the instrumental signal for the thermal lag of the calorimeter can be performed by chemical calibration, through an evaluation of the parameters of Tian's equation, modified for the microcalorimeter. This equation relates the instrumental signal to the actual power developed by the reaction. This paper shows how a continuous exponential gradient method can be applied to the determination of the enthalpy changes of metal-ligand-proton equilibrium reaction in solution. The enthalpies obtained for the protonation of D-cycloserine and for the formation of CuL and CuL_2 complexes in the Cu(II)-Glycine system are in substantial agreement with published values. The computer programs, in BASIC language on an IBM-XT PC, used to process gradient data, are presented.

INTRODUCTION

Heat-leakage flow microcalorimeters are widely used in chemical, biochemical and biological investigations because they present several advantages with respect to other calorimeters (refs. 1-3): simplicity and speed of operation, elimination of the need for equilibration time prior to the experiment, absence of a gaseous phase (particularly relevant when measurements are performed with liquids of high vapor pressure), absence of surface adsorption effects (provided the reactants are allowed to flow until the active centers on the wall are saturated). The LKB 2107 flow microcalorimeter used in this work can operate in either flow-through or mixing mode. In the latter mode, two different solutions are continuously pumped through the measuring cell, where they mix and react.

*For Part I, see ref. 8.

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When proton-ligand-metal (or macromolecule) solution equilibria are studied from a thermodynamic point of view, a large set of experimental points must be collected. The conventional procedure has two serious limitations: *i*) the time required for the point by point determination of the enthalpy changes and *ii*) the quantity of reagents needed to continuously fill up the reaction cell. When the microcalorimeter operates in mixing mode, one of the feeding pumps can be connected to a continuous gradient generating device (refs. 4-8) in order to realize a type of "continuous titration" and to collect a large number of points in a single run.

In Part I (ref. 8), it was shown out how the actual instantaneous thermal output can be extracted from the instrumental signal and correlated with the concentration of the eluted reagent. The experimental parameters affecting the calorimetric response were determined by making a comparison between electrical and chemical calibration. The present paper shows how continuous gradient method can be applied to the determination of enthalpy changes of solution equilibrium reactions.

EXPERIMENTAL

A LKB 2107 flow microcalorimeter in a thermostated room (25.0 ± 0.5 °C) was employed. The control unit was connected to a Hewlett-Packard 3555A digital voltmeter and to a 5150A thermal printer with timer, in order to collect the couples of data (signal-time) of a continuous gradient experiment. The solutions were delivered into the mixing cell by LKB Perspex 10200 peristaltic pumps. The continuous exponential gradient generating device (refs. 4-6) consisted of a magnetically stirred known constant volume vessel, whose cap (fitted with two teflon tubes for the inlet and outlet of reactants) was sealed with epoxidic or siliconic sealant. The instrumental deflections were measured by referring to a base line obtained from a background solution (KCl 0.1 mol/L) flowing at the same rate as the reaction liquid. The reactant solutions were introduced by switching three-way HPLC stopcocks, which meant that it was possible to make experimental corrections for dilution heats at the operative ionic strength. The chemical calibration data were obtained through a strong acid-strong base neutralization reaction (HCl 0.01 mol/L and KOH 0.02 mol/L in KCl 0.1 mol/L) at the same flow rate as the reaction.

D-cycloserine (D-4-aminoisoxazolidin-3-one) Fluka was used without further

purification: a slightly alkaline solution of the ligand (0.03 mol/L) was titrated with HCl (0.03 mol/L) in order to evaluate the protonation enthalpies.

The experiment for determining the formation enthalpies of Cu(II)-Glycine complexes was carried out by titrating with HCl (0.02 mol/L) a slightly alkaline solution of metal (0.01 mol/L) and ligand (0.02 mol/L, Glycine C. Erba recrystallized from water/methanol). The hydrochloric acid was diluted with the background solution of KCl 0.1 mol/L, the dilution starting when the steady state signal was perfectly stable.

MICROCALORIMETRIC RESPONSE AND EXPONENTIAL GRADIENT

The relation between the power developed by a given process, W and the calorimetric response, $\Delta = \Delta(t)$ depends on the instrumental apparatus used and is described by Tian's equation. When applied to the flow microcalorimeter, the equation becomes (refs. 7,8)

$$W' = W - \gamma R W = \alpha_0 \Delta + \eta_1 (d\Delta/dt) + \eta_2 (d^2\Delta/dt^2) + \ldots + \eta_n (d^n\Delta/dt^n) \qquad (1)$$

where W' is the effective power detected by the instrument, γ is the instrumental parameter for the power flowing out of the cell per unit of power W and of flow rate R (ref. 8) and α_0 is the calibration constant in static condition. The parameters $\eta_1, \eta_2, \ldots, \eta_n$ depend on the calorimetric time constants and on the exchange coefficients.

When continuous gradient experiments are carried out, Δ is a function of time and of the concentration of the reagent eluted. In order to transform Δ into the effective power W' measured by the instrument, it is necessary to compute the derivatives of the microcalorimetric signal with respect to time and to evaluate the derivative coefficients in eqn. (1). For a sufficiently approximate description of the power generated by a slow process, only one derivative is sufficient, the power being uniformly generated in the calorimetric cell. For fast reactions (as in the present case) at least two derivatives are needed. In order to evaluate W, the actual power developed by the electric source or by the chemical reaction, the value of γ was determined in separate experiments (ref. 8). We chose to evaluate the parameters of Tian's equation by chemical calibration, instead of determining the dynamic properties of the microcalorimeter by conventional procedures (ref. 9 and refs. therein). In order to emphasize this choice we substitute W_e for W in any expression involving the

real power developed by a chemical reaction.

CHEMICAL CALIBRATION

In steady state conditions, the total chemical power W_c generated by the calibration reaction (the strong base is fed in excess, the strong acid at constant flow rate F and concentration C) is given by

$$W_c = F C \Delta H_w \tag{2}$$

where ΔH_w is the enthalpy change of the reaction $H^+ + OH^- = H_2O$. The value of ΔH_w° at 25 °C and $I = 0.1$ mol/L (KCl), experimentally determined by the same flow microcalorimeter, was -56.39 ± 0.10 kJ/mol.

When the titrant solution is diluted according to a continuous exponential gradient, the concentration C at each time t is given by

$$C = C_0 \exp(-F(t - t_0)/V) \tag{3}$$

where C_0 is the initial concentration of the titrant, t_0 is the starting time for the dilution, V is the volume of the constant volume vessel and F is the flow rate (ref. 5). At each time t, the value of W_c for the calibration reaction results

$$W_c = F C_0 \Delta H_w \exp(-F(t - t_0)/V) \tag{4}$$

In order to obtain the effective power W' detected by the instrument, the chemically produced power W_c must be corrected for the heat lost with the liquid flow (ref. 8)

$$W' = W_c (1 - \gamma_c R) \tag{5}$$

where γ_c is the value of γ in eqn. (1) in the case of the chemical reaction. In these calibration experiments, the couples of data W' (theoretically computed from eqns. (3-5) and Δ (the corresponding experimental signal) are collected.

COMPUTER PROGRAMS AND DATA PROCESSING

A set of programs expecially designed for processing gradient data was written in BASIC and run on an IBM XT Personal Computer. Fig. 1 shows a

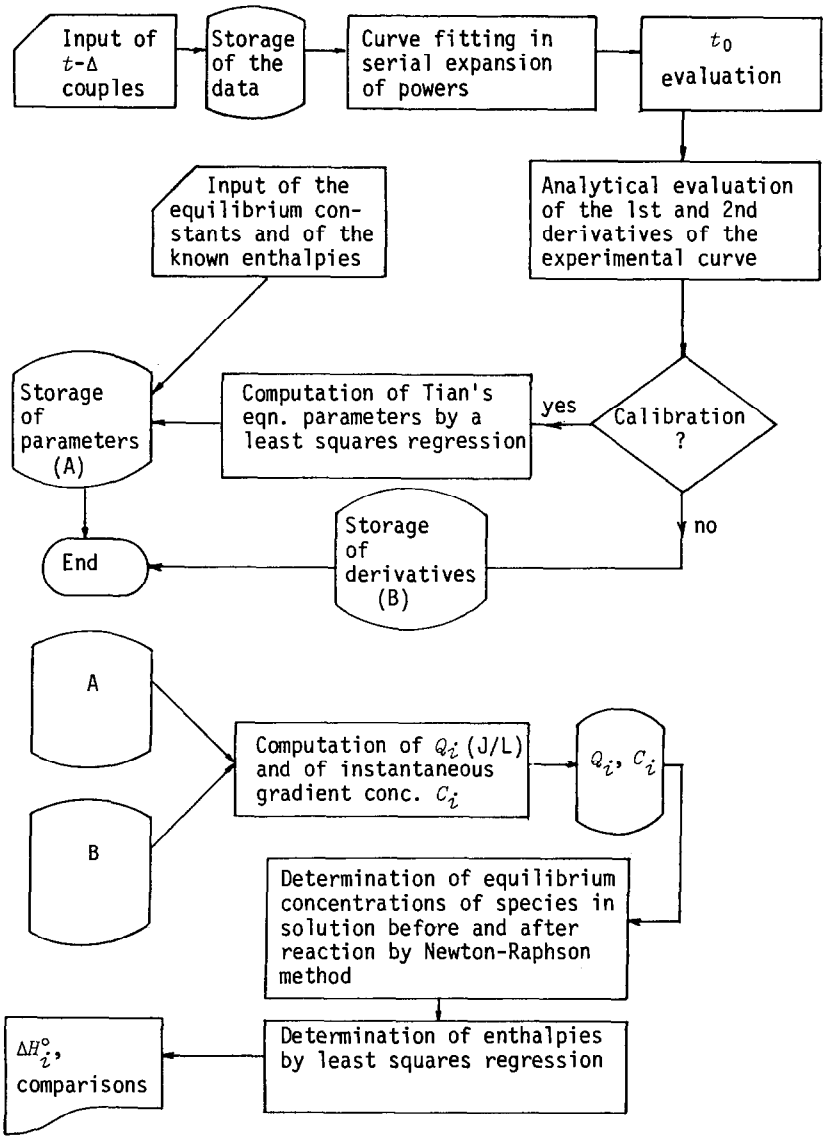

Fig. 1. Schematic flow diagram of the programs for the evaluation of the enthalpies of equilibrium reactions in solution through a continuous gradient experiment.

schematic diagram of the programs. The procedure is as follows: the experimental curve is fitted in a serial expansion of powers of $1/t$ and the first and second derivatives are analytically computed. The instrumental starting time for the dilution, t_0, is mathematically evaluated by extrapolating the initial part of the decay curve to the value of the steady-state signal. For the calibration, the coefficients of the linear function $W' = W'(\Delta,\Delta',\Delta'')$ in eqn. (1) are evaluated by a least squares multiple linear regression. For the reaction under study, there are evaluated: i) C (the instantaneous concentration of the reactant diluted) from eqn. (3), ii) the first and second derivatives of the calorimetric signal as a function of time, iii) the power W_c generated into the cell at each time t, by means of eqn. (1). For each experimental point, the composition of the system at the equilibrium before and after reaction is estimated by solving the system of mass balance equations using the iterative Newton-Raphson method. The formation enthalpies are computed by a least squares regression (ref. 10).

These programs are available upon request.

RESULTS

The reliability of the experimental method applied to the determination of enthalpies of equilibrium reactions in solution and the validity of the computer programs were tested by determining the enthalpies of D-cycloserine protonation and of Cu(II)/Glycine complexation.

In part I (ref. 8), we reported the protonation enthalpies of D-cycloserine as obtained with and without electrical calibration, by using Tian's equation parameters in steady state conditions. In the present work, the continuous gradient data were collected in a single experimental run, starting from a solution at pH 7.4 to a pH after reaction between 4.5 (beginning of the dilution of titrant acid) and 7.2 (end of the dilution). The results are reported in Table 1, in comparison with the values obtained for the same system by conventional techniques (refs. 8,11).

For the Cu(II)-Glycine system, the most relevant species are CuL and CuL_2 (L^- indicates the glycinate anion; the charges are omitted for simplicity) (ref. 12). Recently (ref. 13) two other complexes, CuLH and CuL_3, were identified in particular conditions: the former (quantity always less than 10%) is present in acidic solution and can be neglected at pH > 3; the latter is formed in

TABLE 1

Protonation enthalpies of D-cycloserine anion (L^-) obtained by conventional techniques and by the continuous gradient method in aqueous solution at 25 °C, I = 0.1 mol/L (KCl).

Reaction	$\Delta H°$ / (kJ/mol)		
	LKB 8700[a] titration calorimeter	LKB 2107 flow microcalorimeter	
		steady-state[b] mode	gradient[c] mode
$L^- + H^+ = HL$	-32.25 ± 0.15	-33.23 ± 0.22	-30.80 ± 0.10
$HL + H^+ = H_2L^+$	-14.52 ± 0.15	-15.19 ± 0.25	-15.08 ± 0.13

[a]ref. 11.

[b]15 experiments, ref. 8.

[c]130 points from one experiment, this work.

significant amount only at ligand to metal ratio (L/M) greater than 4.

The determination of the formation enthalpies of CuL and CuL_2 was carried out according to the experimental procedure suggested by Ting Po I and G. Nancollas (ref. 14) but modified for the flow microcalorimeter. The following procedure was adopted: *i*) KOH was added to the solution containing the ligand and the metal (L/M= 2) until an approximately neutral pH was reached, *ii*) the resulting solution was then titrated with HCl, diluted by the exponential gradient generating device, *iii*) the data were collected within a range of pH variations (ΔpH = pH before reaction - pH after reaction) in the interval $4.6 > \Delta pH > 0.7$. The protonation and complexation constants and the protonation enthalpies at I = 0.1 mol/L and 25 °C used to process the calorimetric data were taken from the published sources (ref. 12). The results obtained by the continuous gradient method (130 points in one experiment) are shown in Table 2, in comparison with the literature values (ref. 12).

CONCLUSIONS

The agreement between the enthalpy values obtained through the continuous gradient method and those obtained through other methods is good for D-cycloserine protonation and very satisfactory for Cu(II)/Glycine complexation, thus demonstrating the reliability of the method and of the computational scheme

TABLE 2

Thermodynamic parameters for the Cu(II)-glycine-proton equilibria in aqueous solution at 25 °C and I = 0.1 mol/L (KCl), (L^- = glycinate).

Reaction	Literature values[a]		This work
	log K	$\Delta H°$ / (kJ/mol)	$\Delta H°$ / (kJ/mol)
$L^- + H^+ = HL$	9.57 ± 0.04	-44.35	-
$L^- + 2H^+ = H_2L^+$	11.93 ± 0.04	-48.53 ± 0.40	-
$Cu^{2+} + L^- = CuL^+$	8.15 ± 0.09	-26.4 ± 2.0	-26.39 ± 0.25
$Cu^{2+} + 2L^- = CuL_2$	15.03 ± 0.10	-55.6 ± 2.0	-54.40 ± 0.18

[a]ref. 12.

adopted. It must be pointed out that the values shown in Tables 1 and 2 were obtained in a single experimental run, the small standard deviation being due to the large number of points collected. These results confirm the validity of the model assumed for the microcalorimetric response and the possibility of evaluating the dynamic corrections by means of a chemical calibration. It must also be stressed that the continuous gradient method allows homogeneous exploration of a broad range of pH and of reagent concentration: this aspect is extremely important when multispecies solution equilibria are studied. The thermal data thus obtained could be of great assistence in the choice of the correct model for proton-ligand-metal equilibria when potentiometric data are not sufficient for an univocal definition of the system composition in solution. Moreover, much experimental time is saved and the amounts of reagents needed for a complete microcalorimetric determination is significantly reduced. The latter aspect is obviously of great importance when expensive materials (e.g. highly purified biochemical compounds) are used.

Nevertheless many experimental difficulties cannot be disregarded. The flow rate constancy of the feeding pumps must be carefully controlled and the evaluation of t_0 is not an easy task.

Our microcalorimetric system is now in the process of being connected to a personal computer so as to permit automated data collection.

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