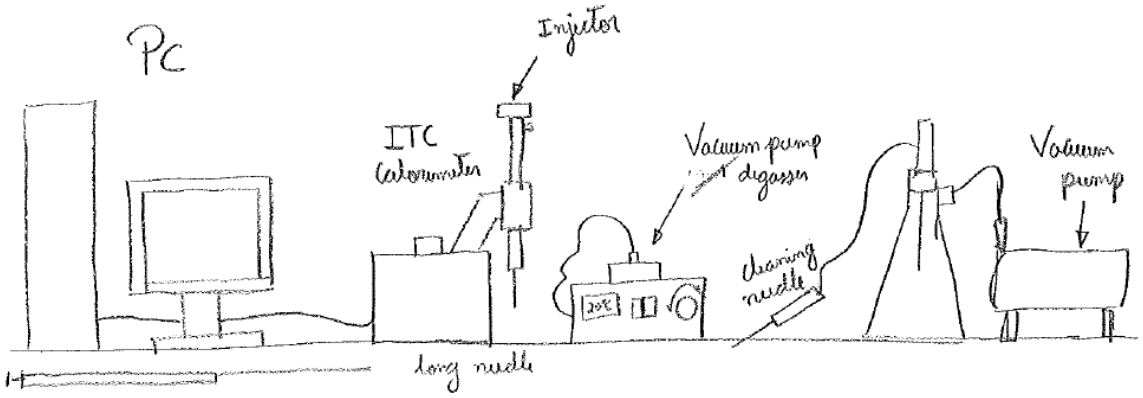
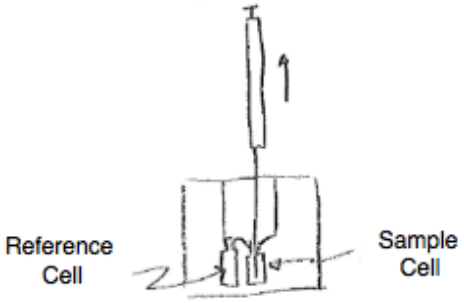
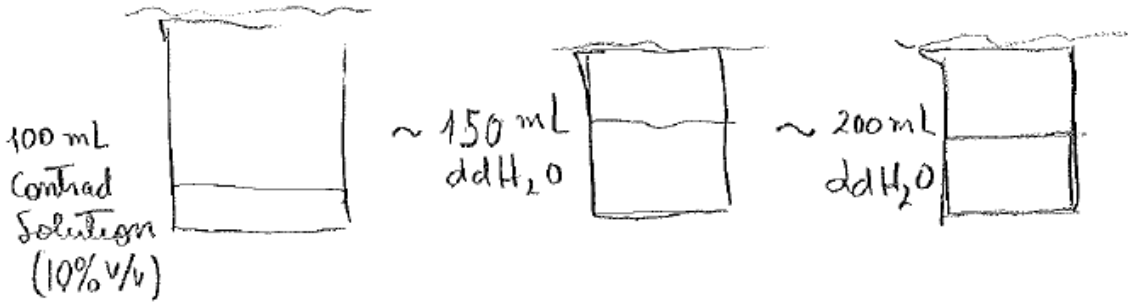
ITC Cleaning Instructions: should be done before and after every use



1. Turn on the computer controller and then turn on the instrument and open the VPViewer program.
2. This step is optional additional cleaning step. Discard any content in the sample cell and fill it with ~1.8 mL of 10% (v/v) filtered Contrad solution. Set the temperature of the sample cell to 40 °C for ~1 hr. After 1 hr, return the temperature of the sample cell to 25 °C or to the temperature of the experiment.

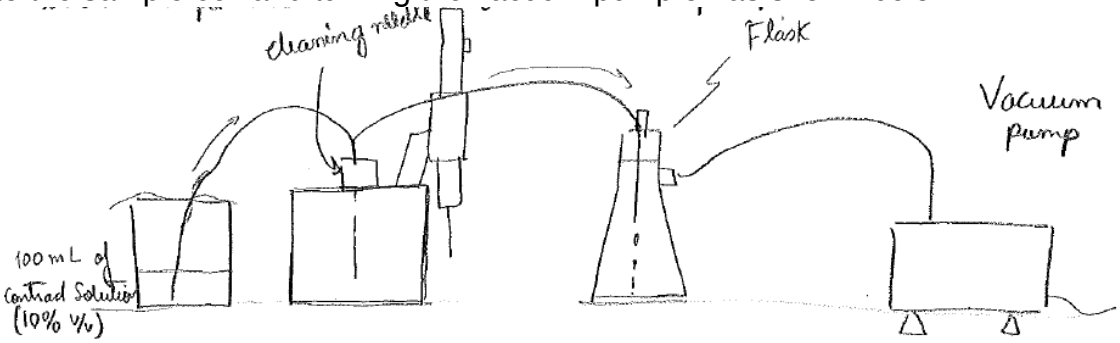


3. To clean the sample cell, pour the contrad solution and filtered ddH_20 into 3 different beakers as shown below. Make sure to cover the beakers to avoid introducing dust.

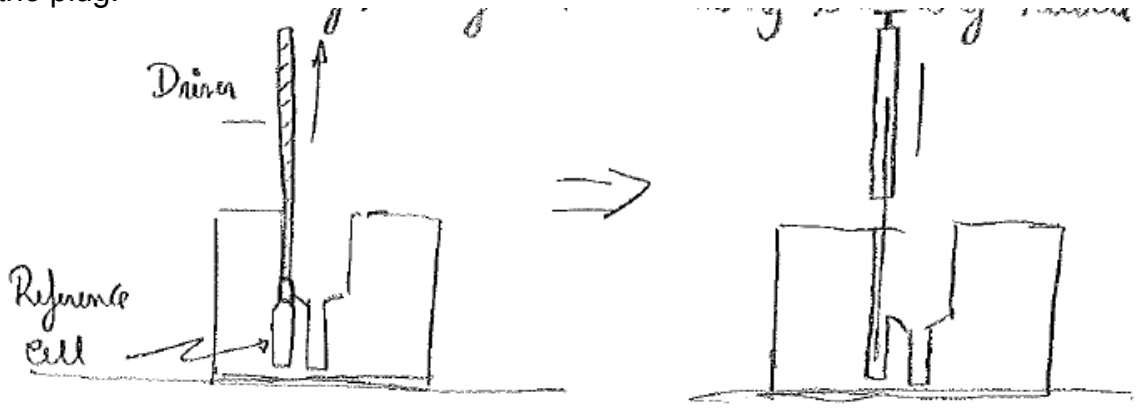


4. Discard the contents of the sample cell.

5. Wash the sample cell with Contrad solution by placing the cleaning needle into the sample cell and turning the vacuum pump on as shown below.



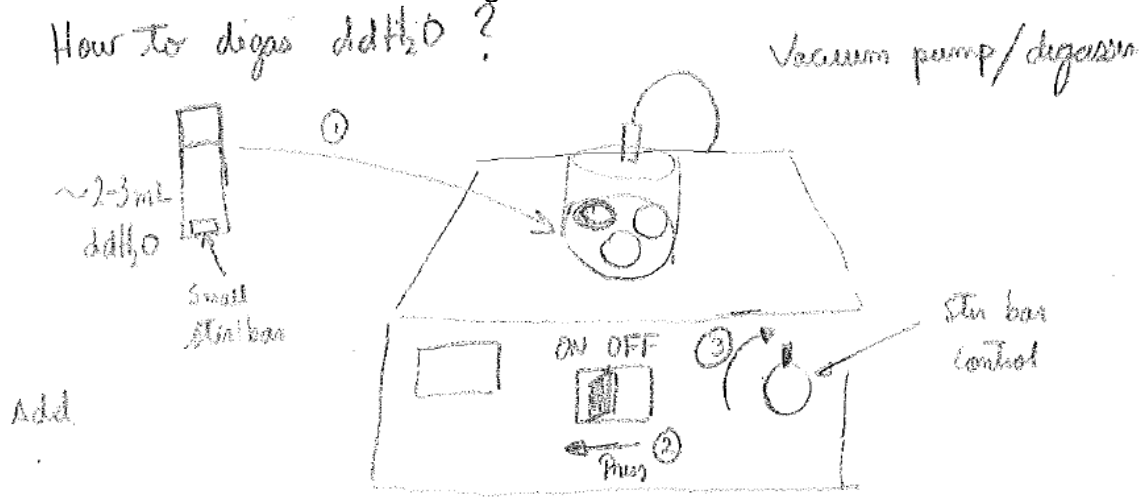
6. Repeat steps 3 and 4 with 150 mL ddH_2O and then with 200 mL ddH_2O. Leave water in the sample cell.
7. Clean the reference cell at least every two weeks. Uncap the reference cell with the reference plug driver and discard the contents of the reference cell. Fill the cell with 1.8 mL degased filtered ddH_2O and replace the plug.



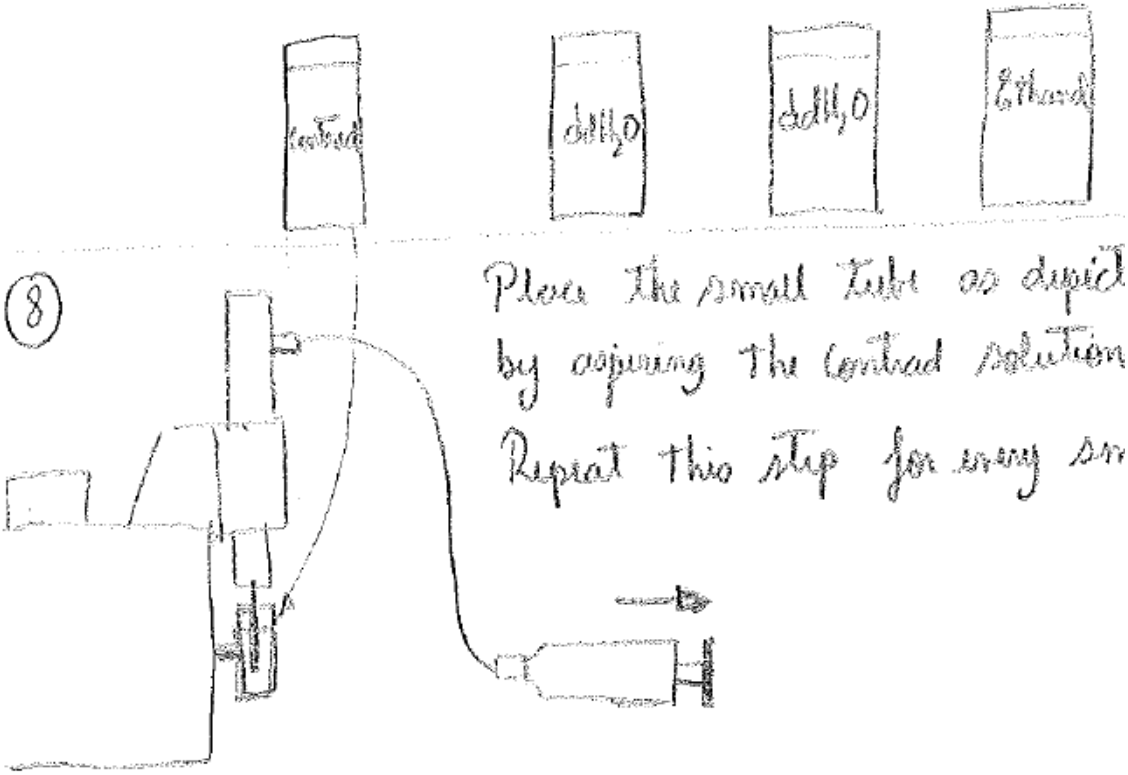
How to load the reference and sample cell:

1. Draw at least 1.8 mL of degased solution into the filling syringe, and remove all air from the syringe.
2. Insert the syringe into the cell and gently touch the bottom of the cell with the tip of the syringe needle. Lift the tip of the needle about 1 mm off the bottom of the cell.
3. Slowly inject the solution into the cell until it begins to spill out of the top of the cell. Finish the filling by abruptly injecting 0.25 mL of solution. If possible, repeat the abrupt injection of the solution to help dislodge any bubbles.
4. To drain off the solution overflow, gently place the tip of the needle on the tiny ledge where the cell stem meets the cell port and draw up the excess solution.

To degas ddH_2O:



8. To clean the injector, fill four small tubes with ~3 mL Contrad solution, ddH_2O, and 100 % ethanol. In the ITC controls window in VPViewer, click on the Open Fill Port button. Attach the tube of the plastic filling syringe to the filling port of the injection syringe.



9. Carefully move the tube of Contrad solution to the holder below the injection syringe and draw up the solution using the plastic filling syringe. Repeat with the tubes of water and 100 % ethanol.
10. Remove the plastic syringe and dry the injection syringe under vacuum for 5-10 minutes.

Instructions for loading sample into the cell and injection syringe

To load macromolecule into the sample cell:

1. Remove contents of sample cell.
2. Gently rinse cell with filtered buffer using filling syringe.
3. Load the macromolecule sample into the cell following the instructions above for how to use the filling syringe to load the sample cell.

To load titrant into the injection syringe:

1. Make sure the fill port is open. Attach the tube of the plastic filling syringe to its port.
2. Place the tube of titrant in the holder to submerge the injection syringe tip in the titrant.
3. Slowly withdraw the plunger of the injection tube just until the titrant solution exits the top filling port. Click Close Fill Port button on VPViewer.
4. Remove the hose of the plastic filling syringe. Click Purge -> Refill on the VPViewer. Once the syringe is filled, repeat the Purge -> Refill again.
5. Remove the pipette from its stand by picking it straight up. Hold it directly over the sample cell and insert slowly.