

Pressure Perturbation Calorimetry

Purpose: To determine the coefficient of thermal expansion as a function of temperature for an organic sulfonate in aqueous solution. The temperature variation is used to determine if the organic sulfonate is a structure maker or a structure breaker in aqueous solution.

Introduction

Molecular binding events control most of the processes in living cells. Binding interactions include enzyme substrate binding, allosteric control of enzyme activity, protein nucleic acid binding, and protein-protein binding. Protein-protein binding is important because most of the enzymes in the cell function as a part of a protein complex and are not active as individual molecules. Molecular binding is very specific. Biomolecules have evolved over time to interact only with specific substrates or other biomolecules. This specificity is achieved through careful control of molecular recognition. Molecular recognition is the result of specific intermolecular forces. These forces include, in order of strength, hydrogen-bonding, charge-charge interactions (salt bridges), dipole-dipole interactions, π - π interactions, and hydrophobic interactions. The strength of all these interactions also depends critically on interactions with the solvent. For example, hydrophobic interactions are completely solvent driven. It is the central role of the solvent that this lab will explore.

Pressure perturbation calorimetry, PPC, is a very new technique for exploring the solvent environment of a molecule. PPC can be used to follow protein folding, denaturation, and binding. In this lab, to make the solutions easier to handle and less costly, we will use examples from small-molecule guest-host chemistry. We will study the solvation of sulfonates that are used as guests for cyclodextrin. For example, you may have done the thermometric titration or ITC titration of 2-naphthalene sulfonate with β -cyclodextrin. You may also study calixarenes that are used as hosts in a similar fashion as cyclodextrins. The same principles and procedures apply equally as well for large biomolecules. The experiment will be faster with the smaller molecules.

The effect of solvation on molecular recognition can be striking. For example, the hydrogen bond that forms in proteins, the peptide hydrogen-bond, Figure 1a, is quite strong in the gas phase. The gas phase interaction energy is roughly 20-40 kJ/mol. However, in aqueous solution the peptide hydrogen-bond strength is much weaker, less than ~ 5 kJ/mol¹.

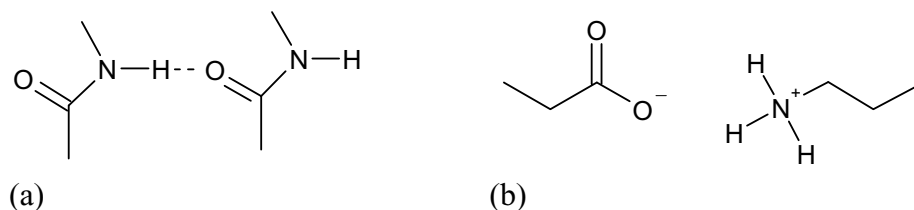


Figure 1. (a) Peptide hydrogen bond, (b) Salt bridge between glutamate and lysine.

Salt bridges in proteins are another example of strong solvation effects. Salt bridges form in proteins from the electrostatic interaction of acidic and basic amino acid side chains. The salt bridge between glutamate and lysine is a common structural element, Figure 1b. Salt bridges in

proteins can be either stabilizing or destabilizing depending on the desolvation Gibbs Free energy of the ions and the environment of the salt bridge in the folded protein. In short, it is impossible to study molecular recognition without a detailed knowledge of solvation.

Solvation

The oceanography community has long recognized two extremes for the solvation behavior of ions in solution, called kosmotropes and chaotropes, or structure makers and structure breakers, respectively. Recent studies using molecular dynamics simulations have also shed light on the solvation of non-polar molecules in solution, which is called hydrophobic hydration. The details of these three extremes are under great debate, but these categories are a good starting point for understanding the effects of the solvent on molecular recognition. Real molecules will be a compromise of these extremes.

Kosmotropes and chaotropes -- Structure Makers and Structure Breakers:

Water molecules can form four hydrogen-bonds. Bulk water has a tetrahedral network of hydrogen-bonds that are dynamically forming and breaking due to thermal motion. The presence of a solute perturbs this hydrogen-bond network. The solvation of an ion in solution can be divided into three concentric spheres. The boundaries between the regions are diffuse and very dynamic.

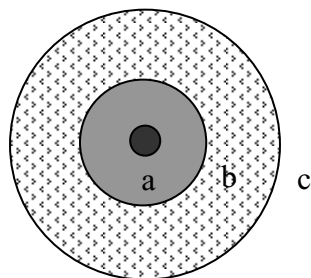


Figure 2. Solvation environment for an ion. (a) primary solvation sphere, (b) secondary solvation sphere, (c) bulk of the solution.

(a) The primary solvation sphere is a layer of 4-8 tightly associated waters. For alkali metals, the interactions in the primary solvation sphere are strong ion-dipole interactions. In transition metal ions the primary solvation sphere may be considered as directly bonded ligands for the metal. In the primary solvation sphere the tetrahedral hydrogen-bonding network typical of the bulk of the solvent is completely disrupted. (b) In the secondary solvation sphere, the hydrogen-bonding network may be more or less ordered than the bulk. For structure makers the secondary solvation sphere is more ordered than the bulk. For chaotropes, or structure breakers, the secondary solvation sphere is less ordered than the bulk. (c) The third region is the bulk of the solution. The difference between structure makers and structure breakers for small ions can be rationalized on the basis of charge to size ratio. Large charge to size ratio ions are structure makers (Chaplin). Good examples are large charge-small size ions that have strong, stabilizing interactions with

water that enhance the hydrogen bonding: SO_4^{2-} , HPO_4^{2-} , Mg^{2+} , Ca^{2+} , Li^+ , OH^- and HPO_4^{2-} . Small charge to size ratio ions, such as H_2PO_4^- , HSO_4^- , HCO_3^- , I^- , Cl^- , NO_3^- , NH_4^+ , Na^+ , K^+ , Cs^+ , and tetramethylammonium ions are or structure breakers. Structure breakers don't form strong interactions with water and destabilize the hydrogen bond network. The structure making or breaking ability of an ion is determined by the nature and size of the secondary solvation sphere.

Hydrophobic Hydration:

Careful molecular dynamics simulations and neutron diffraction studies have shown that non-polar molecules are structure makers. That is, the hydration sphere is more ordered than the bulk solvent. The desolvation of non-polar compounds is entropically favored, and the change in entropy is the major driving force in the hydrophobic interaction.

As mentioned above, ions that form weak interactions with water (weaker than water-water) are expected to be structure breakers. Ions that form strong interactions with water and hydrophobic molecules that form weak interactions with water are found experimentally to be structure makers. It is clear that non-ionic molecules need to be considered separately from small ion solutes. The difference is that non-ionic molecules don't have a tightly structured primary solvation sphere. The water molecules near the surface of the non-ionic solute have a large imbalance in forces—Van der Waals and dipole-dipole on the molecule side and hydrogen bonding on the bulk solution side. However, the solvated waters must at the same time have the same chemical potential as the bulk of the solvent, since they are in equilibrium with the bulk solvent. The net result is that hydrophobic solutes are structure makers.

The order or disorder in a region of solvent is a measure of the average number of hydrogen-bonds. Each water molecule can form four hydrogen-bonds. However, in bulk water the thermal motions are constantly breaking and then remaking these interactions so the average number of hydrogen-bonds is less than four. In the solvation sphere of non-polar molecules and other structure makers the increased order is reflected in a larger number of hydrogen bonds on average. The larger number of hydrogen bonds decreases the enthalpy of the solvated water, which is favorable, and decreases the entropy, which is unfavorable. The net result on the Gibbs Free Energy is zero, $\Delta_{\text{sol}}G = \Delta_{\text{sol}}H - T\Delta_{\text{sol}}S$, with a decrease in entropy.

Pressure Perturbation Calorimetry:

As more is learned about solvation, the distinction between structure makers and breakers becomes more confused. There are many arguments in the literature concerning the distinction. One difficulty is the experimental determination of the solvation properties. The early evidence concerning ionic solvation was gleaned from partial molal volume, viscosity, and ionic conductivity measurements. The different techniques can disagree and the behavior of a given ion can be a strong function of concentration and ionic strength. However, PPC has emerged as a more definitive technique for the characterization of ionic solvation. Figure 3 shows the behavior of the side chains for several amino acids from PPC.

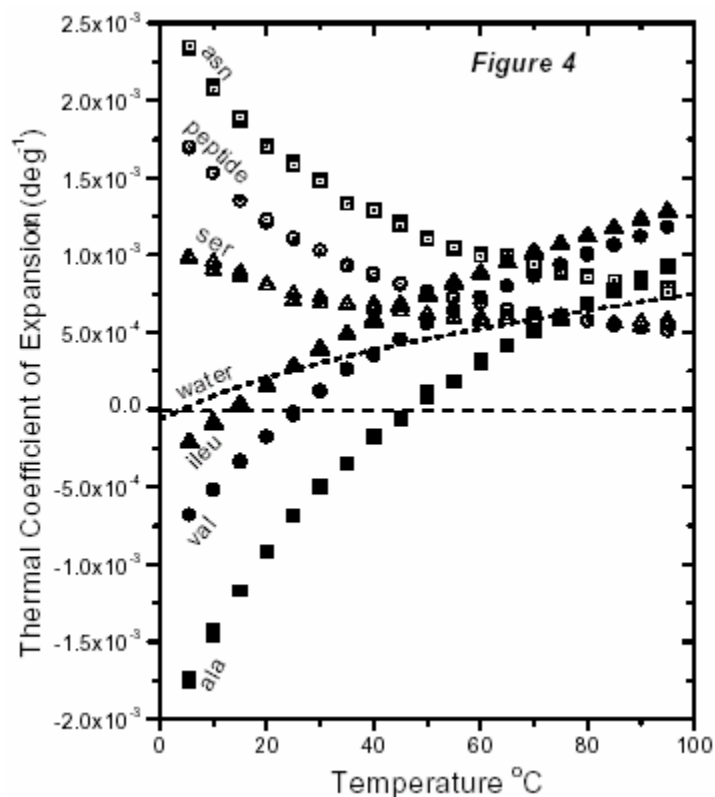


Figure 3. PPC scan for the side chains of several amino acids, a short peptide, and water.

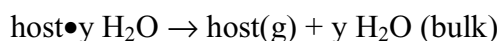
The structure makers are the hydrophobic side chains and the structure breakers are the ionic side chains. The structure makers show an increase of the coefficient of thermal expansion with temperature and the structure breakers show a decrease. The zwitter-ionic backbone for each amino acid is a structure-breaker.

Solvation and Molecular Recognition:

What effect does the structure making or breaking ability of a solute have on molecular recognition? At first sight the formation of a complex is expected to be considerably entropically unfavorable, since in binding the translational and rotational degrees of freedom of the guest are being lost. In other words, two particles are combining to give one particle, the complex. However, the solvation of the separated guest and host and the guest-host complex must also be considered. When a guest binds to a host, water molecules are displaced at the interface. The binding affinity depends on the interaction of guest with the host and the difference in the interactions of solvated water and water with the bulk solvent. The loss of solvated water from the first two solvation spheres is called desolvation. The change in Gibbs Free energy in this process is often unfavorable and is called the "desolvation" penalty. For the guest the Gibbs Free energy of desolvation corresponds to the process:



For the host the Gibbs Free energy of desolvation corresponds to the process:



The desolvation Gibbs Free Energy for the guest-host complex involves fewer waters of solvation than the separated guest and host combined:



The net process is then:



where $(x+y-z)$ molecules of water are removed from the binding interface. For polar solutes the enthalpy change for each of these processes is expected to be small, because although the desolvation of the solute involves breaking strong interactions to water, the released water forms strong hydrogen bonds as they enter the bulk of the solution. The desolvation and reincorporation of the water molecules into the bulk tend to cancel out. The entropy change of the desolvation of the interfacial water can then be a dominating factor. For structure makers the release of solvated water increases the entropy and is favorable, since the secondary solvation sphere is more ordered than the bulk of the solvent. This increase in entropy may overcome the intrinsic entropy penalties in binding. For structure breakers the release of solvated water decreases the entropy and is therefore unfavorable, since the secondary solvation sphere is less ordered than the bulk.

Applying these principles to the peptide hydrogen-bond, it is found that the strength of the hydrogen bond is entropy driven. The release of a few hydrogen-bonded waters from the separated molecules as the new hydrogen-bond forms is entropically favorable.

Amino acids have solvation shells with intermediate characteristics, intermediate between hydrophobic and hydrophilic and intermediate between structure-making and structure-breaking. The zwitterionic amino acid backbone is ionic and therefore hydrophilic. The side chains on amino acids, as discussed above have a wide variety of characteristics. The net result is difficult to predict, but the overall character has an important impact on molecular recognition. The sulfonates in this laboratory also have varying amounts of hydrophobic and hydrophilic behavior. The solvation behavior of these compounds is difficult to predict. The entropy effects will probably dominate binding.

Pressure Perturbation Calorimetry

PPC Theory

The entropy is defined as:

$$dS = \frac{dQ_{\text{rev}}}{T} \quad (1)$$

Differentiating with respect to P at constant temperature gives:

$$\left(\frac{\partial Q_{\text{rev}}}{\partial P}\right)_T = T \left(\frac{\partial S}{\partial P}\right)_T \quad (2)$$

Using the Maxwell relation:

$$\left(\frac{\partial S}{\partial P}\right)_T = - \left(\frac{\partial V}{\partial T}\right)_P \quad (3)$$

and substituting into Eq. 2 gives:

$$\left(\frac{\partial Q_{\text{rev}}}{\partial P}\right)_T = -T \left(\frac{\partial V}{\partial T}\right)_P = -TV\alpha \quad (4)$$

since the coefficient of thermal expansion, or isobaric thermal expansivity, is defined as:

$$\alpha = \frac{1}{V} \left(\frac{\partial V}{\partial T}\right)_P \quad (5)$$

Integration of Eq. 4 for changes in pressure gives:

$$\int dQ_{\text{rev}} = - \int TV\alpha dP \quad (6)$$

Assuming the volume and α are constant for the small pressure change in these experiments gives:

$$Q_{\text{rev}} = -TV\alpha \Delta P \quad (7)$$

A differential scanning calorimeter may be used to measure the heat flow after a change in pressure for a sample held at constant temperature in the calorimeter. The heat flow is directly related to the thermal expansion coefficient. Eq. 7 requires that the process be reversible. The reversibility of the process is verified by measuring the heat flow with both a positive pressure change and then while returning back to the original pressure. If the process is reversible, the heat effects should be equal in magnitude but opposite in sign. Then the temperature can be changed and the pressure changes repeated to find α at the new temperature.

Eq. 7 applies to a pure substance. To apply Eq. 7 to a solution, we must introduce the use of apparent specific properties. Eq. 7 applies to solutions if the volume V is the apparent specific volume for the solute and α is the apparent specific thermal expansion coefficient. In other words the quantities in Eq. 7 are just the effective values per mole of solute.

Apparent Molal and Apparent Specific Quantities^{2,3}

The experimental determination of partial molal volume and apparent molal volume of the solute, ϕV , involves the careful measurement of the densities of solutions of known concentrations. Consider the volume of a solution as n_2 moles of a solute are added to a fixed n_1 moles of solvent. The volume of the solution might change as shown in Figure 2. The volume due to the added solute (per mole) is called the apparent molal volume ϕV and the partial molal volume of the solute, V_2 , is the slope of the curve at the desired concentration.

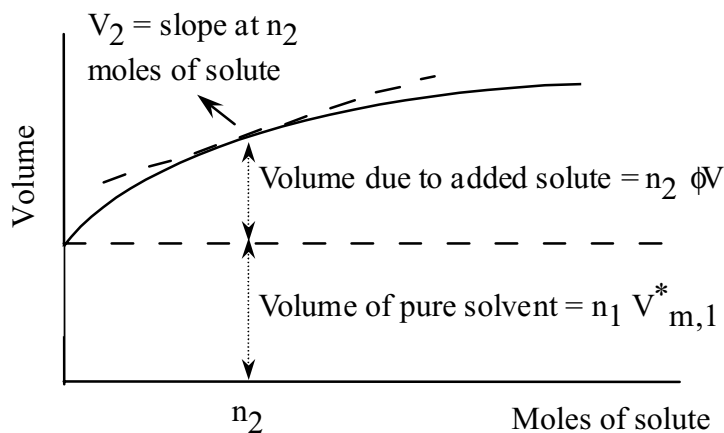


Figure 2. The total volume of a solution V depends on the volume of the pure solvent and the apparent molal volume of the solute ϕV .

Figure 2 shows that

$$\phi V = \frac{V_{\text{solution}} - V_{\text{solvent}}}{\text{moles of solute}} \quad 11$$

or

$$\phi V = \frac{V - n_1 V_{m,1}^*}{n_2} \quad 12$$

Thus, in Figure 2, the volume V of the solution at any particular added n_2 moles of solute is given by a rearrangement of Equation 12:

$$V = n_1 V_{m,1}^* + n_2 \phi V \quad 13$$

Equation 13 shows that the apparent molal volume ascribes all of the change in volume of the solution to the solute. The effective volume of the solvent, somewhat artificially, is assumed to be the pure molar volume of the solvent, $V_{m,1}^*$. The apparent molal volume includes the volume of the solute and the change in volume of the solvent caused by the interactions of the solute with the solvent. The partial molal volume, on the other hand, shares the change in volume between the effective volume of the solute and the effective volume of the solvent, both of which change with concentration. The apparent specific volume, \bar{V}_s , is the apparent volume per gram instead of per mole; the difference being just multiplication by the molar mass of the solute:

$$\phi V = \bar{V}_s \mathbf{M}_B \quad 14$$

The apparent specific volume can also be calculated directly from the density of the solution by

$$\bar{V}_s = \frac{V - (V d_{\text{soln}} - g_s)/d_1}{g_s} \quad 15$$

where d_1 is the density of pure solvent and g_s is the weight of the pure solute in the solution with volume V . For organic ionic compounds, such as amino acids, the apparent specific volume is often quite close to 0.7 mL/g.

In Eq. 13, then the pure molar volume of the solvent is replaced by the specific volume of the solvent, V_o :

$$V_{m,1}^* = V_o \mathbf{M}_A \quad 16$$

The specific volume is just the volume per gram. And the total volume of the solution is given as:

$$V_{\text{total}} = g_o V_o + g_s \bar{V}_s \quad 17$$

where g_o is the mass of the solvent. The α values determined by PPC are apparent specific thermal expansivities:

$$\alpha_s = \frac{1}{\bar{V}_s} \left(\frac{\partial \bar{V}_s}{\partial T} \right)_P \quad 18$$

This relationship can be visualized by changing the vertical axis in Figure 2 to the α for the solution as a whole and then experiment determines the difference in α due to the presence of the solute, on a per gram basis.

The α values determined by PPC are apparent specific values because of the differential nature of the measurement.⁴ The differential mode of operation is required to achieve sufficient sensitivity to measure the very small heat transfers. To show how the differential measurement is done, first take the derivative of Eq. 17 with respect to temperature at constant pressure:

$$\left(\frac{\partial V_{\text{total}}}{\partial T}\right)_P = g_o \left(\frac{\partial V_o}{\partial T}\right)_P + g_s \left(\frac{\partial \bar{V}_s}{\partial T}\right)_P \quad 19$$

The calorimeter determines:

$$\left(\frac{\partial Q_{\text{rev}}}{\partial P}\right)_T = -T \left(\frac{\partial V_{\text{total}}}{\partial T}\right)_P \quad 20$$

Substitution of Eq. 19 into Eq. 20 gives:

$$\left(\frac{\partial Q_{\text{rev}}}{\partial P}\right)_T = -T \left(g_o \left(\frac{\partial V_o}{\partial T}\right)_P + g_s \left(\frac{\partial \bar{V}_s}{\partial T}\right)_P \right) \quad 21$$

The relationship to the α values can be facilitated by the manipulation:

$$\left(\frac{\partial Q_{\text{rev}}}{\partial P}\right)_T = -T \left(g_o \frac{V_o}{V_o} \left(\frac{\partial V_o}{\partial T}\right)_P + g_s \frac{\bar{V}_s}{\bar{V}_s} \left(\frac{\partial \bar{V}_s}{\partial T}\right)_P \right) \quad 22$$

and substitution of the definition of the α values:

$$\left(\frac{\partial Q_{\text{rev}}}{\partial P}\right)_T = -T (g_o V_o \alpha_o + g_s \bar{V}_s \alpha_s) \quad 23$$

Integrating this equation over the change in pressure during the perturbation, assuming that the specific volumes don't change significantly over the pressure range gives:

$$Q_{\text{rev}}(\text{sample}) = -T (g_o V_o \alpha_o + g_s \bar{V}_s \alpha_s) \Delta P \quad 24$$

For the differential measurement the sample is placed in the sample cell and the pure solvent is placed in the reference cell. The volume of the pure solvent in the reference cell can be thought of as being in two parts. The number of grams of solvent in the sample cell for the solution is g_o and the contribution to the total α is $g_o V_o \alpha_o$. In the reference cell, pure solvent occupies the corresponding volume and the associated contribution is also $g_o V_o \alpha_o$. The rest of the sample cell is filled with solute, which occupies the volume $g_s \bar{V}_s$. In the reference cell the corresponding volume is occupied by pure solvent instead of solute and the corresponding contribution is $g_s \bar{V}_s \alpha_o$. The Q_{rev} for the pure solvent in the reference cell is then:

$$Q_{\text{rev}}(\text{ref}) = -T (g_o V_o \alpha_o + g_s \bar{V}_s \alpha_o) \Delta P \quad 25$$

Notice that α_o appears in both terms, since both apply to the solvent. The instrument then determines the difference in heat flow:

$$\Delta Q_{\text{rev}} = Q_{\text{rev}}(\text{sample}) - Q_{\text{rev}}(\text{ref}) = -T (g_s \bar{V}_s \alpha_s - g_s \bar{V}_s \alpha_o) \Delta P \quad 26$$

Solving Eq. 26 for α_s gives:

$$\alpha_s = \alpha_o - \frac{\Delta Q_{\text{rev}}}{T \Delta P g_s \bar{V}_s} \quad 27$$

explicitly giving the apparent specific isobaric thermal expansivity.

If pH control is important, buffer is substituted for pure solvent in the reference cell, while the sample solution contains an identical buffer as the solvent. In actual practice, the cell volumes of the sample and reference cell are not exactly equal. To account for instrumental artifacts of this type several background runs are taken, including water/water, water/buffer, buffer/buffer, and buffer/sample, where the reference cell is listed first and the sample cell is listed second. A spreadsheet-based algorithm is then used to solve for the α_s value using Eq. 27 after background correction.

Procedure

A reference PPC run with water in both cells is needed for the calculations. Degas some reagent grade water using the Microcal vacuum degasser (see Appendix). Fill the reference and sample cells with degassed water. Use the following settings in the Microcal PPC control software: set the prescan equilibrium as 5 minutes, the pulse duration as 90 seconds, the filter period as 1 second, the feedback as “medium”, and the iso-scan mode as “low noise.” Do the experiment at 10°C intervals from 20-60°C. Adjust the nitrogen regulator to about 70 psi. The calorimeter measures the exact pressure.

Make 10 mL of a 10 mg/mL solution of the sodium salt of 2-naphthalene sulfonic acid in water. remember to record the exact values. Degas the sample. Fill the sample cell with your sulfonate salt. Use the same settings as the water-water reference run.

During the PPC runs determine the density of the sodium 2-naphthalene sulfonate solution (see Appendix). Briefly degas your sample before determining the density to avoid bubble formation in the density meter. Calculate the apparent specific volume immediately since you need this value to analyze the data from the PPC runs.

Report

Report the density and pure molar volume of water that you used in your calculations. Report the density of your sulfonate solution and its uncertainty. Report the apparent specific volume, apparent molal volume, and associated uncertainties propagated from the uncertainty in the density. Assume the density meter is uncertain to ± 3 in the last digit. Enclose the plot of the isothermal compressibility versus temperature. Is your compound a structure maker or breaker? Comment on the high temperature value of alpha; that is, is the high temperature value positive or negative? Does the curve approach the high temperature value asymptotically (smoothly to a more or less constant value)? Does the curve pass through a maximum or minimum? Small inorganic salts like sodium chloride typically have a minimum in the plot of alpha versus temperature; does your sulfonate act like a small inorganic ion? Which part of the molecule, the hydrophylic part or the hydrophobic part dominates the solvation? Will this structure making or

structure breaking behavior aid or hinder interactions with an aqueous host like cyclodextrin or calix[6]arene sulfonate? What changes to the functional groups or the molecular skeleton would you make to produce a guest that would bind even more strongly to an aqueous host? Give a single, specific example.

Literature Cited:

1. Martin Chaplin, London South Bank University, <http://www.lsbu.ac.uk/water/kosmos.html>.
2. D. H. Williams, M. S. Searle, J. P. Mackay, U. Gerhard, R. A. Maplestone, "Toward an Estimation of Binding Constants in Aqueous Solution: Studies of Association of Vancomycin Group Antibiotics," *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 1172-1178.

Appendix: Operations Instructions

I. How To Use The ThermoVac- Degassing Samples

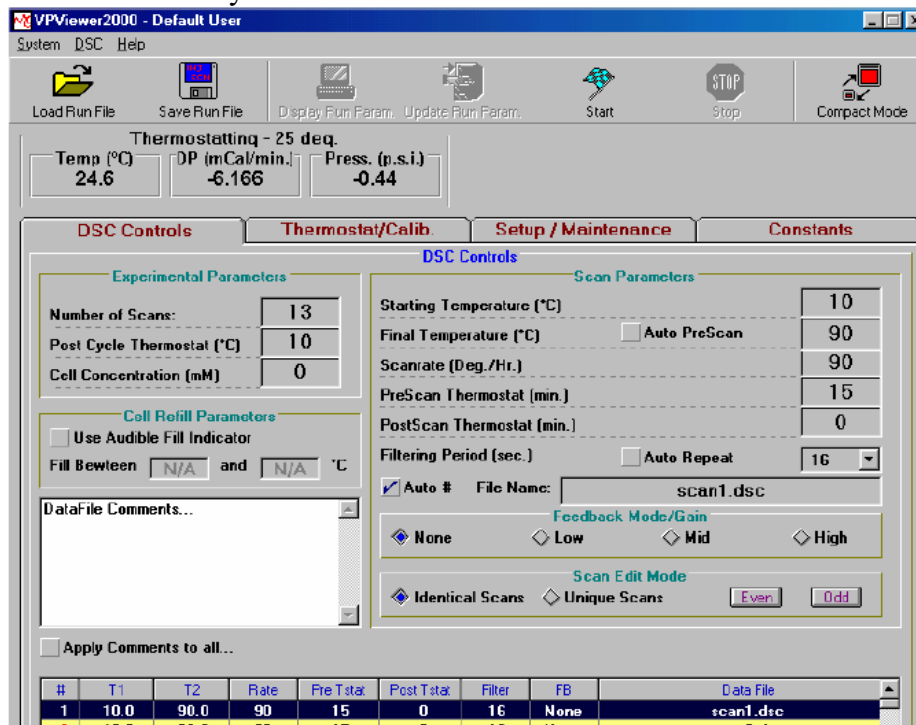
Before placing the samples into the cells or injection syringe, please do the following:

1. Turn on the Power Main Switch.
2. Set the desired temperature to about 5-10°C below your starting temperature.
3. Place the solution to be degassed into a plastic vial, add a small stir bar and place the vial into one of the open cylinders of the Tube Holder insert.
4. Turn the stirring on and adjust the speed. Open the needle valve on the vacuum cap.
5. Turn on the vacuum.
 - a) Push the switch to the right to activate the vacuum for a preset (ca. 8 minutes) duration.
 - b) Push the switch to the left if you wish to manually control the time for the vacuum.
6. Place the Vacuum cap on top of the sealing o-ring. Close the needle valve until the sound of the vacuum pump changes pitch to indicate the vacuum has sealed the Cap to the o-ring. Once the vacuum has sealed, the Vacuum Cap will be held firmly in place, till the vacuum pump shuts off. Carefully observe your sample to detect bubbles. Continue closing the needle valve until bubbles begin to form at the surface of your sample. Wait a minute or so then continue to close the needle valve until just closed. No force should be applied to the needle valve; the valve does not need to be tightened. If your sample begins to bubble again, slightly open the needle valve until bubbling ceases.
7. Wait for at least four minutes, then open the needle valve. Turn the vacuum pump off, if you are not using the pre-set automatic mode.
8. For the first few minutes after the vacuum pump is turned off, the vacuum in the sample degassing chamber may remain fairly tight making the removal of the Vacuum Cap difficult. During this period, the easiest way to release the vacuum is to remove the tubing from the rear Vacuum Port or to twist off (turn counter-clockwise) the in-line filter.

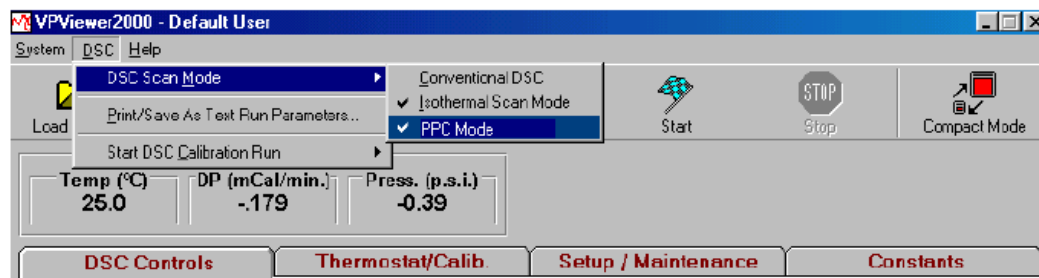
II. PPC Instructions

Activating The PPC Mode

To carry out PPC scans, VPViewer must first be put into the PPC Mode of operation. There are four window tabs available within VPViewer, each of which will display different content in the main window when selected. Select the left most window tab labeled DSC Controls and you will see a window very similar to that shown below:

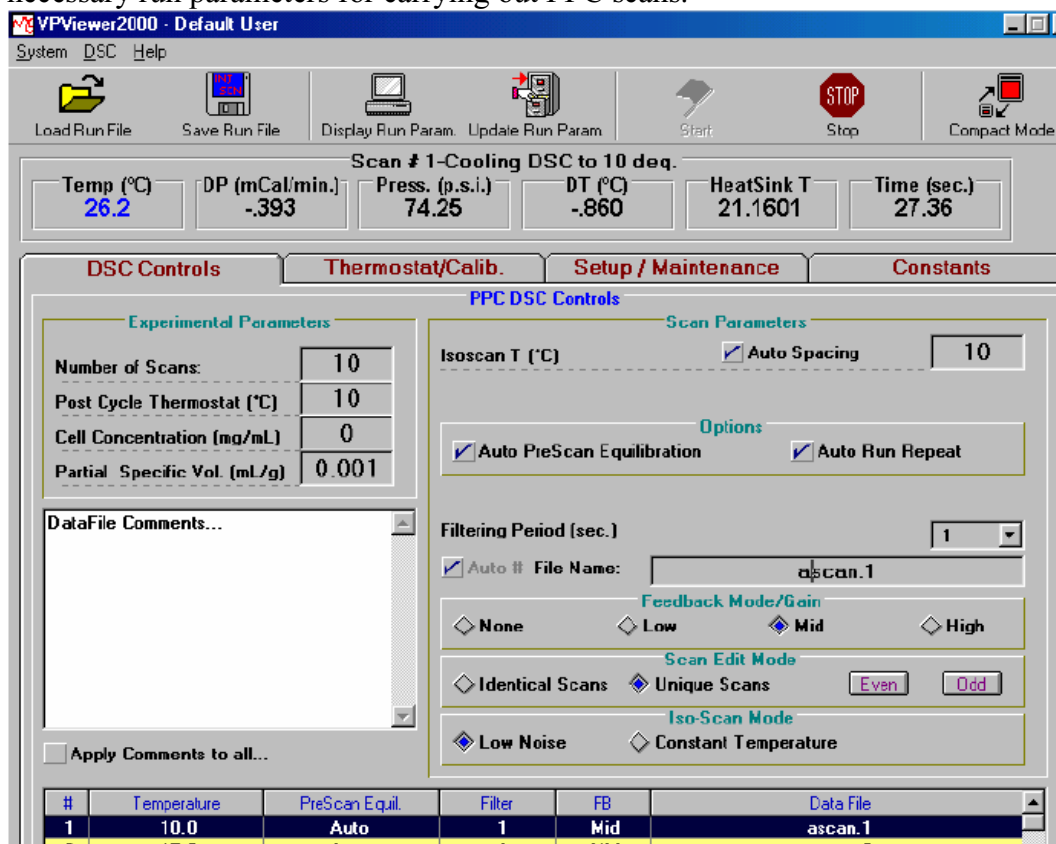


By default, the run parameters for Conventional DSC applications are displayed. There are additional modes of operation available through the drop down menus at the top of the main window. Refer to the picture below to assist in switching the DSC Controls Window into PPC Mode for use with the PPC Accessory.



By selecting the main menu DSC|DSC Scan Mode, you will see a list of the three available modes of operation with the VP-DSC. Begin by selecting the menu DSC|DSC Scan Mode|Isothermal Scan Mode and a check mark will appear next to that menu option. Additionally, the DSC Controls window will be altered to display only those run parameters that are relevant to Isothermal operation of the VP-DSC.

Once DSC Scan Mode is set to Isothermal, the Compressability Mode menu option will become available. Select the main menu DSC|DSC Scan Mode|PPC Mode and a check mark will appear next to that menu option, indicating that the software is now ready to be programmed for PPC scans to study the effects of pressure on biopolymers at constant temperature. Again, notice that the content of the DSC Controls Window has changed again, now displaying all necessary run parameters for carrying out PPC scans.



Looking in the upper left area of the DSC Controls Window, there are 4 run parameters grouped in the Experimental Parameters frame. These run parameters are set once for an entire series of PPC scans and are independent of the individual scan details. Each of these run parameters are described in detail below:

Number of Scans: Determines the number of PPC scans to be carried out. Each scan consists of a decompression and compression cycle of the cell solutions, as well as the measured heat effect, all at constant temperature. Frequently, each individual scan is carried out at a unique temperature, with incremental increasing of the temperature prior to each scan.

Post Cycle Thermostat (Deg. C): The desired temperature of the DSC cells after completion of the last programmed PPC scan. This parameter will typically be set back to the temperature of scan #1, to facilitate unloading and possible reloading of sample, all in preparation for the next set of PPC scans.

Cell Concentration (mg/mL): The concentration, in milligrams per milliliter, of the sample cell solution. VP-DSC users should realize that **the units for entering cell concentration for PPC applications are different than for the other modes of operation (Conventional DSC Scans and Isoscans use mM)**. The value entered here will be automatically carried

into and used in the PPC Data Analysis session. If the Cell Concentration is not entered here prior to starting the scans, or if the value entered is found to be incorrect, that entry can be changed during the PPC Data Analysis session within Origin™.

Partial Specific Volume (mL/g): The partial specific volume of the solute (e.g. protein) in the sample cell, in milliliters per gram. The value entered here will be automatically carried into and used in the PPC Data Analysis session. If the Cell Concentration is not entered here prior to starting the scans, or if the value entered is found to be incorrect, that entry can be changed during the PPC Data Analysis session within Origin™.

Located below the box for entering the Partial Specific Volume is the **Data File Comments** box. Comments entered in this box will be saved as part of the data file header and can be retrieved during the data analysis session, or read directly from the data file in any ASCII text editor. Data file comments can be applied to individual scans by first highlighting the desired scan(s), then entering the comments into the data file comments box. Comments can be applied to all scans by checking the option box labeled *Apply Comments To All...*, located just below the data file comments box.

Setting The Scan Parameters & Options

The right hand side of the DSC Controls Window contains the run parameters for each individual PPC scan, as well as some options which will control the entire series of scans and how they will be carried out. The PPC Scan list box at the bottom of the DSC Controls Window displays the run parameter settings for all individual PPC. Run parameters for individual (or multiple) PPC scans are changed by clicking on one or more (with Ctrl key pressed) scans in the list box, then changing the entry in a parameter box. The 'Scan Edit Mode' will determine how changes entered into the run parameter boxes affect the individual PPC scan parameters. A detailed description of the 'Scan Edit Mode' is found below:

Scan Edit Mode

Identical Scans: When this is the chosen scan edit mode, any changes to PPC Scan parameters will be applied to all PPC scans. This includes only the parameters IsoScan T, Filter Period and FB Mode.

Unique Scans: When this is the chosen scan edit mode, any changes to PPC Scan parameters will be applied only to PPC scans which are highlighted in the PPC Scan list box. This includes only the parameters IsoScan T, Filter Period and FB Mode. To highlight a PPC scan in the listbox, click on the row for that scan. Multiple selections can be achieved by pressing the Ctrl key while clicking on a scan's row.

PPC Scan Parameters

A detailed description of the PPC scan parameters can be found below:

IsoScan T (Deg. C): The desired temperature of each PPC scan, in degrees Celsius.

Auto Spacing (Option): When this option is active, indicated by a check mark in the adjacent box, the IsoScan Temperature for all scans will be automatically spaced. When making any changes to the desired IsoScan Temperature for scan #2 or higher, the same spacing in temperature will be applied to all higher numbered scans. As an example, begin by setting the desired IsoScan Temperature for PPC scan #1 to be 10 degrees. Next, select the 'Auto Spacing' option by clicking on the adjacent option box so that a check mark appears in it. Now, highlight scan #2 in the PPC Scan List Box by clicking on it once and note that the

'IsoScan T' parameter box will display the current IsoScan Temperature setting for scan #2. Enter 15 into the 'IsoScan T' box and notice that all PPC scans are now spaced by 5 degrees, the same spacing as between scans #1 (10 deg.) and scan #2 (15 deg.).

Filtering Period (sec.): The time, in seconds, between data points saved to disk.

Feedback Mode/Gain: The selection of the feedback mode will determine the method and magnitude of cell-cell compensation, in response to temperature differences between the reference and sample cell. Both the response time of the DP signal as well as the short term baseline noise are affected by the feedback mode being used. Of the four available feedback modes, only passive mode does not use heat to the cell(s) to actively compensate for temperature differences between them. The passive feedback mode provides for the smallest amount of baseline noise with the tradeoff being a relatively slow response time. The low, mid and high gain feedback modes are all active compensation modes, each providing a different magnitude of power compensation to the cell(s) for any given temperature difference. The higher the gain of the feedback mode (low, mid or high), the faster the DP (differential power) response time and the higher the short term noise. Another consideration is that the higher the feedback mode/gain, the larger the range of measurement for the DP signal. For most PPC applications it has been determined that the mid or highgain feedback modes are most appropriate.

File Name: The data file names for the individual PPC scans are entered here. All individual PPC scans within a single series of scans will have identical file names, but different file extensions. The file extension will be the PPC scan number, in the series of scans. This cannot be changed.

For example, a series of 10 PPC scans are programmed. The user then highlights any of the 10 scans in the PPC Scan list box and types a file name into the File Name parameter box. All 10 PPC scans will be assigned that same file name. Additionally, each file name will be assigned a unique extension, the scan number. The end result is 10 data files of the same name, with each file extension being unique. This file naming protocol is necessary to link each of the individual PPC data files of a series. Upon reading the PPC data files into Origin for data analysis, users will select the first file from a series of scans (will have a .1 extension) and all other data files for that series (.2, .3, .4...) will be found using the common file name and unique extension.

Options

Auto Prescan Equilibration: When this option is selected (checked) then the DP prescan equilibration criterion will be determined by VPViewer, automatically. The prescan equilibration occurs immediately after the PPC IsoScan Temperature is achieved. VPViewer will implement a slope check on the DP signal and will determine when it is sufficiently equilibrated, or flat. Once VPViewer determines that the DP signal is sufficiently flat it will proceed to the next step in the scan process. This option will provide users with the fastest possible throughput without compromising the quality of the data. Since some proteins may suffer from degradation over time, minimizing the time spent at each temperature becomes critical.

Prescan Equilibration (min.): If the Auto Prescan Equilibration option is not selected then there is one additional parameter box present in the DSC Control Window. This box is used to enter a precise equilibration period, in minutes, as an alternative to the automatic slope checking mentioned above.

Auto Run Repeat: When carrying out PPC scans it is sometimes desirable to repeat the same series of PPC scans, using the same samples already loaded in the cells. This can be used to quantify the reversibility of the volumetric parameters obtained from the first series of PPC scans. When the Auto Run Repeat option is selected (checked), VPViewer will automatically repeat the programmed series of PPC scans with no further user interaction. The data files for the repeat series of scans will be the same as used in the original series, but with an 'x' appended to all file names. The data file extensions will follow the same convention of using the scan number as the extension.

Isoscan Mode

This option allows users to select between one of two fundamental modes of operation for all programmed PPC scans, of a series. The two Isoscan Mode options are defined in the DSC Controls Window as being 'Low Noise' and 'Constant Temperature', as described below:

Low Noise: Provides for the most sensitive calorimetric measurements. Users can expect very slight temperature changes using this mode, less than 0.05 degrees over the entire PPC scan. The 'Low Noise' mode option is the recommended choice for all PPC scans.

Constant Temperature: Provides for the most stable cell/jacket temperature during an Isoscan. This mode uses active regulation of the jacket temperature during the scan. This active temperature regulation will result in a higher short term noise than would be received if the 'Low Noise' mode was selected. The 'Constant Temperature' mode is recommended when each Isoscan is expected to be very long in duration (> 1 hour).

In general, the 'Low Noise' Isoscan mode option will be the best choice and is recommended. This mode will provide for the greatest calorimetric sensitivity due to the smaller short term baseline noise as compared to data collected while using the 'Constant Temperature' mode. For Isoscans which require very long equilibration times, users may consider using the 'Constant Temperature' mode to achieve better temperature constancy throughout the duration of the IsoScan.

III. Density Procedure

Degass the sample for about a minute to avoid bubble formation in the density meter. Suspend the flask in the 25°C bath to reach constant temperature.

The densities of the solutions are measured with a Mettler density meter that has been calibrated by determining the density of water (see tables in the CRC or page 6 of the meter instructions) at the temperature of the measurement using the following instructions. First, rinse the meter with a few portions of reagent grade water using the following steps. Fill the meter by dipping the Teflon inlet tube into the liquid and pulling the liquid into the oscillator U-tube using the syringe. Carefully examine the U-tube to ensure that there are no bubbles. Select "DENS" as the measured value by pressing the "MEAS" key and then pressing the Δ or ∇ . Monitor the density reading for 30 sec. If the reading doesn't stabilize there is an air bubble in the measurement U-tube: empty and refill if necessary. Press the "ENTER" and "CALIB" key simultaneously. The "CALIB" symbol will be flashing and "AUTO" is displayed. The automatic calibration is at an end when "MEAS" is again displayed, which can take a minimum of one minute and a maximum of 15 minutes.

Check the calibration by emptying the measuring tube and refilling with reagent grade water. If the difference between the measured and tabulated values is greater than 0.001 g/cm^3 , check to see that the measuring U-tube is clean and repeat the measurement. If the difference is less than 0.001 g/cm^3 , repeat the determination two more times and use the average difference between the measured and tabulated values to correct all subsequent readings.

Exercise extreme care in filling, rinsing, cleaning, and handling the meter.

Measure the density of the your organic sulfonate solution, taking the same care used in calibrating the meter. Keep the sample flask immersed in the thermostat bath while filling the meter.