

Thermometric Titration of β -Cyclodextrin¹

Purpose: Determine the stoichiometry and enthalpy of binding of β -cyclodextrin and the sodium salt of 2-naphthalene-sulfonic acid. This reaction is a good example of a guest-host complex.

Introduction

Titrations can be done in a solution calorimeter². The temperature of the vessel is monitored as a function of added titrant. Such titrations are called thermometric titrations. Thermometric titrations have become a commonly used method for analytical² and reaction enthalpy determinations³. Thermometric titrations have become especially important in studies of protein and nucleic acid binding. For example, the enthalpy of binding of an inhibitor to an enzyme is a common determination. In this lab we will study the binding of a cyclic-polysaccharide to the sodium salt of 2-naphthalene-sulfonic acid.

The polysaccharide is β -cyclodextrin, β -CD. Cyclodextrins are often used as active site analogs for enzymes. Cyclodextrins are used to aid the absorption of drugs in the body. Other uses for cyclodextrins include the petroleum industry for separating aromatic hydrocarbons and in agriculture to reduce volatility of insecticides. Cyclodextrins are natural products produced by bacteria from starch. β -CD is made from seven D(+)-glucopyranose units linked through α -(1->4) glycosidic bonds⁴, Figure 1.

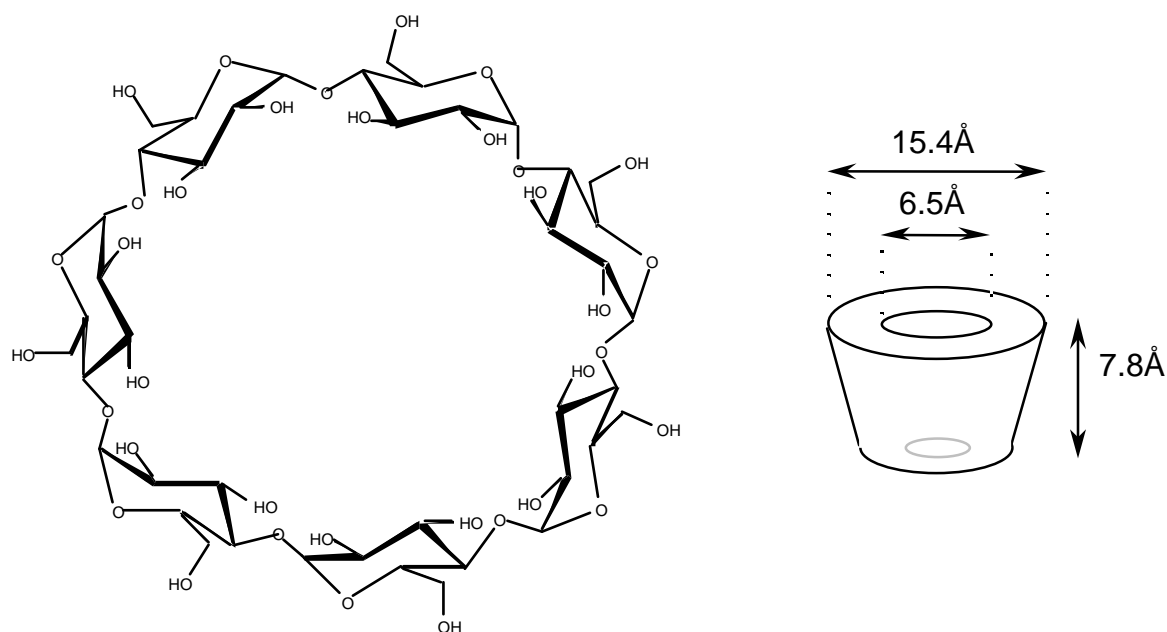


Figure 1. β -cyclodextrin (cycloheptaamylose).

In aqueous solution the CH bonds on the rings point inward producing a hydrophobic cavity inside a cylinder of diameter 15.4 Å. The OH groups extend from the top and bottom of the cylinder, providing sites for strong hydrogen bond formation. On average about 11 water

molecules fit inside the cylinder. The cavity volume is 0.14 mL/g. Cyclo-dextrins bind with a wide variety of substances. Such complexes are examples of guest-host complexes, where cyclodextrin is the host.

At pH 7.2, 2-naphthalene-sulfonic acid is predominately in its deprotonated form, Figure 2 ($pK_a = 0.6$). 2-Naphthalene sulfonate is expected to bind to β -CD because it has a hydrophobic side chain that will fit into the cyclodextrin cavity, while the $-\text{SO}_3^-$ group can participate in hydrogen bonds with the sugar OH groups. The reaction stoichiometry is not known, however, the binding is probably 1:1 based on the size of the cavity:

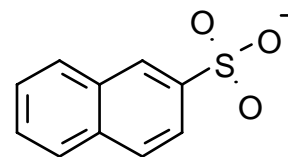
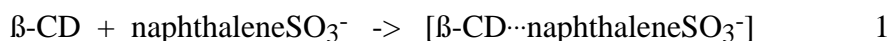


Figure 2. 2-Naphthalene sulfonate ion.



Also remember that some water molecules originally in the cavity will be excluded in the complex. This change in the number of water molecules in the cavity has an important effect on the binding enthalpy and entropy¹.

The analytical sensitivity of thermometric titrations is linearly related to concentration, in contrast to the logarithmic relation to concentration that exists for many other analytical methods, e.g., potentiometric methods. A linear relation is an advantage when very dilute solutions or solutions with high concentrations of interfering ions are being analyzed. For example, pH titrations of pyridine at concentrations below 0.05 M give poorly defined end points, while the end point of a thermometric titration is well defined.

Any calorimetry experiment consists of two parts. The first is the determination of the heat capacity of the system and the second is the determination of the heat effect of the reaction of interest. The heat capacity of the system is determined by passing a known amount of energy through a heater immersed in the solution and measuring the corresponding temperature change. The heat transferred is equal to the electrical work done by the heater

$$Q_{\text{el}} = -w_{\text{el}} = \int_0^t V I dt \quad 2$$

where V is the voltage across the heater and I is the current through the heater. The heat capacity of the system is then

$$C_{\text{sys}} = \frac{Q_{\text{el}}}{\Delta T_{\text{el}}} \quad 3$$

where C_{sys} is the heat capacity of the apparatus and solution and ΔT_{el} is the temperature change during the heating.

The second part of the calorimetry experiment is the determination of the enthalpy of the reaction of interest. If the change in the temperature for the reaction is ΔT_{rx} , then the heat produced is:

$$Q_{\text{rx}} = C_{\text{sys}} \Delta T_{\text{rx}} \quad 4$$

Since our solution calorimeter is at constant pressure, the molar reaction enthalpy is

$$\Delta_r H = \frac{Q_{rx}}{n_{rx}}$$

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where n_{rx} is the number of moles of the limiting reactant in solution.

Calorimeter

A constant rate buret, an insulated titration vessel, a stirrer, a heater, and a recording Wheatstone bridge are needed for this experiment. A schematic diagram of the apparatus is shown in Figure 3. All electrical and mechanical parts enter the vessel through the lid. The volume of the dewer is only four mL. The small volume is specifically designed for biochemical studies, where reagents are expensive and in short supply. A motor is used to drive the stirrer. The buret and reaction vessel are held in a constant temperature bath.

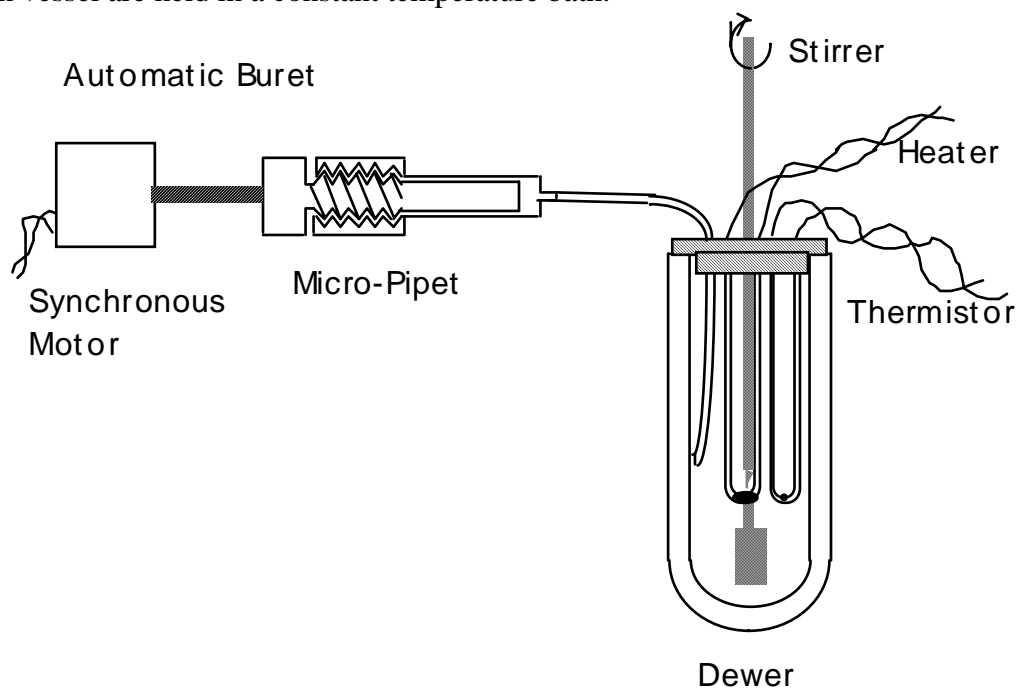


Figure 3. Tronac titration micro-calorimeter.

A thermistor is used to measure the temperature using a Wheatstone bridge circuit. The circuit diagram for the recording Wheatstone bridge is given in Figure 4.

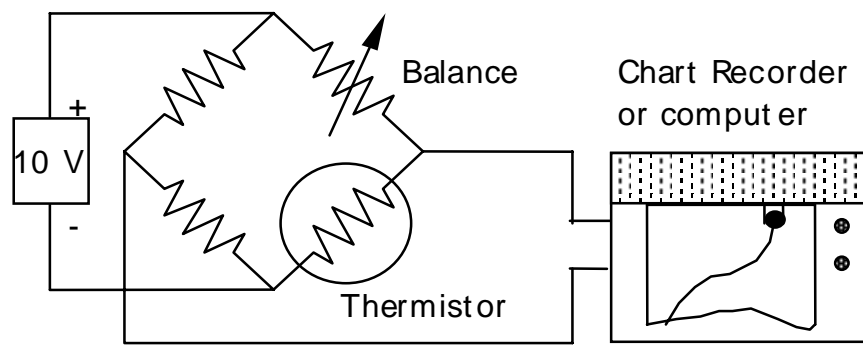


Figure 4. Recording Wheatstone bridge.

The response of the thermistor is not linear with temperature; it varies as

$$R = B \exp (A/T) \quad 6$$

where A and B are constants, and R is the resistance of the thermometer. However, over the narrow range of temperatures in this experiment, the variation of the bridge output with temperature is approximately linear. The constant rate buret is a commercial micro-buret. The buret is driven by a synchronous motor, to provide a constant titrant delivery rate.

To calculate Q_{el} from Eq. 2, the voltage and current must be monitored during the heating run. Rather than doing the integral, we will calculate the average voltage, V_{av} , and current, I_{av} , and then approximately:

$$Q_{el} = V_{av} I_{av} t \quad 7$$

Theory

Analytical Experiment

A typical titration curve obtained for a thermometric titration followed by a heating run to determine the heat capacity of the system is shown in Figure 3. 2-Naphthalene sulfonate ion is the guest and is added as the titrant. Cyclodextrin is the host and starts in the dewer. In the following discussion the symbol of the type A-B means "the line between points A and B".

The data acquisition is started without running the buret to establish the drift rate of the temperature; this gives R-S. If the calorimeter is hotter than the surroundings, the drift will be negative; if it is colder than the surroundings, the drift will be positive. After starting the buret at S, the upward line, S-A, indicates an exothermic reaction of the guest with the host, with the end point occurring at A. Between A and B, additional titrant is added with no further chemical reaction occurring. The heat effect for A-B is just the enthalpy of dilution of the titrant. At point B, the buret is turned off. Data acquisition is continued for a short time longer to establish the drift rate before turning on the heater. At C the heater is turned on, as is allowed to run to produce a temperature change that is roughly comparable to the temperature change in the titration. At D the heater is turned off. Data acquisition is continued for a short time longer to establish the drift rate at the final temperature. For an endothermic reaction, the S-A line segment would show a decrease in temperature.

The titrant must be standardized so that the concentration, M_{guest} , is well known. We wish to know the number of guest molecules that bind to the host to verify the stoichiometry in Eq. 1. The number of molecules that bind will be given by

$$\frac{\text{moles guest}}{\text{moles host}} = \frac{M_{\text{guest}} V_{\text{guest}}}{M_{\text{host}} V_{\text{host}}} \quad 8$$

where the M's are molar concentrations and the V's are the volumes of added guest and host, respectively. For this titration, $V_{\text{guest}} = V_A - V_S$. We only have a rough estimate of the M_{host} , because the extent of hydration of β -CD is variable. Once the stoichiometry of the reaction is known, the exact concentration of the host can be calculated in the normal fashion for a titration. Assuming 1:1 stoichiometry:

$$M_{\text{guest}} V_{\text{guest}} = M_{\text{host}} V_{\text{host}} \quad 9$$

Eq. 9 is solved for M_{host} .

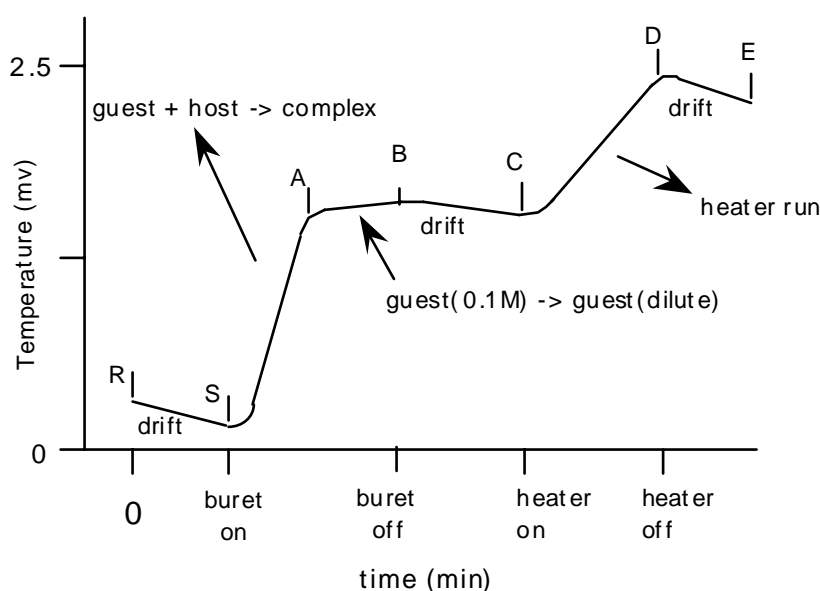


Figure 3. A typical thermogram for a simple titration followed by a heating run to determine the heat capacity. An exothermic reaction is illustrated.

Physical Experiment

The rate of rise of the temperature, as indicated by the slopes of the lines in Figure 3, is proportional to the enthalpy of the reaction involved, if the slopes are corrected for the rate of heat gain or loss caused by the surroundings, the stirrer, the "heater" effect of the thermistor, and the enthalpy of dilution of the titrant^{5,6}.

The correction for heat loss or gain to the surroundings, the stirrer, and the "heater" effect of the thermistor is made for all regions of the titration by using Newton's Law of cooling and slopes R-S, B-C, and D-E⁷. The first step in this correction is to determine the slope of the titration curve subsequent to point A if the titration had been stopped exactly at the endpoint. This slope is indicated as A-A' in Figure 4. The calculated slope is then used to find the temperature change caused by the reaction by extrapolating both the fore-and after- period slopes to the midpoint of the reaction region to find the temperature change by difference, ΔT_{rx} ⁸.

These calculations are done as follows. Let t_R through t_E be the times corresponding to the lettered points in Figure 4, and let T_R through T_D be the corresponding temperatures, measured in millivolts (arbitrary units) from the thermogram. We need to calculate the slopes of each of the line segments; the slopes are indicated by the symbol "M". The slopes R-S, B-C, and D-E are:

$$M_{\text{RS}} = (T_S - T_R)/(t_S - t_R) \quad 10$$

$$M_{\text{BC}} = (T_C - T_B)/(t_C - t_B) \quad 11$$

$$M_{\text{DE}} = (T_E - T_D)/(t_E - t_D). \quad 12$$

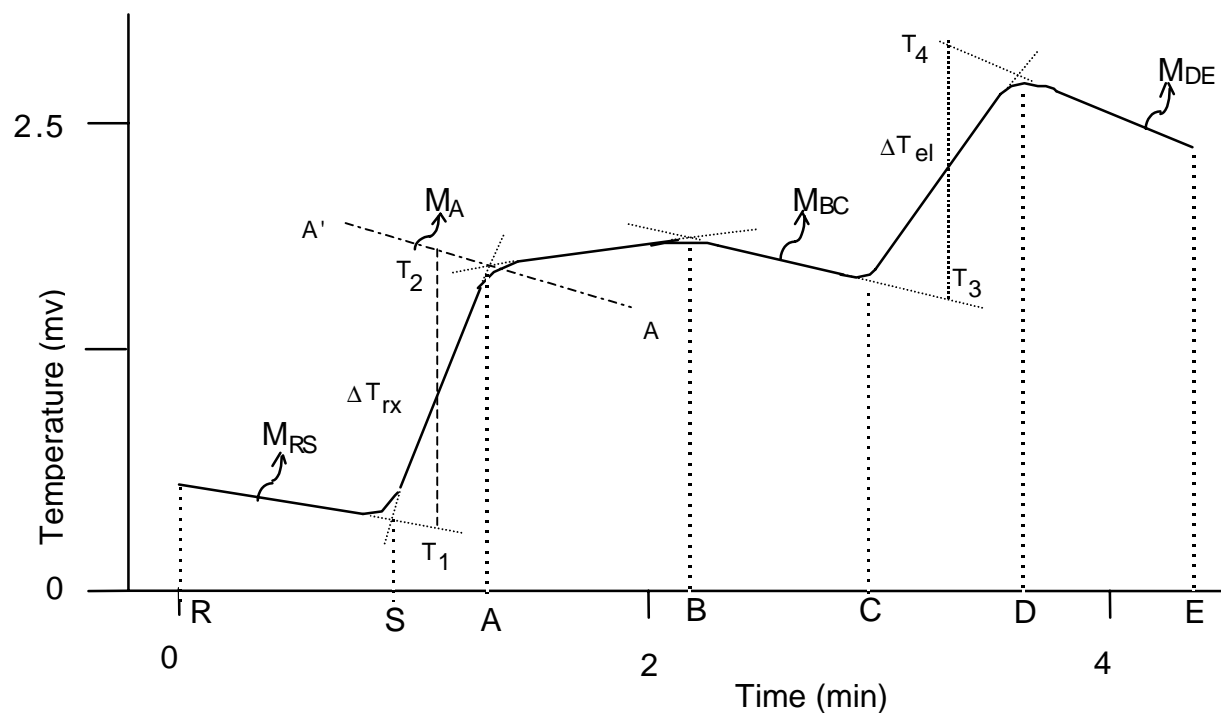


Figure 4. Derived quantities.

We estimate the slope at A-A' as a weighted sum of the above slopes.

$$M_A = M_{RS} + (M_{BC} - M_{RS})(T_A - T_S)/(T_B - T_S) \quad 13$$

Then extrapolating forward to the midpoint of the reaction

$$T_1 = T_S + M_{RS} (t_A - t_S)/2. \quad 14$$

Extrapolating backward along A-A' gives

$$T_2 = T_A - M_A (t_A - t_S)/2. \quad 15$$

Finally

$$\Delta T_{rx} = T_2 - T_1. \quad 16$$

Similarly

$$T_3 = T_C + M_{BC} (t_D - t_C)/2 \quad 17$$

$$T_4 = T_D - M_{DE} (t_D - t_C)/2 \quad 18$$

and

$$\Delta T_{el} = T_4 - T_3. \quad 19$$

The amount of heat liberated in the heater is given by Eq. 2 or 7, which gives the heat capacity of the system at B or C using Eq. 3 as:

$$C_{\text{sys,B}} = \frac{Q_{\text{el}}}{\Delta T_{\text{el}}} \quad 20$$

We must determine the heat capacity at A indirectly, knowing that the heat capacity of the added titrant between A and B is essentially that of water, 4.184 J/mL/deg. For this calculation we need to know the buret delivery rate, R, in mL min⁻¹ and the sensitivity of the Wheatstone bridge, S, in degrees millivolt⁻¹. The heat capacity at B is the sum of the heat capacity at A plus the heat capacity of the added titrant solution (the change of heat capacity of the solutions due to the reaction is negligible). This sum gives

$$C_{\text{sys,B}} = C_{\text{sys,A}} + (t_{\text{B}} - t_{\text{A}})R (4.184)(S). \quad 21$$

We then solve Eq. 20 for C_{sys,A}. The heat evolved during the titration is given using Eq. 4 as

$$Q_{\text{rx}} = C_{\text{sys,A}} \Delta T_{\text{rx}} \quad 22$$

The molar enthalpy of the reaction is then given by Eq. 5, where n_{rx} is the number of moles of host determined from the volume of titrant added at the endpoint.

Procedure

Prepare the solutions: Place about 25mL of 0.1 M 2-naphthalene-sulfonate in pH 7 buffer and about 10 mL of 10mM β-cyclodextrin in pH 7 buffer in a 25°C constant temperature bath.

Prepare the recorder : The constant temperature bath will be stabilized when you come in to lab. The system will be set at standby. Turn on the power to the chart recorder and set the chart rate at 5 cm min⁻¹ and the sensitivity at 5 mv full scale. Turn on the chart drive to make sure the paper moves at a steady rate. Turn the monitor mode switch on the calorimeter console to "RV temp"; the digital voltmeter will now display the voltage output of the Wheatstone bridge. In this mode the digital volt meter and the chart recorder are both recording the same thing, the temperature of the reaction dewer.

Determine the delivery rate: Determine the delivery rate of the buret, R, by placing water in the buret and weighing the amount of water delivered for 1 minute. Use the chart drive on the recorder to determine the time. The units will be ml/min. Do three replicates and average the result. To fill the buret, lift the calorimeter insert up from the water bath. Remove the teflon tube from the tip of the buret. Loosen the bolt at the top of the micro-buret. Fill the buret by turning the plunger barrel. Expel any air by turning the buret upside down and turning the plunger barrel. Reattach the buret to the motor shaft. Leave the calorimeter in the up position during your volume calibration runs. Remove the reaction dewer flask and place a pre-weighed 10-mL beaker under the delivery tube. Start the chart recorder, when the pen passes a calibration line on the paper turn on the buret drive. When the chart indicates that 1 minute has passed, turn off the buret drive and reweigh the beaker. Remember to convert from weight to volume using the density of water from the CRC density tables.

Determine the bath temperature: Next we need to determine the bath temperature accurately. With the reaction dewer still off, lower the calorimeter insert onto the two grey metal spacers. This position allows the thermistor to be immersed in the constant temperature bath. Adjust the fine bridge balance control so that the pen is near, but not at, the top of the chart paper. The position of the pen now shows the bath temperature. Note the voltage on the digital volt meter that corresponds to this temperature, you will need this reading later. Raise the calorimeter insert out of the bath.

Fill the buret : Expel any remaining water from the buret. Be careful not to lower the buret plunger to the point where the glass barrel begins to curve in; this may damage the plunger. Rinse the buret twice with small amounts of 2-naphthalene-sulfonate solution. While rinsing, invert the buret and retract the plunger so the walls of the buret are rinsed well. Fill the buret with 0.100 M 2-naphthalene-sulfonate solution in pH 7 buffer. Expel any air and reattach. Run the buret for a short time to flush out the delivery tube. To keep the titrant solution from coming into contact with the host solution before the titration is to start, using the chart recorder for timing, switch the buret drive into the reverse position and run the buret backwards for two small divisions on the chart paper. There should now be a small bubble in the end of the delivery tube. When you determine the time $t_A - t_S$ to get to the endpoint of the titration, remember to subtract these two divisions from the time, to take into account this bubble. Switch the buret drive back to the forward direction. Rinse the delivery tube, stirrer, thermistor, and heater with a stream of reagent grade water in a wash bottle. Carefully use a ChemWipe to blot any water droplets from the end of the delivery tube, stirrer, thermistor, and heater.

Fill the reaction dewer: Accurately pipet 2.00 mL of 10mM cyclodextrin in pH 7 buffer into the reaction dewer. Attach the dewer to the calorimeter. Turn on the stirrer and make sure that the stirring rate is constant. Lower the calorimeter insert all the way into the constant temperature bath.

Heat the reaction dewer to the bath temperature: At first the solution in the dewer will be below the bath temperature and the drift rate will be too large. We need to start at a temperature much closer to the bath temperature. We will use the heater circuit to heat the solution to the bath temperature. Make sure the stirrer is on. Set the heater power to 20 mcal/sec. Turn on the heater and monitor the voltage, which corresponds to the reaction vessel temperature, on the digital volt meter. The voltage will increase slowly; when the voltage approaches the value you read above, turn off the heater. The pen on the chart recorder should now respond. Switch the heater power to 5.0 mcal/sec. Turn on the heater for short periods of time until the pen is near the position you set for the bath temperature.

Do the titration: Switch the monitor mode switch to "V heater." The digital volt meter will now display the voltage across the heater. Turn on the chart recorder to begin the R-S segment. Monitor the temperature drift for one minute. The slope of the line should not be steep, but rather show less than 0.1 mV min^{-1} drift. Turn on the buret, and mark the chart at this time. Let the buret run about 30 sec after the end point is complete to yield the A-B segment. Turn the buret off to begin the B-C segment. Monitor the temperature drift for one minute or so. Check that the heater power is 5.0 mcal/sec. Turn on the heater and mark the chart. While the heater is running, **record the voltage and current on the heater** as read from the digital volt meter. You will need to switch the monitor mode switch from "V heater" to "I heater" and back, repeatedly. Read the

voltage and current every 10 sec. When the temperature change caused by the heater is about the same as the temperature change for the titration, turn off the heater. This step completes the C-D segment. Leave the chart recorder drive on for about one minute more to monitor the drift rate for the D-E segment.

Repeat the titration three times: Rinse out the reaction dewer and dry. Rinse off the thermistor, heater, stirrer, and titrant delivery tube with a stream of deionized water from a wash bottle. Blot any remaining drops of water with a ChemWipe. Check the buret to make sure that you have enough titrant for another run. Repeat the above instructions. After you are finished with three runs, remember to clean out the micro-buret and rinse with deionized water. Leave the buret filled with deionized water. Clean out the reaction dewer and rinse off the thermistor, heater, stirrer and titrant delivery tube with a stream of deionized water. Turn off the chart recorder and digital volt meter. Leave the constant temperature bath on.

Extrapolate each section of the thermogram to find the endpoints. To do this, use a straight edge and sharp pen or pencil. You will also need to know the temperature sensitivity of the Wheatstone bridge. The sensitivity is defined as

$$S = \Delta T(\text{in degrees})/\Delta T(\text{in millivolts}). \quad 23$$

The measurement of the sensitivity takes awhile, so the sensitivity will be measured for you. Make sure to record the value listed in the front of the calorimeter operators manual. You will also need to record the value of the heater resistance, which is also listed in the operators manual.

Calculations

Knowing the titrant concentration and the volume added from points S to A calculate the reaction stoichiometry using Eq. 8. Then use Eq. 9 to calculate the exact concentration of the host in the stock solution. Report the average concentration for the host and the standard deviations of the trials.

Calculate M_{RS} , M_{BC} and M_{DE} from Eq. 10-12. Calculate M_A from equation 13. Calculate T_1 and T_2 using equations 14 and 15, and then ΔT_{rx} from Eq. 16. Calculate T_3 and T_4 from equations 17 and 18, and ΔT_{el} from Eq. 19.

To calculate the electric energy delivered to the system, note that the "I heater" setting of the monitor gives the voltage across a standard 100 ohm resistor and not the current directly. To convert your voltage readings in the "I heater" mode to a current, divide by 100. Average the voltage across the heater and the current through the heater during each run. Then use Eq. 7 with $t = (t_D - t_C)$. (You can double check your V and I readings using $V=IR$, where R is the resistance of the heater, to look for unit and data errors.)

The heat capacity at A is then found from Eqs. 20 and 21. Finally, calculate the molar enthalpy of reaction, $\Delta_r H$, using Eq. 22 and 5. Do your calculations for each run separately and average your final $\Delta_r H$ values. Calculate the standard deviation of the trials. An EXCEL spreadsheet will make your calculations much easier.

Discussion

In your discussion use A-A', extrapolating forwards, and B-C, extrapolating backwards, to estimate the enthalpy of solution of the 2-naphthalene-sulfonate titrant in the calorimeter. You don't need to do the calculations, just get a rough qualitative estimate. Determine if this enthalpy of solution has any effect on the enthalpy calculated for the reaction. What does the stoichiometry and $\Delta_r H$ of the reaction tell you about the complex? Why does the complex form? Please include a table containing all of the temperatures and times for R through E, n_{rx} , the slopes, the heat capacities, T_1 , T_2 , T_3 , T_4 , ΔT_{rx} , ΔT_{el} , V_{av} and I_{av} for each run. Also include R, S, the resistance of the heater, and the concentration of your titrant.

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