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AUTOMATIC BATCH CALORIMETRY: APPLICATION TO THE DETERMINATION OF THE THERMODYNAMIC PARAMETERS OF Co(II)-5′ ADENOSINE MONOPHOSPHATE COMPLEX FORMATION

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ABSTRACT

A simple system for automatic data acquisition, representation and processing of LKB batch microcalorimetric measurements, based on the use of IBM PC or compatible computers, a digital potentiometer with RS232C interface and a batch microcalorimeter, is described.

This system was applied to the direct determination of the enthalpy of complex formation between Co(II) and 5′AMP using a microcalorimetric method.

INTRODUCTION

Many thermodynamic studies on the formation of complexes of adenine nucleotides with divalent metals have been carried out using techniques such as spectroscopy, pH titration, ion-exchange resins and calorimetric methods. A great deal of work has been focused on the magnesium–adenine nucleotide system because of its importance in many biological reactions. The adenine nucleotide cobalt complexes have been studied only rarely [1,2]. In this paper, we report the direct determination of the enthalpy of complex formation between Co(II) and 5′ adenosine monophosphate (Co5′AMP) using a microcalorimetric method and the values of ΔS and ΔG.

Because manual batch calorimetric measurements are both very tedious and time consuming, the development of an automatic data acquisition and processing system could be very interesting.

The production of microcomputers has increased considerably, and their cost has been largely reduced due to the development of microelectronics. Personal microcomputers provide several advantages to the user in addition to their low cost. They are easily programmed in BASIC and abundant

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software is available that can be connected to scientific instrumentation (for instance, by a RS232C interface). Although the data processing capability of microcomputers is not very large, they are very useful for data acquisition and control tasks and several programs have been described for these purposes [3,4]. In order to facilitate both experimental data acquisition and computing work in micorocalorimetric methods, a very simple and flexible system has been developed by interfacing the computer to a digital potentiometer which acts both as an A/D converter and RS232C interface connecting an analogue output of a batch LKB microcalorimeter with the personal microcomputer.

The software consists of a program called BATCHCAL which allows calorimetric data to be acquired, processed and represented. The temperature curve obtained can be integrated and thus the ΔH values can be derived.

EXPERIMENTAL

All experiments were performed at 25 ° C. The medium was buffered with triethanolamine hydrochloride–NaOH at a pH of 7.5 and the ionic strength was kept at 0.1 M by the buffer without addition of supporting electrolyte.

Apparatus

The following apparatus was used: a batch 2107 LKB microcalorimeter with a registered output of 1 V full scale, (the total volume of reactants used was 6 ml); a portable IBM PC microcomputer provided with 256 Kb of RAM memory and graphics capability with a resolution of 620 × 200 points, and equipped with an RS232C interface; a Crison Digilab 517 potentiometer with an RS232C interface (precision, ±0.1 mV); a Fujitsu 2200 printer; an LKB 2210 recorder (1-Channel); a Hetofrig cooling bath type CB7.

Reagents

5′AMPNa$_2$ was purchased from SERVA and was used without further purification. Triethanolamine (TEA) hydrochloride and $CoCl_2 \cdot 6H_2O$ were obtained from Merck.

DESCRIPTION OF THE AUTOMATIC SYSTEM

Hardware

The block diagram of the system is shown in Fig. 1.

The system consists of an IBM PC connected through a serial port (RS232C interface) to the potentiometer which is used as an A/D converter.

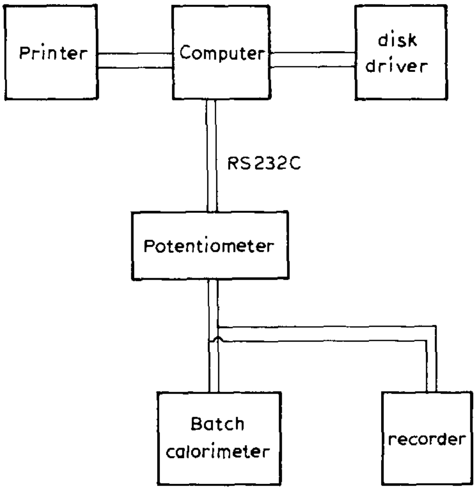


Fig. 1. Block diagram of the calorimetric system.

The LKB microcalorimeter output is connected to the electrodes' input of the potentiomenter and to a register.

Software

The program BATCHCAL was written in BASIC and then compiled. It was specially designed with the following considerations in mind.

(1) Ease of operation (the program is organized in a number of basic blocks and the user can select the different options by means of a menu).

(2) Data storage and optional printer output.

(3) Data processing (it includes baseline interpolation and peak area determination, as well as optional plotting of curves on the monitor screen or printer).

The flow diagram of BATCHCAL is shown in Fig. 2. After the program presentation, the main menu is presented on the screen, allowing several options to the user: (1) to execute the experimental task; (2) to retrieve data of previous experiments from the disc; (3) to end the program.

When option (1) is selected, the general conditions of the experiment, such as ionic strength, remarks, time between readings, maximum slope allowed for baseline, etc., are requested and stored in a selected name data file. After these questions, the experimental task begins by opening the communication channel and by reading several data until the baseline has a slope lower than a prefixed value (0.00001 mV s^{-1} in our experience). Then the mixing process is started manually and the calorimetric data are taken until the curve returns to the previous baseline. The temperature curve remains stored in the active computer memory. Once the baseline has been

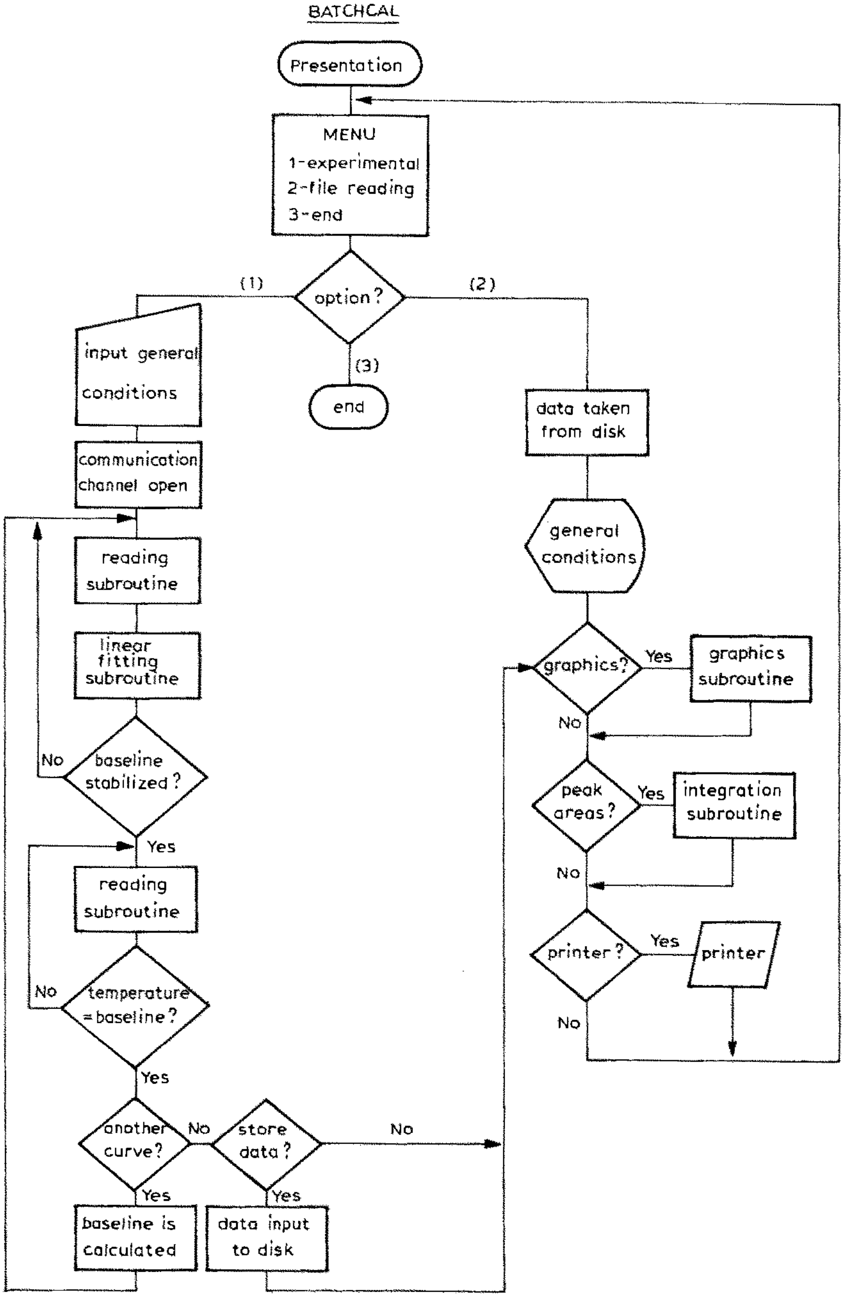


Fig. 2. Flow chart of BATCHCAL program.

reached, the user can either obtain a new calorimetric curve (to obtain the heat of friction, for instance) or finish the experimental task. If the first option is chosen, the process re-starts and the new curve is registered following the previous curve. The process can be repeated a number of times. When finishing, the data are stored in a data file.

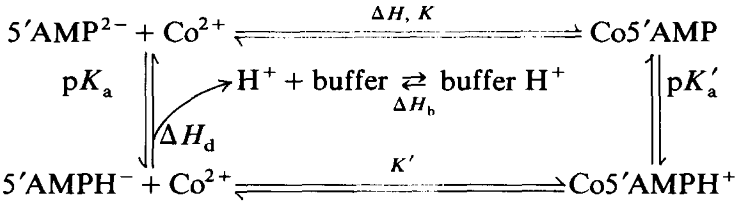
Once the experimental data have been automatically introduced with option (1) or (2), several other options are presented in a successive order: the temperature curve can be displayed on the screen and a hard copy can be obtained, the peak areas can be calculated by integration, the data set can be listed and, finally, all results can be optionally printed. When integration is required, the baseline is subtracted from the peak data points to produce a zero baseline peak.

RESULTS

Theory

For our experimental conditions we can assume that only the 1 : 1 5′AMP : Co(II) complex exists.

The equilibria involved in the bimolecular complexes of 5′AMP with Co(II) are



where ΔH is the enthalpy of formation of the complex 5′AMPCo to be determined; ΔH_d is the enthalpy of ionization of $5'AMPH^-$ (1.9 kcal mol^{-1} at 25 °C and ionic strength 0.1 [1]), ΔH_b is the enthalpy of the ionization of the triethanolamine buffer ($dpK_b/dT = -0.02$ [5], +8.1 kcal mol^{-1} at 25 °C and $I = 0.1$ [6]), K is the stability constant of the Co5′AMP formation reaction ($\lg K = 2.56$ at 25 °C and $I = 0.1$ [6]) and K_a is the protonation constant of $5'AMP^{2-}$ ($\lg K_a = 6.19$ at 25 °C and $I = 0.1$ [1,6]).

Although for a pH value lower than 8.5 we must consider the 5′AMPH species, at a pH of 7.5 this conjugate acid species constitutes 6% of the initial concentration of 5′AMP. Moreover, the pK_a' value determined by Sigel and Brintzinger [7] at 25 °C and at an ionic strength of 0.1 is 5.52 ± 0.05. Therefore at pH 7.5, the $Co5'AMPH^+$ formed can be neglected and only Co5′AMP complex is formed.

The correction of ΔH takes into account the neutralization heat of the protons which are liberated during the reaction and the heat of dilution of the reagents. If Q is the heat evolved during the reaction, then ΔH is

$$\Delta H = Q/x$$

where x is the concentration of the complex Co5′AMP at equilibrium.

TABLE 1

Dilution enthalpy of Co(II) ($I = 0.1$ M (TEAHCl–NaOH); pH 7.5 (TEAHCl–NaOH); $T = 25 \pm 0.1°$ C)

Experiment	Co(II) (mol × 10^5)	$-Q$ (mcal)	$-\Delta H$ (kcal mol^{-1})
1	4.39	6.2	0.14
2	4.40	5.1	0.12
3	4.41	6.0	0.14
4	4.40	5.9	0.13
5	4.41	5.8	0.13
6	4.39	5.8	0.13
7	4.40	5.6	0.13
8	4.39	5.4	0.12
9	4.43	5.5	0.12
10	4.38	5.1	0.12
			$\overline{0.13 \pm 0.02}$ [a]

[a] 3σ. TEA, triethanolamine.

The neutralized protons from 5′AMPH are

$$\alpha_{\text{AMPH}} = \frac{[\text{AMPH}]}{[\text{AMPH}] + [\text{AMP}]} = \frac{[\text{H}][\text{AMP}]K_a}{[\text{H}][\text{AMP}]K_a + [\text{AMP}]} = \frac{K_a h}{1 + K_a h} \qquad (1)$$

α_{AMPH} is the quantity of protons evolved per mole of nucleotide during complex formation. The variation in enthalpy ΔH_N associated with this process is

$$\Delta H_N = \frac{K_a h}{1 + K_a h}(\Delta H_d - \Delta H_b) \qquad (2)$$

TABLE 2

Dilution enthalpy of 5′AMP ($I = 0.1$ M (TEAHCl–NaOH); pH 7.5 (TEAHCl–NaOH); $T = 25 \pm 0.1°$ C)

Experiment	5′AMP (mol × 10^5)	Q (mcal)	ΔH (kcal mol^{-1})
1	4.20	1.3	0.03
2	4.21	2.1	0.05
3	4.19	1.8	0.04
4	4.19	1.4	0.03
5	4.23	1.5	0.04
6	4.19	1.6	0.04
7	4.24	2.1	0.05
8	4.22	1.9	0.05
9	4.25	2.3	0.05
10	4.23	2.3	0.05
			$\overline{0.04 \pm 0.02}$ [a]

[a] 3σ. TEA, triethanolamine.

TABLE 3

Results obtained for complex Co5′AMP ($I = 0.1$ M (TEAHCl–NaOH); pH 7.5 (TEAHCl–NaOH); $T = 25 \pm 0.1°$ C)

Experiment	$-Q_m$ (mcal)	Co(II) (mol × 10^5)	5′AMP (mol × 10^5)	$-Q_c$ (mcal)	Co5′AMP (mol × 10^5)	$-\Delta H_c$ (kcal mol^{-1})	pH_f	$-\Delta H_N$ (kcal mol^{-1})	$-\Delta H$ (kcal mol^{-1})
1	36.8	4.45	4.11	32.7	2.31	1.42	7.48	0.30	1.12
2	36.6	4.44	4.10	32.5	2.31	1.41	7.53	0.27	1.14
3	36.5	4.43	4.11	32.4	2.31	1.41	7.50	0.29	1.11
4	34.3	4.43	4.09	30.2	2.30	1.31	7.53	0.27	1.04
5	34.8	4.40	4.08	30.7	2.29	1.34	7.48	0.30	1.04
6	34.9	4.41	4.07	30.8	2.29	1.34	7.47	0.31	1.03
7	35.2	4.44	4.11	31.1	2.31	1.35	7.53	0.27	1.08
8	37.1	4.44	4.20	33.0	2.35	1.40	7.36	0.39	1.01
9	36.5	4.42	4.21	32.4	2.35	1.38	7.36	0.39	0.99
10	33.0	4.38	4.21	29.0	2.33	1.24	7.55	0.26	0.98
									$\overline{1.05 \pm 0.17}$[a]

[a] 3σ. TEA, triethanolamine; m, measured; c, corrected for dilution.

Therefore, the variation in enthalpy measured during complex formation will be

$$\Delta H_c = \Delta H + \frac{K_a h}{1 + K_a h}(\Delta H_d - \Delta H_b) \tag{3}$$

where ΔH_c is the variation in enthalpy of complex formation measured and corrected for the heat of dilution of the reagents.

The initial concentration of nucleotide and Co(II) was about 7×10^{-3} M. The values determined for the enthalpy of dilution of the reagents are listed in Tables 1 and 2.

ΔH was calculated according to eqn. (3). Experimental results and correction values are summarized in Table 3. A ΔH value of -1.05 ± 0.17 kcal mol^{-1} was obtained from these values. This result does not agree with that of Melardi et al. [2] who obtained $\Delta H = -0.1$ kcal mol^{-1} at 25 °C and at an ionic strength of 0.2 using the van't Hoff relationship. However, the result given in this paper is in agreement with that reported by Taqui Khan and Martell (-1.1 ± 0.2 kcal mol^{-1}) determined at an ionic strength of 0.1 and at 25 °C using the van't Hoff relationship [1].

Values of ΔG and ΔS for the reaction were calculated using the thermodynamic relationships

$$-\Delta G = RT \ln K$$

$$\Delta S = \frac{\Delta H - \Delta G}{T}$$

The value of K used above was reported by Smith and Martell [6] (lg $K = 2.56 \pm 0.03$ at 25 °C and $I = 0.1$). The ΔH, ΔG and ΔS values are summarized in Table 4.

TABLE 4

Thermodynamic parameters of Co5′AMP complex ($I = 0.1$ M (TEAHCl–NaOH); pH 7.5 (TEAHCl–NaOH); $T = 25 \pm 0.1$ °C; $\Delta G = -3.49$ kcal mol^{-1})

Experiment	$-\Delta H$ (kcal mol^{-1})	ΔS (cal K^{-1} mol^{-1})
1	1.12	8.0
2	1.14	7.9
3	1.11	8.0
4	1.04	8.3
5	1.04	8.2
6	1.03	8.3
7	1.08	8.1
8	1.01	8.3
9	0.99	8.4
10	0.98	8.4
	1.05 ± 0.17 [a]	8.2 ± 0.5 [a]

[a] 3σ. TEA, triethanolamine.

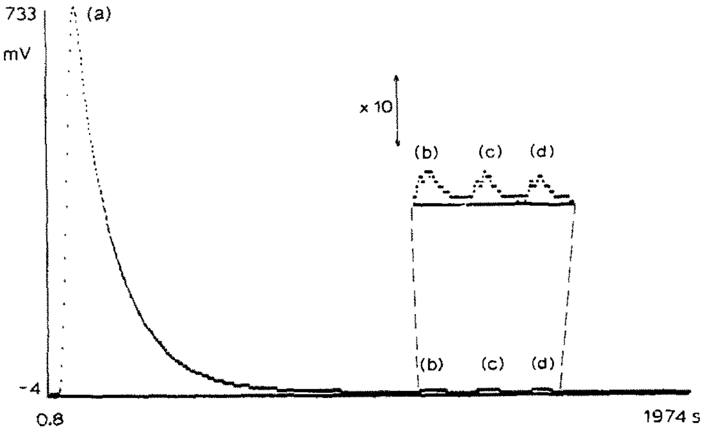


Fig. 3. (a) Experimental curve of formation of Co5′AMP complex; (b), (c) and (d) heat of friction peaks.

The entropy appears to be the principal driving force for the formation of Co(II)5′AMP chelate species in solution.

In Fig. 3 an example of the utilization of the BATCHCAL program for data acquisition and representation of experimental curves can be seen. The peak (a) represents the heat of formation of the Co(II)5′AMP complex. The successive peaks (b), (c) and (d) represent the heat of friction of the microcalorimeter. They have been amplified to improve their resolution.

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