

ITC


Start up protocol

Please note: this is a **very basic** handout to give the user a “jump start” while setting the experiment. There is an extra copy of the complete manuals for ITC and ThermoVac that can be borrowed from the Biophysical Resource for limited time to produce additional copies; alternatively, the entire manual can be downloaded from:

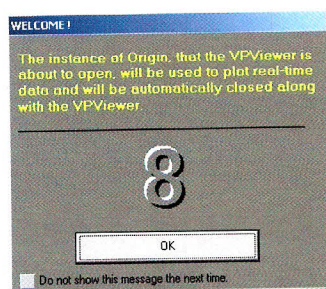
http://info.med.yale.edu/wmkeck/biophysics/VP_ITC_MANUAL_11_20_02a.pdf
(follow the link from the main ITC page).

Each user is strongly advised to read the full manual before operating the ITC instrument independently.

1. Turn on power on VP-ITC (back of the instrument).

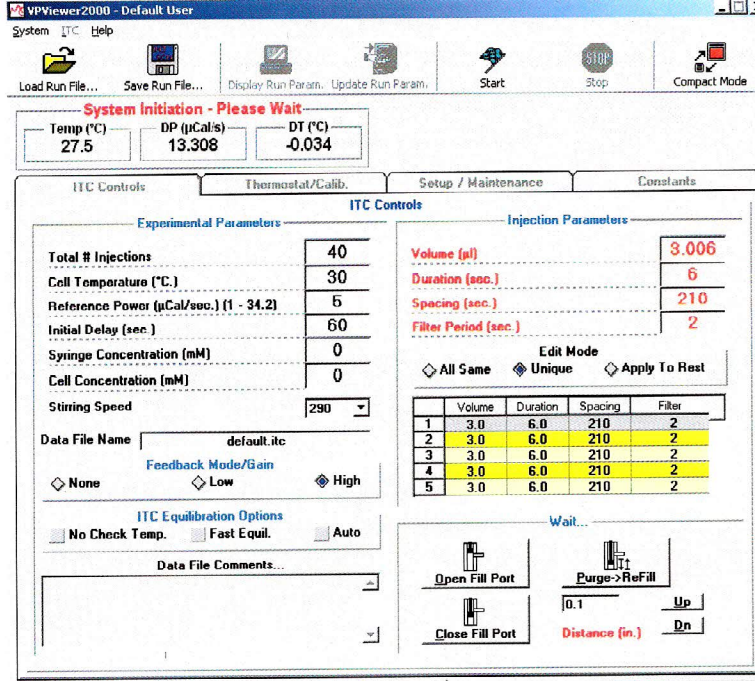
2. Launch the VPViewer 2000 ITC (double click on the  icon).

3. The following window appear:

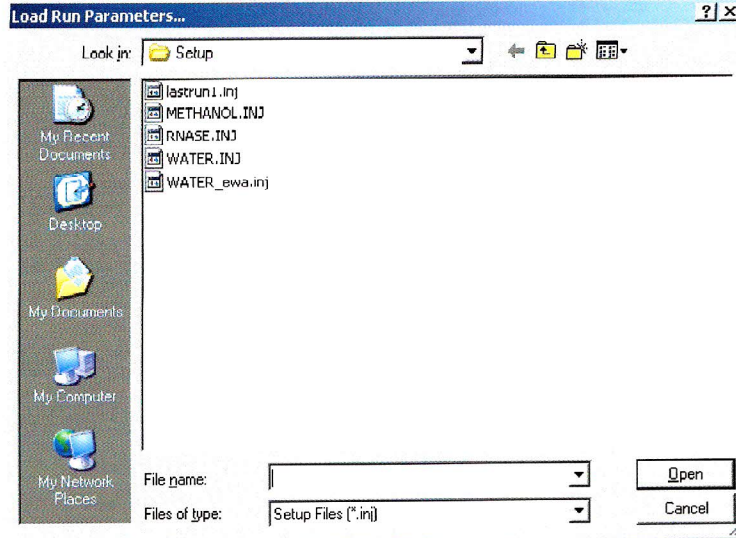


4. Click OK.

5. The following window appears:



6. This window illustrates most of the parameters that define how the ITC experiment will be performed. The parameters are loaded from the recently used file, so they might be quite different than the ones that a given user had used previously. *During your training session a “default” experiment will be created for you and saved in your directory, so you can load your settings by choosing “Load run setup file” (top left corner- first button).*
7. The following window appear:



8. Find your directory and load appropriate settings.

9. The following window appears:

VPViewer2000 - Default User

System ITC Help

Load Run File... Save Run File... Display Run Param. Update Run Param. Start Stop Compact Mode

Final Baseline Equilibration...

Temp (°C) 30.0 DP (µCal/s) 4.401 DT (°C) 0.000

ITC Controls Thermostat/Calib. Setup / Maintenance Constants

ITC Controls

Experimental Parameters

Total # Injections 40

Cell Temperature (°C.) 30

Reference Power (µCal/sec.) [1 - 34.2] 5

Initial Delay (sec.) 60

Syringe Concentration (mM) 2.74

Cell Concentration (mM) 0.05601

Stirring Speed 290

Data File Name Rnase112402414pm.itc

Feedback Mode/Gain None Low High

ITC Equilibration Options

No Check Temp. Fast Equil. Auto

Available Titrant 275.19 µl

Injection Parameters

Volume (µl) 3

Duration (sec.) 6

Spacing (sec.) 210

Filter Period (sec.) 2

Edit Mode All Same Unique Apply To Rest

	Volume	Duration	Spacing	Filter
1	3.0	6.0	210	2
2	3.0	6.0	210	2
3	3.0	6.0	210	2
4	3.0	6.0	210	2
5	3.0	6.0	210	2

Ready

Open Fill Port Close Fill Port

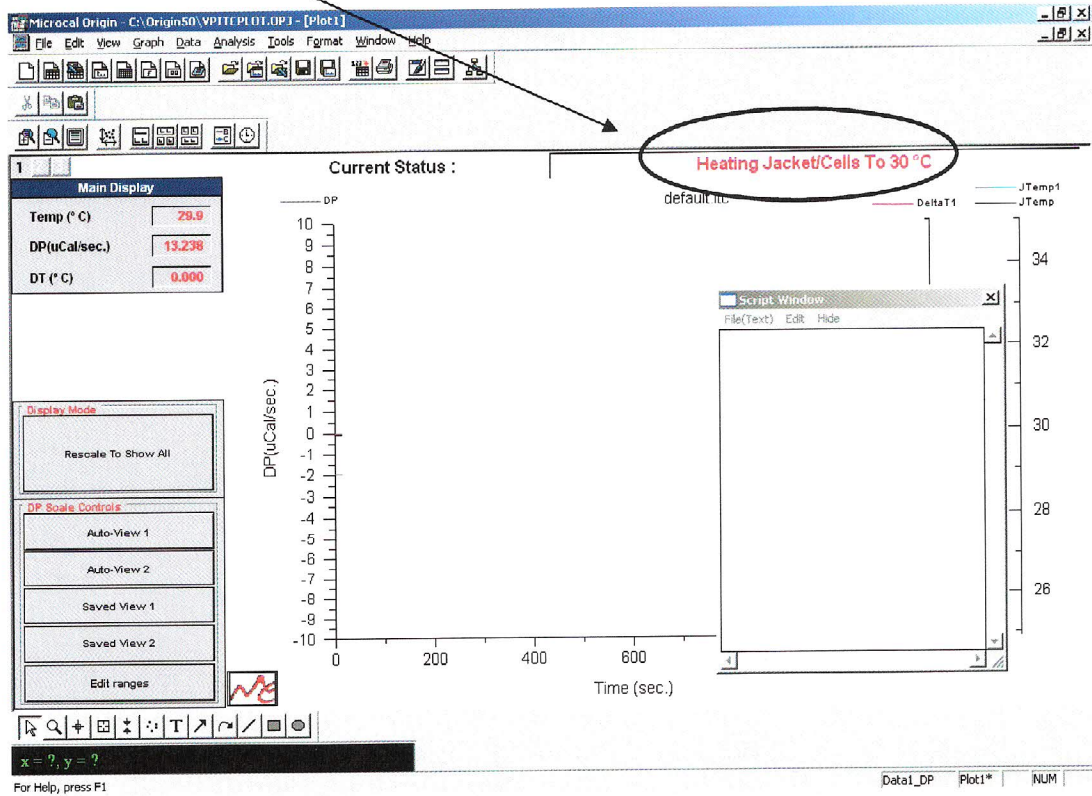
Purge->ReFill 0.1 Distance [in.] Up Dn

10. The experimental setting could be modified at this time. Please update the file name (“ITC Control” Tab, Data File Name) and the path for data storage to be sure that the data file will be saved in your directory (“Set Up/Maintenance” Tab, Data File Path). Check the concentrations to be sure they reflect the currently performed titration. **NOTE:** the experimental settings can also be modified during the execution of the titration; for example, a slow progress of binding might justify increasing the injection volume. To implement a newly modified settings when the titration is in progress choose: Update Run Param.
11. Most commonly, the **FeedBack Mode/Gain** will be set at “High”, **ITC Equilibrium Option** will be set at “Auto” and the **Stirring Speed** will be 290 (chosen from the pull down menu).
12. Update the Injection Parameters. **NOTE:** the injection could be all identical, or the injection volume, speed et.c. can be set uniquely for each injection.

CAUTION: double-check that the reference cell is filled with degassed water, the ITC cell is filled with macromolecule solution and that the Auto-Pipette is loaded, *disconnected from the filling syringe* and has been purged. .

13. If the sample cell and syringe are ready, you may start the run by clicking the “START” bottom.
14. The following window appears, which is a real time display of progress of ITC titration.

ITC cell status



15. The status of the ITC cell will change from “Heating/Cooling Jacket/Cell” to “PreRun Thermostat”, “PreStirring Equilibration”, “Final Baseline Equilibration” and eventually “Running” as illustrated below.



Main Display

Temp (°C) **30.0**

DP(uCal/sec.) **-35.889**

DT (°C) **0.2001**

Display Modes

Rescale to show all

DP Scale Controls

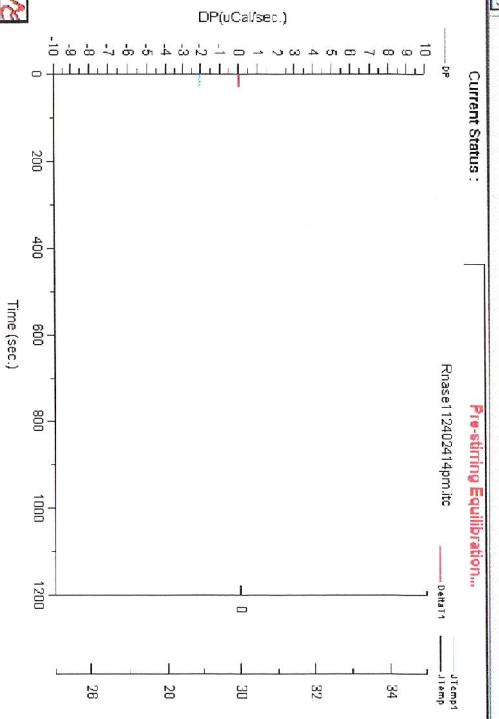
Auto-View 1

Auto-View 2

Save View 1

Save View 2

Edit ranges



X = 9, Y = 7

For help, press F1

Data_DP Peak* NUM



Main Display

Temp (°C) **30.0**

DP(uCal/sec.) **4.540**

DT (°C) **0.2000**

Display Modes

Rescale to show all

DP Scale Controls

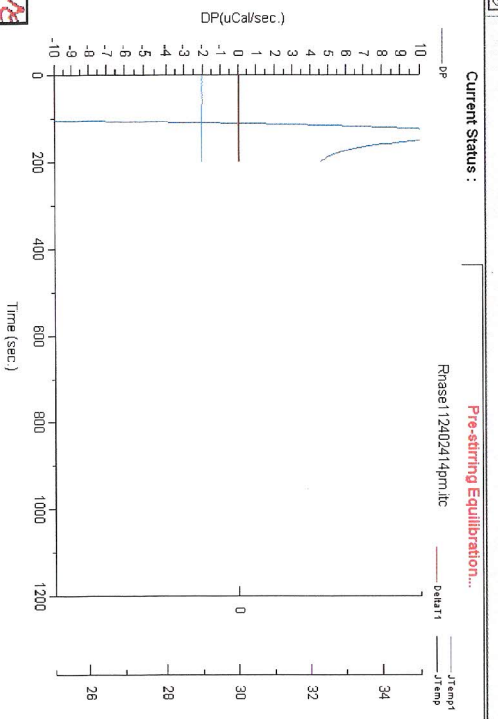
Auto-View 1

Auto-View 2

Save View 1

Save View 2

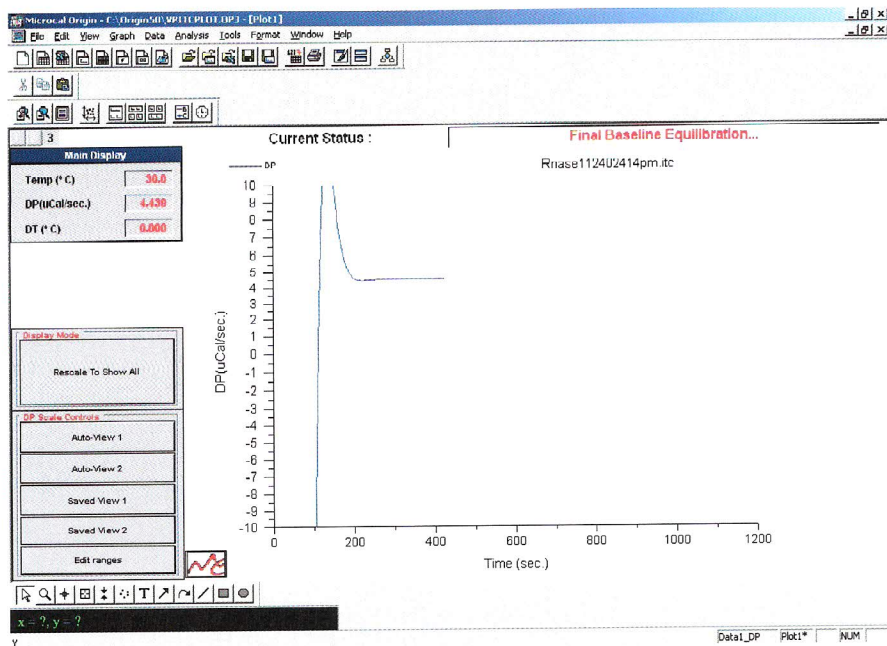
Edit ranges



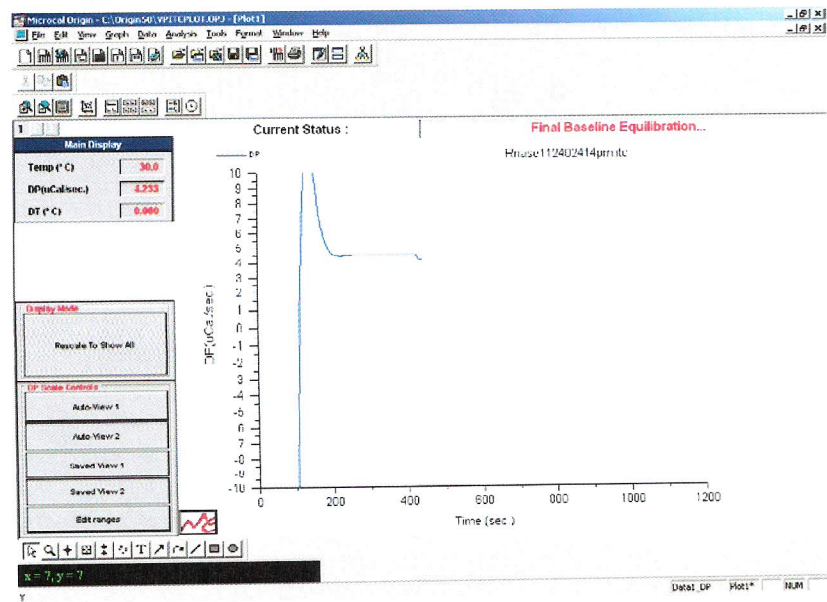
X = 9, Y = 7

For help, press F1

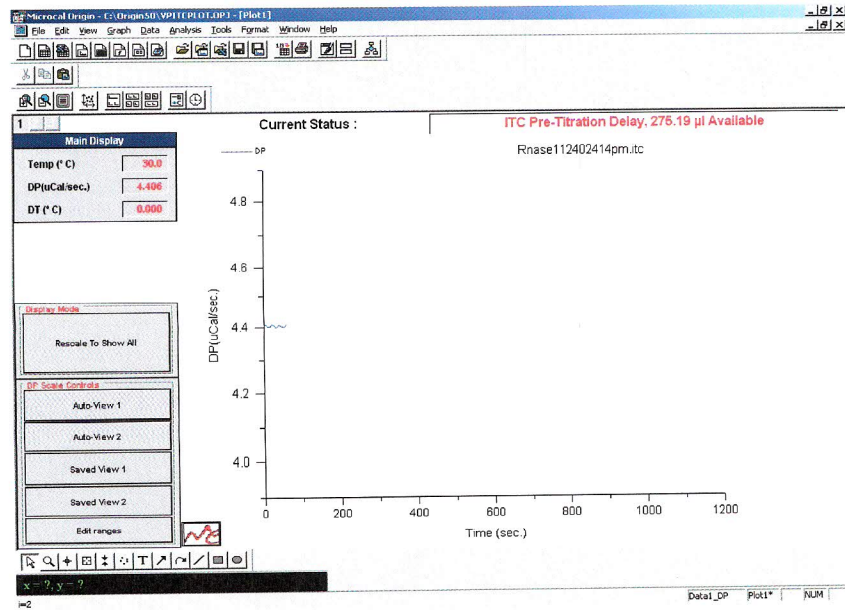
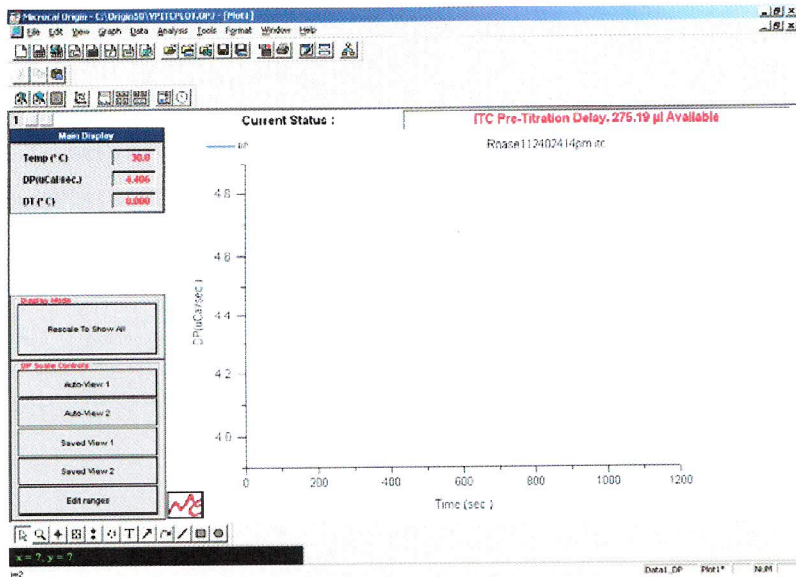
Data_DP Peak* NUM



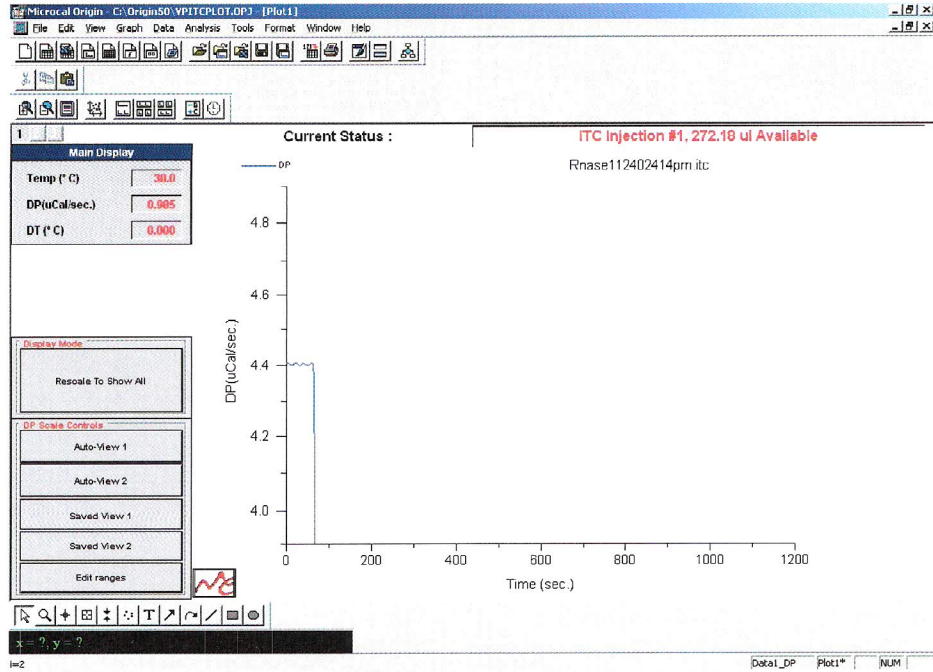
stirring begins



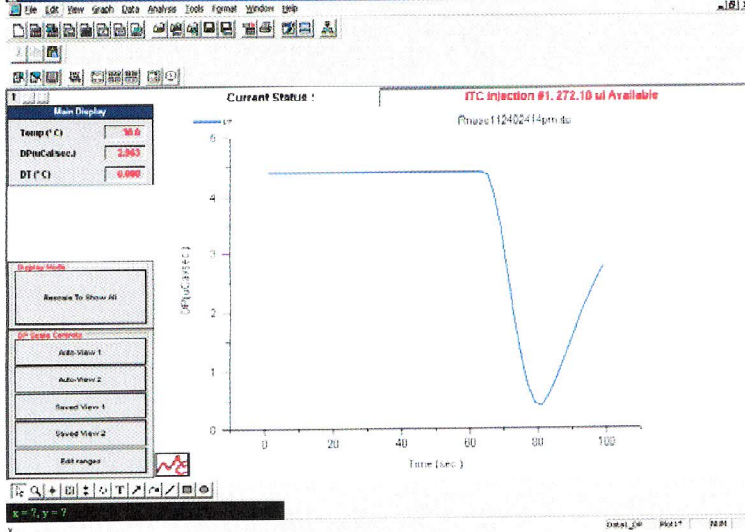
stirring continues



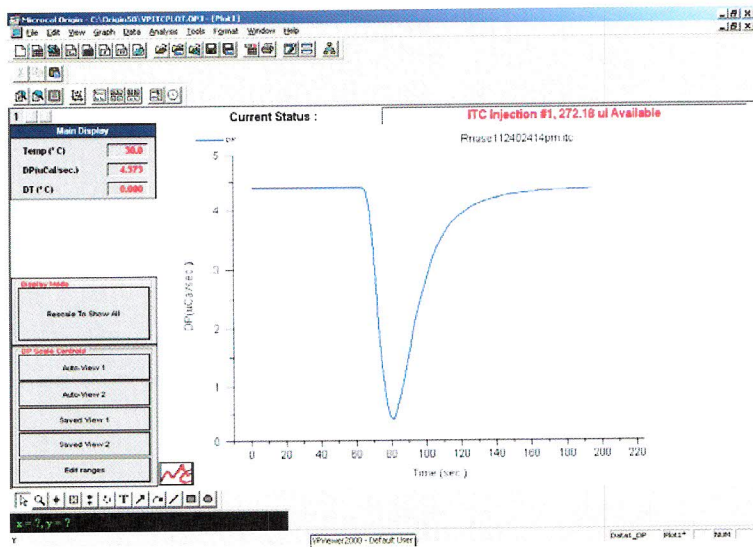
initial injection



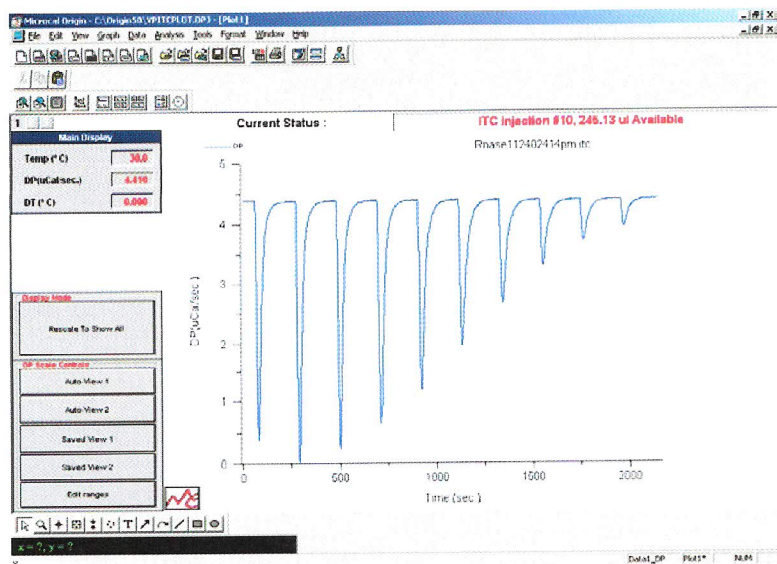
after “autoscaling” click ‘Rescale to Show All’



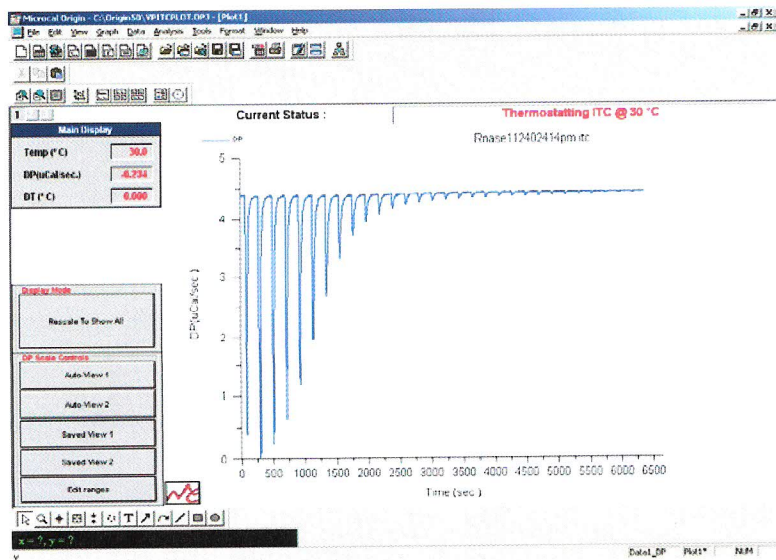
titration continues



Please note that the ITC cell status indicates which injection is being completed.



16. Once the titration is completed the ITC cell is thermostated and a full titration curve is displayed.



17. At this point the data are saved and can be processed using ORIGIN.