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Ernesto Freire

Ernesto Freire, Major Professor

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Date November 27, 1985

DEVELOPMENT OF A MICROCOMPUTER-CONTROLLED, ISOTHERMAL TITRATION MICROCALORIMETER

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Glen Douglas Ramsay

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ACKNOWLEDGMENTS

I would like to express my gratitude to Dr. Ernesto Freire for his guidance, insights, and support during my stay at U.T.K. I would also like to thank my parents for their omnipresent love. Furthermore, recognition should be given to the crew at the U.T.K. Physics Department instrument shop for the construction of much of the calorimeter system.

ABSTRACT

The ability of a calorimeter to directly measure heat makes it a very desirable instrument when performing thermodynamic studies. A titration microcalorimeter is particularly suited for measuring the heat evolved in biochemical reactions. Unfortunately there does not exist a commercially available instrument with sufficient sensitivity, so it was decided that a batch type microcalorimeter would be modified to allow the injection and mixing of reactants.

The microcalorimeter chosen was a LKB 2277 BioActivity Monitor. In addition to the modifications to the calorimeter a titration system was built so that the injection of reactants and data collection would be computer controlled. Computer software was written to manipulate the data and make the necessary calculations. The result of this work is a titration microcalorimeter approximately ten times more sensitive than commercially available instruments.

To check the feasibility of the instrument the interaction between cytochrome b_5 protein and phospholipid membrane vesicles was investigated. The measured molar enthalpy was -227 kcal/mole protein. The association and insertion of the protein into the membrane was found to be a slow process characterized by a half-time of 8.6 seconds.

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CHAPTER 1

INTRODUCTION

The ability of a microcalorimeter to measure directly the heat effects associated with biological reactions makes it the instrument of choice when performing thermodynamic and bioenergetic experiments. Although titration techniques utilizing spectrophotometers, fluorometers, and other instruments are available they are not capable of directly measuring the energetics of reactions. Because the heat produced is often rather small due to the small quantities of biological material available or the inability to produce a sufficiently concentrated solution, a calorimeter of high sensitivity is necessary. For these reasons and because such an instrument has not been available commercially it was decided that a microcomputer controlled titration microcalorimeter should be built.

Principle of Operation

The titration microcalorimeter developed in our laboratory has been designed to be used with a batch type isothermal calorimeter (LKB 2277 BioActivity Monitor, LKB-Produkter AB, Bromma, Sweden). The heart of the instrument are Peltier (thermoelectric) elements, which generate a voltage proportional to the temperature gradient across them. The reactants are placed in a stainless steel ampoule which is positioned in the calorimeter in thermal contact with the Peltier elements. The ampoule is thermally isolated from the rest of the calorimeter so that any heat produced (or absorbed) in the ampoule will be transmitted

across the Peltier elements. The Peltier elements are in turn connected to metal heatsinks which are maintained at a constant temperature ($\pm$ 0.0002^{o}C over an eight hour period) by a 25 liter water bath. This enables the instrument to be sensitive to less than 0.15 uWatts and capable of maintaining baseline noise of less than 0.1 uWatts when used as a batch type microcalorimeter.

Unfortunately, when used as a batch type microcalorimeter the LKB 2277 BioActivity Monitor is not useful for measuring the heat effects associated with biochemical reactions due to the instrument requiring at least a hour to fully equilibrate. Only very slow reaction processes (such as microbial fermentation) would still be producing heat after the equilibration time.

The LKB microcalorimeter can also be equipped with a flow/mixing cell in which two continuous streams of reactants are mixed. This configuration is capable of measuring the heat resulting from fast reactions, but has the disadvantages of having a reduced sensitivity and of being susceptible to obstruction by particulates, thus precluding its use with natural or artificial membrane suspensions. Therefore an instrument which is capable of measuring the heat from fast reactions but without the above problems is needed. This instrument is a high-sensitivity, isothermal titration microcalorimeter.

CHAPTER 2

DESIGN AND CONSTRUCTION

A block diagram of the calorimeter system is shown in figure 1.* Briefly, the calorimeter system was constructed to make use of a commercially available batch type microcalorimeter, the LKB 2277 Bio-Activity Monitor. The stainless steel ampoule in which the reactants are placed was modified to allow injection of fluids and insertion of a stirring mechanism (figure 2). The cap of the ampoule was ground flat and three holes were drilled through it. The center hole accommodates the stirring shaft. The second hole contains a nylon set screw which anchors a two inch stainless steel tube to which teflon tubing is connected. The other end of the tube is immersed in the solution in the ampoule. The third hole in the cap is connected to a Plexiglas rod which is used to raise and lower the ampoule in the calorimeter (in figure 2 it has been replaced with a set screw). The ampoule can contain 1-4 ml of liquid sample.

The stirrer mechanism consists of a teflon paddle connected to a stainless steel tube (Small Parts, Inc., Miami, FL). The tube is connected to a D.C. motor (Polk's Hobbies Intl., Jersey City, NJ) powered by a 12V battery (Sears, Roebuck and Co.) (figures 1 and 2). The motor rotates the paddle at 90 r.p.m. and it can be switched on and off either manually or by a relay connected to the computer. The rate of mixing of this configuration was measured by replacing the ampoule with

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*All figures appear in the Appendix.

a glass test tube and placing the titration apparatus in a spectrophotometer. Concentrated bromophenol blue was injected and the optical density of the solution was monitored. The half-time for complete mixing was found to be 4.0 $\pm$ 0.5 seconds.

Injection of solutions is accomplished by a 0.5 ml gas tight syringe with a threaded plunger (Hamilton Co., Reno, NE) (figure 1). The syringe is connected to a 35" length of teflon tubing (0.02" O.D.) which leads to the ampoule. The plunger is rotated by a stepper motor (Superior Electric Co., Bristol, CN) which is powered by a stepper motor controller (Model SCM 102A, Maxwell Electronics, Inc., Martinsville, NJ). The controller is interfaced with the computer via a RS-232 serial interface. This type of interface was chosen so that the system can be easily switched between different microcomputer systems. The volume of reactant injected is under computer control. In this dissertation injection volumes of 25 ul were used. The quantity being delivered was measured by making a injections onto a weighing tray and weighing on an analytical balance. The average of 3 injections was 25 $\pm$ 0.1 ul.

The computer used in these experiments is a TEC-86 (TecMar Inc., Cleveland, OH) equipped with an A/D and D/A board. This board converts the analog voltage from the calorimeter to digits for data storage on floppy disks and it converts digits to the analog voltage necessary to switch the stirrer motor on and off. One thousand values are read from the A/D board and their average is recorded as the calorimeter output. Software has been written which controls 1) the number of injections that are made, 2) the volume of the injections, 3) the time between

injection, 4) the duration of stirring, 5) how often data is to be collected from the calorimeter, and also analyzes the data and generates titration curves in the form of heat vs. total concentration of added reactant.

In a typical experiment the ampoule is loaded with 3 ml of solution and the syringe and tubing are loaded with 0.6 ml of degassed reactant. The ampoule is placed in a pre-equilibration position for 30 minutes to begin thermal equilibration and then it is lowered to the measuring position. A steady baseline is reached in about 2 hours. The computer is started and up to 20 injections of 25 ul can be made. Data is collected every 10 seconds and injections are made at intervals ranging from 45 minutes to several hours depending on the nature of the reaction under study.

CHAPTER 3

MATERIALS AND METHODS

Electrical Calibration

The LKB 2277 is equipped with an electrical calibration unit which is accurate to within 1%. The unit supplies a constant voltage to a small heating coil located on the ampoule cup. By knowing the electrical resistance of the heating coil and the voltage applied the power output can be calculated:

$$\text{Watts} = \text{Volts}^2 / \text{Ohms} \quad (1)$$

By applying the voltage for a known time interval the amount of heat produced can be easily calculated:

$$\text{Joules} = \text{Watts} * \text{Seconds} \quad (2)$$

One joule is equal to 4.184 calories.

For the electrical calibration the sample and reference cells were filled with 3 ml of water, the syringe and tubing were filled with degassed water and the apparatus was allowed to sit for several hours until a stable baseline had been reached. The calibration unit was ran for 2790 seconds at 3 uWatts. The calorimeter was set at 25^{o}C for this and all other experiments.

Chemical Calibration

The heat produced by the reaction between a strong inorganic acid and base is typically -13.5 kcal/mole (Vaughan, 1973). For the chemical calibration, standardized 2.0 N HCl (Sigma, St Louis, MO) was diluted to 2.5 mM with boiled, deionized, distilled water and 3 ml of this solution was placed in the sample ampoule. A 1.0 N solution of diagnostic grade KOH (Sigma) was diluted to 25 mM with boiled, deionized, distilled water and was loaded into the syringe. After equilibration, injections of 25 ul were made every 45 minutes.

Experiments

EDTA/$MgCl_2$: EDTA will strongly chelate many divalent cations, often with a sizable molar enthalpy (Jordan and Alleman, 1957). EDTA binds to Mg^{++} in a 1:1 ratio with an association constant of 6×10^8 (Peters et al., 1974). For the experiment, standard 1.0 M $MgCl_2$ (Sigma) was diluted to 50 mM with buffer (5 mM phosphate, 5 mM NaCl, pH 7.2) and loaded into the syringe. The concentration of the $MgCl_2$ was checked by Eriochrome Black T colormeteric titrations. Eriochrome Black T is an azo dye which will bind Mg^{++} with an equilibrium constant of 5.4 at pH 10.0 (Diehl and Lindstrom, 1950). The free dye is blue and in the presence of Mg^{++} it is violet. Reagent grade (99% pure) disodium EDTA (Sigma) was used to make a 3.5 mM EDTA solution in the same phosphate buffer and 3 ml of this mixture was placed in the sample ampoule. Every 45 minutes an injection of 25 ul of $MgCl_2$ was made.

Cytochrome b_5: Cytochrome b_5 was kindly supplied by Peter W. Holloway and was prepared by the method originally described by Ozols

(1974) and modified by Leto (1980). This membrane protein has a molecular weight of 16,700 (Spatz, 1971) and is found mainly in the endoplasmic reticulum of liver cells (Jarasch et al., 1979). Injections of 25 ul of 0.85 mM cytochrome b_5 (10 mM Tris, 0.1 mM EDTA, pH 8.1 buffer) were made into 3 ml of either 15 mM POPC vesicles or buffer. The vesicles were prepared by suspending a dried film of lipid in the same Tris buffer, sonicating in a bath type sonicator for 1/2 hour followed by 5 minutes of centrifugation (15,000 g). The top 2/3 of the supernatent were used and the remainder was discarded. During the preparation the POPC was kept at or above room temperature. The vesicles were used immediately after preparation.

CHAPTER 4

CALCULATIONS

The interaction of one reactant with another will often generate or absorb heat and this enthalpy change can be measured by a calorimeter. The heat source which is of interest is that of the resulting reaction(s); however, the dilution of the reactants during the injection may also be accompanied by heat effects. In addition there is also a constant quantity of heat of mixing produced by the stirring and the injection process. These heats are be summed up in the equation:

$$q(i) = q(r) + q(d) + q(m) \qquad (3)$$

where q(i) is the measured heat, q(r) is the heat of reaction, q(d) is the heat of dilution, and q(m) is the heat of mixing (mechanical heat).

The signal from the calorimeter is in the form of the instantaneous power output (uWatts or W) of the ampoule. To calculate q(i) the signal must be integrated with respect to time:

$$q(i) = \int_0^\infty W \, dt \qquad (4)$$

Because the calorimeter output is recorded as discrete units at constant time intervals by the computer, equation 4 is simplified to:

$$q(i) = \Delta t \sum_{i=0}^{\infty} W_i \qquad (5)$$

where Δt is the time interval of data collection. The heat of reaction q(r) is obtained from q(i) after subtracting q(d) and q(m). The sum of q(d) and q(m) are obtained by injecting the reactant into an ampoule containing only buffer and q(m) is obtained by injecting buffer into buffer.

The heat q(r) per injection will be equal to the molar enthalpy of the reaction multiplied by the moles of product formed:

$$q(r) = [\text{Product}]\ V\ \Delta H \qquad (6)$$

where V is the reaction volume.

The sum of the heat released (or absorbed) is:

$$Qr_j = \sum_{i=1}^{j} q(r)_i \qquad (7)$$

where j is the number of injections made. If all of the reactant(s) initially in the sample ampoule have been consumed then upon subsequent injections q(r) is equal to zero. At this point Qr should be constant:

$$Qr_{Max} = \sum_{i=1}^{\infty} q(r)_i \qquad (8)$$

and the molar enthalpy of the reaction can be calculated:

$$\Delta H = \frac{Qr_{Max}}{\text{Moles Product}} \qquad (9)$$

If the amount of product formed can be measured or estimated independently, then the molar enthalpy can be calculated from the heat of a single injection. An important situation occurs when the reactant in the ampoule is in excess and the association constant is very large. Under these conditions essentially 100% of the reactant injected participates in the reaction and:

$$\Delta H = \frac{q(r)}{\text{Moles Reactant Injected}} \qquad (10)$$

Thus, isothermal titration calorimetry allows a direct determination of the enthalpy of a chemical reaction without any prior knowledge of the molecular mechanism of the reaction. Since an isothermal titration curve also produces a ligand binding isotherm, this curve can be further analyzed to obtain the equilibrium constant. By knowing H and Keq, G and S for the reaction can be calculated.

Consider the reaction in which a ligand molecule binds to a macromolecule:

$$M + X \rightleftharpoons MX \qquad (11)$$

the chemical equilibrium constant is:

$$K_{eq} = \frac{[MX]}{[M][X]} \qquad (12)$$

where [M] and [X] are the concentrations of free macromolecule and ligand. Examples of the expected heats for binding reactions with the

same molar enthalpy but different Keq are shown in figure 3. The concentration of bound ligand, [MX], can be determined by the equation:

$$[MX] = [M]_T F_B \qquad (13)$$

where F_B is the fraction of macromolecule bound and $[M]_T$ the total macromolecule concentration. F_B is a function of Keq and the concentration of free ligand:

$$F_B = \frac{Keq[X]}{1+Keq[X]} \qquad (14)$$

The concentration of free ligand can be calculated from the available data:

$$[X] = [X]_T - [M]_T \frac{Qr}{Qr_{Max}} \qquad (15)$$

where $[X]_T$ is the total ligand concentration.

If a macromolecule has n number of binding sites which are identical and independent, then equation 13 becomes:

$$[MX] = [M]_T \, n \frac{Keq[X]}{1+Keq[X]} \qquad (16)$$

Because the quantity of heat released is proportional to the quantity of product formed equation 16 can be rewritten as:

$$Qr = Qr_{Max} \frac{Keq[X]}{1+Keq[X]} \quad (17)$$

Given Qr, Qr_{Max}, and [X], Keq can be calculated if equation 17 is rearranged as:

$$\frac{1}{Qr} = \frac{1}{Qr_{Max}} + \frac{1}{Qr_{Max}K_{eq}[X]} \quad (18)$$

and 1/Qr is plotted vs. 1/[X]. Figure 4 represents the linear transformation of the data shown in figure 3. The Keq will be equal to:

$$K_{eq} = \frac{1}{Qr_{Max}Slope} \quad (19)$$

Given the molar enthalpy and Keq of a reaction a complete thermodynamic picture can be obtained. The molar Gibb's Free Energy is:

$$\Delta G = -RT\ln(Keq) \quad (20)$$

and the change in entropy is:

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad (21)$$

where T is degrees Kelvin and R is the gas constant (1.987 cal/(deg. mole)).

CHAPTER 5

DATA AND RESULTS

Electrical Calibration

The results from the electrical calibration are shown in figure 5. The heat produced for the duration of the calibration was -2000 ucal (the convention is to list exothermic activities as negative and endothermic as positive). This known amount of heat can be used to calculate the calorimeter calibration constant.

The baseline noise was $\pm$0.05 uWatts, which is an improvement over the $\pm$0.1 uWatts listed by the LKB literature. This improvement is due to the data manipulation and not any changes made in the calorimeter. By recording the average of 1000 values read over a 2 second period the effect of high frequency noise is reduced.

The relaxation constants of the instrument can be obtained be analyzing the decay of the electrical signal, as is shown in figure 6. The relaxation constants were calculated using a nonlinear least squares computer program for the curve fitting and the results for various experiments are shown in table 1.* The values given are the measured decay constants and the effect of the instrument's intrinsic decay constant has not been eliminated. The instrument's intrinsic decay was found to be monoexponential and characterized by a relaxation constant of 270 seconds.

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*All tables appear in the Appendix.

Chemical Calibration

The results of the HCL/KOH titration are shown in figures 7 and 8. The measured Qr_{Max} for the experiment was -95 mcal while the expected result on the basis of the electrical calibration constant was -101 mcal. This result indicates that the electrical and chemical calibration differ by approximately 6%. Differences up to 10% are common for these configurations and can be attributed to the different geometrical locations of the heat sources.

A chemical calibration produces heat at the same location as a real experiment (inside the reaction ampoule), however, the results are susceptible to variations of the concentration of the reactants used to perform the calibration. On the other hand, electrical calibrations provide an accurate heat source but the source of heat is an electrical resistor surrounding the ampoule vessel and the thermal contact with the system is different. In general the average heats obtained by several chemical calibrations provide more accurate calibration constants than electrical calibration. Concentration errors are normally distributed and tend to cancel out after several experiments, whereas the error due to the geometrical location of the electrical heater is not random.

Experiments

EDTA/$MgCl_2$: The results of the calorimetric titration are shown in figures 9 and 10. The molar enthalpy calculated from the sums of all of the heats (equation 8) is +7.4 kcal/mole while the value from the average of peaks 3-7 is +7.3 kcal/mole (equation 6). These values

compare favorably with the value of +7.6 kcal/mole reported by Rialdi (1972), and they certify that the instrument calibration has been done properly.

Cytochrome b_5: The results for the injections of cytochrome b_5 into POPC vesicles or buffer are shown in figure 11. The heat measured for the individual injections are shown in table 2. The molar enthalpy for the binding of cytochrome b_5 to POPC vesicles is calculated to be -227 $\pm$ 13 kcal /mole protein (average of 2 injections). This value is large compared to the enthalpy changes for acid/base neutralization or EDTA chelation, however the binding of a protein to a membrane vesicle is a complex interaction involving several processes. This will be considered further in the discussion.

The binding of cytochrome b_5 with POPC vesicles had a decay constant of 740 sec., which is significantly larger than the instrument's intrinsic decay constant of 270 sec. This experiment indicates that the binding of cytochrome b_5 is a slow process characterized by a half-time of 8.6 minutes.

CHAPTER 6

DISCUSSION

We have been able to build a computer-controlled, isothermal titration calorimeter with a sensitivity better than 0.1 uWatts. This sensitivity is about one order of magnitude better than can be obtained with commercially available instruments.

Experiments

Cytochrome b_5: Cytochrome b_5 will insert into membrane vesicles of POPC when incubated at room temperature (Freire et al., 1983). Previous studies have shown that there are 14 $\pm$ 1 phospholipid molecules located in the boundary layer of lipid surrounding the inserted protein (Freire et al., 1983). These lipid molecules are unable to participate in the gel to liquid-crystalline phase transition. Furthermore these molecules are in a relatively disordered state and they remain so above and below the phase transition.

The lipid vesicles are in the liquid-crystalline phase (POPC, T_m= -2.6^{o}C) (Davis et al., 1981) prior to the insertion of the protein. The insertion of a protein molecule into a membrane results in the pertubation of the physical state of the lipid molecules (Chapman et al., 1979; Curatolo et al., 1977; Marsh et al., 1982). This pertubation is accompanied by heat effects. If the physical state of the boundary layer is intermediate between the gel and liquid-crystalline phases, then the enthalpy of pertubation should be of smaller magnitude than that of the overall phase change. Furthermore the sign of the enthalpy

change in going from the gel to boundary layer should be endothermic ("partial melting") while going from liquid-crystalline should be exothermic ("partial freezing").

The molar enthalpy for the binding of cytochrome b_5 to POPC vesicles calculated on a per protein basis is -227 kcal/mole. If calculated on a per boundary lipid basis the enthalpy change is -16.2 kcal/mole (14 lipids per protein). However the molar enthalpy for the complete phase transition of POPC is -5.4 kcal/mole (Davis et al., 1981), so although the enthalpy contributed by the lipid pertubation is most likely a major contributor to the total binding enthalpy, it cannot be identified as the sole source.

When a hydrophobic molecule is introduced into an aqueous environment the water molecules must be arranged around the intruding molecule to accommodate it. This adjustment results in a loss of entropy and also disrupts hydrogen bonding between water molecules. By packing the hydrophobic molecules together their interfering influence is reduced. Furthermore ionic bonding and Van der Waal forces between the hydrophobic molecules can be maximized. The sum of these interactions is called "hydrophobic bonding," and it is the driving force behind the separation of hydrophobic and hydrophilic molecules (Davidson, 1967).

The interactions of proteins with water can be complicated. Because the different amino acids have different solubilities in water a protein can have domains of varying hydrophobicity. Furthermore, regardless of how hydrophobic a protein is, the peptide bonds linking the amino acids will always be capable of forming hydrogen bonds with water (Alvarez and Biltonen, 1973). For this reason there will be a

sphere of hydration surrounding a protein molecule in an aqueous environment. When a protein binds to a membrane there must be a redistribution of water molecules across the surfaces of the protein molecule and the membrane. This process results in a net gain of H-bonds, and consequently negative enthalpy changes.

The transfer of organic molecules from an aqueous to a hydrophobic environment is accompanied by negative enthalpy changes (Tanford, 1980). The enthalpy change due to hydrogen bond formation can range between -4 to -7 kcal/mole per bond (Stryer, 1975), which is small compared to the enthalpies given earlier, but when the number of the hydrogen bonds is considered the total enthalpic contribution could be substantial. The losses and gains in enthalpy and entropy must all add up to give a net loss in Gibb's free energy for binding to occur. It appears that the major contributors to the enthalpy of association between membrane protein and lipid are the formation of the boundary layer of lipid and the enthalpy of transfer of hydrophobic protein domains from water to a hydrophobic environment.

CHAPTER 7

CONCLUSION

In this dissertation the construction and testing of a high-sensitivity, computer controlled, isothermal microcalorimeter has been reported. This instrument is approximately 10 fold more sensitive than currently available commercial instruments and should prove extremely important when measuring the heats of reactions in dilute biological materials.

With this instrument the heat of binding of cytochrome b_5 to membrane vesicles composed of POPC at 25^{o}C was measured. The enthalpy of binding was found to be -227 kcal/mole protein. In addition the process was found to be very slow and characterized by a time constant of 740 seconds.

The ability of this instrument to measure minute quantities of heat makes it a prime choice when doing thermodynamic studies on biological materials. This instrument makes possible experiments which previously could not be performed. Experiments with myelin basic protein and cholera toxin have also been performed and other experiments are currently under investigation.

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APPENDIX

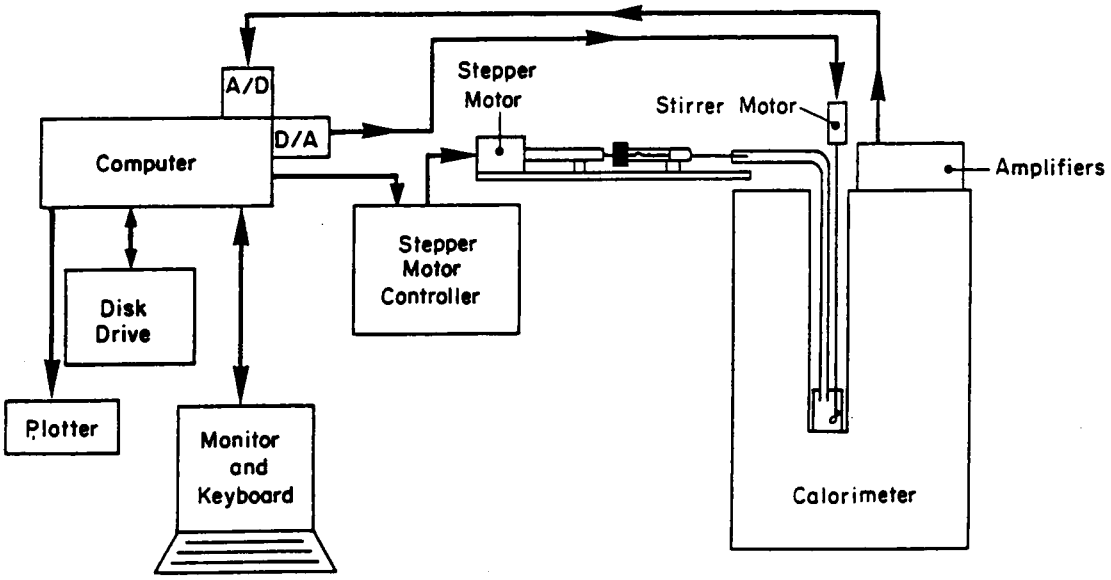

Fig. 1. Block diagram of the calorimeter system.

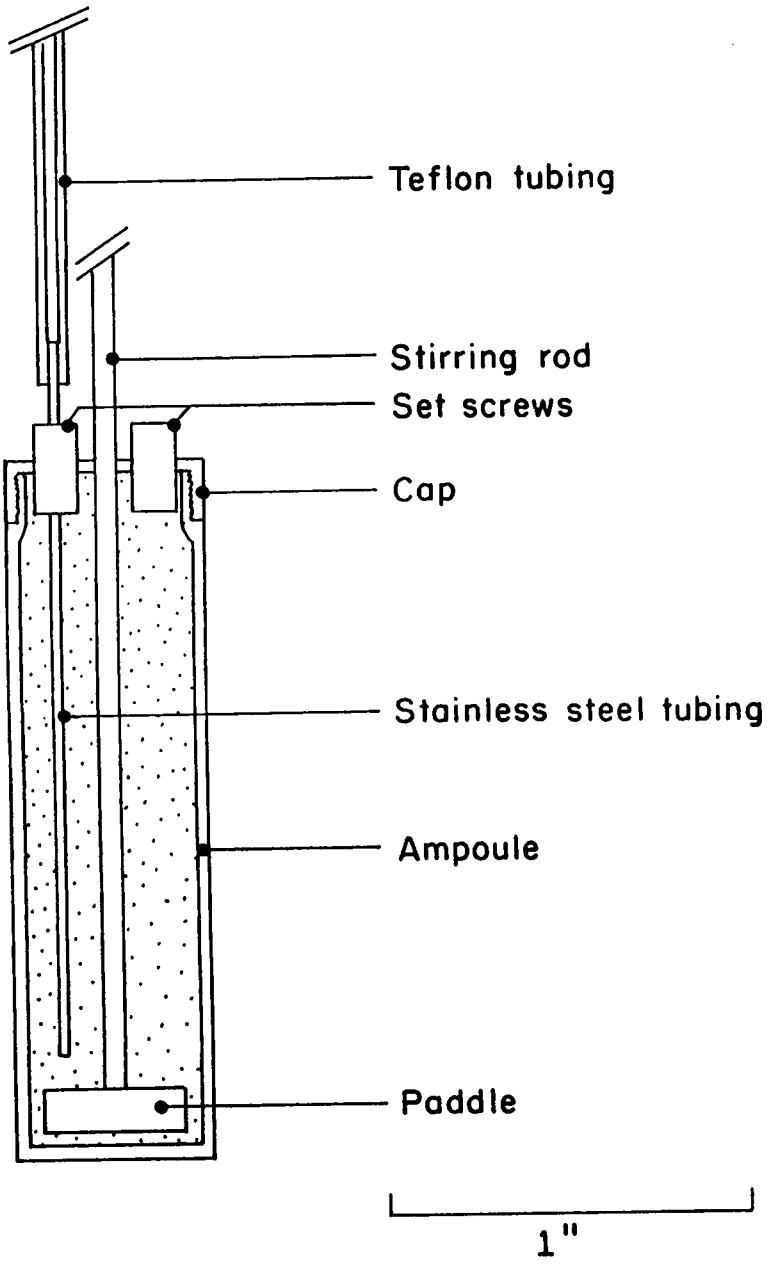

Fig. 2. Schematic of modified reaction ampoule.

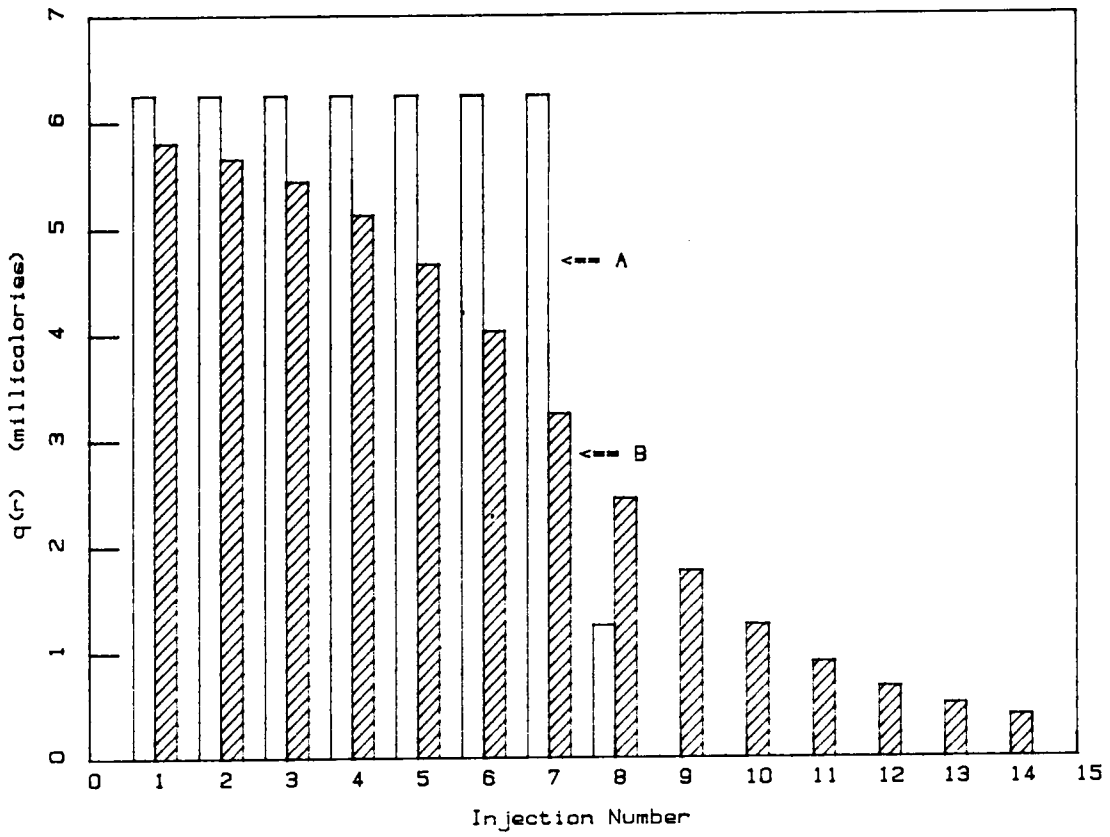

Fig. 3. Expected heats resulting from the injection of 25 ul of 25 mM of ligand into 3 ml of 1.5 mM macromolecule. The molar enthalpy in both cases is -10 kcal/mole and the Keq for case A (open bars) is 10^9 and 10^4 for case B (hatched bars).

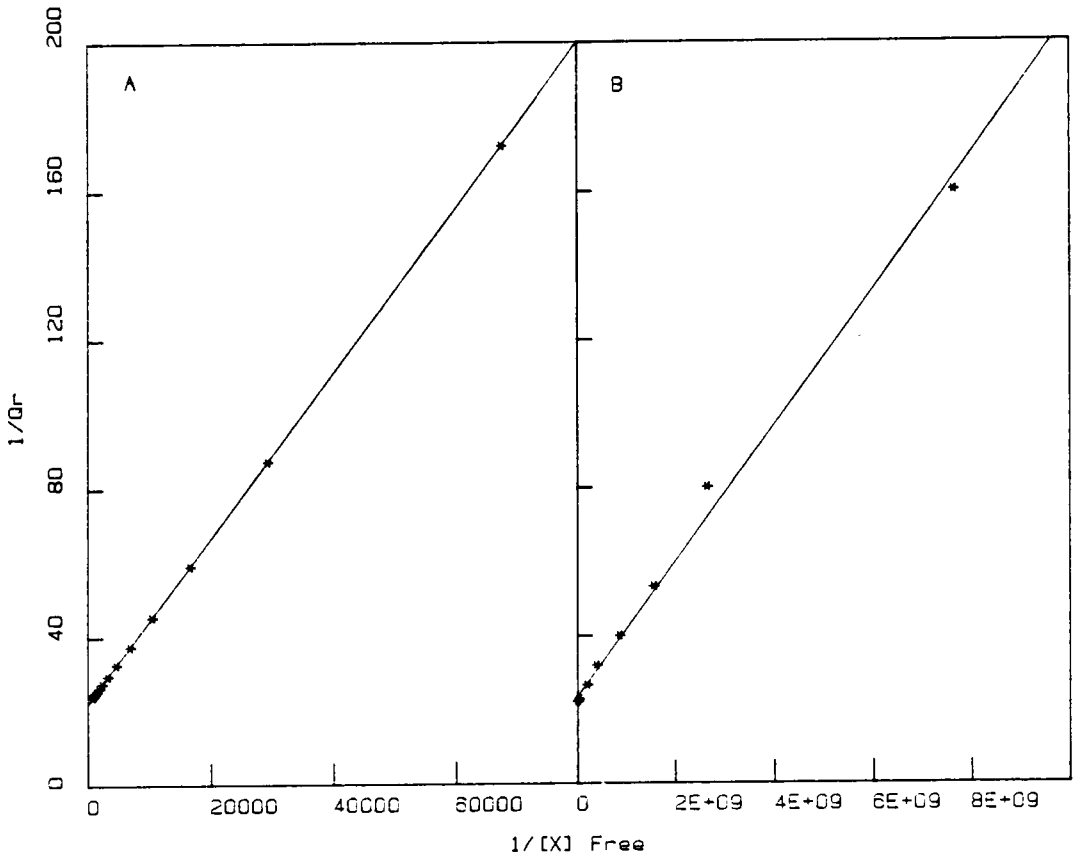

Fig. 4. Determination of Keq from isotherm. Data from figure 3 was used. Keq calculated for panel A was 10^4 and the actual Keq was 10^4. For panel B the calculated Keq was $1.3 * 10^9$ and the actual value was 10^9.

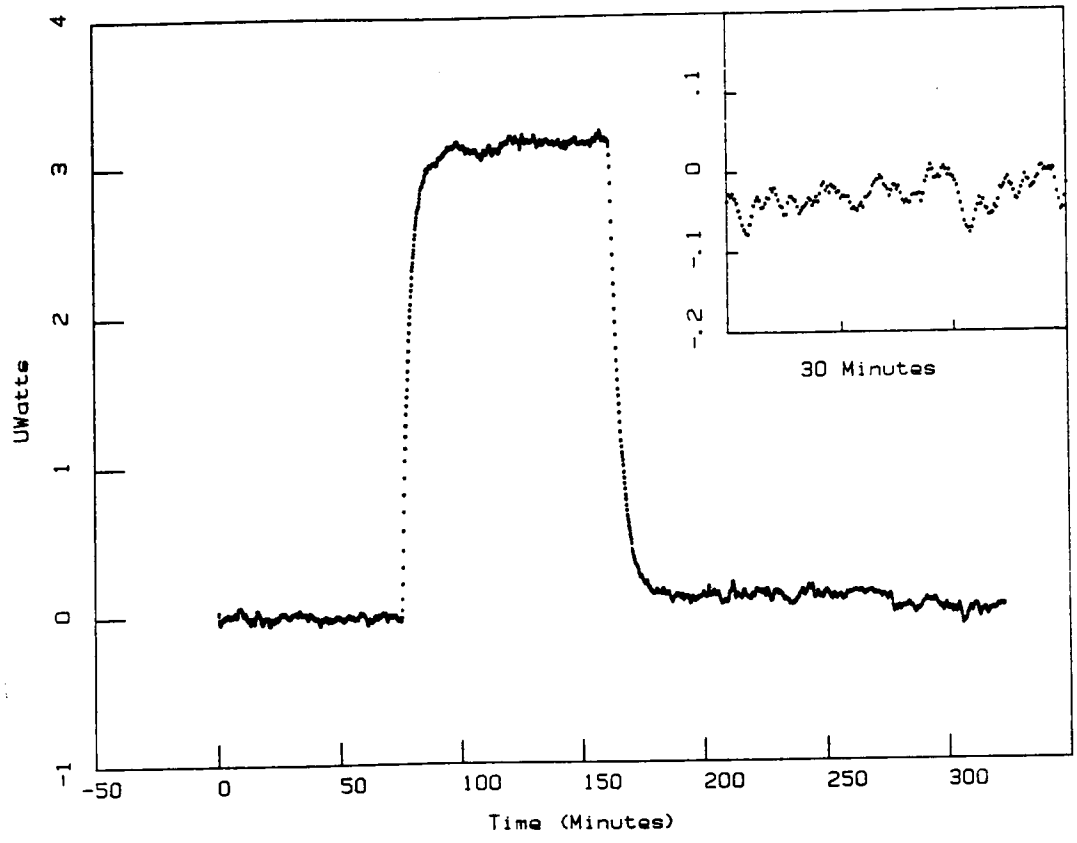

Fig. 5. Electrical calibration of calorimeter. The insert is an enlargement of the baseline prior to the peak.

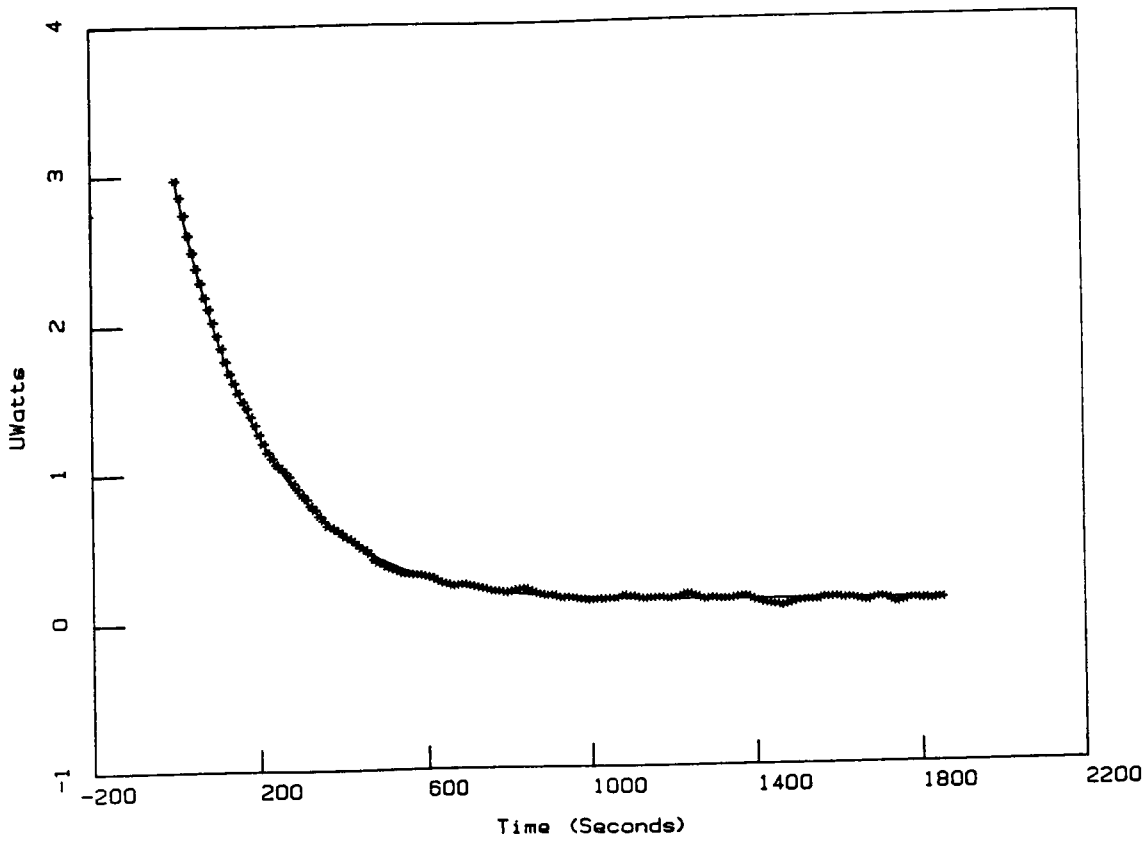

Fig. 6. Fit of a monoexponential curve to the decay of the electrical calibration. The equation uWatts = $A_1 + A_2 e^{-t/tau}$ was used. The *'s are the actual data while the solid line is the fitted curve.

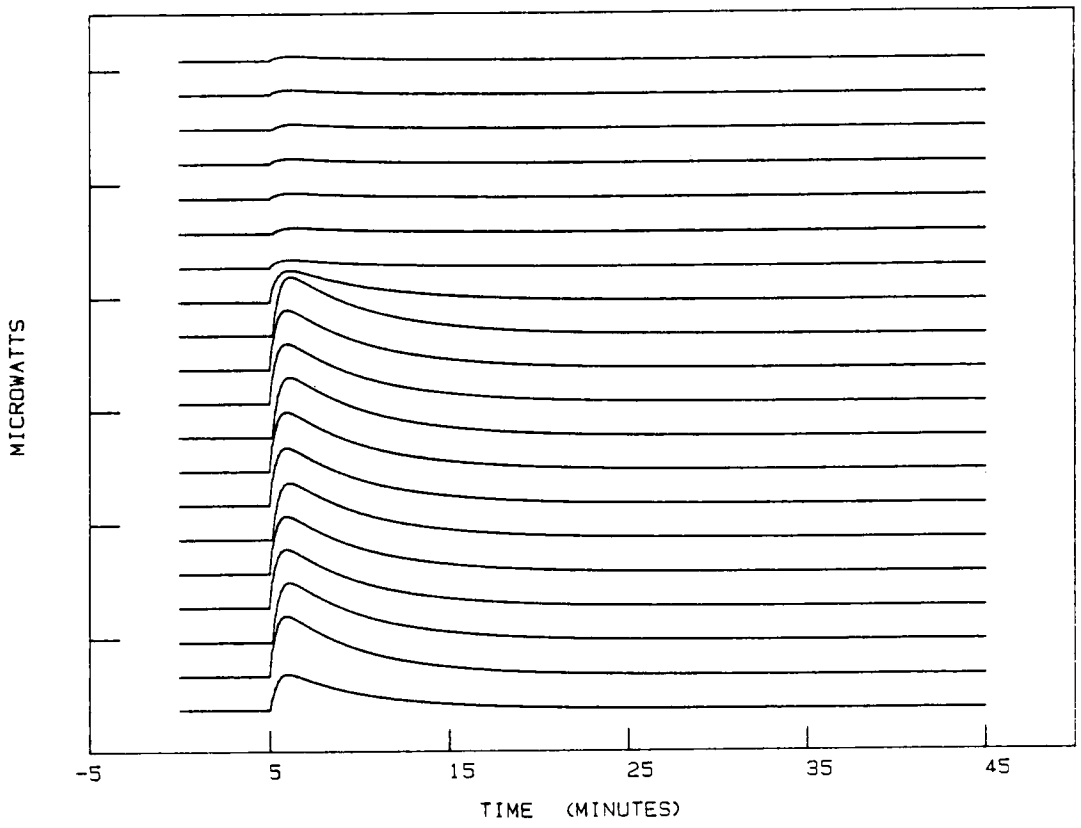

Fig. 7. Results from the injection of 25 ul of 25 mM KOH into 3 ml of 2.5 mM HCl. The first injection is on the bottom and the last is on the top. The microwatts scale has the increments of 10 uWatts.

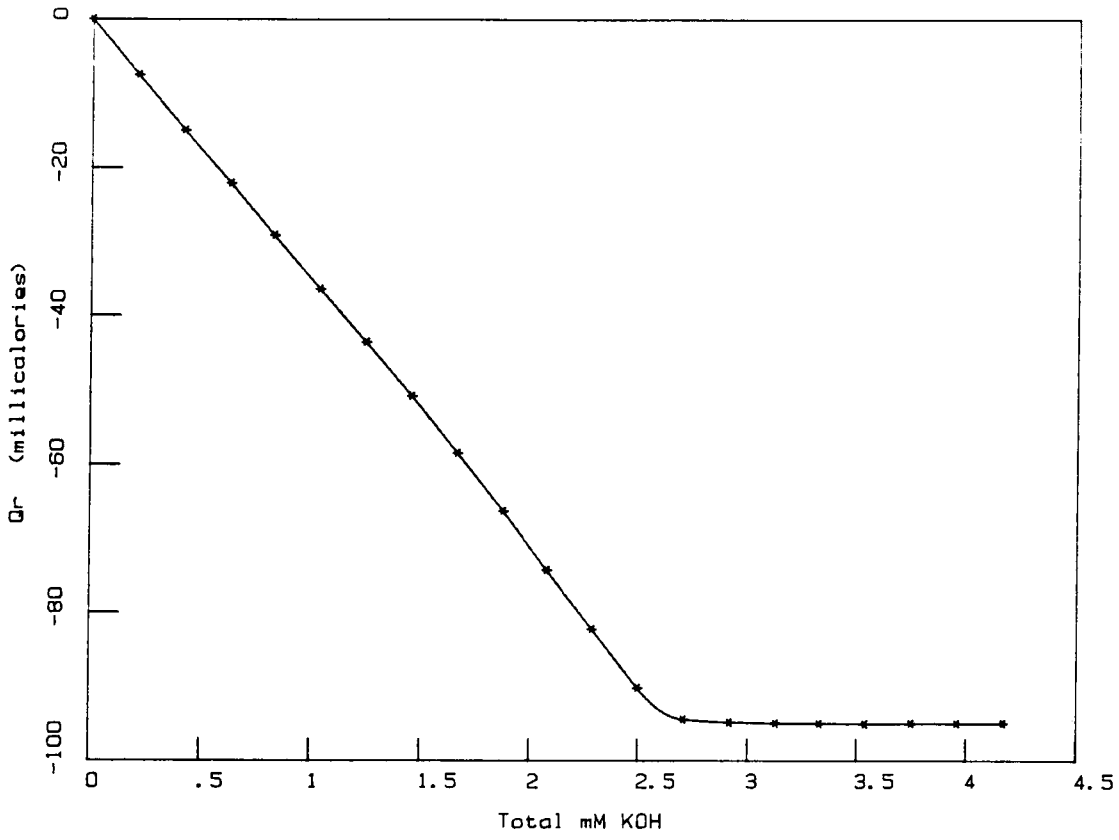

Fig. 8. Plot of Qr vs. the total concentration (neutralized plus free) of KOH in the ampoule.

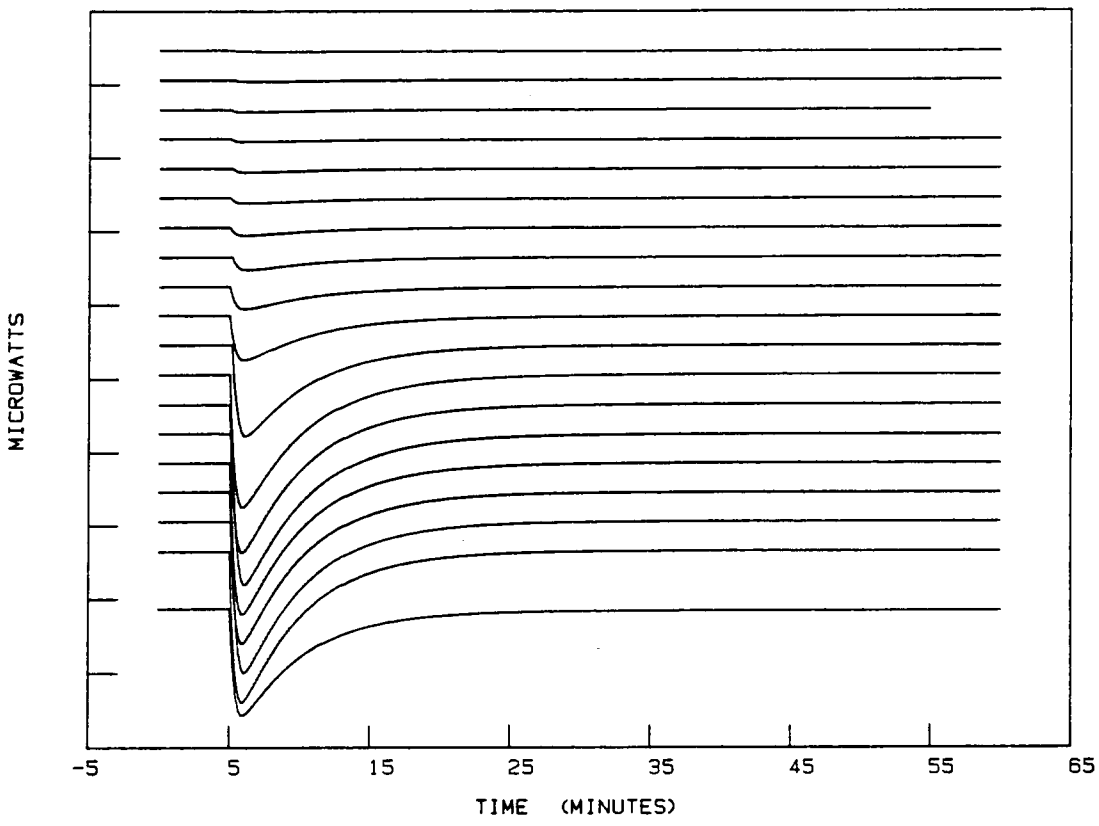

Fig. 9. Results from the injection of 25 ul of 50 mM $MgCl_2$ into 3 ml of 1.5 mM EDTA. First injection is on the bottom and the last is on the top.

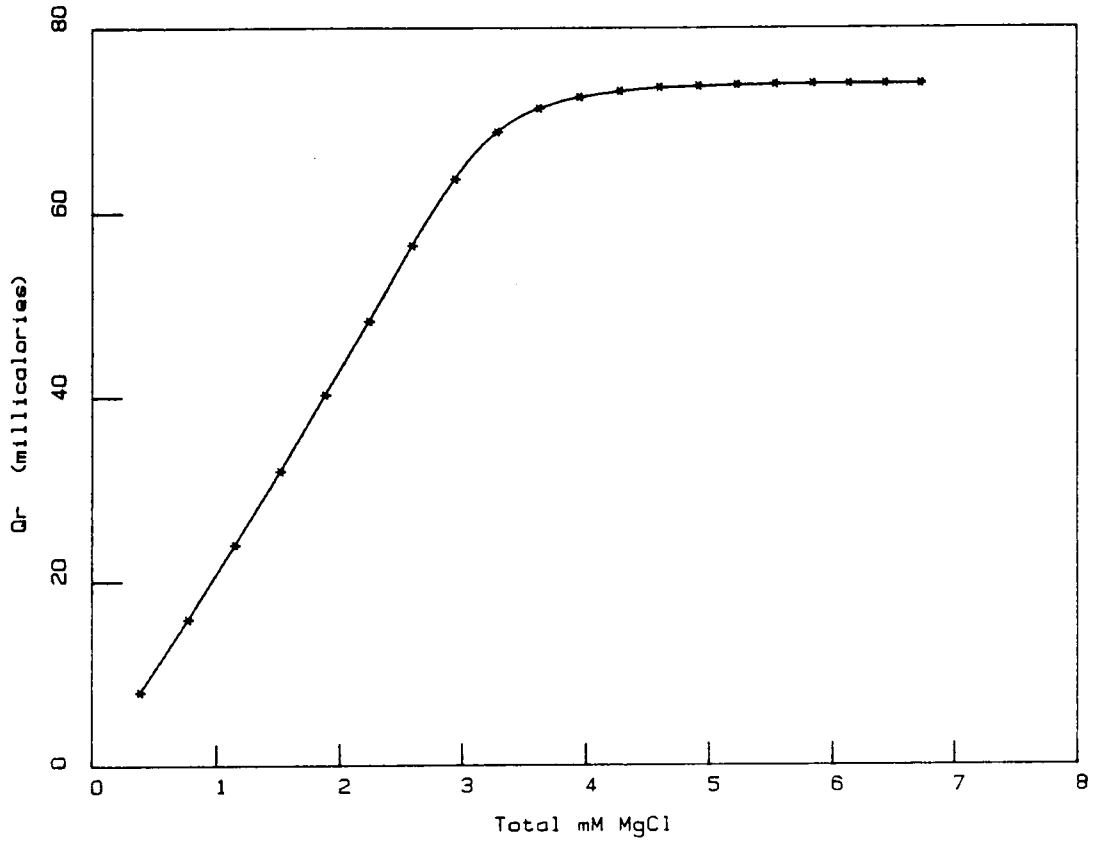

Fig. 10. Plot of Qr vs. the total concentration (chelated plus free) of $MgCl_2$ in the ampoule.

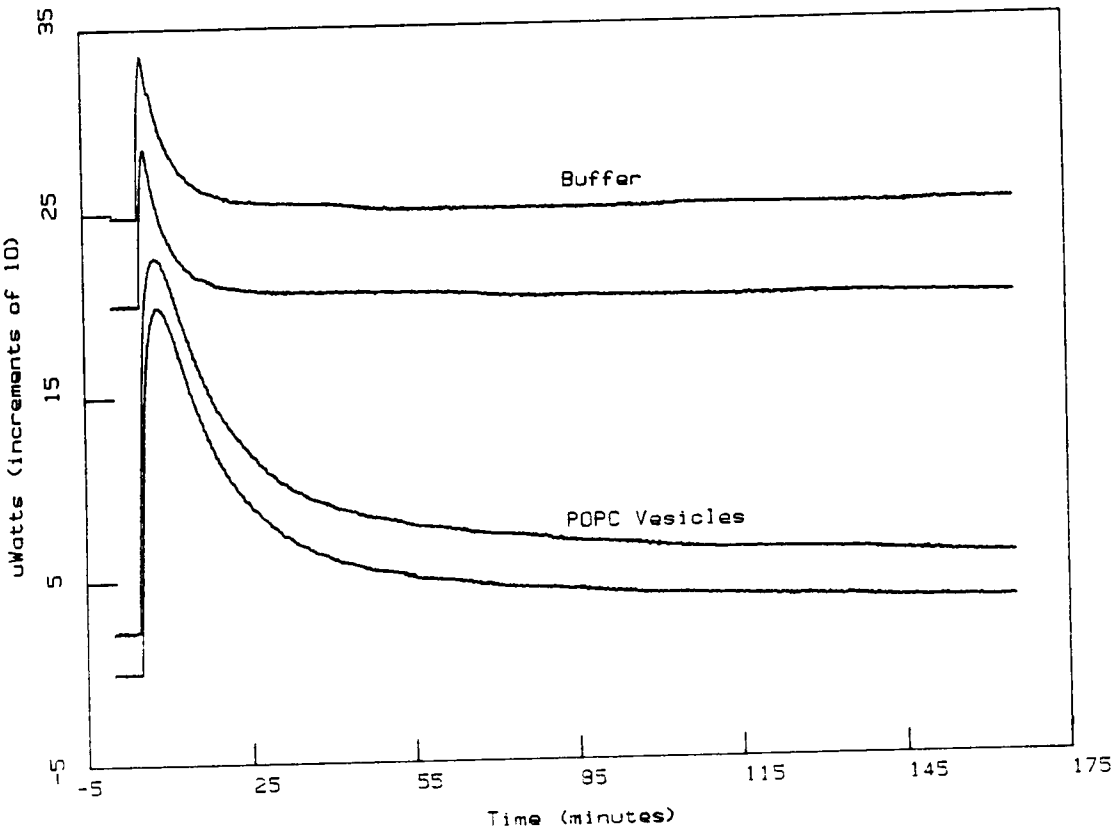

Fig. 11. Results from the injection of 25 ul of 0.85 mM cytochrome b_5 into 3 ml of either buffer or POPC vesicles. First injection is on the bottom.

Table 1. Relaxation times for various experiments.*

Experiment	tau (sec.)
Electrical Calibration	288
KOH into HCl	273
Cytochrome b_5 into Buffer	248
Cytochrome b_5 into POPC Vesicles	740

* The equation fitted was: uWatts = $A_1 + A_2 e^{-t/tau}$ where A_1 is constant value at infinite time, $A_1 + A_2$ is the initial value, and tau is the relaxation constant.

Table 2. Measured heats from the injection of cytochrome b_5.

Experiment	Microcalories	
	Injection: 1	2
Water into Water	-246 average	
Protein into Buffer	-596	-597
Protein into POPC Vesicles	-5154	-5564

VITA

Glen Douglas Ramsay was born in Hobbs, New Mexico on March 16, 1959. He attended elementary school in Tulsa, Oklahoma and later in Lafayette, California. He then moved with his family to Littleton, Colorado where he attended high school. In fall of 1976 he went to Colorado State University at Fort Collins and received his Bachelor of Science degree in Microbiology with a minor in Biochemistry in the spring of 1982. In the fall of 1983 he enrolled with the University of Tennessee, Knoxville in the Biochemistry department and subsequently received his Master of Science degree in the fall of 1985. At the time of this writing he is continuing his studies at U.T.K. in pursuit of his Doctor of Philosophy degree.