Contents

Section 1

The Omega ITC System

Section 1.1

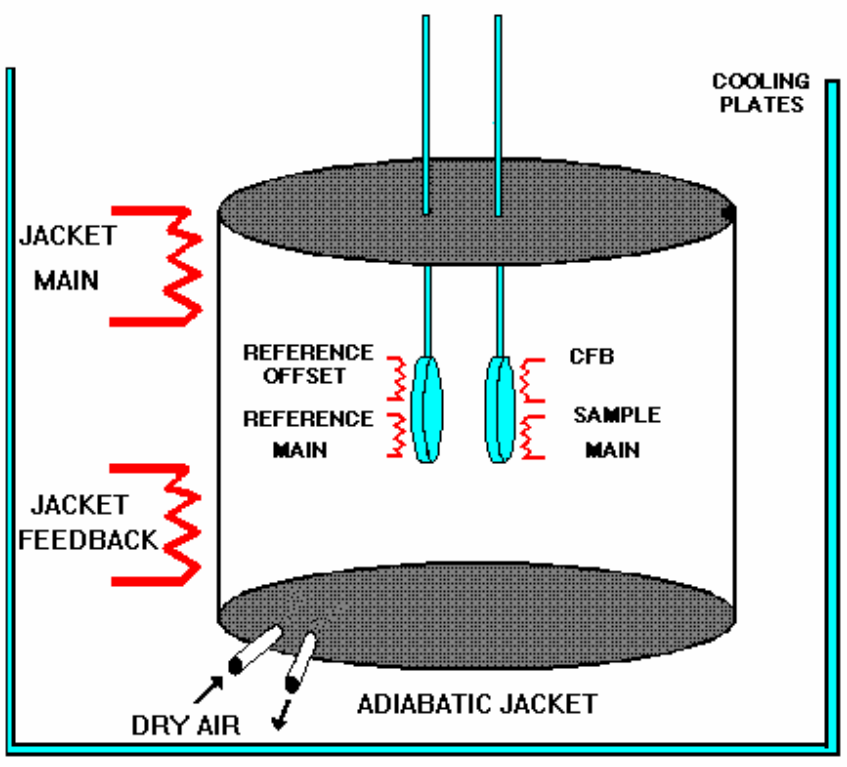
Omega - Isothermal Titration Calorimeter

The Omega ITC Unit directly measures heat evolved or absorbed in liquid samples as a result of injecting precise amounts of reactants. A spinning syringe is utilized for injecting and subsequent mixing. Spin rates are user selectable.

The normal operating range is 0 °C to 80 °C.

Wetted surfaces are Hastelloy-C alloy.

Sample and reference cells are accessible for filling and cleaning through the top of the unit. The sample cell is on the right as one faces the front of the unit.



A pair of identical coin shaped cells are enclosed in an adiabatic jacket. Access stems travel from the top exterior of the instrument to the cells. Both the coin shaped cells and the access stems are totally filled with liquid during operation. This requires approximately 1.7 ml. per cell.

During an experiment the reference cell is heated by a very small constant power, the reference offset. The temperature difference between the two cells is constantly measured and a proportional power is increased or reduced to the sample cell by the cell feedback (CFB) system to keep the temperature difference very close to zero. A signal proportional to that Cell feedback is called dCp, and with the instrument temperature and time, constitutes the

instrument's relevant raw data. The dCp is calibrated in units of μCal/sec. The operating range is ca. -200 to +230 μCal/sec.

The instrument is calibrated by applying a known power input to the sample cell and measuring the resulting deflection in the dCp power signal.

An injection which results in the chemical evolution of heat within the sample cell causes a negative change in the dCp power since the heat evolved chemically provides heat that the cell feedback is no longer required to provide. The opposite is true for endothermic reactions. Since the dCp has units of power, the time integral of the peak yields a measurement of thermal energy, ΔH.

If the cell feedback is negative there is no feedback power applied to the sample cell and no measurements of thermal energy are being made. If the cell feedback saturates positive, the corresponding peak will appear clipped. Either can happen during an experiment if a sample generates too large a signal. It is then useful to be able to offset the cell feedback level within its operating range. This is easily done with the BASE LINE control switch.

A jacket feedback system constantly monitors the temperature difference between the cells and the jacket. Power applied to the jacket resistive heater is increased or reduced to keep the temperature difference small.

This scheme can only function when the instrument is above ambient temperature since temperatures are controlled only by applying heat in various amounts. In order to work below room temperature, water cooled plates surround the core instrument, and permit simulation of lower ambient temperatures. A refrigerated circulation bath provides fluid to the plates, and hose connections are made at the rear of the Omega Cell Unit.

Section 2

Omega ITC - Hardware

Section 2.1

Omega - Instrument Assembly

NOTE: Those users who have purchased an Omega accessory cell (to be hooked up to an existing MC-2 system) may find it necessary to replace their current MC-2 Control Module PC board with a new PC board designed to operate with both the Omega and MC-2. This new PC board has been shipped along with your new Omega accessory cell and consists of a small (approximately 4.5" square) printed circuit board which has two gray cables attached to it (one thin cable ending in a white two-prong plug, the other a thicker cable ending in a 15-pin connector). Instructions on changing the Control Module PC Board can be found in the Appendix section of this manual.

Motor Stand: The motor stand is mounted to the top of the Omega cell unit (Figure 3) with a single screw on the right-hand side. Proper tension on the screw allows the stand to swing forward and back. A thumbscrew is used to lock the stand in place during experiments. A cable labeled "**To Motor Stand**" (C6 in Figure 3) exiting the rear of the Omega connects to the motor stand (C4 in Figure 3).

Electrical Connections: A Switch Box is included to interface the Control Module to both DSC (MC-2) and Omega cells. This is shown in Figure 2. For those users with a stand-alone Omega system (no MC-2 cell with the system), a small Gain Box replaces the Switch Box. The FEEDBACK GAIN adjustments on the Switch or Gain Box allow cell feedback gain changes. The gain setting with the blue box MicroCal Nanovolt Preamplifier is **100** on the **100 X** scale (set the **FINE** dial for **100** with the **COARSE** knob set for the **100 X** scale). If using the built-in pre-amplifier, set the **FINE** dial for **100** with the **COARSE** knob set for the **1 kX** scale.

Setting up the Omega stand-alone instrument (no MC-2 cell with the system), please refer to figures 2, 3 and 4 (pages 10, 11 and 12):

1. Connect **E** (18-pin cable socket on Control Module) with **F** (18-pin cable socket on Omega Cell Unit) using the 18-pin cable.

2. Connect **C1** (Jacket TP cable from Control Module) to **H** (Jacket TP socket on Omega). Be sure the largest prong is seated in the largest hole.

3. Connect **C2** (Cell TP cable from Control Module) to **I** (Cell TP socket on Omega). **Note:** For those users with an external nanovoltmeter, the nanovoltmeter's TP cable connects to **I** on the Omega and **C2** is left unconnected. The output cable from the nanovoltmeter is then connected to **X** on the Control Module.

4. Insert **C5** (the 6-blade plug from the Omega) into the matching socket (**R**) on the small Gain Box.

5. Plug **C3** (PC Board cable connector) into **O** on the small Gain Box.

6. Connect **C7** (Omega main heater plug) into socket **G** on the back of the Omega. Twist the outer ring to lock the plug into place after insertion.

7. Plug **C6** (motor stand control cable connector) into its matching socket (**C4**) on the motor stand base.

8. With the computer off, insert the gray ribbon cable (**C8**) into the connector of the **DT2801** board (which should already be in your computer - see your computer manual for information on inserting an option card if you still need to install the DT2801). The red stripe on the ribbon cable should match up with the top of the DT2801 board's connector. You may have received an additional gray ribbon cable with your shipment - this is merely an extension cable which can be used to connect the DT2801 and the Omega's gray ribbon cable (**C8**) more easily.

Setting up the Omega cell with the MC-2 system, please refer to figures 2 - 5 (pages 10 - 13):

1. Connect **E** (18-pin cable socket on Control Module) with **L** (18-pin cable socket on Switch Box) using one of the 18-pin cables. Now connect **F** (18-pin cable socket on Omega) with **M** (18-pin cable socket for Omega on Switch Box) using another of the 18-pin cables. One end of the last 18-pin cable should be plugged into **N** on the Switch Box (MC-2 18-pin cable socket on Switch Box), while the other end of that cable should be connected to **C** (18-pin cable socket on MC-2).

2. Connect **C1** (Jacket TP cable from Control Module) to **H** (Jacket TP socket on back of Omega cell unit). The TP extension cable marked "MC-2 JACKET" should be left unconnected till you wish to make the MC-2 the active cell. Be careful to insert the TP cable plugs right-side up - one of the two prongs on each plug is larger than the other and one of the two holes on the matching socket is slightly larger than the other. Take care to match the larger prong with the larger hole when inserting the TP plugs.

3. If you are using the MicroCal Nanovolt Preamplifier connect the white two prong input TP plug into **I** (Cell TP socket on the back of the Omega cell unit). The black phono type output plug is then connected to **X** on the Control Module. If you are not using the MicroCal Nanovolt Preamplifier connect **C2** (Cell TP cable from Control Module) to **I** (Cell TP socket on back of Omega cell unit). Again, take care that the plugs are being inserted right-side up (see above in 2.)

4. Insert **C5** (the 6-blade plug from the Omega) into the matching socket (**R**) on the Switch Box.

5. Plug **C3** (P.C. Board cable connector) into **O** on the Switch Box.

6. Connect **C7** (Omega main heater plug) into socket **G** on the back of the Omega. Twist the outer ring to lock the plug into place after insertion.

7. Plug **C6** (motor stand control cable connector) into its matching socket (**C4**) on the motor stand base.

8. For MC-2 users, Plug **C9** (the Relay Box 25-pin cable connector) into **D** (the Control Module 25-pin socket marked **"FOR COMPUTER USE ONLY"**). If you do not have a Relay Box, leave the 25-pin "dummy plug" on this socket.

9. You should have received an additional gray ribbon cable with your shipment - this is merely an extension cable which can be used to connect the DT2801 and the appropriate gray ribbon cable (**C8** - Omega or **C10** - MC-2) more easily. With the computer off, insert the gray ribbon cable extension into the connector of the **DT2801** board (which should already be in your computer - see your computer manual for information on inserting an option card if you still need to install the DT2801). The red stripe on the ribbon cable should match up with the top of the DT2801 board's connector. Now insert the gray ribbon cable from the Omega (**C8**) into the other end of the extension cable, making sure the red stripes line up. Make sure the cables are all connected as firmly as possible.

10. The three toggle switches of the front of the Switch Box should be flipped to the Omega position for Omega use.

Switch between DSC (MC-2) and Omega operation by:

1. With all power off, switch three toggles to all up (Omega use) or all down (MC-2 use), as marked on the Switch Box.

2. Plug appropriate 50 conductor ribbon cable into extension ribbon cable already plugged into computer.

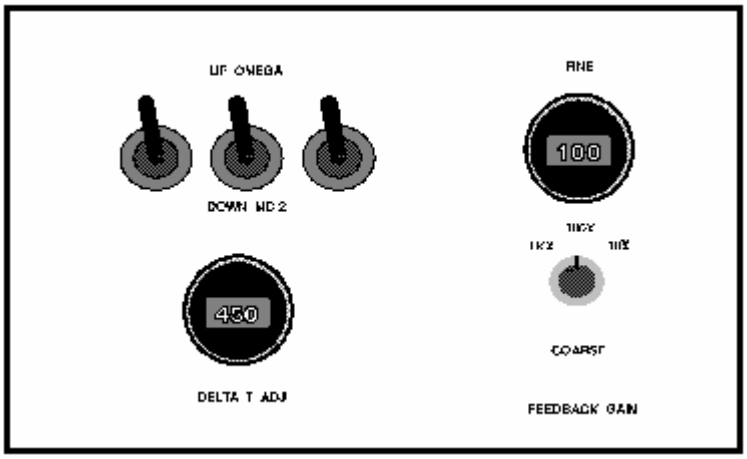
3. When the Omega is being used with the nanovolt preamplifier connect its input cable to **I** on the Omega cell unit and leave C2, the Cell TP cable disconnected, if the nanovolt preamplifier is not being used connect C2, the Cell TP cable, to **I.** Connect the Jacket TP cable to **H** on the Omega cell unit. When the MC-2 is being used with the nanovolt preamplifier connect its input cable to **B** on the MC-2 cell unit and leave C2, the Cell TP cable, disconnected, if the nanovolt preamplifier is not being used connect C2, the Cell TP cable, to **B.** Connect the Jacket TP cable to **A** on the MC-2 cell unit.

KEY TO FIGURES 2 - 5

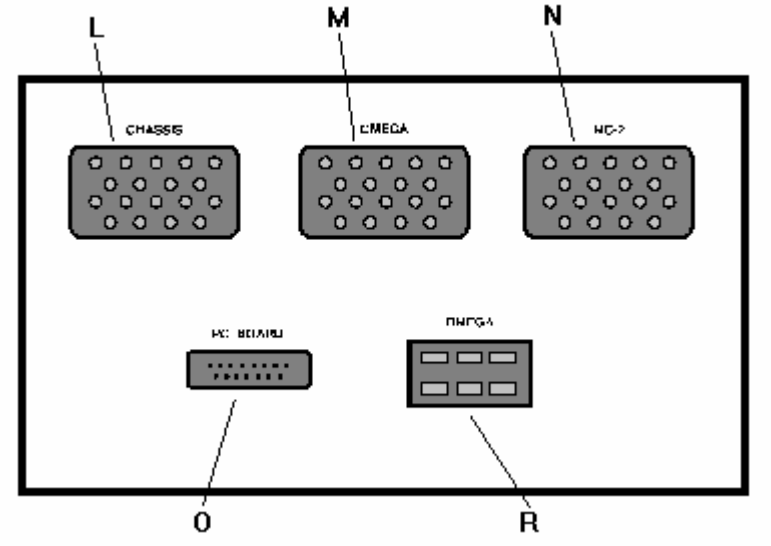
C1: Jacket TP Plug on Control Module (goes to H on Omega Cell or A on MC-2 Cell depending on which cell is currently active)
C2: Cell TP Plug on Control Module (goes to I on Omega Cell or B on MC-2 Cell, if the MicroCal Nanovolt Preamplifier is being used this plug is left unconnected)
C3: P.C. Board cable 15-pin plug on Control Module (goes to O on Gain/Switch Box)
C4: Omega Motor stand cable socket (connects to C6)
C5: Gain/converter box 6-blade plug (connects to R)
C6: Omega Motor stand control cable plug (connects to C4)
C7: Omega Main heater cable plug (connects to G)
C8: Omega Motor box 50-conductor "ribbon" cable (connects to DT2801 interface board socket on computer)
C9: MC-2 Relay box 25-pin cable plug (for MC-2 only - connects to D on Control Module)
C10: MC-2 Relay box 50-conductor "ribbon" cable (for MC-2 only - replaces C8 at computer when MC-2 is used)
C11: MC-2 Cooling Solenoid control cable (connects to U on Control Module)

A: Jacket TP socket on MC-2 (connect to C1 - Jacket TP Plug on Control Module when MC-2 is active cell)
B: Cell TP socket on MC-2 (connect to MicroCal Nanovolt Preamplifier or C2 Cell TP Plug on Control Module when MC-2 is active cell)
C: 18-pin cable socket on MC-2 cell
D: "FOR COMPUTER USE ONLY" 25-pin socket on Control Module (MC-2 use only - MC-2 Relay Box connector C9 attaches here)
E: 18-pin cable socket on Control Module
F: 18-pin cable socket on Omega cell
G: Main heater socket on Omega cell
H: Jacket TP socket on Omega cell (connect to C1 - Jacket TP Plug on Control Module when Omega is active cell)
I: Cell TP socket on Omega cell (connect to MicroCal Nanovolt Preamplifier or C2 Cell TP Plug on Control Module when Omega is active cell)
L: 18-pin cable socket on Switch Box for Control Module 18-pin cable
M: 18-pin cable socket on Switch Box for Omega 18-pin cable
N: 18-pin cable socket on Switch Box for MC-2 18-pin cable
O: 15-pin socket on Switch/Gain Box for P.C. Board cable connector (C3)
R: 6-blade socket on Switch/Gain Box for Omega 6-blade cable connector (C5)
U: MC-2 Cell Solenoid cable socket on Control Module (connects to C11)
X: External nanovoltmeter socket on Control Module

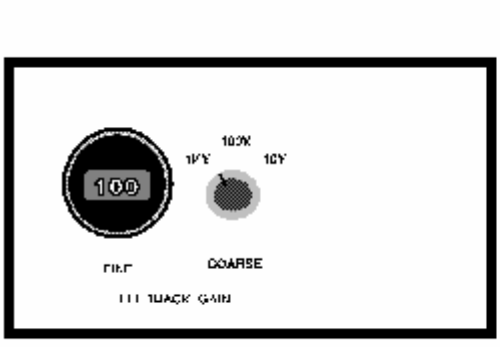
Figure 2
SWITCH BOX and GAIN BOX
(Omega & MC-2 SYSTEM)



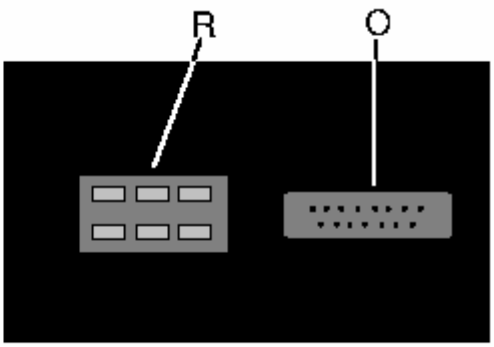
FRONT VIEW OF SWITCH BOX



BACK VIEW OF SWITCH BOX



FRONT VIEW OF GAIN BOX



BACK VIEW OF GAIN BOX

Figure 3

Figure 4

Figure 5

Section 2.2

HAAKE F3-CH External Water Bath

This section of the manual is intended to familiarize the user with the Haake external water bath's controls and connectors. If you do not have a Haake water bath you may skip this section. This is not intended to, nor does it suffice as a replacement for the user's manual which you have received with the Haake. Please refer to the following diagrams for a minimum description of the Haake F3-CH connections and controls.

1) Please insulate the water bath hoses from the Omega with the insulation provided or that supplied with the Haake bath.

2.) Connect the hose from the Omega marked 'water in' to the Coolant Out Port (12) on the rear of the F3 controller and the hose marked 'water out' to the Coolant in Port (13), with the appropriate hose clamps.

3.) Make sure the control cable from the socket of refrigerated bath is connected to the socket labelled 4 (10) on the rear of the controller.

4.) Selector switch (16) should be set to internal.

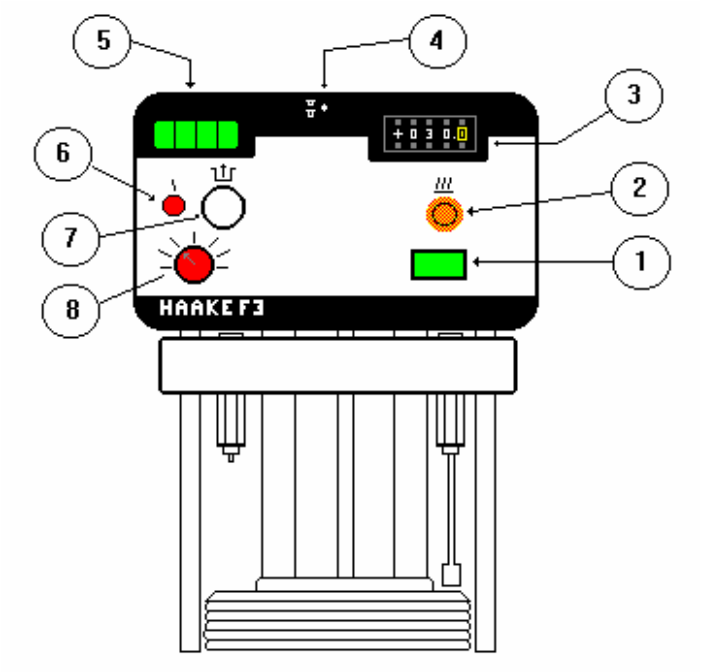
5.) The switch on the back of cooling unit/water reservoir should be set on.

6.) Reversing Switch (*intern/extern*) (4) on front of controller should be set for *internal* (button OUT position) and adjust the digital switch (3) to desired setting. WARNING — do not set the bath temperature to below the freezing point of the liquid of the cells or the cells will be irreparably damaged.

7.) Set temperature limiter switch (9) on front of controller to 80 °C. WARNING — the maximum temperature the jacket shield of the Omega can tolerate is 80 °C. Should this temperature be exceeded the cell assembly may be irreparably damaged.

8.) Fill the bath reservoir with distilled water.

Identification of Parts-Front Side:

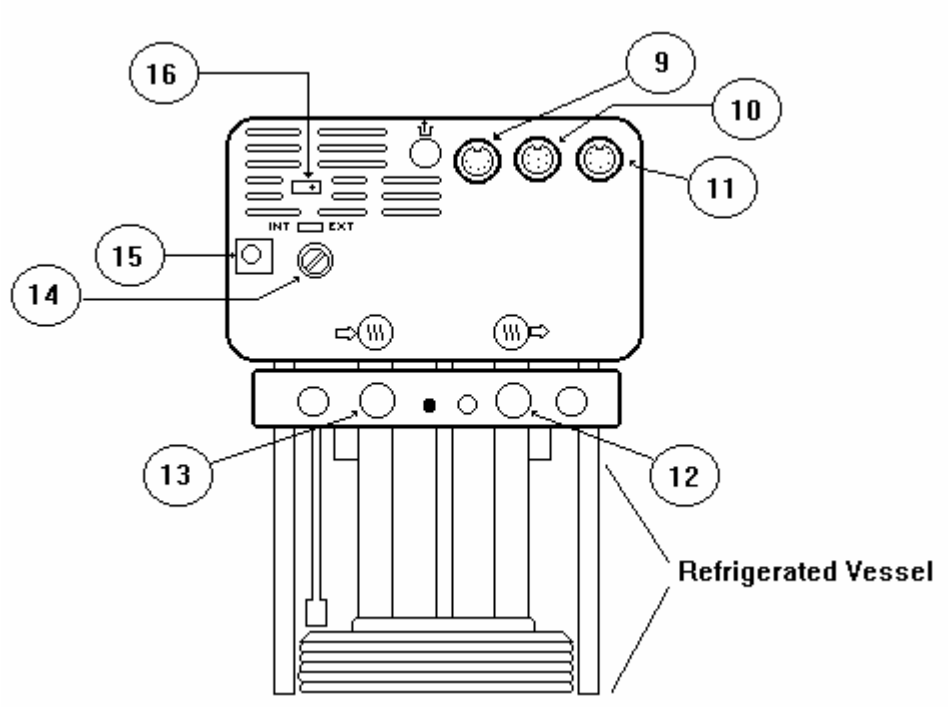


FRONT VIEW

(1) Main Switch with Power Control Light
(2) Heater Control Light
(3) Digital Switch for Preset Temperature Setting
(4) Reversing Switch (Internal/External)
(5) Digital Display for Current Temperature of Circulating Coolant
(6) Malfunction Indicator
(7) Release Switch
(8) Excess Temperature Limiter

Note: The Reversing Switch (4) should be set for the Internal mode, with a non-freezing temperature setting, when the bath is on and the computer is turned off or not running the DSCITC program. Otherwise there is no control for the bath and the bath will be set for 0 (zero) degrees C. Which may cause freezing of the cells!

Identification of Parts-Rear Side:



REAR VIEW

(9) Socket for connecting external temperature controller
(10) Socket for connecting control cable to a refrigerated bath vessel
(11) Socket for monitoring functions of safety elements
(12) Coolant Out Port
(13) Coolant In Port
(14) Fuses
(15) Main Power Cable
(16) Selector Switch - Control Mode (INT/EXT)

Coolant Composition & Water Bath Level

The choice of coolant composition represents a compromise between having a high boiling point and low freezing point on the one hand, and achieving rapid flow of fluid and reducing tendencies for agglomeration on the other hand. In the User's Manual we have suggested 50% ethylene glycol/50% distilled water. In our infinite wisdom, we have changed our minds! We now recommend 30% Prestone anti-freeze/70% distilled water to achieve a higher flow rate, reduce the tendency for agglomeration, and take advantage of the anti-corrosion additives in the commercial anti-freezes. If user's note a problem in baseline stability of the DSC unit when scanning above 105 °C, then increase to 40% Prestone and see if this corrects the problem. Also be aware that coolant composition will change with time, due to evaporation, particularly in the case of a DSC used often at high temperatures. It is a good idea to purchase an inexpensive freezing-point meter at an auto supply store and monitor changes in the composition of your coolant to determine when it should be changed.

User's may increase the cooling rate of their water bath by decreasing the volume of coolant within the bath vessel. Though the bath does require a minimum volume to function (low liquid level indicator will sound), by using as little volume as required sufficient gains will be made in both cooling your DSC Cell Unit after a high temperature scan, as well as in downscan operation. With the ITC, these factors are unimportant and a high liquid level is best.

DSC & ITC Coolant Lines

The refrigerated baths circulate coolant through narrow veins machined in aluminum (i.e., located in the jacket of the DSC cell and in the outer coolant plates of the ITC). It is possible for the narrow veins to clog so periodically the coolant flow should be checked to be certain that flow is consistently strong and no constriction of the narrow veins is occurring. We are aware of two factors which might cause constriction and loss of flow: 1) Using tap water to fill the bath. Only distilled water should be used. 2) Letting the instruments sit idle for long periods of time with the circulating bath turned off. If the cell is not to be used for an extended period of time the coolant lines should be flushed with distilled water and drained of all coolant.

If poor circulation arises then the coolant lines should be cleaned. If the constrictions are caused by ethylene glycol agglomerations, then these can usually be removed by circulating hot tap water through the system and back to a sink at moderately high flow rate. Be sure that hose clamps are used, and with the DSC, the solenoids must be open before flow is started. Salt or other deposits can usually be cleared by using a solution containing 5% acetic acid and 5% EDTA, heated to 50 °C, and pumping it slowly through the coolant lines. Typically, 4-5 liters of this cleaning solution will suffice.

Section 2.3

GRANT LTD 6 - External Water Bath

This section of the manual is intended to familiarize the user with the Grant external water bath's controls and connectors. If you do not have a Grant water bath you may skip this section. This is not intended to, nor does it suffice as a replacement for the Instructions printed on the side of the Grant. Please refer to the following diagrams for a minimum description of the Grant LTD-6 connections and controls.

1) Please insulate the water bath hoses from the Omega with the insulation provided by MicroCal.

2) Remove the blank Bypass Cover Plate (13) and screw in the Pump Connector which is supplied with the bath. Insure that the sealing rings are properly fitted.

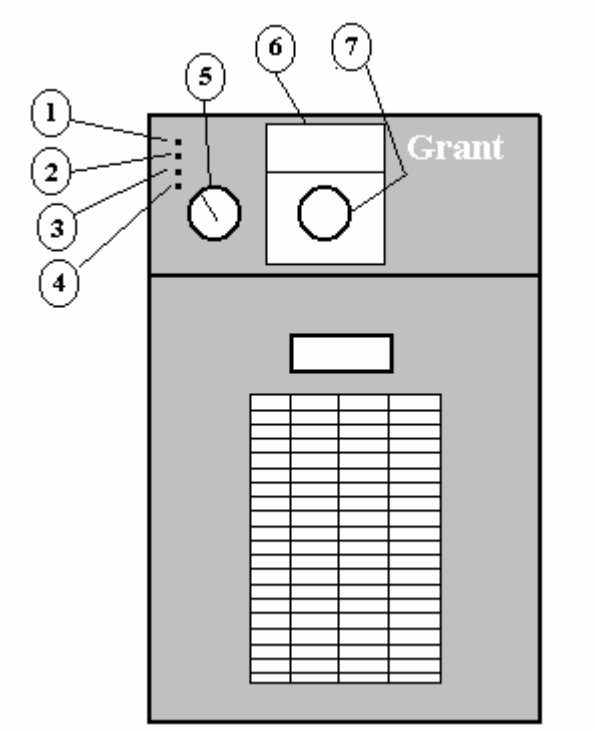
3) Connect the hose from the Omega marked 'water in' to the Coolant Out Port (14) on the rear of the F3 controller and the hose marked 'water out' to the Coolant in Port (15), with the appropriate hose clamps.

4) To allow the bath to cool below room temperature, turn the Compressor On/Off Switch(3) to on.

5) To prevent the bath from freezing or going below 5 °C, turn the Cooling Below +5 °C Switch(4) on.

6) Fill the bath reservoir with distilled water.

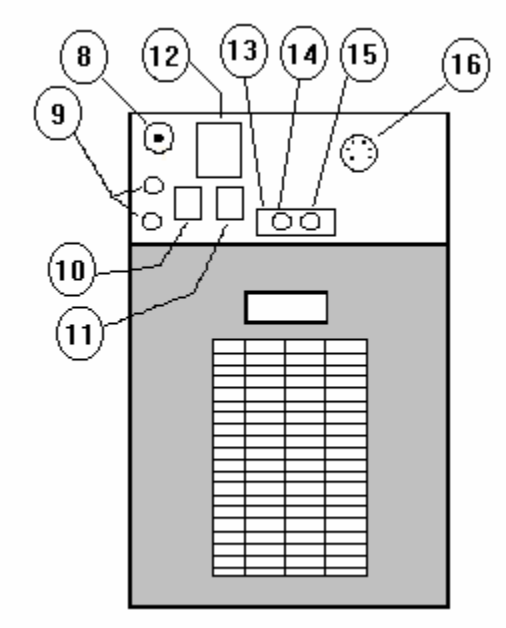
Identification of Parts - Front Side:



Front View

(1) Power On Indicating Light.
(2) Heater On Indicating Light.
(3) Malfunction Indicating Light.
(4) Cooling On Indicating Light.
(5) Upper Temperature Set Control.
(6) Digital Temperature Display.
(7) Temperature Set Point.

Identification of Parts - Rear Side:



Back View

(8) Main Power Cable.
(9) Fuses.
(10) Cooling Compressor On/Off Switch.
(11) Cooling Below + 5 °C Switch.
(12) Main Power Switch.
(13) Bypass Cover Plate or Pump Connector
(14) Coolant Out Port
(15) Coolant In Port
(16) Socket for Connecting External Control

Section 2.4

DT2801 I/O BOARD

If you purchased a computer system from MicroCal, the DT2801 board should already be configured, calibrated and installed in your computer and you may ignore this section.

The DT2801 boards are plug-in analog and digital I/O boards for the IBM PC/XT/AT and compatibles. They are electrostatic sensitive so always hold the boards by the edges and ground yourself to the computer before handling. If you purchased a DT2801 board for your system from MicroCal, it should already be configured and calibrated for use with your Omega and ready to insert into your computer. The board can be installed in any full-length PC/XT/AT expansion slot. Always insert boards with the computer turned off and unplugged from ac power. Check your computer's manual for further details on adding expansion cards, if you encounter trouble.

The inputs/outputs and base address are jumper selectable, so if you purchased the board from another supplier, it may need to have its jumpers adjusted. For Omega compatibility, jumpers **W1, W3, W4, W7, W12, W16, W19, W23, W26** and **W27** should be ON; all other jumpers should be removed. These jumpers should be adjusted BEFORE putting the board into the computer.

It may happen that after inserting the DT2801 board you try to run the DSCITC software program and the computer does not seem to be reading the correct data, controlling the instrument correctly or another card in your system is not behaving as before. This may indicate a addressing conflict between two different devices in your computer (this is especially true if you have two DT2801 boards in the same computer). The DT2801 board is factory configured for the base address 748 (hexadecimal 2EC), if you feel there is a conflict you may wish to change the base address of the jumpers. We have found the address 758 to be compatible when 748 is not. This involves moving the jumper from W1 to W2 and moving the jumper W19 to W18. **You must also change the software constant in the Omega.sp file** to reflect the new address number, 758 (refer to **Section 3.3** and **Appendix A** on how to change the address). Please refer to the DT2801 manual for different base addresses.

Section 2.5

MicroCal's Nanovolt Preamplifier

The nanovolt preamplifier is an isolated ultralow noise dc amplifier with a fixed gain of 10,000. It is powered by a rechargeable battery which provides 15 hours of operation on a 12 hour charge.

Connections to Calorimeters:

The input cable should be plugged into the matching female socket on the right rear of the cell unit. The output cable taken from the rear of the nanovolt preamplifier has a headphone jack on the end and plugs into the rear of the MC-2 control chassis. Omega users should set the CFB gain to **100** on the fine adjust dial and **100x** on the adjacent switch. Y-axis calibrations should be checked whenever making changes to the gain settings.

Operation:

The unit is charging whenever the AC power cord is connected as indicated by the red light on the front of the unit, if the power cord is disconnected the unit is operating under battery power alone. A full charge will be reached after approximately 12 hours if the unit is turned off while charging. This time is lengthened to about 18 hours if the unit is on while charging. The unit is normally operated with the rear AC power cord connected, if it is suspected that the unit is exhibiting excessive amount of noise due to AC power disturbances then the AC power cord can be disconnected from the rear, the unit will then operate under battery power providing the highest degree of electrical isolation. The user should be aware that if the batteries are allowed to drain down too low they may experience a polarity reversal and they can not be charged up to the full voltage, if this happens the unit will not operate correctly and the battery pack will have to be repaired or replaced.

A three position rotary switch on the front of the unit controls the meter display. The center position is the normal setting and causes the meter to display the input voltage multiplied by the gain of 10,000. Offscale inputs are indicated by a non-numeric display. The right and left switch positions cause the meter to display the negative and positive battery voltages divided by 10 respectively. The batteries reach a full charge of about

-10v and +10v and so are indicated as -1.00v and +1.00v. The point at which the unit must be recharged is when either battery voltage drops below 7.5v (.75 on meter display). The positive battery voltage will normally drop faster than the negative.

Reduce the susceptibility of the unit to interference by keeping it away from any high energy fields especially fluctuating magnetic fields.

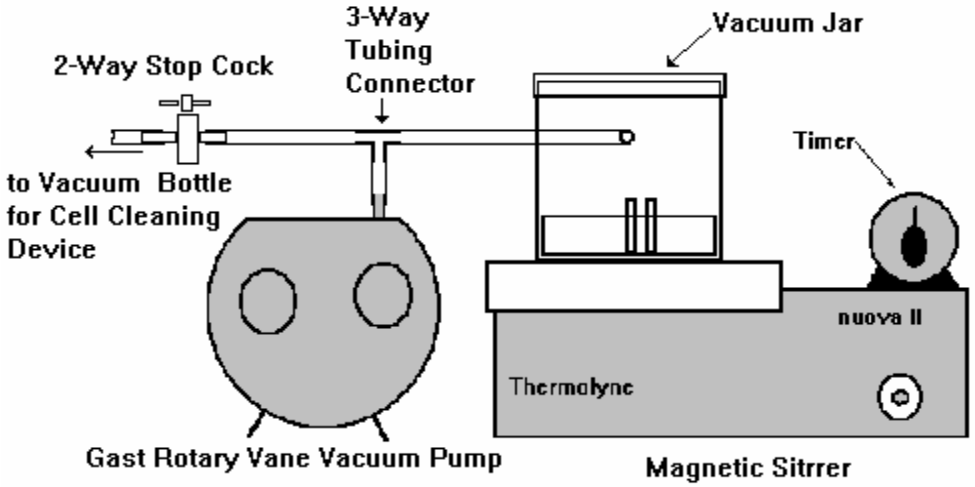
Section 2.6

Degassing System

To facilitate degassing samples and cleaning the cells, you have been provided with a degassing system (please refer to **Sections 4.2 and 4.5** for more information on sample preparation and cell cleaning). The unit has been equipped with a 30 minute timer to control the on/off cycle of the rotary vane vacuum pump and magnetic stirring plate.

To minimize noise and vibration the rotary vane vacuum pump should be mounted on a solid surface that will not resonate. To prevent interference with floppy and hard disks, the magnetic stirring plate should be placed a few feet away from the computer and all floppies stored away from the unit.

1.) Connect a length of the tubing from the hose fitting of the vacuum jar to one arm of the 3-way tubing connector.
2.) Connect another length of tubing from the 3-way tubing connector to the vacuum port of the Gast vacuum pump.
3.) Connect another length of tubing from the 3-way connector to the 2-way stop cock.
4.) The other end of the 2-way stop cock should be connected to the 1 liter side arm flask, used for the cell cleaning device (please refer to **Section 4.5 Cell and Syringe Cleaning**).
5.) The AC power cord for the Gast pump should be plugged into the back of the timer (the power switch for the pump should be turned on) then the timer should be plugged into an AC circuit.



Degassing: Place the test tube with solution and magnetic stir bar in the test tube rack inside the vacuum jar and place the cover on the jar. Turn the stop cock off, turn the timer on for 5 -10 minutes and adjust the stir rate so the there will ample mixing without turbulence due to over stirring.

Cleaning: Insure the cover of the vacuum jar is in place and turn the stop cock to allow air flow to the side arm vacuum flask. Follow the instruction in **Section 4.5** for setting up the cleaning device, then turn on the timer when you are ready.

Section 2.7

Control Module

The MicroCal CONTROL MODULE was designed to control either the Omega ITC or the MC-2 DSC. Therefore there are switches and dials which are only required for the MC-2 and not the Omega. Below is a brief description of the controls used by the Omega, followed by a brief description of the controls used only for the MC-2 or for debugging.

POWER

Main power switch for the control module. We recommend that this switch be turned on at least 2 hours before the start of any experiment to allow the electronics to warm up to there operating state. When using the Omega constantly, you may wish to leave the POWER on all the time.

RESET

This switch is used for the MC-2 and should be off for an Omega experiment. It is turned off by setting the SHUT OFF dial to its lowest setting.

RUN

This switch is used to turn on the heaters to the cell and must be on for an Omega experiment. If you will not be using the Omega for an extended period of time (e.g. overnight) this switch should be turned off, as the small amount of heat it provides to the cells may over time cause the cells to heat up to temperature high enough to damage them. **The RUN switch should always be on whenever the SCAN ENABLE is used (see Section 2.7)**.

Y SUPPRESSION

This dial is used to adjust the Y axis offset voltage. When doing a baseline run the user is advised to adjust this so that the dCp values as read on the computer and plotted in Origin are between +/- 10 μcal/sec. It is usually only required to adjust this when the BASELINE setting is changed (of Section 2.7) on the Omega Reaction Cell.

TEMPERATURE METER

The temperature in °C of the jacket of the Omega. This is also the cell temperature during an experiment, after the cells and jacket are thermally equilibrated.

ΔT

When this button is depressed, the difference between the jacket temperature (which is read on the TEMPERATURE meter) and the cell temperature is displayed. The instrument is not equilibrated until this meter is reading ca. 0.00. Please Note:

this is not an exact measurement, but if you divide the ΔT reading by 1.3 you will obtain a measurement closer to the real difference in deg C.

CELL FEEDBACK METER

This is a measure of power going into the sample cell heater. During an experiment this should never go negative or saturate positive (ca. 120-125), as this means that the sample cell will not be in thermal equilibrium with the reference cell.

JACKET FEEDBACK METER

This is a measure of power going into the jacket heater to maintain temperature equilibrium with the cells. During an experiment it should always be positive. The higher the temperature of the cells is above ambient the higher the jacket feedback readings will be.

The following controls are used for the MC-2 DSC cell only and are listed for reference purposes only. They have no effect on the Omega operation.

SCAN RATE

This switch is used for setting the scan rates for the MC-2.

CALIBRATION

This button is used only for calibrating the MC-2 when it is used without a computer.

SLOPE +/-

This dial and switch are used for adjusting the baseline slope of the MC-2.

TEST JACKS (A-L)

These are used only for debugging purposes, as directed by a MicroCal engineer.

CELL FEEDBACK BALANCE

This button is used only for MC-2 operation to hasten equilibration when the cell feedback is pegged in the negative position.

BASELINE SHIFT

This dial is used for adjusting the cell feedback levels for the MC-2.

SHUT OFF

This dial is a high temperature safety shut off for the MC-2 and should always be set to its lowest value (see RESET above) for the Omega.

JACKET FEEDBACK BALANCE

This button is used only for MC-2 operation to hasten equilibration when the jacket feedback is pegged in the negative position.

JACKET FEEDBACK ADJUST

This slotted knob is used only for MC-2 operation for adjusting the jacket feedback level and the scan rates.

Section 2.8

Omega Reaction Cell

The controls located on the Omega Reaction Cell front panel face set the cell feedback level, the temperature of the cell and the stirring rate of the injection syringe. These controls require that the POWER and RUN switches of the CONTROL MODULE are on.

POWER

This is the main power for the Omega Cell Controls. We recommend that this switch be turned on at least 2 hours before the start of any experiment to allow the electronics to warm up to their operating state. When using the Omega constantly, you may wish to leave the POWER on all the time.

BASELINE

This switch is used to set the cell feedback readings. Only the positions 0-5 are active with 0 being off. Throughout an ITC Omega experiment, a small constant amount of power is continuously supplied to the reference offset heater of the reference cell which in turn activates the feedback heater of the sample cell. Higher settings lead to higher power levels being applied to the reference heater and hence higher cell feedback readings being observed on the front panel meter of the Control Module. The 2 position gives a feedback reading of ca. +20 after equilibrium and is satisfactory for almost all experiments. For strongly exothermic reactions it might be necessary to go to the 4 or 5 setting while the 1-3 positions are appropriate for smaller exothermic or strongly endothermic reactions. It is easy to tell if your setting is appropriate by watching the cell feedback readings during an injection. This reading should never go negative nor should it be pegged at the extreme positive position (ca. 120-125) during an injection. It should be noted that as the setting is increased to the higher values more power is being supplied to the cells which will cause the cell temperature to increase very slowly (ca. 0.1 - 0.2 deg/hr) during an experiment so it is best to use the higher settings only when required.

BASELINE ENABLE/SCAN ENABLE

When this switch is in the SCAN ENABLE position **(the RUN switch should always be on whenever the SCAN ENABLE is used)**, the cell will heat up in temperature till it reaches the temperature as set by the TEMPERATURE SHUT-OFF dial. The final temperature reached will be closest to the dial setting, if both the reference and sample cell are filled with solution before scanning is commenced. The cell and jacket feedback meters will be in a pegged position while the instrument is scanning up in temperature. When the desired temperature has been reached, move the switch to the BASELINE ENABLE position. The system will then begin to equilibrate. The progress of equilibration may be followed by pressing the ΔT button on the Control Module front panel. The number displayed is near zero (0.0-0.3) at

equilibration, but may be as high as 2.0 just after scanning stops. As equilibrium is approached, the jacket feedback will reach a constant reading in the +10 to +80 range, and the cell feedback will become stable in the +10 to +100 range depending on the BASELINE setting being used. This equilibration period is about 15-20 minutes when working within 10 degrees of the room temperature and longer for higher temperatures. The system, once equilibrated, will thermostat at the equilibrium temperature.

TEMPERATURE SHUT-OFF

This dial sets the equilibrium temperature for the SCAN ENABLE switch. The dial reads in °C times 10 (i.e. a setting of 275 indicates 27.5 °C).

ON/OFF

This switch will turn the stirring motor on or off. Turning the stirring on will cause an immediate large decrease in the cell feedback. The cell feedback should return to within 5 μcal/sec of the 0 RPM baseline within 2-3 minutes. If the cell feedback goes to negative numbers upon stirring or if stirring causes a larger than normal offset from the 0 RPM baseline, see the section on troubleshooting.

RPM

By turning this dial you can adjust the speed of the stirring motor and observe the speed in RPM by watching the value on the meter. Normal operation is at 400 RPM. Stirring above 500 RPM may introduce unnecessary noise into the baseline and is not recommended.

Section 2.9

Precautions

There are a few precautions of which the user should be aware, to guard against faulty operation or permanent damage to the instrument.

Injection/Stirrer Syringes: The Omega depends on straight injection/stirrer syringe needles for proper operation. Bent needles may contact the cell walls or access tubes and generate thermal noise.

Overheating: The Omega reaction cell CANNOT be allowed to reach a temperature of 80^{o}C without incurring damage. Likewise, exposure to low temperatures that would lead to freezing of cell contents must be avoided. **It is therefore necessary to turn the RUN button off at the end of the days experiments.**

Cell Care: The cells and their access tubes are made of Hastelloy-C alloy. A booklet detailing the corrosive susceptibility of Hastelloy-C is included with the instrument. Strong acid solutions **cannot** be added to the cells, and the corrosion booklet should be checked before adding any other potentially corrosive substances.

Dry Nitrogen Purging: The Omega has two hoses which connect to the adiabatic shield (jacket). The hoses are used to fill the jacket of the Omega with dry nitrogen (1 atm, dew point -50 °C) for below room temperature operations. The following procedure is best accomplished at room temperature or above.

Attach the dry nitrogen source (5-7 PSI) to one hose (#1) and release the clamp on that hose. Now, carefully release the clamp of hose #2 and allow the nitrogen to flow through the jacket for ten minutes or so. Clamp off hose #2 and then hose #1 (connected to the nitrogen source) once the period of flow is complete. Remove the nitrogen source and release any excess pressure by unclamping one hose briefly.

During operation of the Omega, the hoses must be kept clamped off to prevent air currents from adversely affecting the baseline.

Stirring problems: Baseline aberrations can occur from problems in stirring arising from bent syringe needles, improper syringe height above the bottom of the cell, poor alignment of the Teflon barrel, or bubbles in cell resulting from poor filling. Stirring problems usually occur only when a syringe is actively stirring the solution in the sample cell. If the problems in the baseline disappear when the stirrer is shut off, it is most likely stirring-related trouble. Try another syringe and check the one that was being used for bends in the syringe needle. If the problem persists, try refilling the sample cell. If that fails, try varying the height of the syringe tip above the bottom of the cell - improper positioning can cause stirring trouble. See **Section 4.9 Troubleshooting** for more information concerning stirring problems.

Filling problems: Baseline aberrations can also be due to bubbles in either the stirring syringe or the cell, or insufficiently degassed solution in the cell. Use care when filling the cells and make sure your solutions are fully degassed and bubble-free.

Section 3

Omega - Software

Microsoft Windows™ is not a true multitasking environment in that it is not preemptive. The system resources must be shared by each application which is running. There is also a hierarchy of functions which will command processor time, including but not limited to the following:

- loading a large application or a large file into a running application
- long mathematical calculations
- screen refreshing such as scaling the axis or clicking between applications
- exiting to the DOS prompt

It is for this reason, the user is **cautioned against running other applications** while actively collecting data. Although the program may appear to be running smoothly, you may find gaps in your data point spacing, due to the fact that the data collection routine did not have the focus while the Windows™ environment was processing information in another application. Downscanning with the MC-2 and short filter times normally used in the Omega data collection are particularly demanding of computer time. When time constraints on the computer are high the filter period spacing of data points will not be exactly as entered; that is if a 2 second filter period is entered the actually data point spacing may be between 1.9 and 2.2 seconds.

Since the DSCITC program is a single application designed to utilize the Dynamic Data Exchange (DDE) of Windows™ to put the data into Origin, there is no problem with collecting and viewing data simultaneously with two instruments connected to the same computer. However, the user is cautioned against analyzing the data for one instrument while actively collecting data with another instrument.

The following section contains information about the DSCITC software program.

Overseas Users of Origin

If you have set the International setting to your own country (i.e., under the Windows: Control Panel: International Option) and find you have difficulty in reading DSC or ITC data files into Origin, then you will have to go back to the International dialog box, and edit the List Separator option so it has a comma rather than the default setting (space, period, semicolon, etc.). Although this will allow you to read data files with no difficulty, it does create another minor problem. If you should ever wish to import a file which has previously been saved by Origin as an ASCII file, then the List Separator will have to be changed to a space. These problems will be fixed in the future and those experiencing the problem will be updated.

Section 3.1

DSCITC Software Setup

Installation

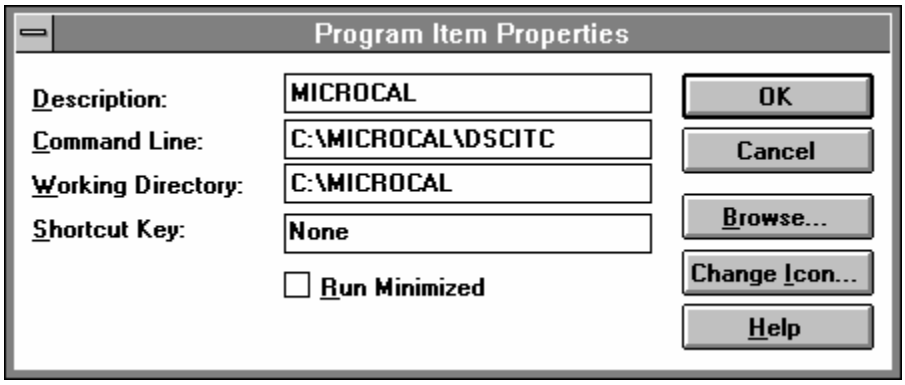
If you purchased your computer from MicroCal, all necessary software will already be installed. If not, you should install your software by running the software installation program that came with your DSCITC distribution disk. The installation program creates a directory named MicroCal and a sub directory of MicroCal called itcdata, then copies all of the necessary files onto your hard disk.

To Install DSCITC

- Launch Windows.
- Insert the DSCITC setup diskette into drive A.
- Select **File:Run** from the Program Manager menu bar. Type **a:install** and click **OK**.

To Create the DSCITC program icon:

- Click on the Program group box (to make it active) in which you want the DSCITC icon to appear.
- Select **File:New:Program Item** from the Program Manager menu bar.
- The Program Item Properties dialog box will appear. Enter the information as shown below.



The following is a description of the files that were copied to your hard disk during the installation process.

WARNING

Altering any of the files provided with the DSCITC may cause problems with using the system. Care should be taken.

Directory C:\MICROCAL

DSCITC.EXE

This is the data collection program. It provides a user interface which will input the run parameters from the user and on command will start the experiment. It then reads the data from the computer interface board which is connected to the Omega instrument, and through Dynamic Data Exchange (DDE), will then enter the data into an Origin worksheet.

CELL.CNF

The first line of this file contains the configuration of the software determined by the instruments which are connected. If you have only one Omega cell connected to the system the first line should be **itc**. If you have an Omega and a MC-2 the first line should be **DSCITC** (case insensitive) . If you have two Omegas connected to the same computer the first line should be **itcitc**.

Omega.SP

This file contains calibration constants which will convert the voltages read by the computer I/O board into calibrated data. Refer to Appendix A for more information on this file.

DEFAULT.INJ

This file contains default values for an Omega experiment that the DSCITC program will use when the Injection Matrix is selected from the main menu. The values in this file can be easily change by entering new values in the matrix and then saving the data with the name DEFAULT.INJ. Refer to section 3.5, Injection Matrix, for more information about injection parameters and saving files.

DEFAULT.CAL

This file is similar to the DEFAULT.INJ file in that this file contains default values for an Omega calibration run that the DSCITC program will use when the Calibration Matrix is selected.

DT.DLL

This is a Dynamic Link Library (DLL) created to convert the data read and written to the DT2801 I/O board into data which can be read and written by the DSCITC program.

VBRUN100.DLL

This is a Visual Basic run-time file used by the compiled DSCITC program to run outside the Visual Basic environment in which it was written.

DSCITC.HLP

This is a compiled help file for accessing on-line help about the DSCITC program.

Directory C:\ORIGIN

DSCITC.ORG

If you have both the Omega and MC-2 connected to your computer, this Origin document will be used for viewing the Omega data from the DSCITC program. Users should not alter any of the worksheet or plot window names contained in this document, as this will prevent the data exchange between the DSCITC program and Origin. This document has been saved as a READ ONLY file to prevent saving any changes to the file.

ITCITC.ORG

If you have two Omega instruments connected to your computer, this Origin document will be used for viewing the Omega data from the DSCITC program. Users should not alter any of the worksheet or plot window names contained in this document, as this will prevent the data exchange between the DSCITC program and Origin. This document has been saved as a READ ONLY file to prevent saving any changes to the file.

ITC.ORG

If you have only a single Omega instrument, this Origin document will be used for viewing the Omega data from the DSCITC program. Users should not alter any of the worksheet or plot window names contained in this document, as this will prevent the data exchange between the DSCITC program and Origin. This document has been saved as a READ ONLY file to prevent saving any changes to the file.

Directory C:\MICROCAL\ITCDATA

This sub directory is created for the saving of data.

Section 3.2

DSCITC - Data Collection Software

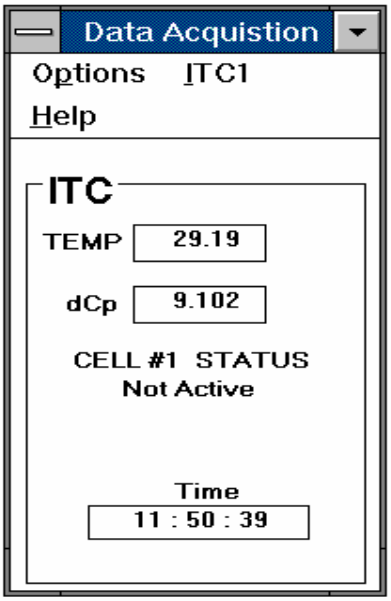
You may start MicroCal's data acquisition and instrument control for the Omega from the Windows™ program manager or from the DOS prompt:

From Windows™ program manager:

- Click on the icon as shown above.

From DOS with the keyboard:

- Enter the command **Win C:\Microcal\DSCITC** at the DOS prompt.

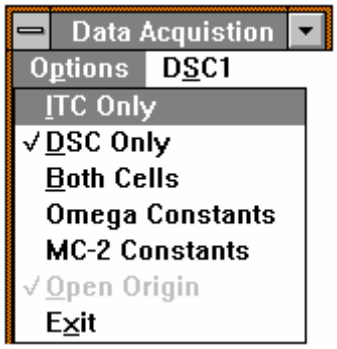


After launching the DSCITC program, the short menu version of Origin™ will be loaded with the document ITC. Then the data collection form, as viewed on the left, will be loaded. This process will take approximately 30 seconds, if Origin has not already been loaded with the document ITC. If Origin is already loaded with a different template then a new copy of Origin will be loaded. Two copies of Origin should not be running simultaneously so exit the copy running that does not have the ITC document.

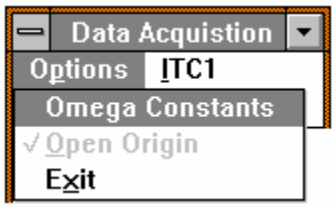
Section 3.3

Options

If you have both the Omega ITC and the MC-2 connected to the same computer you will have the following menu pop down when you click on **Options.**



Users with only the Omega ITC connected to the computer will view the following menu when they click on the **Options** menu.



ITC Only

Click on this menu when you wish only to view the Omega ITC data and do not care about the MC-2 DSC data.

DSC Only

Click on this menu item when you are only running the MC-2 DSC.

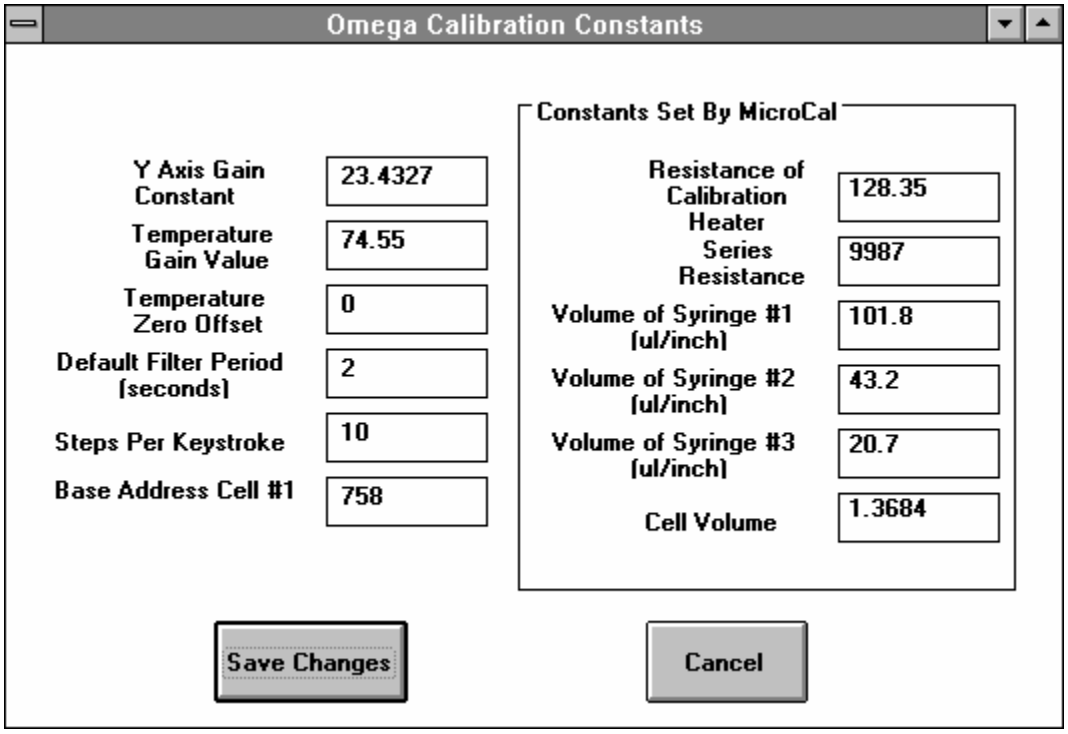
Both Cells

Click here when you wish to view data from both cells simultaneously.

Omega Constants

This Option will allow you to view and change calibration constants used by the DSCITC program for running the Omega and collecting the data. Please use care

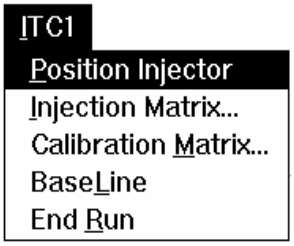
when entering changes as it will directly affect your data. Please refer to the **Appendix A and section 3.6**, Calibration Matrix, for more information on calibration constants. The following window will appear when you click on this option.



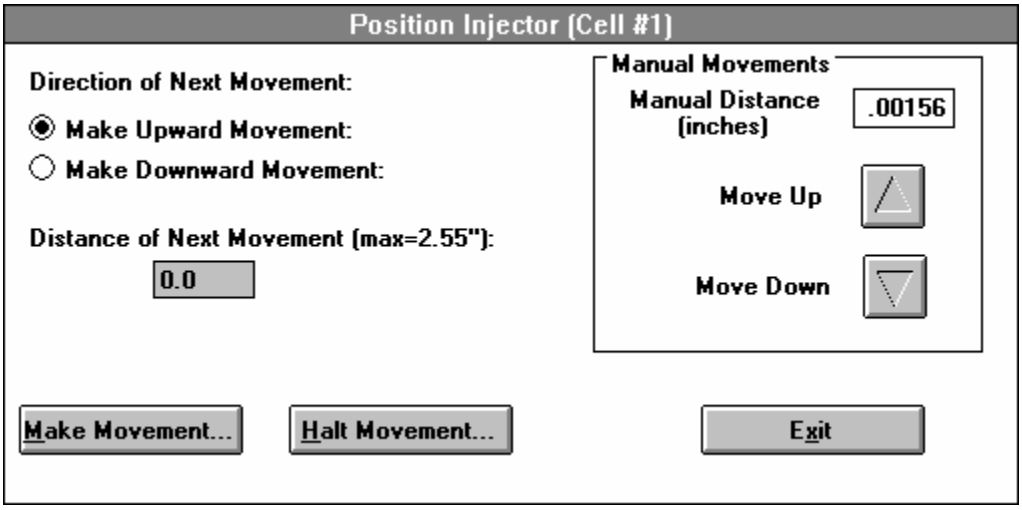
Section 3.4

Positioning the Injector

To access the window for positioning the injector:



- Click on **ITC1**
- Then click on **Position Injector**



Direction of Next Movement:

- Select either the **Make Upward Movement** or **Make Downward Movement** option to determine the direction for the injector travel.

Distance of Next Movement (max=2.55"):

- Enter in the box the distance (in inches) of travel for the injector. There is a maximum distance of 2.55 inches which can be entered at any one time.

Make Movement:

- Click on this button (or from keyboard press and hold **Alt** and then press the **M** key) to start the injector moving.

Halt Movement:

- Click on this button (or from the keyboard press and hold **Alt** and then press the **H** key) to stop the injector from moving before the end of its travel.

Manual Movements:

For the final positioning of the injector shaft to abut the syringe plunger you may wish to implement a few small incremental distances. This is easily done by entering a small distance (.00156 is a typical value) in the **Manual Distance** box. Then each time you click on the **arrow** button the injector will move that distance.

- Enter distance for the injector movement or use the default value of .00156 inches.
- Click on the **Move Down** or **Move up** arrow

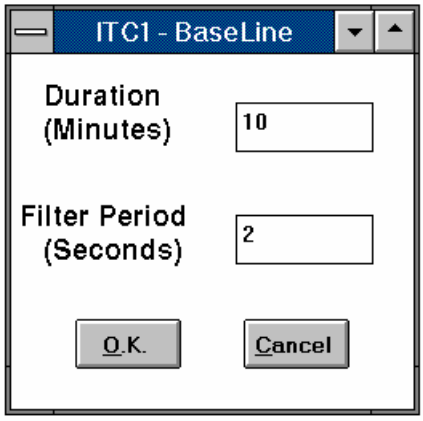
Exit:

- Click on the Exit button (or press and hold **Alt** then press **X**) to exit the Position Injector routine and return to the main Data Acquisition window.

Section 3.5

Baseline

- Select **BaseLine** from the **ITC1** menu.



The purpose of this routine is to observe the baseline after the injection syringe has been entered into the sample cell and stirring has commenced. The positive cell feedback caused by the relatively cold needle being inserted into the cell will be superimposed on the decrease in cell feedback caused by starting the stirrer. When the sum of the two has died out the baseline will become flat and an experiment can begin. After equilibrium, check the Y axis value (dCp) on the Data Acquisition window. This reading should always be in the range -10 to +10 before starting an experiment. If it should be outside that range, bring it into range by using the Y axis suppression knob on the panel face of the Control Module.

Duration:

- Enter in the box, or use the default value, for the length of time in minutes you wish to observe the baseline run (you may stop the baseline at any time, by selecting ITC1 : End Run).

Filter Period:

- Enter in the box, or use the default value, for the spacing of the data points in seconds (e.g. the computer samples the calorimeter signals as rapidly as it can and will average the values sampled over the time specified to store one data point). A 2 second filter constant is fast enough for almost all cases.

O.K.:

- Click on **O.K.** to return to the Data Acquisition window and to start the baseline run with the plotting of data in Origin.

Cancel:

- Click on **Cancel** to return to the Data Acquisition window without starting a baseline run.

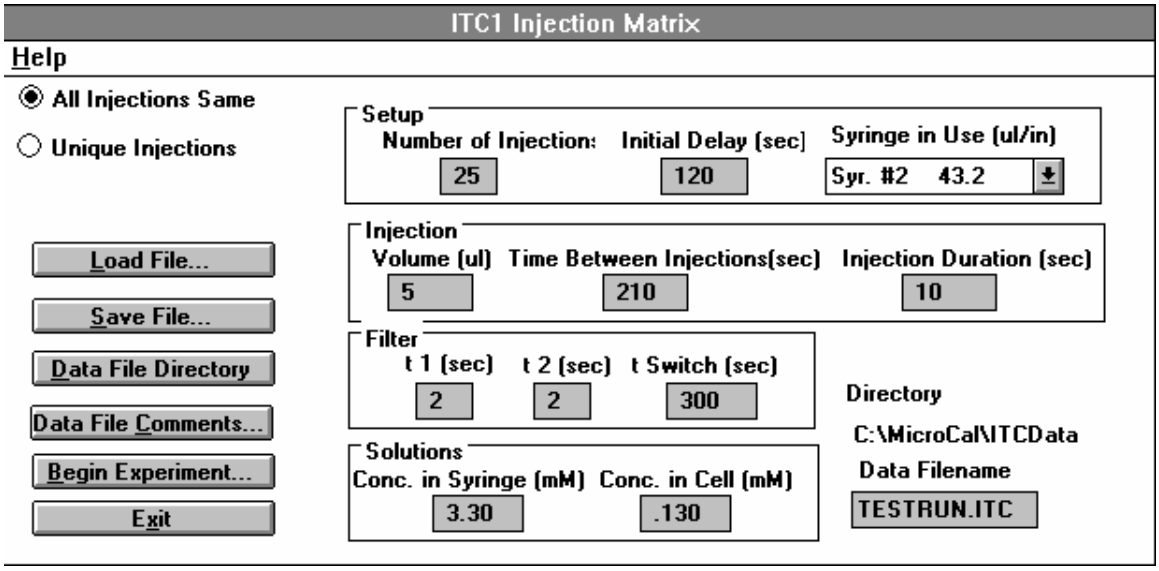
RMS Noise:

- When doing baselines this button will appear in the Origin ITC Plot Window. Clicking on this button will give an RMS value for the noise level over the past 60 seconds of data collection. This value should be less than .015 μcal/sec, if you are using the MicroCal nanovoltmeter.

Section 3.6

Injection Matrix

- Select **Injection Matrix** from the **ITC1** menu.



Setup

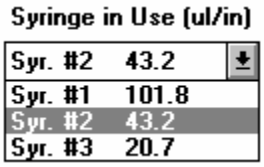
Number of Injections:

- Enter the total number of injections (50 is the maximum) for the experiment.

Initial Delay (sec.):

- Enter the time in seconds from the start of the experimental data collection till the time of the first injection. This time should be at least 60 seconds to establish enough baseline before the first injection.

Syringe in Use (µl/in):



- From the drop down list select the syringe you wish to use for the experiment from its volume displacement capacity. The maximum plunger travel in the barrel is 3 1/4 inches. The syringe we designate as 250 µl has a volume capacity of 101.8 µl/in so that the maximum volume which can be injected is actually 330 µl. Likewise the 100 µl syringe has a volume displacement of 43.2 µl/in and a maximum volume of 140 µl

and the 50 µl syringe has a volume displacement of 20.7 µl and a maximum dispensing volume of 67 µl.

Injection

Volume (µl):

- Enter the volume you wish to inject during a single injection.

Time Between Injections (sec):

- Enter the time, in seconds, from the start of one injection till the start of the next injection.

Injection Duration (sec):

- This is the time, in seconds, for the titrant to be injected. Normally, this might be in the range of 20 seconds. Note that the maximum speed of the injector motor is about 1 in/min, so the minimum time for the duration is dependent on the injection volume and syringe size. The program will warn you if you enter a time less than the minimum time.

Filter

The filter period is the time over which the computer samples the data and averages these values to plot one data point. If it so happens that the reaction has two different kinetic phases, perhaps a fast then a slow reaction, the user may wish a short filter period (**t 1**) for the fast reaction and a longer filter period for the slow reaction (**t 2**). **t Switch** specifies the time from the start of the injection, when the t 1 filter period is being used, after which the **t 2** filter period will be used. This will keep the data file from becoming much larger than necessary. The minimum filter period is one second, and a period of 2 seconds is ideal for most fast reactions.

t 1 (sec):

- Enter the time in seconds for the initial filter period of the injection.

t 2 (sec):

- Enter the time in seconds for the second filter period of the injection.

t Switch (sec):

- Enter the time in seconds from the start of the injection till the filter period is switched to **t 2**. The user may prevent any filter switching by entering a t Switch value that is greater than the **Time Between Injections**.

Solutions

The concentrations entered here are used by the Origin program for data analysis. These values can be edited in Origin if any errors are made.

Conc. in Syringe (mM):

- Enter millimolar concentration of the solution in the syringe.

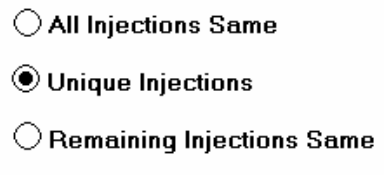
Conc. in Cell (mM):
- Enter millimolar concentration of the solution in the cell.

Directory:

Please refer to the next section on the command button **Data File Directory** for information on how to change the directory for storing data.

Data Filename:
- Enter the prefix for the filename using DOS convention of eight characters maximum. The program will automatically add .ITC extension so that the raw data file will be recognized by Origin as an Omega data file. Do not begin any filename with a number or include any dashes in it, as Origin will not accept it.

Unique Injections:



The Unique Injections option allows you to set up injections using different parameters. You may customize each injection schedule to have different volumes of injections, injection durations, filter periods etc.. When you click on Unique Injections a drop down list of the Injection Schedule, as shown below, will appear at the bottom of injection matrix window.

Injection Schedule:

Injection #	Volume (ul)	Duration (sec)	Time Between (sec)	Filter 1 (sec)	Filter 2 (sec)	Filter Switch (sec)
#1	10	20	30	2	2	300
#2	9	20	30	2	2	300
#3	8	20	30	2	2	300
#4	7	10.49	30	2	2	300
#5	6	10	30	2	2	300

- Click anywhere on the row of the injection number for which you wish to edit the run parameters. The values will then appear in the respective boxes for you to edit. Use the scroll bar to access hidden injections you wish to edit.

Remaining Injections Same:

If you wish only the first few injections to have unique injection but all the last ones to have the same parameters. You may select this option.

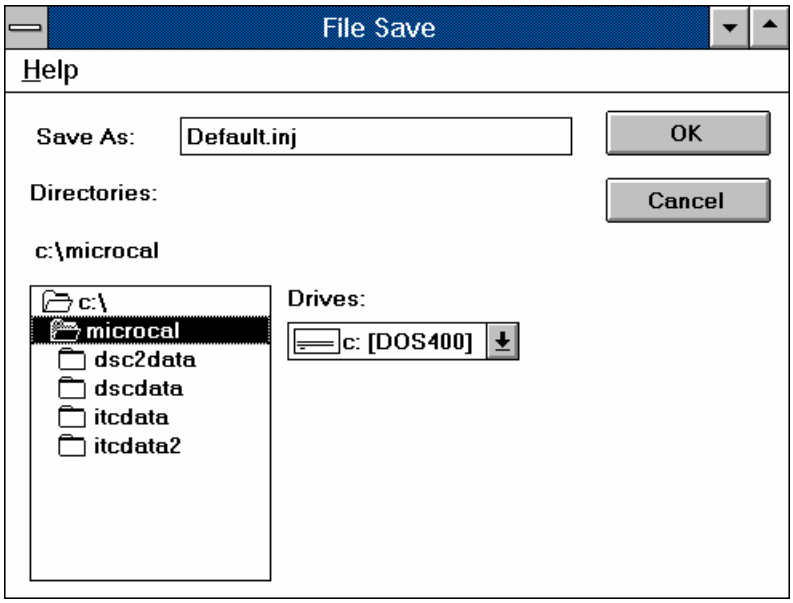
- Click on the row which you want all the remaining injections to match.

Injection Matrix Command Buttons:

Save File:

This command will allow you to save the injection matrix run parameters, so that you may call it up later with the **Load File** command to run a new experiment with the exact injection schedule as the one saved. The filename is saved with the filename extension .INJ.

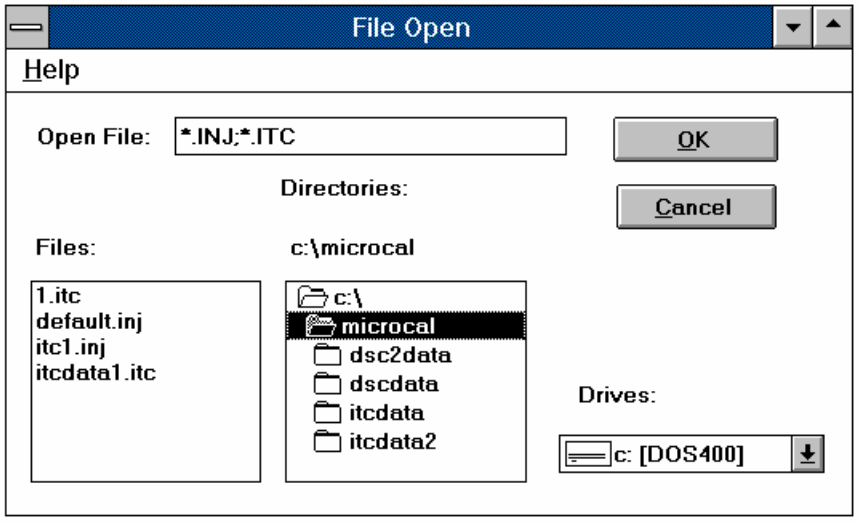
- Click on the **Save File** command button to call the following form.



Load File:

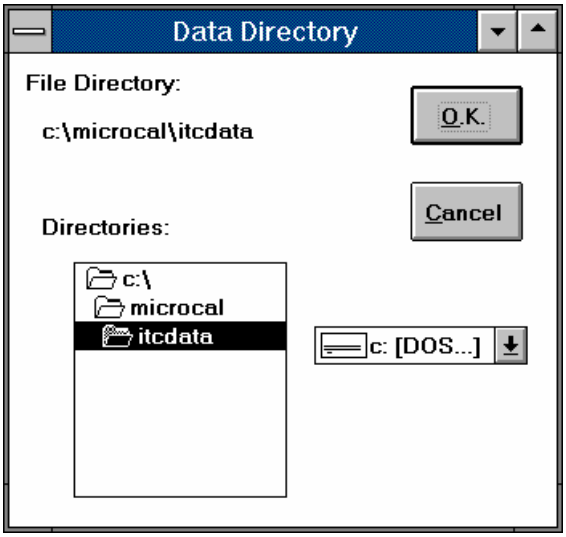
This Command will allow you load an injection matrix with the same parameter as a previous injection run (filename extension .ITC) or from a Injection matrix (filename extension .INJ) saved with the **Save File** command.

- Click on **Load File** command button.



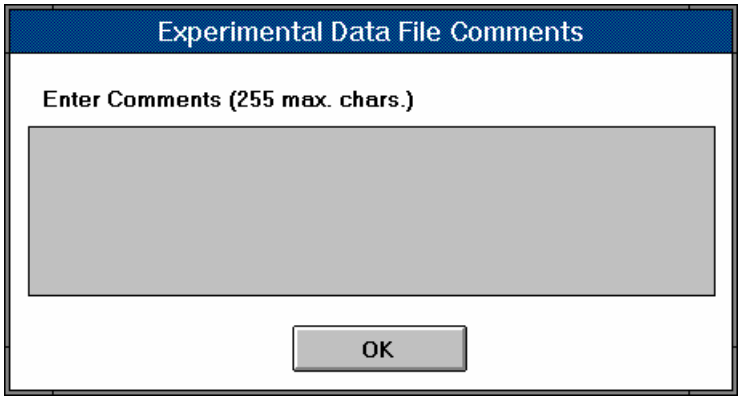
Data File Directory:

- Click here to bring up the dialog box for changing the directory for saving the experimental data.



Data File Comments:

- Click on this command and the following dialog box will pop up. You may enter any comments about your experiment to be saved with the data file for future reference.



Begin Experiment:

- Click on this command, and the program will check all entries in the Scan Matrix Table to insure legal values, then exit the scan matrix form and return to the ITC main form. The injection run will begin immediately.

Exit:

- Click on this command to exit the scan matrix form without starting the experiment. This command is also useful for exiting the dialog box after the run has already started.

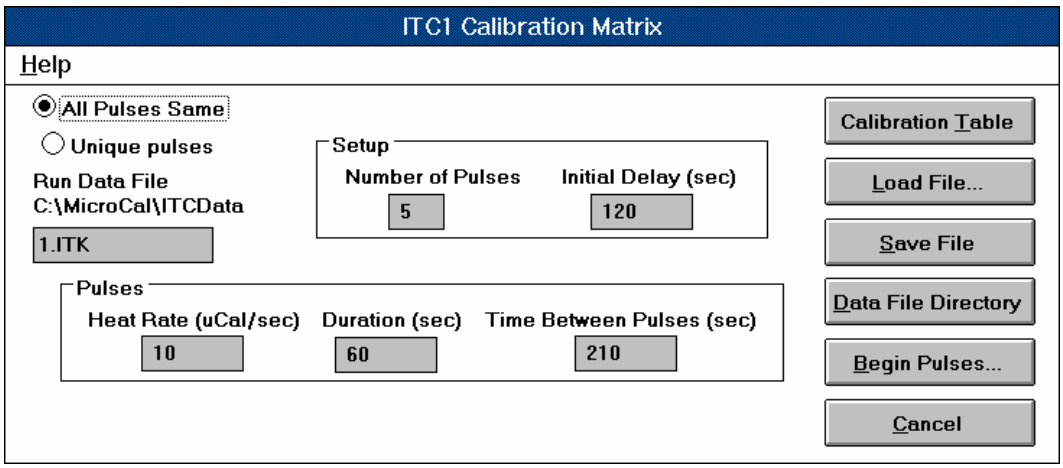
Section 3.7

Calibration Matrix

The calibration procedure should be run every month or two for checking the accuracy of the Y axis to insure it is within a percent or so. The calibration pulse can be selected in the range from 0 to 30 µcal/sec for a chosen time period. Most accurate calibration is obtained for pulses of 200 µcal or larger (e.g. 10 µcal/sec for 20 sec = 200 µ cal). The total heat of the pulse (µcal) can be displayed after the run by clicking on the **Calibration Table** command button. This known heat, obtained from this table should agree with the heat obtained by finding the area in Origin. If the area obtained, Q_A, disagrees with the known heat injected, Q_K in the calibration table by more than 2 percent then a new calibration constant should be determined. Please refer to **Section 5.1 Y Axis Calibration** at the end of this manual for more information.

Note that whenever calibration data is called into Origin, it is automatically interated and normalized on concentration. If Origin cannot find a concentration value in the file header, it assigns a value of 1.0, to avoid dealing with zeros. The integrated data obtained after concentration normalization has an **.ndh** extension, while the integrated data before concentration normalization has a **.dh** extension. For checking calibration areas, you must always work with the **.dh** file, since it will be in µcal and can be compared directly to the selected size for the calibration pulse.

- Select **Calibration Matrix** from the **ITC1** menu.

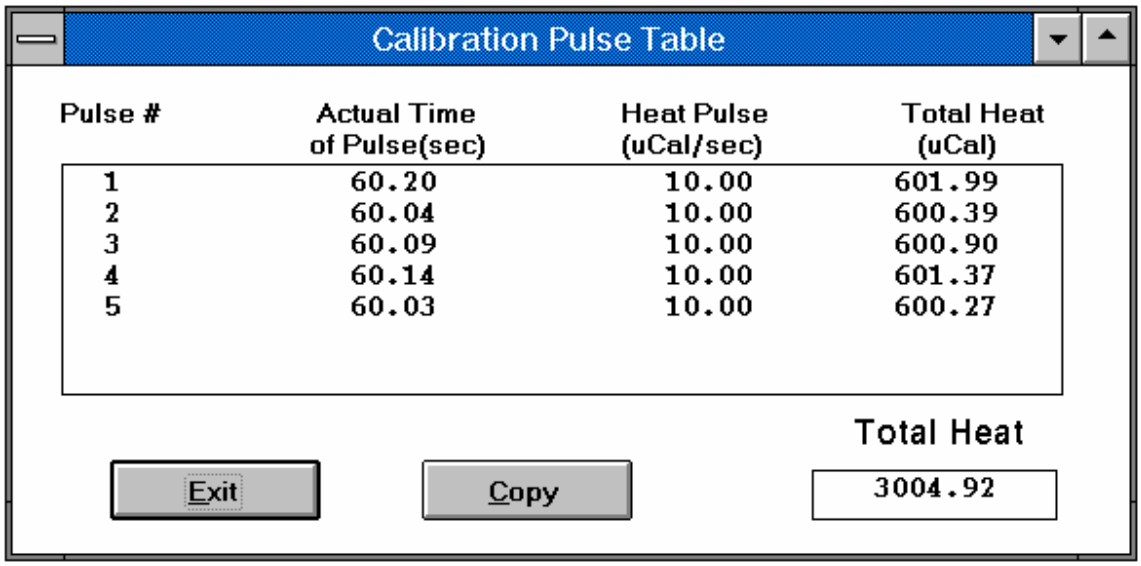


The commands are similar to the those used for the injection matrix form.

Calibration Table:

Because Windows™ is not a true multitasking system but must share resources, the time of the applied pulse cannot be controlled accurately. Therefore the actual time of the applied pulse is recorded in the **Calibration Table**. From this table the applied heat can be determined and recorded. The **Copy** command can be used to put this data into the Windows™ Clipboard so that it can be easily pasted into Origin for verification of the applied pulse vs. the measured pulse.

- Click on the **Calibration Table** command button in the **Calibration Matrix** form.



- Click on the **Copy** command and the following list will be copied into the Clipboard for easy pasting into Origin.

Pulse # 1 601.99
Pulse # 2 600.39
Pulse # 3 600.90
Pulse # 4 601.37
Pulse # 5 600.27
Total Heat = 3004.922

Note: The Calibration Pulse Table is complete for copying only **after** the calibration experiment is completed, and you need not be concerned that the listings are incomplete and/or inaccurate while you are setting up the calibration experiment.

Section 4

Running an Omega ITC Experiment

Section 4.1

Designing ITC Experiments

For a ligand **X** binding to a single set of **n** identical sites on a macromolecule **M**, i.e.,

$$M + X = MX$$

$$MX + X = MX_2$$

$$\vdots \qquad \vdots$$

$$MX_{n-1} + X = MX_n$$

the single-site binding constant is

$$K = \frac{[\text{filled sites}]}{[\text{empty sites}][X]}$$

and

$$\Delta G^o = -RT \ln K = \Delta H^o - T\Delta S^o$$

Where ΔG^o, ΔH^o and ΔS^o are the free energy, enthalpy, and entropy change for single site binding.

By non-linear least squares fit of calorimetric titration data, the parameters **K**, ΔH^o, and **n** are determined directly in a single experiment and ΔG^o and ΔS^o may then be calculated. Titration calorimetry is the only technique capable of defining all of these parameters in a single experiment resulting in nearly complete thermodynamic characterization of the interaction. Measuring the binding isotherm at a second temperature allows additional determination of the change in heat capacity of binding through the relation:

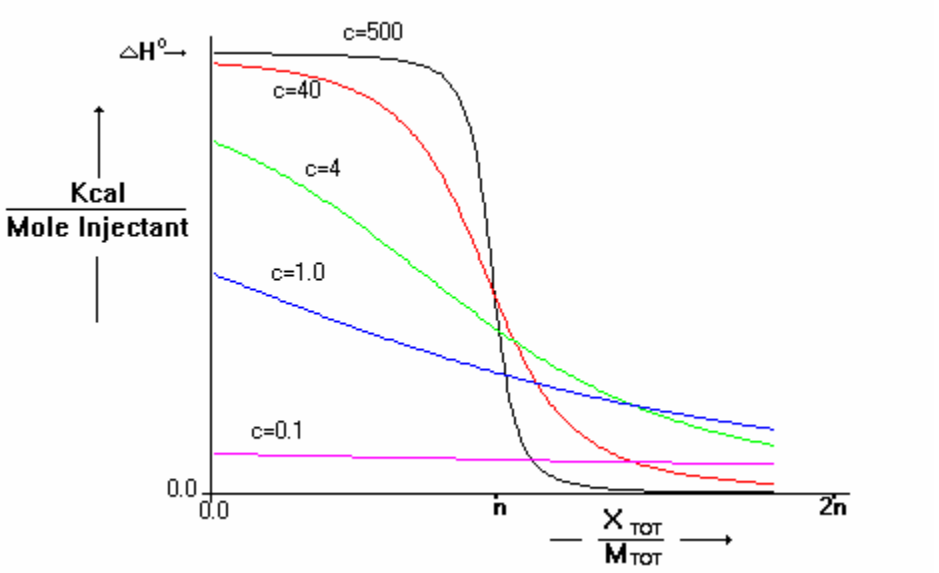
$$\Delta C_p = \frac{\Delta H^o_{T2} - \Delta H^o_{T1}}{T2 - T1}$$

It is well-known that ΔC_p is a good indicator of changes in hydrophobic interactions with binding, being negative if hydrophobic bonds are formed and positive if they are broken.

The critical parameter which determines the shape of the binding isotherm is the unitless constant c, which is the product of the binding constant K times the total macromolecule concentration in the cell at the start of the experiment, M_{tot} , times the stoichiometry parameter, n.

$$\mathbf{c = KM_{tot}n}$$

Very large c values lead to very tight binding and the isotherm is rectangular in shape with the height corresponding exactly to $\mathbf{\Delta H^o}$ and with the sharp drop occurring precisely at the stoichiometric equivalence point n in the molar ratio X_{tot}/M_{tot} . The shape of this curve is invariant with changes in K so long as the c value remains above ca. 5000. As c is reduced by decreasing M_{tot} (i.e., holding K, $\mathbf{\Delta H^o}$ and other parameters constant), the drop near the equivalence point becomes broadened and the intercept at the Y axis becomes lower than the true $\mathbf{\Delta H^o}$. In the limit of very low initial M_{tot} concentration (cf., c=0.1), the isotherm becomes featureless and traces a nearly horizontal line indicative of very weak binding. It is apparent from looking at these isotherms that their shape is reasonably sensitive to binding constant only for c values in the range $1 \leq c \leq 1000$, corresponding to binding of intermediate strength. We will refer to this range as the "experimental K window". When available, the middle of the window from c = 5 to 500 is most ideal for measuring K.



The correct choice of macromolecule concentrations for an experiment depends both on the objective of the experiment (i.e., whether you wish to determine a ΔH^o of binding only, or whether you wish to determine n and K in addition to $\mathbf{\Delta H^o}$) and upon the magnitude of K. While considering your choice of starting concentration, it must also be remembered that the limiting Omega ITC sensitivity is ca. 0.5 µcal so for precise measurement each injection should have an average of at least 5 -10 µcal of heat absorbed or evolved into the 1.4 ml cell.

How these factors impinge on your choice of macromolecule concentration can be seen by considering a particular example of the binding of 2'CMP to ribonuclease A, where

the binding constant is approximately 1 x 10^6 M^{-1} and the ΔH^o is approximately -15000 cal/mole for the single binding site.

A. Measuring ΔH^o, K and n by deconvolution of total binding isotherm.

For a K of 10^6, RNASE concentrations are in the experimental K window for the Molar range $10^{-6} \leq M_{tot} \leq 10^{-3}$. It requires at least 10 separate injections to define the total binding isotherms and each injection must average ca. 10 μcal, so the total heat Q required in the 1.4 ml cell is 100 μcal, i.e.,

$$Q = 100 \times 10^{-6} \text{ cal} = (15000 \text{ cal/mole}) (M_{tot} \text{ moles/l}) (1.4 \times 10^{-3} \text{ l})$$

Solving this equation for M_{tot} gives a minimum concentration of ca. 5 x 10^{-6} M. This concentration is larger than the lowest concentration, 1 x 10^{-6} , in the experimental K window so the concentration range available in the K window becomes 5 x $10^{-6} \leq M \leq 10^{-3}$. Although any value within this range is acceptable, it would lead to better estimates of parameters to choose concentrations higher than the minimum of 5 x 10^{-6} so that Q signals will be larger and c values will be in the ideal range between 10 and 100. (i.e., $10^{-5} \leq M_{tot} \leq 10^{-4}$).

B. Measuring only ΔH^o by single ligand injection into excess macromolecule.

To measure ΔH^o by a single injection (i.e., without deconvolution of the total binding isotherm) requires a c value large enough so the experimental intercept on the isotherm intercepts the Y axis very close to the true ΔH^o, i.e., $c \geq 100$. This means $M_{tot} \geq 10^{-4}$. Since there will be excess macromolecule in the cell, the experimental heat Q will be determined by the amount of ligand injected, i.e.,

$$Q = (15{,}000 \text{ cal/mole}) (\text{syringe conc.}) (\text{inj. vol.})$$

For example, a 10 ml injection of a 7 x 10^{-5} M ligand solution would give the minimal 10 μcal of heat. It is also possible to measure ΔH by injecting excess ligand into a very low concentration of macromolecule.

Referring to case A above, once you have chosen M_{tot} you must select the ligand concentration X_{tot} for the solution to be loaded into the syringe. This will depend on which syringe you plan to use. For c values larger than ca. 10, the final concentration of ligand in the cell after all injections are completed should be ca. 1.5 times the total concentration of macromolecule binding sites in the cell at the beginning of the experiment,

$$\text{i.e., } X_{tot} \times \Delta v/V = n \times M_{tot} \times 1.5,$$

where Δv is the total volume of injectant to be used, V is the cell volume (ca. 1.4 ml), and n is the ligand/macromolecule stoichiometry. For cases where n=1, the ligand concentration, X_{tot}, should be ca. 42 times M_{tot} (50 μl syringe), 21 times M_{tot} (100

μl syringe) or 8.5 times M_{tot} (250 μl syringe). If the c value for your system is lower than 10, you may wish to increase the final ligand/macromolecule ratio from 1.5 up to 2.0 or even 2.5 as is evident by referring back to the previous figure showing binding isotherms as they depend on c. It should be realized however that accurate curve fitting is possible even when saturation of sites is not achieved.

There may be other factors, specific to your system, that are important considerations in experiment design, such as the total amount of macromolecule or ligand that is available for the experiment and/or solubility restrictions on the macromolecule or the ligand. Concerning the latter, if you are doing a total binding isotherm using the 100 μl syringe, then the initial ligand concentration in the syringe must be ca. 20 times larger than the concentration of macromolecule sites in the cell. This can lead to ligand solubility problems, especially if the ligand is another macromolecule. Thus, the concentration of macromolecule in the cell could be chosen close to the low end of the available range, and/or the 250 μl syringe could be used instead of the 100 μl syringe to overcome this problem.

Several other experimental design problems should be mentioned. First, the buffer in which the ligand is dissolved should be an **exact match** (i.e., pH, buffer concentration, salt concentration, etc.) to the buffer in which the macromolecule is dissolved, or else large spurious heat effects from buffer mixing will result. For example, if the ligand is dissolved directly into the buffer which was dialyzed against the macromolecule solution, the exact pH of the ligand solution may change due to titration of ionizable groups on the ligand. If this happens, then the ligand solution should be back-titrated carefully until the pH is identical to that of the macromolecule solution before doing the experiment.

Second, control experiments (i.e., ligand solution added to buffer in cell without the presence of macromolecule) to determine the heat of dilution of ligand should be carried out in the same way as the experiment with macromolecule present, and these heats of dilution should be subtracted from the corresponding injection into the macromolecule solution. You will usually find these heats of dilution to be small and frequently negligible (unless the ligand dimerizes or aggregates with itself!) but they should be checked as a precaution.

Finally, you may find occasionally that the first injection in a series of injections shows a smaller heat effect than it should. This can result from bending the syringe needle a little when seating the injector into the barrel, or leakage resulting from having the syringe in the cell a long time before the first injection is made (particularly if it is stirring all the while). It you find this to be a persistent problem with certain systems, even when care is taken to avoid the aforementioned factors, you may wish to make a small first injection (e.g. one 1 μl injection followed by ten 10 μl injections) and then delete the first data point before doing curve-fitting in Origin.

Because of release or uptake of protons during many biological binding reactions, the observed heats of binding may be strongly dependent on which buffer is used. In fact, certain binding reactions which have extremely small ΔH and produce virtually no signal in buffers with small ΔH_{ion} (e.g., phosphate) can sometimes be studied nicely in buffers with a large ΔH_{ion} (e.g., tris) where the signal will be much larger.

Section 4.2

Sample Preparation

Degas cell and syringe samples that may contain dissolved gas to insure bubble free loading of each. This is particularly important if samples recently were at refrigerator temperatures. A diaphragm or rotary vane pump (ca. 27" Hg vacuum) and plastic vacuum chamber placed atop a magnetic stirrer may be used for degassing without excessive boiling. Normally only 5 minutes of vacuum is required to degas if the sample is being stirred but much longer without stirring. Allow solutions to warm up to room temperature before degassing.

If volatile buffers or ligands are being used then solutions should be prepared from degassed or pre-boiled water and stored air-free.

If sample solutions contain any undissolved solutes or extraneous solid material of other types, they should be filtered before use.

Section 4.3

Cell Loading

The cells are filled using the long needled 2.5 ml glass syringe, filling from the bottom of the cell to the top. The top of the access tubes are visible in the floor of the injection system. The sample cell is in the center and the reference is offset to the left, as viewed from the front of the instrument.

Fill slowly until liquid can be seen overflowing the access tube. There is a tendency for a small bubble to be left near the top of the cell. Finish the filling by adding an abrupt spurt of about .25 ml to dislodge any bubbles.

Fill the reference cell with water. There is no need to refill the ITC reference with each experiment. A water reference may be good for a week or two with no attention if the water was thoroughly degassed before filling. A small stopper and insertion device has been provided to cap the reference cell access tube after filling, if desired.

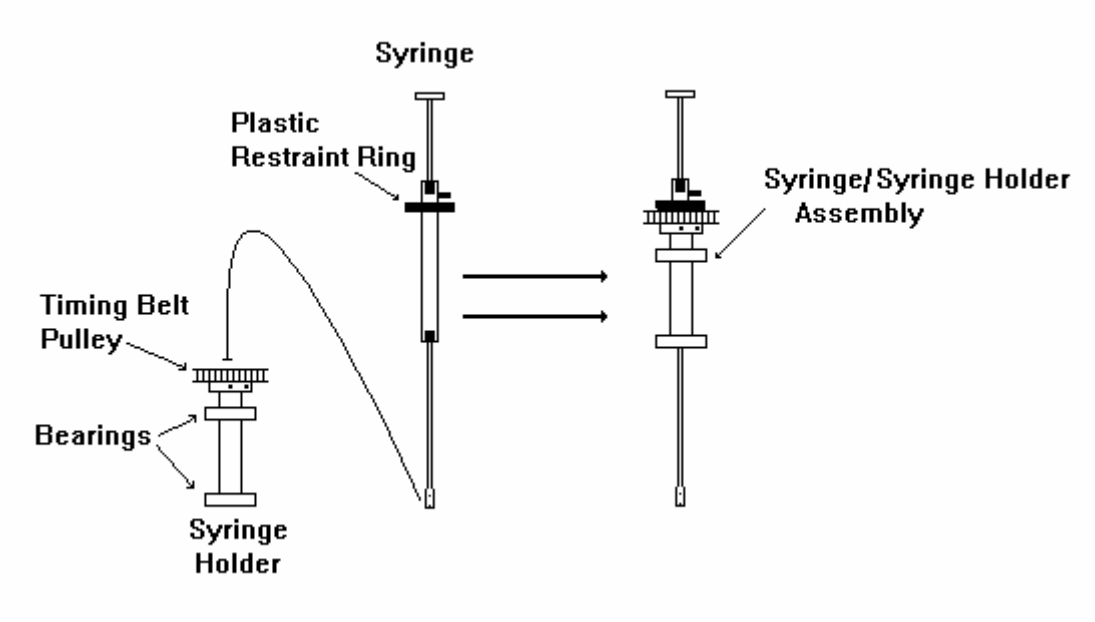
Remove any excess solution from the floor of the reservoir after filling, following withdrawal of the filling syringe.

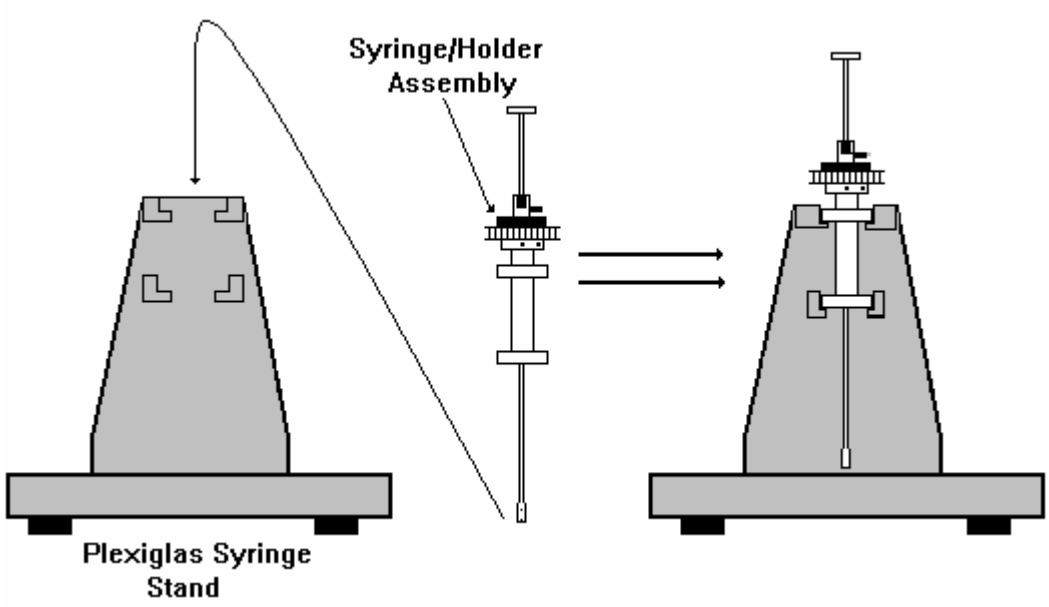
Section 4.4

Injection Syringe Filling

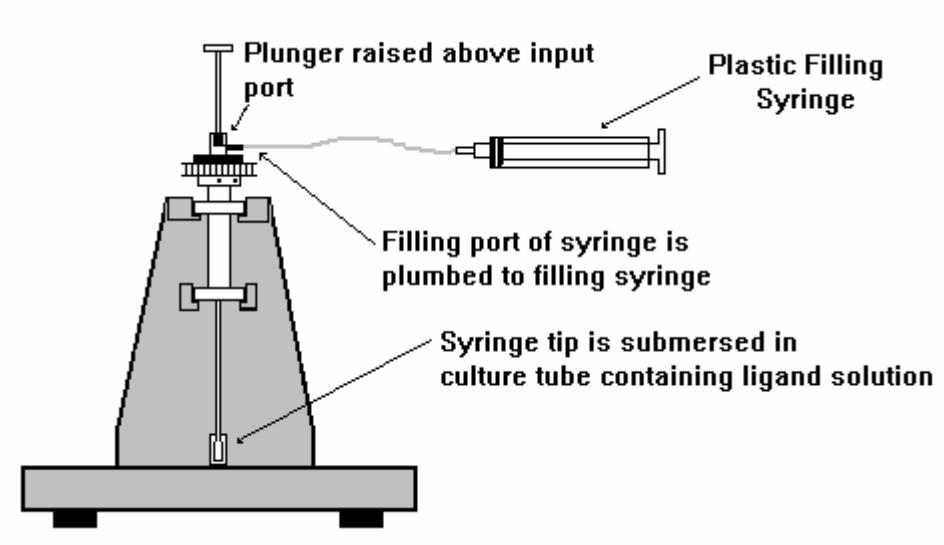
The syringe capillary must be completely dry before filling (e.g., methanol rinse followed by 5 minute purge with dry air) or well rinsed with the ligand solution.

It is very important to load the injection syringe with the complete absence of bubbles. You will fill the injection syringe while it is in the metal syringe holder, and your filling configuration should be as pictured below.





After seating the syringe into the syringe holder, the black disk at the top of the syringe should be firmly seated against the surface of the timing belt pulley of the syringe holder, since this controls the final distance between the stir paddle and the bottom of the cell. This black disk has been positioned correctly, and secured with the set screw, by MicroCal. If you need to reposition this, see the Troubleshooting section.



The syringe's plunger is pulled up to the high edge of the metal filling port located near the top of the syringe. A second syringe is attached to the filling port with a short length of thin plastic tubing. This is used to suction solution into the injection syringe, removing all of the air between the plunger and the solution. Once the solution begins to exit the Filling Port, the syringe plunger is depressed below the filling port. The plastic tubing may now be removed from the filling port.

Depress the plunger sharply with the stirring paddle still immersed in sample and then draw solution slowly back up. Repeat this procedure. This insures that any small bubbles formed in loading will be expelled out of the long needle.

After filling, rinse the tip of the syringe lightly with water from a washbottle, carefully pat dry with a lint free tissue wiper and seat it back into the syringe stand.

A syringe loaded with titrant must not be flexed or titrant will be displaced from the needle, resulting in an injection volume error for the first injection.

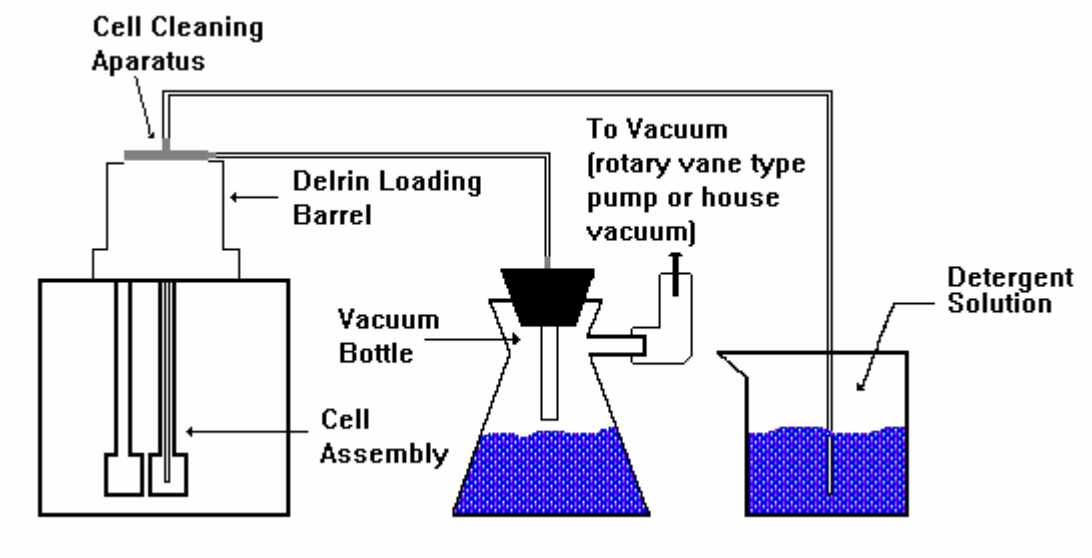
Section 4.5

Cell and Syringe Cleaning

The Omega ITC reference cell doesn't require special cleaning but should be rinsed occasionally using long needle syringes.

The Omega ITC sample cell need not be cleaned with detergent solution after each experiment, since rinsing with water or buffer from a syringe is often adequate. However, after every 3-5 experiments solids which cannot be removed by rinsing will adhere to the cell interior and these can cause baseline problems if not removed periodically by a thorough cleaning with detergent solution.

A cell cleaning apparatus is provided for that purpose.



The device is inserted into the sample cell and seals the sample cell overflow reservoir in the floor of the injection system. A vacuum trap beaker is connected to the output and a beaker of cleaning solution to the input of the cell cleaning apparatus. A hot solution (~75 °C) of 25% Top Job detergent with water is recommended.

Once assembled as above, the vacuum is activated. Allow ca. 500 ml of the detergent solution to flow through the cell and then remove the tubing from the detergent solution. Rinse the end of the tubing with a wash bottle to remove all traces of the detergent solution. Then dip the tubing into another beaker containing rinse water and allow ca. 500 ml of flow before removing the tubing from the beaker. Remove the cell cleaning apparatus from the cell, and drain remaining water from the cell using the long-needled syringe.

We do not recommend drying the cell before filling with your sample solution, but it should be rinsed twice with the buffer you are using for the experiment. Finally, it should be completely drained and filled with your sample solution in the manner described earlier.

Because a small amount of the buffer used for final rinsing will adhere to the walls of the cell and act to dilute your sample solution, you may wish to correct for this by lowering

your sample concentration by 2% if you measured concentration before the sample was introduced into the cell.

Injection syringes may be cleaned with detergent solution and rinsed with water using the same method as described earlier for filling the injection syringe by drawing liquid upward through its filling port and into the plastic syringe. If you have sufficient ligand solution for rinsing the syringe following the water rinse, then it is recommended before final filling. If you do not have extra ligand solution for rinsing, then follow the water rinse with a methanol rinse and dry the syringe of methanol by purging with air. If **any** methanol is left in the syringe before filling with ligand solution, then your experiment will be unsuccessful since the heat of dilution of aqueous methanol into water is very large.

In the event of clogging, small cleaning wires have been provided for cleaning solids from the injection ports of the stir paddle.

WARNING:

The cells of the Omega ITC are fabricated from Hastelloy C, and should **never be cleaned with strong acids** of any kind. Please consult the booklet provided on corrosion resistance of Hastelloy C before using any substances which have corrosive properties.

Section 4.6

Below Room Temperature Operation

Since heat can only be added to the cells and jacket of the Omega (i.e., there is no direct way to cool these components), the operational temperature for an experiment must be at least several degrees higher than "ambient temperature". The purpose of the refrigerated circulating bath is to bring about a decrease in the effective ambient temperature to permit studies below actual room temperature. Coolant from this bath circulates through metal plates which surround the cell and jacket assembly. It is important to realize that about 2.5 inches of polyurethane foam insulation are sandwiched between the metal plates and the jacket, which makes heat transfer between the plates and jacket very slow. **In fact, once the temperature of the circulating bath is changed, it requires ca. 8-10 hours for equilibration to take place again and achieve a stable baseline**.

Because of this slow equilibration, the most time-efficient way to conduct a series of experiments at different operating temperatures (some of which are below room temperature) is to avoid changing the temperature of the circulating bath whenever possible. For example, if you wish to carry out titration experiments at 10 °C and 25 °C in one day, then the best way to do this would be to set the temperature of the circulating bath at 5 °C before leaving the laboratory in the evening. When you arrive the next morning, the Omega temperature will be ca. 6-8 °C. Using the SCAN ENABLE procedure, scan the instrument to 10 °C and do the first experiment. After completing that experiment, scan the Omega for a second experiment at 25 °C **but do not change the set temperature of the circulating bath**. It will only require ca. 20 minutes for the Omega to scan from 10 °C to 25 °C and equilibrate at the new temperature. Thus, by working progressively from experiments at the low temperatures to experiments at the high temperatures without changing the temperature of the circulating bath, it is possible to do many experiments at different temperatures in the same day. There are some limitations to this procedure. There tends to be a gradual degradation in baseline quality as the Omega operating temperature moves further and further above the temperature of the circulating coolant. For experiments where maximum performance of the Omega is required, it is best not to do experiments at temperatures which are more than ca. 35 °C higher than the temperature of the circulating coolant.

To achieve the best performance from your Omega (see **Section 4.11 on Maximizing Baseline Stability**), MicroCal recommends that the external water bath be in use continuously since this tends to reduce effects on the baseline which arise from changes in room temperature.

Section 4.7

Far Above Room Temperature Operation

For the reasons mentioned in the previous section, operating far above room temperature also requires an awareness of the possibility of baseline degradation if maximum performance is needed. Thus, if you wish to carry out an experiment at 55 °C, better performance would result if the Omega ITC was first equilibrated overnight with the bath circulator set at 50 °C as opposed to simply scanning the Omega ITC up to 55 °C with the bath not circulating.

Section 4.8

Precautions

The long needle and stir paddle on the injection syringe must not be bent even slightly or baseline stability will be compromised. Exercise care at all times in the handling of the stirring syringes. Exercise care at all times in the handling of the stirring syringes.

Carefully remove the syringe from the instrument after an experiment ends, clean, dry, and store safely.

All ITC purchases include a clear plexiglass syringe stand. This is often used to hold the injection syringe during filling, or merely for safe keeping of syringes. Users should be aware that damage to the injection syringe may be incurred when placing them into the syringe stand, if the syringe height setting (black flange fixed in place with a set screw) is incorrect. Though all syringe height settings will be proper when shipped from MicroCal, it is good practice to insert the injection syringes into the stand while paying attention to the bottom tip of the syringe. If the bottom tip of the syringe reaches the base of the syringe stand, then do not force the injection syringe-syringe holder assembly into it's seat until you are sure that there is clearance between the two.

The user should be aware that the design of the ITC Omega instrument is such that during an experiment the syringe barrel is spinning (usually at ca. 400 R.P.M.'s) while the plunger and teflon tip of the plunger remain stationary. This will eventually cause the teflon tip to wear away so there is no longer a proper seal and the solution in the injection syringe may leak or not be properly injected. The user should note that there is proper resistance between the plunger tip and syringe barrel when loading the syringe and replace the tip when the wear of the tip seems excessive.

Section 4.9

Troubleshooting

Problems may take the form of a short term noise in the baseline, long term drift of the baseline, and erratic peak sizes. Mistakes in technique can cause all of these problems.

Problems are most often due to either cell/syringe filling or stirring.

PROBLEM:

Cell Feedback and/or Jacket Feedback will not go positive

SOLUTION:

Since the Omega ITC cells and jacket cannot be cooled directly, experiments must be carried out at temperatures above the *effective ambient temperature*.

When you are not circulating coolant through the cooling plates, your experimental temperature must be at least 3-4 °C. above room temperature, to allow for heat generated within the Omega ITC unit. When operating below room temperature and flowing coolant from the circulating bath, then the lowest experimental temperature you may use is 4-5 °C. above the bath temperature.

PROBLEM:

Prior to inserting the injection syringe the baseline is not stable (peak to peak noise > .025 μCal/sec, drift > .5 μCal/sec/hour).

SOLUTION:

Higher than normal drift may indicate the presence of moist air in the jacket. Flush the jacket with dry nitrogen as described in Section 2.7. Desiccate for one hour and repeat.

Higher than normal drift is expected for ca. 10 hours after changing the bath set temperature.

Higher than normal drift is expected after increasing the operating temperature. The drift is higher for larger temperature changes. The drift will decrease to zero with time.

Your Omega ITC instrument is responsive to changes in room temperature, but because of the thick thermal insulation which separates the jacket from the room it responds very slowly to these temperature changes. Thus, the normal 10 min. on/off cycle of an air conditioner or heating system has little or no effect on the baseline so long as the average temperature remains constant. However, if the room where the calorimeter is located increases in temperature by several degrees beginning at the start of each working day, then the baseline will slowly drift for many hours before it levels out and becomes flat. The total

change in baseline over this long time period will amount to ca. 0.03 μcal/sec for each degree change in room temperature. This effect may be dampened to some extent, but not eliminated, by always circulating coolant through the cooling plates. The best solution is to make sure the room is thermostatted at the same temperature for 24 hours a day while the instrument is being used (Also see Section 4.11).

PROBLEM:

Baseline is stable without stirring, but becomes unstable after syringe is inserted and stirring begins prior to injections.

SOLUTION:

Indicates a stirring problem. May be debris or bubbles in cell, bent injection syringe needle, improper vertical positioning of the syringe, or injection system alignment problem.

Debris or bubbles - refill cell after rinsing or using cell cleaning apparatus and try again. Extraordinary circumstances may require carefully inverting the entire Omega ITC unit on a suitable stand and flushing the sample cell with the filling syringe to remove solid debris.

If your test samples are suspensions of particles try higher stirring rate to achieve uniform suspension, although the extra noise caused by the increased stirring speed may cause deterioration of the baseline.

Bent injection syringe needle - test by rolling on flat table top. Bent needles can sometimes be straightened by a machinist using a lathe.

Improper injection syringe vertical position - The stir paddle at the tip of the injection/stirrer syringe should be centered vertically in the cell to prevent contact while stirring. Stirring problems can be caused by the stir paddle being either too close to the bottom or top of the cell. With the syringe inserted in the instrument loosen the set screw on black plastic disk on the top of the injection syringe glass and slide up slightly. Hold the syringe holder firmly with one hand and rotate the injection syringe glass so that it may be pushed up and down within its holder. Slowly push the syringe through the holder until the bottom of the cell is felt as an impediment. Raise the position of the syringe 0.1 inches, push the black disk down to the top of the holder and re tighten the set screw.

Injection System Alignment - If all your injection/stirrer syringes cause the same stirring-related problems, the alignment of the syringe mounting barrel may be responsible. The syringe mounting barrel is spring-mounted to allow for angular adjustments. Empty the cell and insert the alignment device. The aluminum disk on the device should fall freely into the top of the syringe mounting barrel when the syringe mounting barrel is properly aligned. Your instrument has had its alignment checked at the factory prior to shipment, so only small adjustments to the alignment should be necessary. Adjust the barrel's three screws a small bit at a time until the alignment device's aluminum disk is centered in the mounting barrel.

The injection motor shaft (the threaded rod running through the top of the motor stand) should be aligned with the injection/stirrer plunger when the motor stand is locked into the operating position with the thumbscrew. This is best checked when the plunger is about a quarter inch from the top of the syringe barrel. If the alignment is off, loosen the four thumbnuts securing the motor to the motor stand to reposition.

PROBLEM:

Titration data shows one or several peaks smaller than the apparent data peak envelope.

SOLUTION:

Likely attributable to bubbles in the injection syringe. Prepare degassed solutions and repeat following loading instructions for the injection syringe.

PROBLEM:

Coolant flow is sluggish through the thermostatting plates of the Omega Cell.

SOLUTION:

It may happen when using hard or unfiltered water or allowing the coolant to sit for an extended period of time in the veins of the thermostatting plates that deposits will form. If the instrument is not going to be used for several weeks or more then the coolant lines should be drained and blown dry. In the event that deposits do form a 4 liter solution of 5% acetic acid and 5% EDTA solution, heated to ca. 50 °C, pumped slowly through the circulating lines should quickly clean the lines.

PROBLEM:

The following error message appears:

SOLUTION:

This message usually occurs when one of the Origin documents (DSC.ORG, ITC.ORG or DSCITC.ORG) used to plot data has be altered by the user. Simply re-install the data collection programs from the distribution disk.

Section 4.10

Omega - ITC Experimental Tutorial

The following experimental tutorial is designed to acquaint you with the basic features of both the hardware and software of the Omega ITC instrument, as well as to provide experience with several manipulations which must be mastered in order to get the highest quality data from your instrument. Rather than beginning experimentation on the Omega ITC using precious biological samples, we strongly suggest that each user of the Omega ITC instrument complete the following tutorial first, using sucrose solutions, so that irreplaceable samples are not wasted while mastering the appropriate techniques.

At this point, your instrument should have been completely assembled according to the directions provided in Section 2, all electrical connections made, and all MicroCal software installed on the hard drive of your computer (this was done for you if you purchased your host computer from MicroCal).

The recommended procedures for preparation of solutions, degassing, filling the cell and injection syringe, etc. have already been discussed in the previous sections and the following tutorial assumes your familiarity with those procedures.

I. Sample Preparation

Begin by degassing (with stirring) ca. 100 ml of distilled water for ca. 5 minutes using your rotary vane vacuum pump (or suitable substitute) and degassing apparatus. This degassed water will be the solution which will go into the sample cell (and also reference cell if it does not already contain water).

Use ca. 15 ml of the degassed water in a test tube and add sucrose to make a 5% sucrose solution, using stirring to be sure all sucrose has dissolved. This solution will be loaded into the injection syringe later.

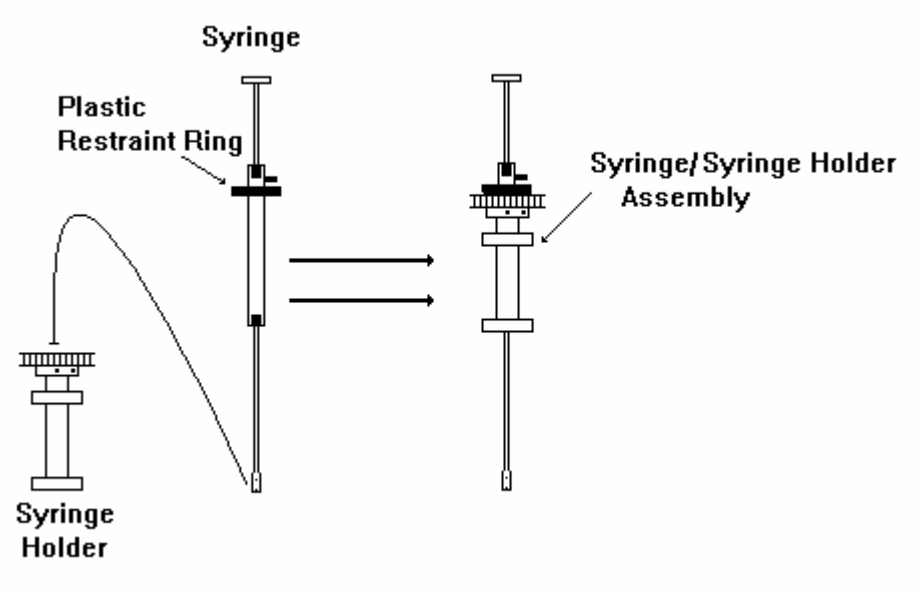
II. Filling The Cells

The Cell Entry Port consists of a 1.0" hole in the center of the white Delrin loading barrel which is for enclosing the bearing assembly for the stirring syringe of the Omega Cell Unit. At the bottom of the port, the small entry tube to the sample cell is in the center and the reference cell is off to the left side (If overhead lighting is poor, you may want to examine these using a flashlight). Load the 2.5 ml glass filling syringe (8" needle) with the degassed water, and tap the syringe bottom after loading so all bubbles float to the top surface. Enter the needle into the sample cell entry tube and carefully slide the needle down the tube until it just touches the bottom of the cell. Begin to slowly depress the plunger so the cell fills from the bottom up. After ca. 1.5 ml of degassed water has been entered, you will be able to see the water come to the top of the small entry tube. Once you see the liquid level, raise the syringe a millimeter or so off the bottom of the cell and depress the plunger very quickly to deliver a spurt of ca. .3 ml. The purpose of the last spurt is to dislodge any bubbles which might be clinging where the entry tube joins the cell.

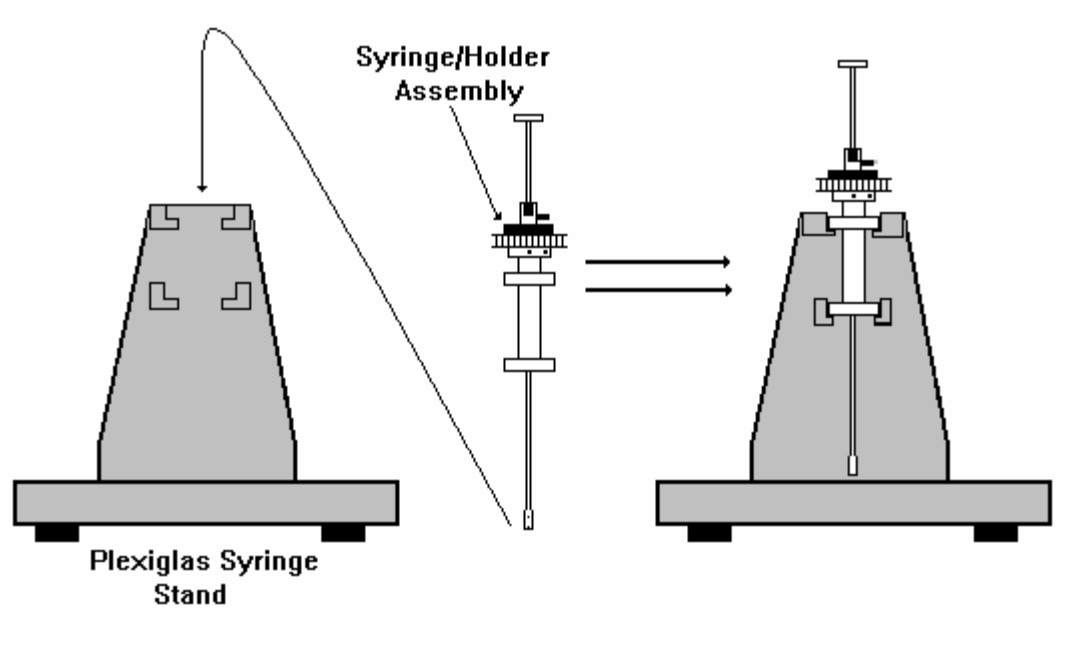
If the reference cell has not previously been filled with water, then carry out the same procedure to fill it. There is a small cap and positioning tool which has been provided that you may use to close off the reference cell if you desire to do this.

III. Assembling and Filling The Injection Syringe

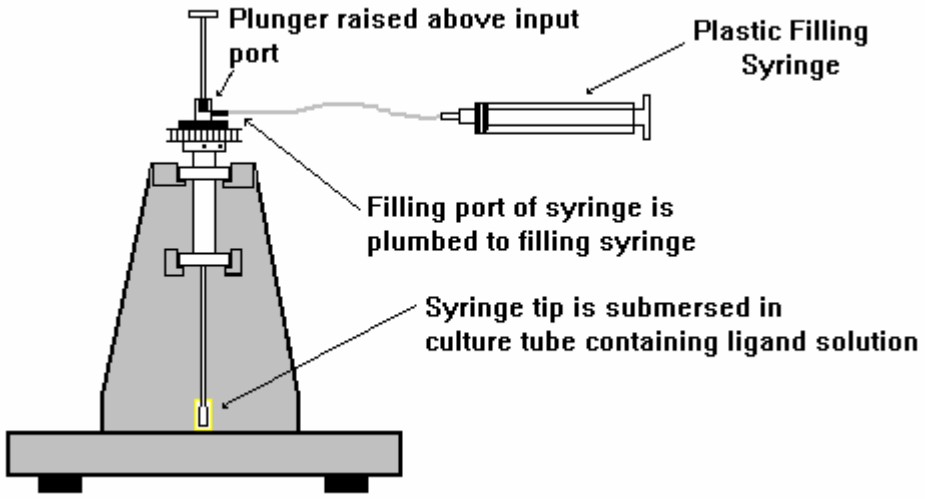
Locate the 100 µl (medium bore) injection syringe with the attached long needle that has a stir paddle at it's tip. Locate the white plastic (Delrin) syringe holder. The syringe holder is expanded at the top with an O ring immediately below the expanded portion. Carefully slide the end of the long syringe needle into the top hole and out the bottom hole of the syringe holder as shown in the diagram below. Push carefully on the black plastic restraint ring at the top of the syringe until it seats firmly against the top timing belt pulley of the syringe holder. (The position of the restraint ring has been fixed prior to shipment so that the stir paddle will be located at the correct height in the sample cell after it has been seated into the barrel of the injection system.)



Locate the clear Plexiglas syringe stand. Seat the assembled syringe/syringe holder assembly into the center of the syringe stand, as shown below.



You are now ready to fill the injection syringe with the sucrose solution you have prepared. Begin by raising the injection syringe plunger to the high edge of the metal filling port located near the top of the syringe, as shown at the top of the illustration below. Now, raise the injection syringe/holder assembly slightly so that you can slide the test tube of sucrose solution underneath the tip of the injection syringe's stir paddle. While holding the test tube with one hand, lower the injection syringe/syringe holder assembly until it is again seated in the Plexiglas syringe stand. The injection syringe's stir paddle should now be completely submersed into the sucrose solution. Now locate the clear plastic filling syringe and the length of clear plastic tubing which came with your instrument. Insert one end of the tubing over the tip of the filling syringe, and the other end over the injection syringe's metal filling port. Your setup should now be as shown below.



By slowly withdrawing the plunger of the plastic filling syringe, you will draw the sucrose solution into the injection syringe. Once the solution begins to exit the top filling port, depress the plunger of the injection syringe until it is slightly below the side-arm filling port, thereby stopping the flow of the sucrose solution. Remove the plastic tubing from the side-arm filling port. With the stir paddle of the injection syringe still immersed in the sucrose solution, depress the plunger sharply and draw it back slowly while being sure not to let the plunger come all the way up to the side-arm filling port. Repeat the procedure a second time to insure that any small bubbles, which might have lodged in the long needle of the injection syringe during filling, are expelled into the excess sucrose solution. Your injection syringe is now filled. Carefully raise the Syringe/Holder Assembly out of the Syringe Stand, remove the vial containing the excess sucrose solution, rinse the stir paddle and lower part of the needle with a wash bottle, and reseat the Syringe/Holder Assembly onto the Syringe Stand. **Anytime that you are handling the injection syringe after it has been filled with titrant solution, you must be careful not to flex the long needle since this will cause some solution to be expelled from the syringe and will result in a poor first injection for your experiment**.

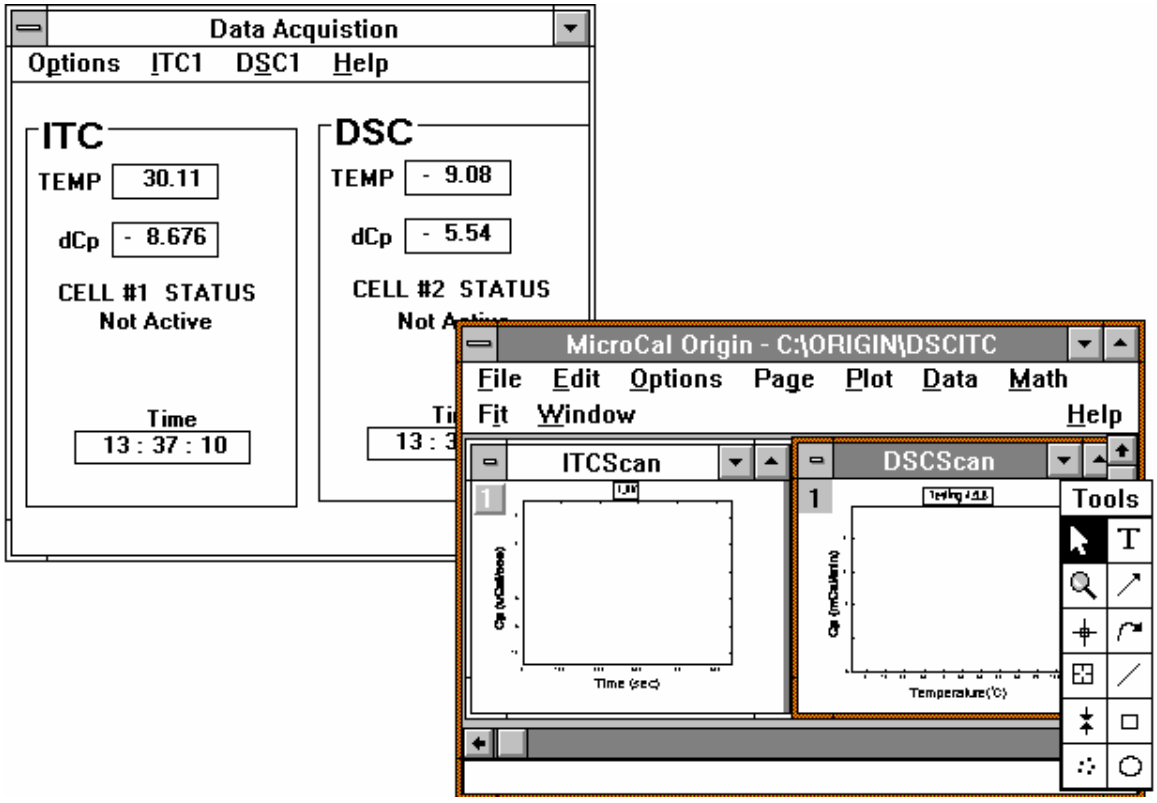
Unlike the present experiment where we are using sucrose solution, there may be times when the titrant you are using is very valuable. Small culture tubes have been provided which minimize the volume of titrant required to load the filling syringe. **It must be recognized however that liquid transfer into and out of the injection syringe occurs through a tiny hole about 1 cm above the bottom of the stir paddle, which must then always be kept below the liquid surface in the culture tube**.

IV. Equilibrating The Cells

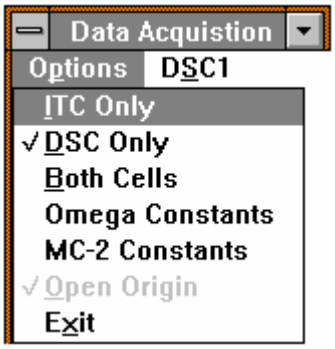
The power should already be on at the Control Module, the Omega front panel and on the nanovolt preamplifier, so now push in the Run button on the front of the Control Module. Typically upon power up the reaction cell will be at room temperature. Since the adiabatic shield will only thermostat above room temperature (i.e., without the circulation of coolant from an external bath) the cell must first be raised to the experimental temperature and allowed to equilibrate. Both cells should be filled with liquid while changing temperatures. Set the desired temperature at the front of the Omega using the TEMPERATURE SHUT-OFF dial. This dial reads in °C times 10 (i.e. a setting of 300 indicates 30.0 °C). If the set temperature is higher than the current temperature (displayed on the Control Module temperature meter) and the toggle switch to the right of the dial is in the SCAN ENABLE mode, the cell will rise in temperature and shut off within several tenths of a degree of the dial setting. Once the temperature rise is complete, the toggle switch must be flipped to the BASELINE ENABLE setting. On the front of the Omega Cell Unit select a BASELINE setting of 2. Turn the SHUT-OFF dial to its counter-clockwise extreme. The system must now be allowed to equilibrate. The progress of equilibration may be followed by pressing the ΔT button on the Control Module front panel. The number displayed is near zero (0.0-0.3) at equilibration, but may be as high as 2.0 just after scanning up. As equilibrium is approached , the jacket feedback will reach a constant reading in the +10 to +80 range, and the cell feedback will become stable in the +10 to +100 range, depending on the BASELINE setting being used (e.g. a BASELINE setting of 2 will have o cell feedback setting between 20 and 30). This equilibration period is about 15-20 minutes when working within 10 degrees of room temperature and longer for higher temperatures. The system, once equilibrated, will thermostat at the equilibrium temperature.

V. Preliminary Operations Using DSCITC Data Collection Program

Start the Omega ITC data collection program by clicking on the icon on the left. If you do not have the DSC MC-2 unit connected, please continue on the next page. If you also have the MC-2 unit connected, upon launching the data collection program Origin will be started and loaded with the document DSCITC.ORG. You will see the main menu program as shown below along with the Origin program. This process will take about 40 seconds. If Origin had previously been loaded, but not with the document DSCITC then a second copy of Origin will be opened. Two copies of Origin running simultaneously is not recommended so please close the copy of Origin which does not contain the document DSCITC.ORG.

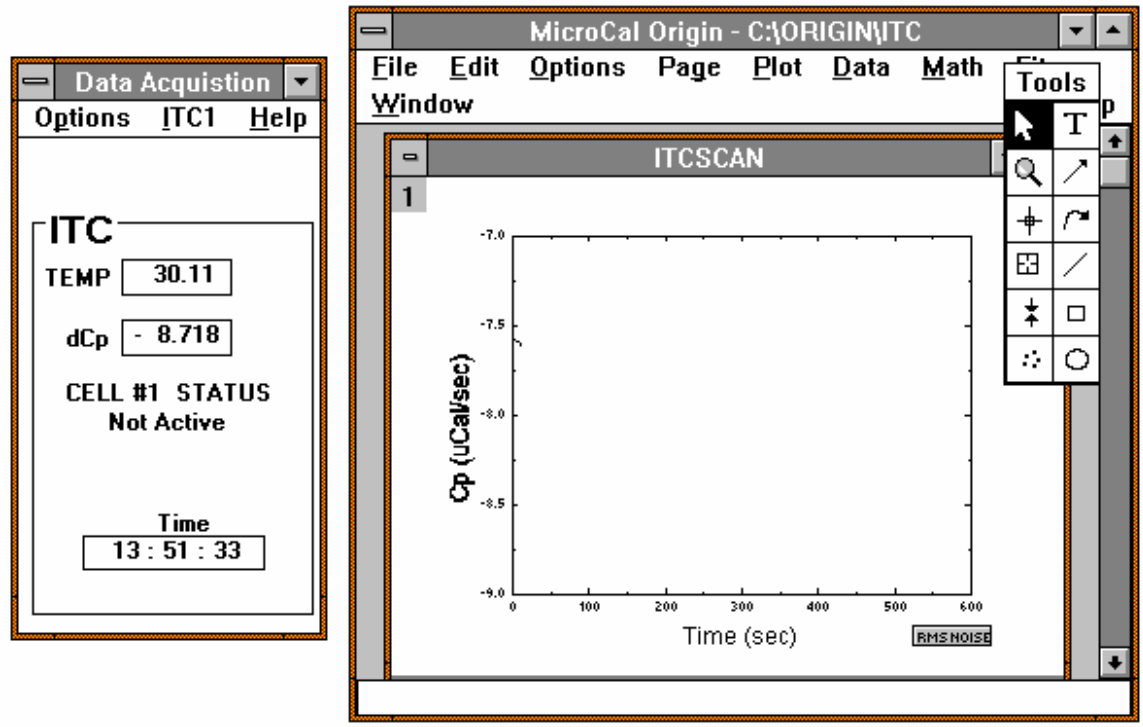


If you have the DSC MC-2 unit connected, you may select ITC Only from the Options menu to show only the ITC menu.



Click on the maximize icon in the upper right corner of the ITCScan window to remove the DSCScan window from the screen.

If you have only the ITC version of the software (or select ITC Only) then the data collection menu will appear as below. All functions of the program are the same for both version, so we will use the ITC Only version to demonstrate the rest of the software.

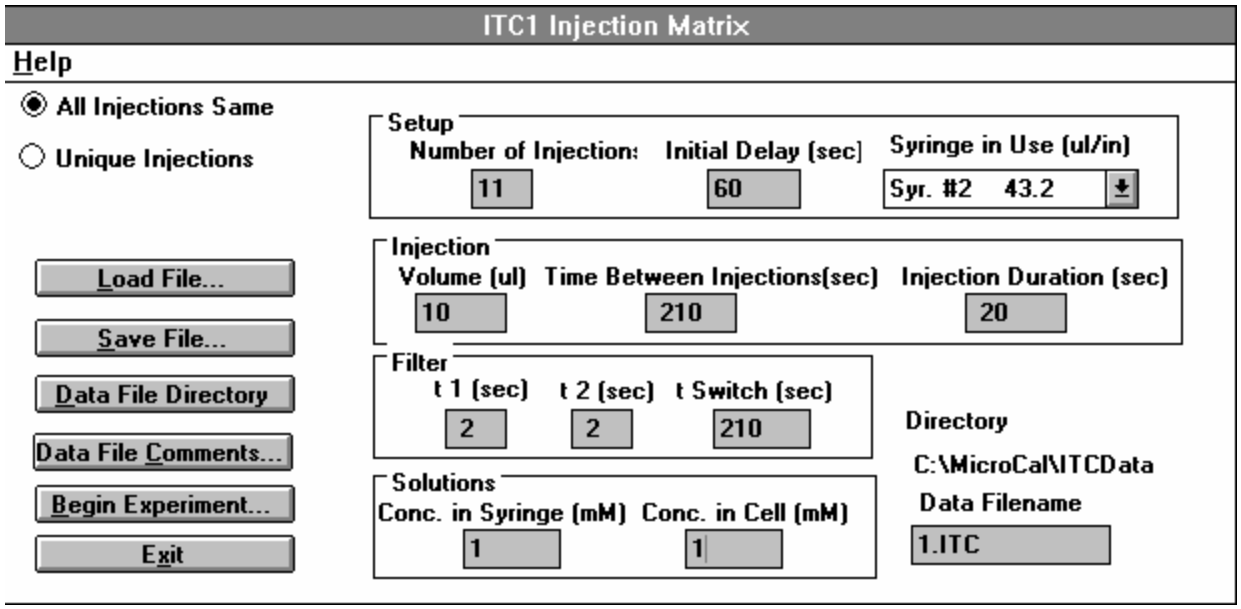


By observing the data channel displays on the main window of DSCITC, you can verify that data is continuously being received from the Control Unit. If you see the values in the text boxes changing approximately every 5 seconds (though only slightly) then you are ready to continue with this tutorial.

Second, move the Origin ITCScan window to the upper right most area of the screen. Put the mouse cursor on top of the lower left corner of the main Origin window so that the cursor becomes a double arrow. Now click the left mouse button and while holding the button down, drag the corner of the window so that Origin's main window occupies most of the video screen. Do not make the Origin window so big that you can no longer see the Data Acquisition window. You now should be able to see both the DSCITC main data acquisition form as well as the Origin plot window where your data will be plotted, as shown above.

VI. Setting Run Parameters

From the main ITC1 menu click on the ITC Injection Matrix menu option. The ITC1 Injection Matrix window will appear. If you are configured for two Omega ITC cells, make sure that you are working with the same cell in both hardware and software.



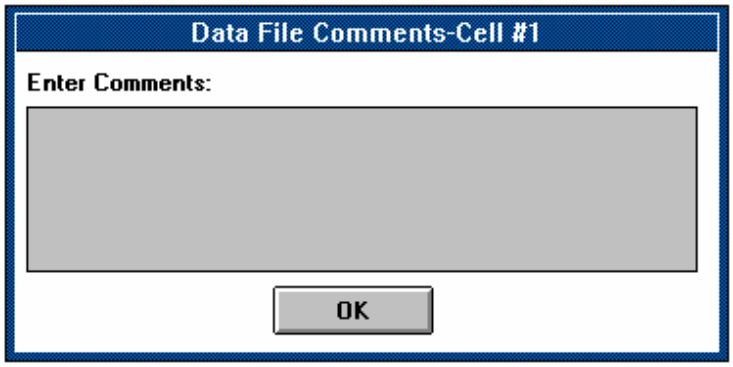
In the upper left corner, click on All Injections Same. The button to the left of the All Injections Same text should be blackened indicating that this is the active choice. Now, working from top to bottom in the text boxes, we will define the experimental parameters.

-Enter 11 in the ***Number of Injections*** text box
-Enter 60 in the ***Initial Delay*** text box.
-Enter 10 in the ***Volume*** text box.
-Enter 20 in the ***Duration*** text box.
-Enter 210 in the ***Time Between Injections*** text box.
-In the ***Syringe In Use*** list box click once on the downward arrow. A list of three syringes will drop down. Click once on the 43.2 µl/in list entry. You may notice that the duration value in the Duration list box changed slightly. That's normal, and you may use the new value if it has in fact changed.
-Type sucr1.itc in the ***Run Data Filename*** text box. You need not type the filename extension, however since regardless of what extension is typed in this text box the datafile extension will be .itc.
-Enter 2 in the ***T1*** text box.
-Enter 2 in the ***T2*** text box.
-Enter 210 in the ***T Switch*** text box.

Since we are not actually working with real biological samples for the tutorial, you may leave the Concentrations In Syringe and In Cell as they are. When working with samples be sure and put the appropriate concentrations for the syringe and cell contents into these text boxes, as they will be used during data analysis calculations in Origin.

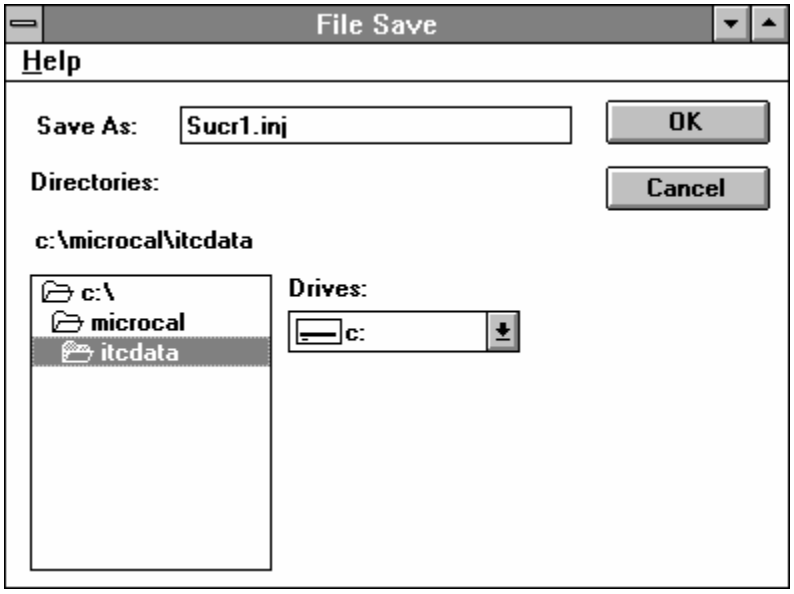
By filling in the ITC Injection Setup as described above, you have indicated that you will be using the medium-sized syringe (delivers 43.2 µl for each inch the plunger is depressed) to deliver 11 identical injections of 10 µl each, with the first being made 60 sec into the experiment and each successive injection spaced at 210 sec following the previous injection. Since t1 and t2 are both 2 sec, all data will be averaged for 2 sec before a data point is stored, which will lead to a total of 1185 data points.

Now, click on the Data File Comments button. The Data File Comments window will open.



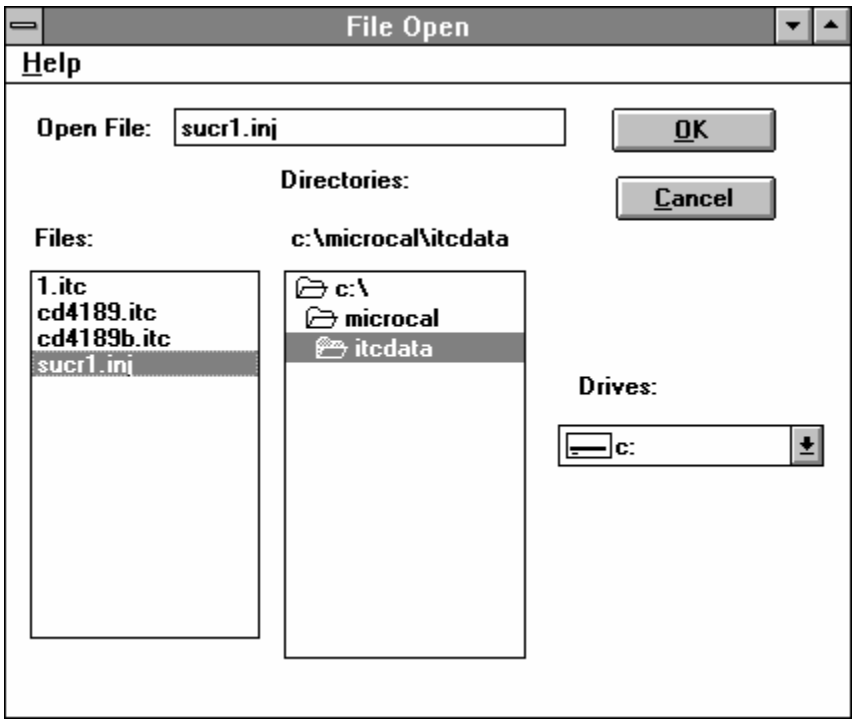
You may type in any comments that you want to be added to the data file header, and click OK.

Now click on the File menu , and then on the Save File sub menu. The Save File window will appear.



Type in sucr1.inj and click on the OK button. You now have saved these Omega ITC run parameters to disk and they are available to you for later use. By default, all setup files like the one that you just saved will reside in the c:\Microcal\itcdata sub directory. Data file comments are not saved to the setup files since they are usually unique to each experiment.

To demonstrate the reuse of setup files do the following: Enter 5 in the # Injections text box. Now click on Unique Injections. An injection summary table will appear on the bottom of the ITC Setup Window, displaying 5 potentially unique injections. Now click on the Load File sub menu. The Load File window will appear, and all of the ITC setup files (.inj extension) will be listed on the left side.



Click on the sucr1.inj entry that you just created and choose 'OK. The parameters from this file will appear in the ITC Setup Window. Confirm that the # Injections text box again displays 10, and that All Injection Same is chosen in the upper left corner. The injection summary table that appeared at the bottom of the window should no longer be visible.

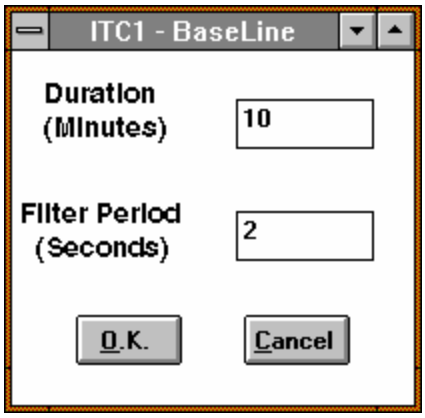
VII. Beginning The Experiment

Now let's begin the ITC experiment. Click once on the Exit button found on the left side of the setup window. This will close the injection matrix and return you to the ITC1 main menu. It should be realized that windows are best to be closed when you are finished using then, rather than simply minimized. Open windows exhaust system resources, and thus should be closed when no longer needed.

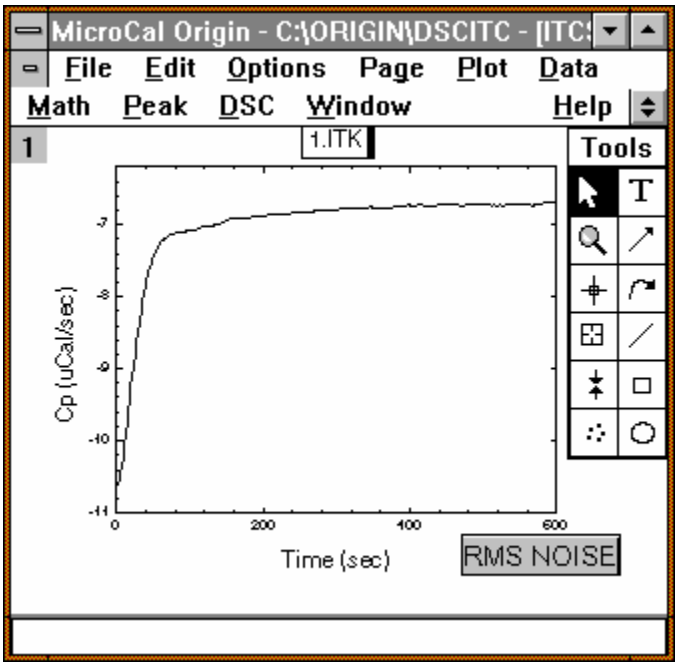
VIII. Observing a Baseline

To observe a baseline, you should wait till the jacket feedback and cell feedback values have reached positive values away from the saturated readings(saturated readings are typically ca. + / - 142). Typical values for the cell feedback when on the BASELINE setting of 2 are ca. 24. Typical for the jacket feedback when the cell temperature is above room temperature by 3 or 4 degrees C. is ca. 18-20.

To start a baseline run select **ITC1:Baseline**, the following window will appear.



A 10 minute baseline run averaging each data point for 2 seconds will give a good display of the shape of the baseline, click o.k.. A typical baseline observed whilel the instrument is still equilibrating is shown below.



Note: If it appears your Y-axis readings are going to equilibrate outside the range from -10 to 10 µcal/sec, then adjust the y-suppression knob on the front of the control panel to bring it into the -10 to +10 µcal/sec range and allow the equilibration to continue.

It will probably happen that the Y-axis data will go off scale while the instrument is equilibrating. The initial Y axis scale for dCp is from -1μcal/sec below the initial data point to +1 μcal/sec above this point, which may be too sensitive and cause the data to go off scale in the Origin window . You may double-click on the Y axis line, which brings the Y Axis Dialogue Box into view. In the upper left corner of this dialogue box, you will see Y axis limit boxes designated as From: and To: The From: box is darkened and ready to edit. Note the present dCp reading in the appropriate Data Acquisition data box. Subtract 1 μcal/sec from that reading and enter the resulting number in the From: box. Press the tab key on your keyboard, which then darkens the To: box for editing. Enter a number in here which is 1 μcal/sec larger than the current dCp reading. Click on OK and the dialogue box closes. Now your data plot should have full scale of 2 μ/sec on the Yaxis and the current dCp data should be centered in the plot. Alternatively you may select **Plot:Rescale to Show All** from the ITCSCAN Origin window which will automatically rescale the axis to view all the data.

You will notice a long 'tail' in the baseline before it becomes flat. You need not observe the baseline for the full 10 minutes, but can exit this routine as soon as it appears to be flat by selecting **ITC1:End Run**.

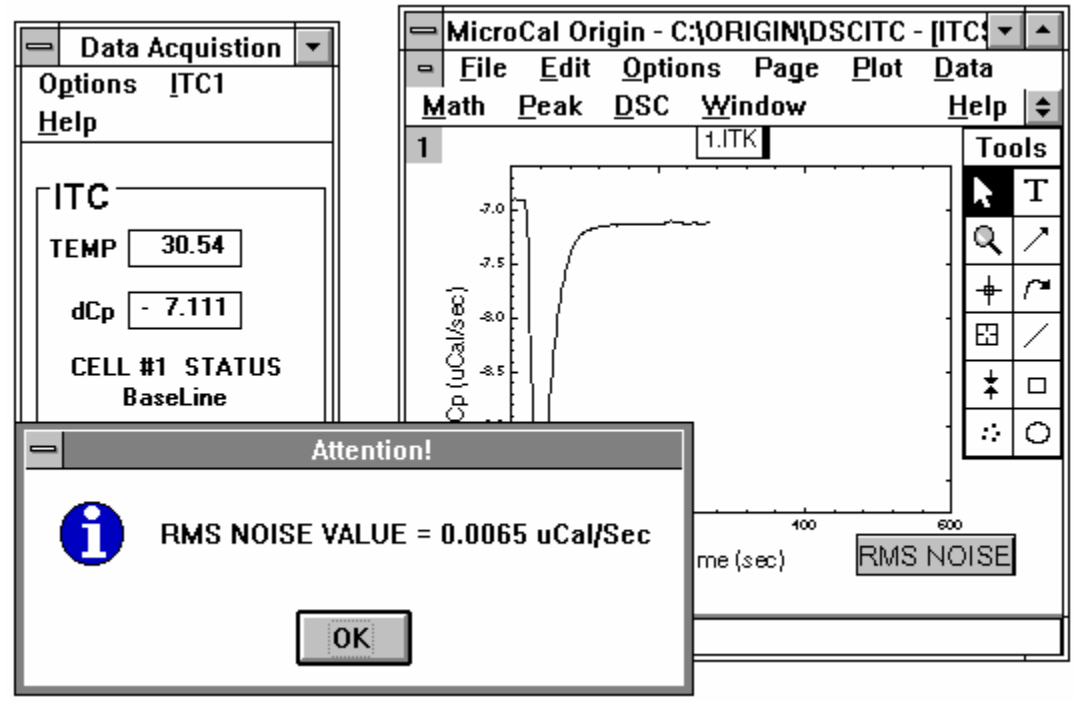
IX. Inserting The Injection Syringe

Carefully remove the Syringe/Holder Assembly from the Plexiglas Syringe Stand and carefully insert the stir paddle end of the needle into the entry port for the sample cell, located in the center of the Cell Port Entry. Lower the syringe slowly until the upper bearing race touches the top of the O ring just below the Cell Port Entry. Push firmly on the top of the upper bearing or timing belt pulley (away from the syringe) until the upper bearing is seated flush with the top of the Cell Port Entry hole. In carrying out the procedure described above, it is important to do it with no flexing of the syringe needle, since if it bends during insertion then liquid will be forced out of the syringe and the volume delivered in the first injection will be incorrect.

X. Baseline Equilibration

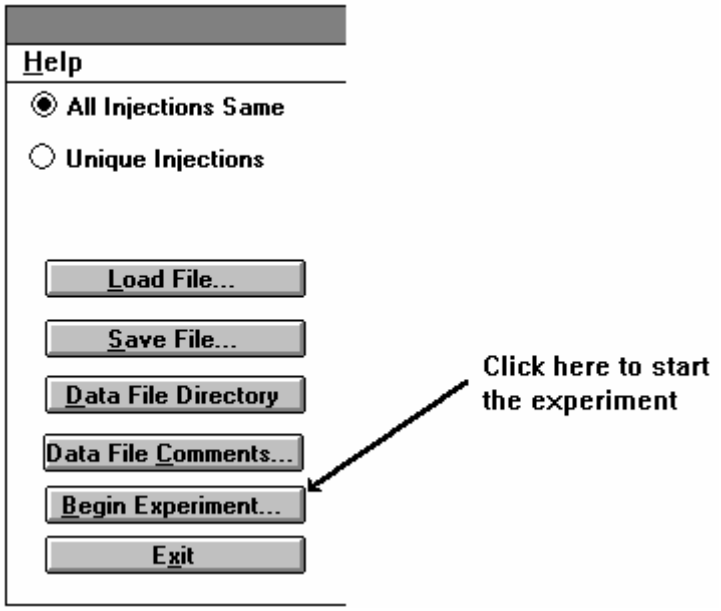
The dCp data is the differential power baseline, and it must flatten out and equilibrate before the injections are started. Turning on the stirrer has caused a disturbance in the dCp but it will stabilize in a few minutes. As before to bring the baseline back into the graph, select **Plot:Rescale to Show All** from the Origin window. If you then wish to zoom in on the data you may select the magnifying glass from the toolbox or double click on the Y- axis to open the Y-axis dialog box and enter the appropriate information into the scale **from** and **to** boxes.

The dCp signal should now be visibly leveling off at this Y- axis scale expansion. The longer you wait, the flatter it will become. Once the dCp signal is changing by ca. 0.1 µcal/sec, or less, over a five minute period then select **ITC1:End Run**. Refer to the ITC Troubleshooting section for a description of problems which may cause unstable baselines, or unusually long baseline equilibration periods.



XI. Data Collection

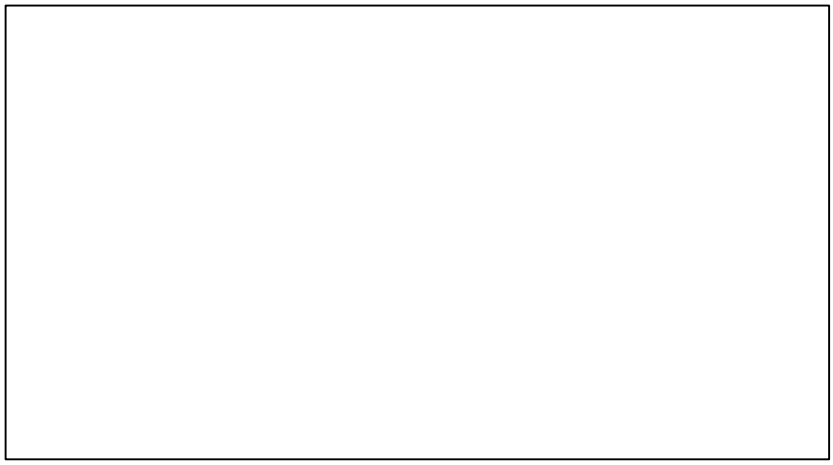
You should now click on Injection Matrix from the ITC1 menu.



The ITC cell will now begin it's initial delay. Within a short time, a new Origin plot window will appear, and all ensuing experimental data will be plotted in it. After the 60 second initial delay, the first injection will be made. You can follow the injection process by watching the Cell Status text box. When the injection is being made, when it has finished, and the data collection period for the injection are all indicated in the Cell Status text box.

In other experiments where you might wish to change run parameters for a run in progress, simply edit the appropriate parameter boxes in the ITC Injection Matrix table and then click on **Exit** and the new parameters will be used for the remainder of the run. For example, if you set up an injection run and after a few injections you wanted to change the injection volume for each of the remaining injection, you would select **ITC1:Injection Matrix**, then enter the new volume in the the Injection Volume box, then click on **Exit**. This new volume would be entered into the cell during each of the remaining injections. (Note: Although the injection volume is stored in the data file for data analysis in Origin, the change is not noted in the header of the data file for future reference (i.e. you will not be able to recall this change of information with the **Injection Matrix:Load File** button)).

After all 11 injections are completed, the data file can be found on your hard drive in the \MICROCAL\ITCDATA sub directory. Carefully remove the Syringe/Holder Assembly from its seated position in the Cell Entry Port and transfer it to its cradle on the Syringe Stand. You will now have the experimental data displayed in Origin. If you wish, double click on the X axis and enter -200 and 2700 in the **From:** and **To:** boxes so all of your data will be displayed. Pictured below is data from a similar sucrose dilution experiment (4.5% Sucrose) generated at MicroCal.



XII. Doing The Control Experiment

Now that data has been obtained for injections of 5% sucrose into water, you should proceed to do the control experiment for 11 equivalent injections of 5% sucrose into 5% sucrose. Since the control experiment involves identical solutions in this case, any heat effects which you see will be the result of very slight temperature mismatch between the solution in the cell and the solution entering the cell from the syringe. Once this control data is subtracted from the original sucrose-into-water data, the difference will be due strictly to the heat of dilution of sucrose.

It should be added that normal control experiments for ligand binding to macromolecule involve injections of the ligand solution into buffer which contains no macromolecule. In these cases, the two solutions are non-identical and control peaks will include effects from heat of dilution of ligand as well as those due to slight temperature mismatch.

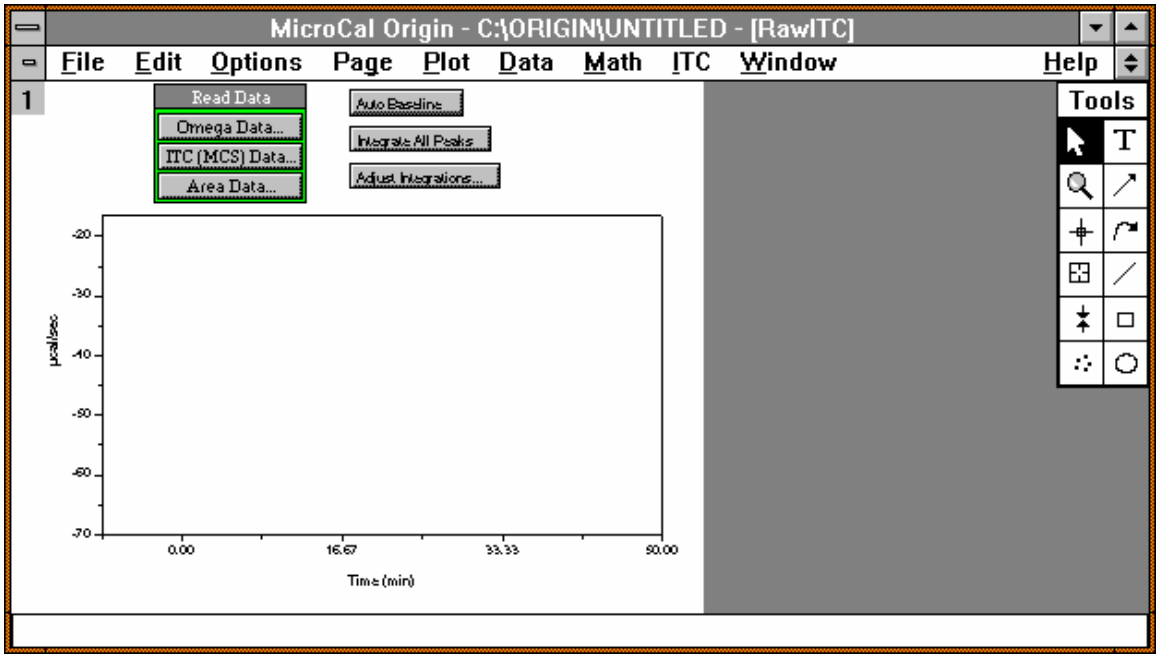
Drain the solution from the sample cell, rinse twice with 5% sucrose and finally fill with 5% sucrose. Refill the syringe to its full position with 5% sucrose. You are now ready to do the control experiment according to the same procedures you used for the sample experiment. Name your data file sucr1ctl.itc for this control experiment.

XIII. Plotting and Analyzing Data In Origin

At this point you should have completed two ITC experiments, the original 5% sucrose into water experiment as well as the 5% sucrose into 5% sucrose control experiment. We can now go to Origin to analyze the data.

If you haven't already done so, you may close the DSCITC program by selecting **Options:Exit**, or you may want to minimize it by clicking on the downward arrow in the upper right corner of the window.

Click on the Origin-ITC Data Analysis icon in Program Manager. You should see the ITC raw data template as pictured below.

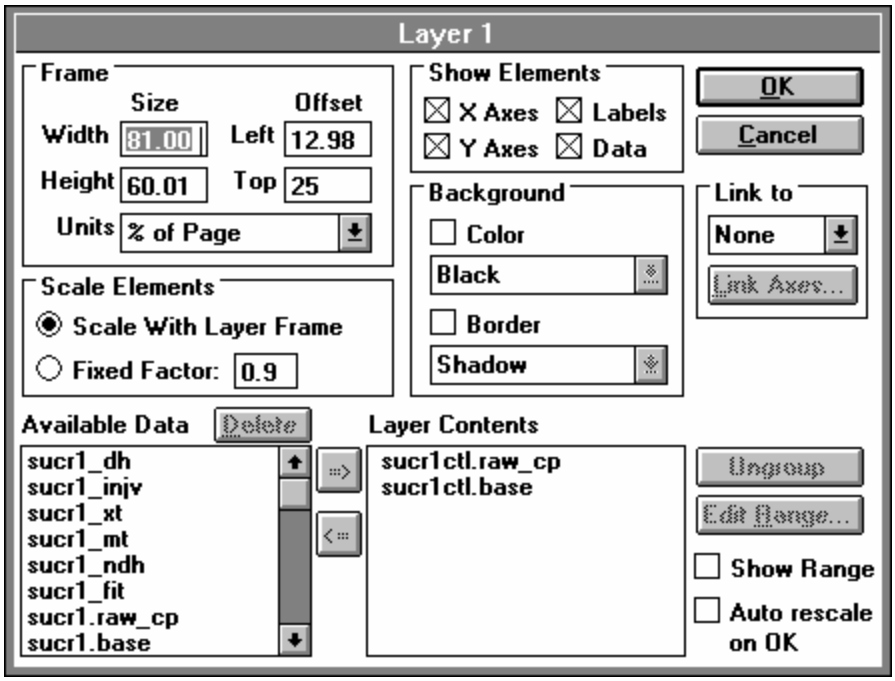


If this is not what you see, then click once on the Origin 'Edit' menu, and then again on the 'Change Menu'\'ITC Data Analysis' sub menu. You may answer yes if prompted to start a new session. You now will be looking at the Raw ITC data template.

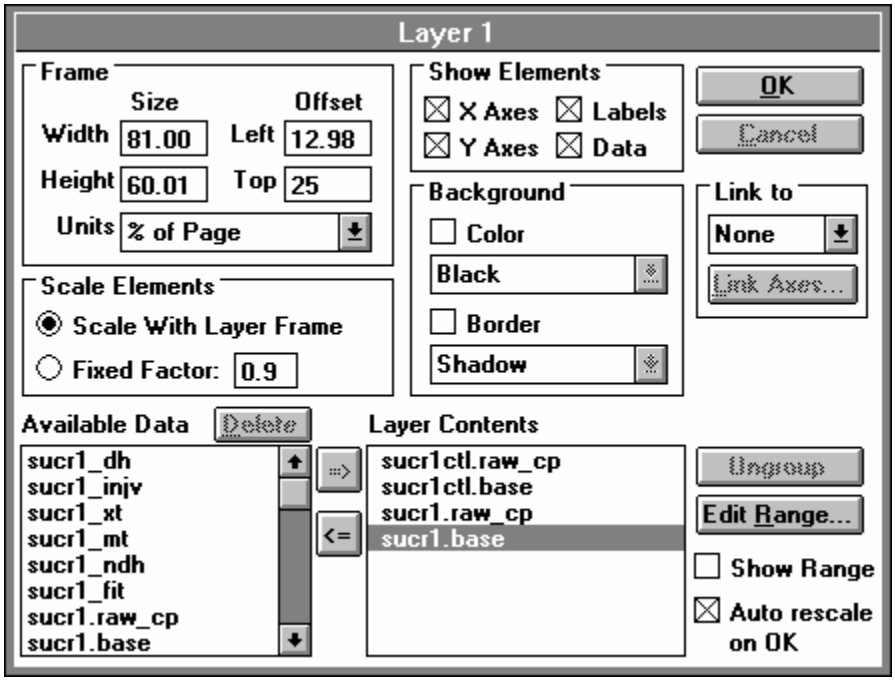
To import your Omega ITC data into the plot window, click on the 'Read (ITC)MCS Data' button. Find your way to the \Microcal\Itcdata sub directory, select the sucr1.itc entry and click 'OK'. Origin will automatically plot the raw data, generate a baseline for the raw data, integrate all peaks, and display the integration results in the Delta H window. We will discuss the Delta H window in some detail later on.

Click on the Raw ITC Data window to make it the active window (or you may select RawITC from the Window menu in Origin). Follow the same procedure as in the previous step to read in the control data (select the control datafile sucrctl.itc this time instead of the sucr1.itc datafile).

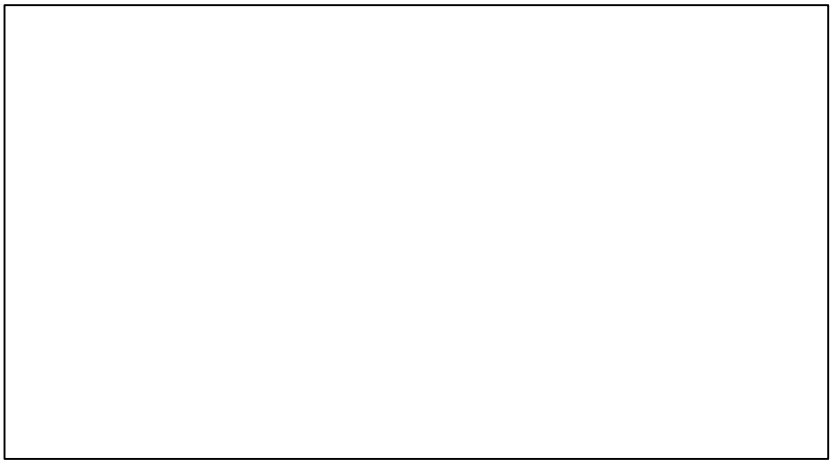
Though Origin now has the raw data from both the experiment and the control, only the control data is displayed in the plot window. Locate the layer icon (a gray box with '1' appearing in it) in the upper left corner of the Raw ITC Data window, and double click on it. The layer control dialogue box will appear.



You can see that the layer contents contains two datasets, sucr1ctl.raw_cp and sucr1ctl.base. Directly to the left is the available data, and by highlighting the desired dataset, and clicking the => button you will move the sucr1.raw_cp and sucr1.base datasets into the layer contents. After doing so, the layer dialogue box should appear as pictured below.



When you have all four datasets included in the layer contents list, click 'OK'. You will return to the Raw ITC Data window, and you should see both raw datasets and their baselines plotted. To gauge the relative success (or failure) of your experiment you can compare your raw data to the plot below.



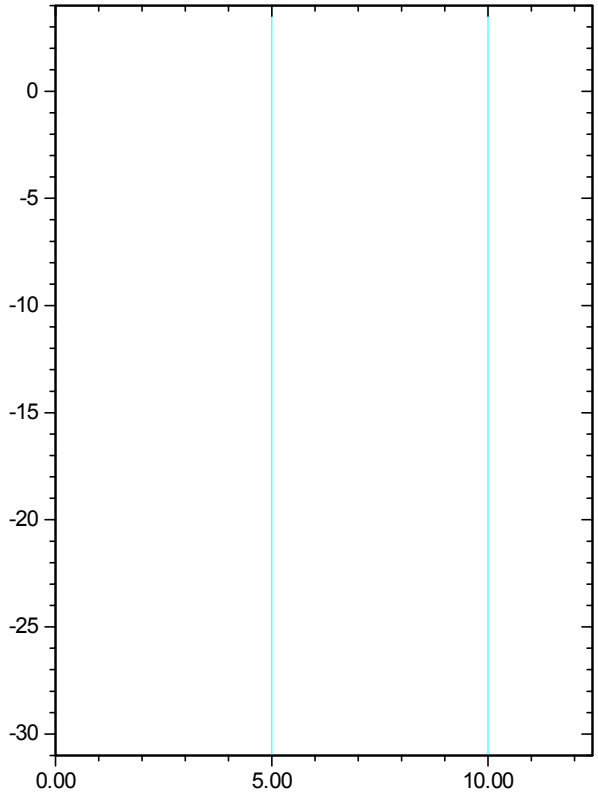
These data were generated at MicroCal on an Omega ITC instrument using a 4.5% sucrose solution and the same run parameters used in this tutorial. Your peak sizes may differ a little (peak size is proportional to ca. the 1.5th power of sucrose concentration). The size of control peaks will also vary a little from instrument to instrument and with actual room temperature. Note also that the above control peaks show an initial exothermic swing followed by a slightly larger endothermic swing. This up-down swing within the same injection peak is not unusual to see for peaks with extremely small net area, but you may or may not see it with your data.

Now click on the DeltaH plot window in Origin or select DeltaH from the Window menu of Origin. Origin automatically plots concentration-normalized areas (kcal per mole of injectant, designated with an .ndh extension) into the DeltaH template whereas what we want to view in this particular instance is the raw data (μcal per injection, designated with a .dh extension). Follow the same procedure as described above to open the layer control dialogue box for the DeltaH plot. Move all .ndh files out of the Layer Contents and move the two .dh files from Available Data to Layer Contents, and click on OK.

Finally, double-click on the numerical tick labels on the Y axis, which causes the dialogue box for the Y axes tick labels to appear. In the Factor box in the upper right you will notice a factor of 1000 (which is normally used to convert from cal to kcal). Edit to put a 1 in the factor box, rather than 1000, and click OK.

Now the two data sets are plotted in terms of μcal per injection on the Y axis versus a number proportional to injection number on the X axis. You may compare your results with those shown below. The scatter of your points from a straight line should not be much worse than these. If you have already been through the tutorial guide for ITC Data Analysis in Origin, then you may wish to integrate all of your peaks one-by-one, rather than rely on the automatic integration procedure, and this will probably reduce scatter.

Again, as a gauge of your success you may compare your results to the plot below.



XIII. The Next Step

If the data you obtained using 5% sucrose look considerably worse than the sample data we have provided for comparison, then you should go back to the beginning of this tutorial and repeat it. Perhaps you might want to read the Troubleshooting section first, if you have not done so.

If you are satisfied with your sucrose dilution data, then you might want to move on to real binding experiments. With your instrument, you received a trial kit which includes solutions of the ligand 2'CMP and the enzyme Ribonuclease A. Before your instrument was shipped from MicroCal, we used aliquots of the same samples to generate a binding isotherm and have provided you with a copy of the results we obtained, including the run parameters and the fitting parameters obtained from data analysis in Origin. If your techniques are good, you should be able now to generate the RNase/2'CMP binding isotherm and obtain fitting parameters very similar to those obtained at MicroCal with your instrument. Note: Since the RNase/s'CMP experiment is strongly exothermic, it is necessary that you use a BASELINE setting of 4 rather than the setting of 2 which was used for the Sucrose experiment.

If everything has gone well to this point, then you should be ready to begin studies on your own samples. Good luck!!

Section 4.11

Maximizing Baseline Stability

For demanding experiments where maximum baseline stability is required, it is necessary that the user be aware of small effects which arise from the *thermal history* of the room in which the instrument is located. It must be recognized that the ITC cell is surrounded by several inches of foam insulation which means that the cell itself is very slow in feeling any effects caused by changes in ambient temperature. For example, the on/off cycles of heating systems and air conditioners, which occur every 10-20 minutes, have almost no effect on baseline position (an exception would be if the air was blowing directly on the instrument). On the other hand, if the room thermostat is turned down several degrees in the evening and then turned back up at the start of the next working day then the baseline will drift very slowly and will not fully stabilize for many hours even though the room temperature remains constant during the entire working day. Although it will vary from instrument to instrument, the change in equilibrium position of the baseline is of the order of 0.03 μcal/sec for each degree change in ambient temperature.

The best way to avoid this problem is to be certain that the room is thermostatted at the same temperature for 24 hours a day when the instrument is being used, if this is possible.

It also helps, but does not completely eliminate, this problem if the circulating bath is used continuously (i.e., even when operating near room temperature and above) since this partially shields the cell from changes in room temperature. If most of your experiments are to be carried out at 25 C and above, then you could set the external bath temperature to 20 C and let it circulate continuously. As implied above and pointed out elsewhere, if the temperature of the circulating bath is ever changed, the instrument should be allowed to equilibrate overnight before beginning experiments at the new set temperature.

Section 4.12

Selecting The Proper Stirring Rate

The stirring rate on your instrument is user-selectable with the RPM dial located on the front of the Omega reaction cell, up to a maximum rate of 700 rpm. Typically selecting a stir rate greater than 500 rpm will introduce excessive noise into the baseline. In selecting the optimum rate, there is a trade-off between mixing efficiency and baseline noise. For almost all your studies, a stirring rates of ca. 400 rpm will give adequate mixing following injections and still provide very high baseline quality.

Section 5

Omega Cell Calibration

Section 5.1

Y Axis Calibration

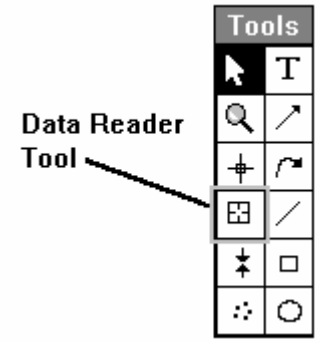
To check the Y axis calibration of the Omega you first do a calibration run from the Calibration Matrix window then analyze the data in Origin. Section 3.5 describes the Calibration Matrix window in detail, and this section assumes your familiarity with it.

All Omega cells have been calibrated at MicroCal, prior to shipment. You may check the calibration by either the Y axis deflection of a single pulse which had a duration period of 4 minutes or longer or by the area of several pulses. It is good practice to verify the calibration of your cell every 3 months by following the procedures discussed in this section. When setting up a calibration experiment, it is best to enter calibration **Heat Rate** on the order of 10 μcal/sec or larger in order to negate any effects from slight baseline drift.

Calibration by Y axis deflection:

To calibrate based on Y axis deflection, enter one or a series of pulses with a **Duration** of 4 minutes or longer, this will allow time for the baseline to re equilibrate itself and to establish a deflected baseline. The size of this baseline deflection is the determining factor for verifying calibration. Users will want to allow enough **Time Between Pulses** to return to the original baseline, this is typically 3 minutes longer than the **Duration**.

Once the Calibration data has been generated and the run completed, inspection of your data will verify calibration of the Omega cell. Users may find it sufficient to verify calibration from within the DSCITC.ORG document (document used to display all Omega data via DDE) using the data reader tool. To do this, simply click on the data reader tool from Origin's toolbox. If you do not see the toolbox in Origin, select the Options:Toolbox menu and it will appear in the upper right corner of the Origin document. The data reader tool is shown below.



After selecting the data reader, click on the dataset in the Origin plot window. Origin will pick the closest point to where you have clicked and display the X and Y

coordinates of that point in the status bar at the top of the window. You may now move through the dataset point by point using the left and right arrows on the keyboard. Move the data reader along the trace until you are at a point that well represents the position of the original baseline (before the pulse was turned on) and record the Y-axis value. Now move the data reader to a point that well represents the position of the deflected baseline after the pulse was turned on and record the Y-axis value at that point. Subtracting the first Y-axis value from the second will yield the magnitude of the baseline deflection.. The result should agree with the pulse size that was entered to within one percent. If the difference is greater than one percent then you may adjust the Y-axis calibration constant by multiplying the current constant by the ratio of the expected deflection to the actual deflection in the baseline, i.e.,

New Constant = Old Constant x (expected deflection/actual deflection)

The Y-axis Gain Constant is accessible from the data acquisition menu by selecting **Options:Omega Constants**.

Calibration by area:

To calibrate based on area, enter a series of pulses with a Duration of 20 seconds or longer and a size of 10 μcal/sec or greater. Because of the shared resources in windows the exact time of each pulse cannot be accurately controlled. Therefore, after the calibration run is completed you may get the exact time of each pulse from the Calibration Table in the ITC1 Calibration Matrix window (see section 3.7). You may load the data file into Origin. Origin will automatically integrate the area and this area can be viewed in the worksheet in the column named .dh.

Once you have the calibration data read into Origin, there are two things that you will need to keep in mind:

1) You need to plot the unnormalized area (*_dh dataset as opposed to the *_ndh dataset) in the Origin Delta H plot window when comparing results.

2) You will need to change the X-Axis from Concentration of ligand to Pulse number

.

By comparing the integrated areas to the expected areas we can confirm calibration of your ITC Omega cell. You can read the integrated area from the Delta H plot window (use the data reader tool), or from the Origin worksheet, **once you have plotted the unnormalized (*_dh dataset) area data versus pulse number**. To compute the expected area simply multiply the Pulse Size by the Actual Pulse Duration i.e.

(μcal/sec) X (sec) = μcal

If you find the areas differing by more than one percent you may adjust the Y-axis gain constant by calculating:

New Constant = Old Constant x (expected area/actual area)

The Y-axis Gain Constant is accessible from the data acquisition menu by selecting **Options:Omega Constants**, from the DSCITC main menu. Save the changes and try the calibration experiment again to assure yourself the the new constant is satisfactory.

Appendix A

Omega.sp Calibration File

The Omega.sp contains various calibration constants used by the DSCITC program. A typical file is reproduced below, the nomenclature is not part of the actual file but is listed for reference purposes only.

23.302	YCAL
128.35	RCAL
9987	RTENK
75.9	TEMPCAL
0	TEMPO
100	SYRING1
42.7	SYRING2
20.7	SYRING3
0	SYRING4
0	'
0	'
0	'
0	'
0	'
0	SYRING10
1.3684	CELLVOL
2	FILTERPERIOD
10	STEPSPERKEYSTROKE
25	YSPAN
1	CURRENTLY NOT USED
758	BASEADDRESS

YCAL

This constant (ca. 23) is used by the program to convert the voltages from the cell feedback circuit into μcal/sec used for the data storage.

RCAL

This constant is ohms resistance (ca. 128Ω) of the calibration heater. It is accurately measured at MicroCal and should not be changed by the user.

RTENK

This constant is the actual ohms resistance of a resistor in series with the calibration heater. It is accurately measured at MicroCal and should not be changed by the user.

TEMPCAL and TEMP0

The TEMPCAL (ca. 74) and TEMP0 (ca.0) constants determine the temperature reading of the cell, calculated from the voltage from the temperature measuring circuit (V2), using the linear equation.

$$°C = (TEMPCAL)(V2) + TEMP0$$

SYRINGE1

This is the dispensing volume (in µl/in) of the 250 µl syringe. The value is typically 101.8 µl/in..

SYRINGE2

This is the dispensing volume (in µl/in) of the 100 µl syringe. The value is typically 43.2 µl/in..

SYRINGE3

This is the dispensing volume (in µl/in) of the 50 µl syringe. The value is typically 20.7 µl/in..

SYRINGE4 - SYRINGE10

These syringe constants are currently not being used by MicroCal.

CELLVOL

This is the working cell volume. It is accurately determined by MicroCal and should not be changed by the user.

FILTERPERIOD

This is the default filter period used for data collection. This is the time in seconds during which the program will sample data before averaging to produce one data point. A 2 second filter period is fast enough for almost all reactions. The minimum time allowed by the program is one second.

STEPSPERKEYSTROKE

This constant is used in the **Position Injector** routine to determine the distance the injector will move when the user clicks on the **manual movement** buttons. The injector screw will travel 6400 steps/in.. Therefore a value of 10 for this constant will move the injector .0016 inches per click on the arrow button.

YSPAN

This is a default value for the full scale Y-axis values. The upper limit for the Y-axis is 2 µcal/sec higher than the value of the first data point. The program will then calculate the lower limit for the axis by subtracting whatever value is stored as YSPAN from the upper limit.

BASEADDRESS

This decimal number selects the base address of the DT2801 series board, which is set by jumpers on the DT2801 board. We ship all instruments with the address decimal number 748 (Hexadecimal 2EC) or 758 (Hexadecimal 2f6). If this number conflicts with another device in your system you may change the number. Please refer to your Data Translation Instruction manual (Hardware Reference : Reference 2: Jumper Configuration) for information on installing or removing jumpers on the board for a different address.

Appendix B

Changing the PC Board

INSTALLING/CHANGING A CHASSIS PC BOARD

Installing or changing the PC (printed circuit) board in the MC-2 chassis is not a difficult task. Items that will be needed for this task are a Philips-head screwdriver, some isopropyl alcohol, and Kim-wipes or other lint-free wipers for use with the alcohol. We recommend that you read through this procedure once before beginning.

The first step is to shut off the POWER (and RUN, if on) button, then remove the four mounting screws, located in the corners of the front panel face, that hold the chassis panel face to the cabinet. Next, the cables on the back of the chassis should be disconnected to allow the panel face and chassis to be pulled forward out of the front of the chassis. These cables include: any recorder cables (both power and signal), the 18-pin cable (this has a gold-colored connector with two thumbscrews, which should be turned simultaneously to prevent jamming), relay box cable (squeeze the buttons in the sides of the connector to release, then pull the cable free while holding in the buttons), solenoid cable (the silver ring on the connector needs to be unscrewed before this cable can be pulled free). Also, the black CELL cable and the gray JACKET cable (both have white, two-prong plugs) should be disconnected from the cell. For Omega users, there is also a fatter gray cable connecting to an external switch box; disconnect this also since it goes to the PC board inside the chassis. Lastly, the POWER cord should be removed from the power outlet.

Taking care that none of the still-attached cables (POWER, CELL, and JACKET) are "catching" on anything, pull the panel face forward out of the front of the cabinet (if things don't seem to be moving, check that all cables are free, and that there is nothing in front of the instrument preventing movement, then try pushing on the lower portion of the chassis (where the connectors are) at the back of the cabinet). The panel face needs to be pulled forward approximately 10-12 inches. A PC board similar in size to the one to be installed should be visible, standing up vertically on the left-hand side of the chassis (in front of a silver box with wires coming out the top). This PC board should have some or all of the following cables/wires attached to it: a green wire (terminating in a green plug)

near the top, a red wire terminating in a red plug, a thin gray cable (this is the JACKET cable that comes out the back of the chassis), and a fatter, gray cable (for an MC-2 chassis already being used with an Omega). Unplug any cables/wires that need unplugging and undo any wire ties holding these cables to the other chassis wiring (at this point all cables attached to the PC board should come free from the chassis when the PC board is removed).

To remove the PC board itself, grasp the board firmly at the top two corners and pull the board straight up. If the board seems "sticky", try rocking it gently from side to side(i.e.. pull up first on the left corner, then the right corner). DO NOT "rock" the board from front to back - this may cause damage to the connector. The board should slip free of its connector without too much difficulty. Once the board is out, pull all attached cables out along with it, taking care that none of the plugs are "catching" on any of the chassis wiring.

Next, take the new PC board and clean the gold "fingers" at the bottom using the Kim-wipes and alcohol (make sure no lint or other particles are clinging to the "fingers" once done). With the solder side facing front (the side with the components on it should be facing the silver box in the chassis), carefully insert the new PC board into the connector in the chassis. Make sure that the board goes down straight into the connector (not cock-eyed) and that it is inserted as far in as it will go. Connect the red and green plugs to the appropriate connectors, then thread the long gray cable(s) out through the back of the chassis and connect to the proper receptacle(s). If there are recloseable cable ties available, secure the new PC board's cables within the chassis in the same manner as the original PC board cables.

The chassis can now be pushed back into the cabinet (take care that no cables are getting "pinched" in the process at the cabinet rear), and the four panel face mounting screws can be screwed back in. All remaining cables (solenoid, recorder, 18-pin, cell plug, etc..) previously removed can now be re-attached. Once everything is back the way it was original