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Matec Applied Sciences

CHDF Software Users Guide

Version 3.5

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About This Manual

This software user's guide is intended to teach you the basics of how to use Matec Applied Sciences CHDF Version 3.2 Microsoft Windows™ based software package. This guide is organized into three chapters. The first chapter covers installation of the software, how to configure your CHDF instrument and autosampler, and introduces you to some important concepts that will help in the use of the software. The second chapter contains step-by-step instructions on how to run a sample manually, how to run an instrument with an autosampler, and how to perform a calibration. If your samples are prepared, and your instrument and software are up and running and configured, you may skip Chapter 1 and go directly to the tutorial. The third and subsequent chapters will consist of a software reference section. All menus and commands in the software will be defined and discussed in this chapter.

Whenever you are requested in the user's guide to use your mouse to click on something on the computer screen, *the instructions will be in italics like this* and each different mouse click *will/be/separated/by/slashes/like/this*. For example, the command to open a fractogram file using the file menu would appear as *File/Open*, which means you should click on the File menu, then click on the Open command. Any commands that you are required to enter from the keyboard will be given **in bold letters like this**. For example, an instruction to type in the name of a fractogram called matec.frm would be "type in the name of the data file **matec.frm**."
If you have any problems or questions about this software or this manual, please call Matec Applied Sciences at the phone number given at the bottom of each page in this manual.
Happy Fractionating!!

FREQUENTLY-ASKED QUESTIONS ABOUT CHDF OPERATION

1. *How frequently should the CHDF GR500 eluant be replaced?*

   The CHDF GR500 eluant degrades with time. It should be replaced every two to three days. Check the conductivity of the eluant in order to determine if it is suitable. The conductivity ranges for the different GR500 types are as follows (in microSiemens per centimeter): 1X-GR500: 7-10; 2X-GR500: 22-27; LA-GR500: 14-18; 1X-GR500 pH9: 13-18. The GR500 eluant absorbs Carbon Dioxide from the ambient which causes its pH to drop, and its conductivity to increase; this results in measured particle sizes smaller than expected. The 1X-GR500 pH9 eluant is especially sensitive to CO2 absorption. Bacterial growth also takes place as the GR500 ages.

2. *I obtain smaller than expected CHDF particle size results when I inject calibration standards.*

Most likely, your GR500 eluant is either degraded or contaminated. See Question 1 above.

3. *How is the GR500 eluant prepared?*

Add 900 ml of deionized water (conductivity should be less than 2 micro Siemens per centimeter) to 100 ml of GR500 concentrate. Eluant degassing by either sonication or vacuum filtration is recommended.

4. *How long does the GR500 concentrate last, and how should it be stored?*

The GR500 lasts about one month. It should be kept refrigerated.

5. *Does the Marker fluid need to be injected at an exact time for every run?*

No. In both manual, and auto-sampler operation, the software detects when the marker is injected. The injection time is recorded; the marker elution time is calculated as the marker exit time minus the injection time. A marker delay time of zero seconds is recommended for auto-sampler operation (this is set under the current method file).

6. *How should the marker fluid be prepared?*

The marker fluid consists of a solution of sodium benzoate in deionized water. Its concentration varies depending on the type of fractionation cartridge used. Typical concentrations are in the range 0.1% to 0.5%. This concentration should be adjusted in order to obtain a reasonable marker peak height (displayed on the CHDF software Raw Data graph) upon marker injection into the CHDF. Marker peaks heights of 10 to 40 mAU's (milli Absorbance Units) for the CHDF-2000 instrument, and 5,000 to 15,000 counts for the CHDF-1100 are acceptable.

7. *Why is the pump pressure fluctuating more than 200 psi during a run?*

Air bubbles are lodged in the pump pistons. Stop the pump; open the pump purge valve (located on the lower right- hand corner of the CHDF-2000; or on the CHDF-1100 pump front panel); insert a 5-ml plastic

syringe into the purge valve, and slowly draw fluid. Air bubbles in the pump and pump-inlet tubing should be removed. Tighten the purge valve and start the pump. If the air bubbles re-appear, the Whatman de-gassing filter on the pump-inlet tubing should be replaced. This filter lasts about one month. Cavitation, which results in air bubble generation, occurs if the Whatman filter needs replacement. For the CHDF-2000, this filter is optional. You may operate your CHDF-2000 without this filter if you so prefer.

8. *Noise and drift are present in the Raw Data baseline*.

This is probably due to a defective Whatman filter. Replace this filter and observe the baseline. This filter must be filled with eluant when in operation. Use the purge knob on the filter is order to draw any air from the filter. Another possibility is that the UV lamp may need replacement if it has been used for more than 1000 hours (see the troubleshooting section of the hardware manual).

9. *How should samples be prepared for CHDF analysis?*

Only aqueous samples can be analyzed on the current CHDF setup. Dry powders must be dispersed in GR500. Sonication should be applied in order to help suspend the particles in the liquid. Concentrated samples should be diluted in GR500 eluant. The solids contents are typically in the range 0.1% to 5% by volume. UV-absorbing samples can be analyzed at lower concentrations while non-UV absorbing samples (such as colloidal silica) must be run at higher concentrations. Sonication may be used in order to break-up particle aggregates. Reasonable sample concentrations should produce peak heights of 20 to 50 mAU's for the CHDF-2000, and 5,000 to 15,000 counts for the CHDF-1100. A syringe filter may be used on the sample syringe in order to remove aggregates from samples prior to injection. Syringe filters may only be used once per sample. Marker, as well as, Rinse filters may be reused until leaks are observed on the filters.

10. *No peaks are detected in the Raw Data graph upon sample, and marker injection*.

The fractionation cartridge is likely plugged. Refer to the troubleshooting section of the hardware manual for cartridge cleaning instructions.

11. *The pump pressure is higher than normal.*

The filter element in the cartridge in-line filter must be either cleaned or replaced. Refer to the troubleshooting section of the hardware manual. The CHDF-1100 is affected by ambient temperature. Low ambient temperatures result in high CHDF-1100 pressures.

12. *What Noise Level should be used under Configure System in the CHDF software?*

30-40 for the CHDF-2000; 400 for the CHDF-1100.

13. *Can any wavelength be used on the CHDF UV-detector?*

The CHDF UV-detector wavelength can be varied from 190 to 380 nm. However, the CHDF PC software only contains particle extinction cross section curves for 200 nm, and 220 nm. One of these values must be selected in the active Method file. Inaccurate results are obtained if the UV-detector, and the software wavelengths differ.

14. *What is the sharp peak in the Raw Data between the sample peak and the marker peak?*

This peak corresponds to UV-absorbing molecular species present in the sample. Molecular species include surfactants, electrolytes, acids, or bases. These molecules have a smaller size than the particles themselves, and therefore exit the capillary after the particles.

15. *A broad peak after the marker peak is displayed in the Raw Data*.

This peak is likely due to non-ionic surfactant. Non-ionic surfactants can continuously adsorb and desorb from the capillary wall as they travel down the fractionation capillary. This adsorption/desorption retards the non-ionic surfactant from exiting the capillary.

16. *Can the marker be blended with the sample so that only one injection is made per sample?*

Yes, in manual operation only. Simply add maker fluid to your samples, and make only the sample injection (do not inject the marker).

17. How long should the instrument be allowed to equilibrate (or warm up) at the beginning of the day before starting sample analysis?

Approximately 30 to 45 minutes.

18. Can the CHDF be turned off at the end of the day, or should it be left running overnight?

The CHDF can be operated in either mode. We prefer to turn it off at the end of the day.

19. How much sample, and marker, should be Loaded into the injection valve?

100 microliters for manual operation. It is recommended that at least 400 microliters be drawn into the syringe in order to minimize the chances of loading air bubbles into the injection valve. If using a syringe filter make sure the filter is filled with sample before loading sample into the injection valve. 80-90 microliters is recommended for auto-sampler operation. This is set under System/Configure Auto-Sampler.

20. Are samples loaded directly into the fractionation capillary?

No. They are loaded into a sample loop (a piece of stainless steel tubing) which is connected to a vent. As sample is pushed into the injection valve, the sample loop becomes filled with sample. Sample exits from the sample loop vent as more sample is loaded. The sample loop becomes connected to the pump, and the fractionation capillary upon switching to Inject.

21. Can the CHDF software be run under Windows9X, NT, and 2000?

Yes, the CHDF2000 software can be run on any Windows operating system software.

22. What solids concentration should be used for calibration standards?

Calibration standards should not be overly concentrated. Solids concentrations should be such that Raw Data peak heights are 10 to 30 mAU's for calibration standards and 20-60 for regular samples. Calibration standards larger than 300 nm should be sonicated prior to use in order to break up aggregates.

23. How frequently should the CHDF capillary be calibrated?

Calibration shifts are related mainly to two factors: (i) changes in GR500 eluant composition; and (ii) changes in ambient temperature (not important for the CHDF-2000). It is recommended that the conductivity of the GR500 eluant be monitored; replace the eluant if the conductivity is outside its normal range. Calibration standards can be run prior to regular sample analysis in order to confirm calibration accuracy. These "control" standards may be added to the calibration curve if re-calibration is deemed necessary.

24. How long does a CHDF cartridge (capillary) last?

Fractionation capillaries do not have an exact life span. Cartridges may last several years since no particle adsorption occurs inside the capillary. Capillary failures are frequently due to plugging right at the capillary inlet. Cutting-off ("snipping")a short piece at the capillary inlet frequently removes the plug. The capillary can also be back-flushed as described in the hardware manual, troubleshooting section.

25. How is the Weight Particle Size Distribution (PSD) calculated?

The particle weight percent is calculated as the Number percent multiplied by the particle diameter raised to the third power: $Wt_1\ \% = N_i\%(D_p)^3$. This assumes that the particle density is the same for all particles in the sample. The area percent is calculated as follows: $A_i\% = N_i\%(D_p)^2$

26. What particle Size Reproducibility should be expected from CHDF

About ± 5%.

27. What is the mean, 25%, median, 75%, and FWHH in the text PSD report?

The 25%, median, and 75% are the 25th, 50th, and 75th percentiles of the particle size distribution (PSD). They are calculated as the particle size evaluated at those values of the cumulative PSD. Physically, for the 25% for example, they indicate that 25% of the particles are below the reported particle size. The FWHH is the PSD Full Width at Half Height. It is the width of the differential PSD at 50% height.

28. What is the list of particle sizes at the bottom of the Text Report?

This list contains a description of all the particle size populations (also referred to as modes) automatically detected by the software in the sample. Each mode is listed by the start and end particle size. The percentages by Number, Area, and Weight are printed. The Area% is the average particle size of each mode (Weight-Weighted). The mode detection sensitivity can be changed in the current method under Optical Mode Detector. Lowering the value of Slope Sensitivity increases mode sensitivity.

29. How can the Particle Size Distribution Report be Customized or Changed?

Open your current CHDF method, and select "Report Setup". The text report, if used, should be placed at the bottom of the report.

30. *What causes noticeable "tailing" in a fractogram?*

    The inlet to the fractionation capillary is likely dirty. Snip the fractionation (top) capillary (see hardware manual section 6.6).

31. *How are odd-shape particles, e.g., discs, rods analyzed?*

    Particles spin in the capillary tube during CHDF fractionation. As a result, particles approach the capillary wall at different orientations. Some positions result in an exclusion layer equal to the longest particle dimension while other positions do the shortest or intermediate dimensions. The resulting elution time is equivalent to an average of all the particle dimensions. Research done at Lehigh University showed that particle doublets generated from partial latex coagulation produce a CHDF particle size equal to one particle diameter multiplied by the square root of two, or 1.41*Dp (instead of 1.5*Dp). Triplets appear to produce one diameter times the square root of 3.

32. *Does the CHDF software extrapolate beyond the two calibration standards at the limits of the calibration curve?*
    The CHDF reports particle size populations in the range specified in the CHDF2000 software under system/Configure-System. On the small particle size end, the CHDF interpolates below the smallest standard and the data point Rf=1, Dp=0, where Rf is the separation factor for a near-zero particle size (Dp) species such as the marker fluid. In fact, if you run the marker as a sample, you obtain Rf=1. Rf is calculated as the particle elution time divided by the marker elution time. Rf increases with increasing particle size as shown in the calibration curve. Elution time is peak exit time minus injection time. For larger particles, the software does extrapolate using a curve-fitting expression of the calibration-standard data points (up to the upper limit specified under System/Configure-System. This curve fit can be a sigmoidal or polynomial fit. Both options have sigmoidal shapes.

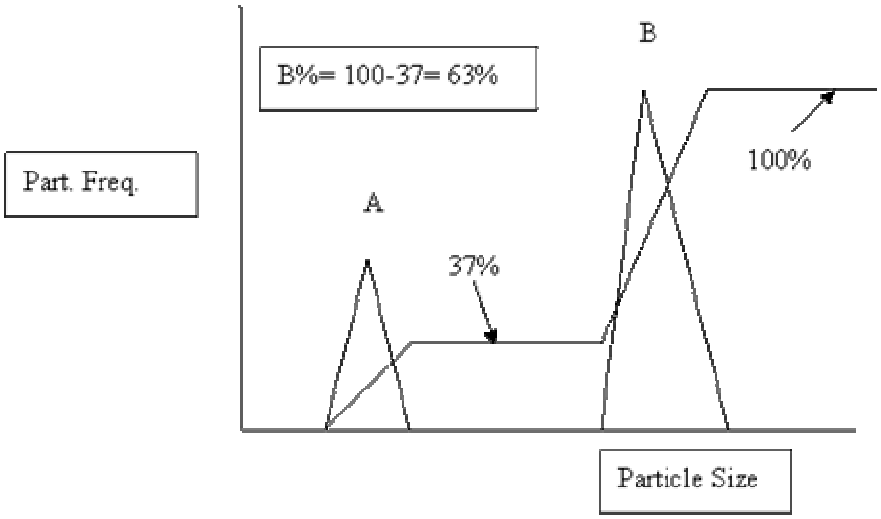
33. ***What is Particle Frequency in the PSD graphs?***
    "Particle Frequency" and "Volume Percentage" are different. The CHDF produces relative PSD's.

    An absolute PSD shows the actual particle volume percentage. However, absolute PSD's are sometimes difficult to compare between different samples (overlay). The reason is that samples with different percent solids will show different PSD-peak heights.

    Relative PSD's as currently shown by the CHDF are normalized so that the tallest peak or peaks have a height of 100 "frequency units". These are not percentage values because there can be two or more peaks with the same 100 value. Also, next to the 100 point, there are values of 98, 92, 86, and so on. If you add all these together, you would obtain much more than 100% for the sample.

    The particle frequency can be used to compare two populations. For example, a 37% peak has half the percentage of a 74% peak.

    If you need to know the percentage of a population, you should use the cumulative distribution (CD). You can subtract the CD values at both ends of each peak. This gives you the percentage of this peak. See example graph below.



34. ***How can the percentage of a particle-size population be calculated?***

See “Particle Frequency” question above.

Chapter 1

Installation and System Configuration

1.1 *Software and Hardware Requirements*

To run the CHDF Version 3.20 software you need an IBM or compatible PC equipped as follows:

1. If you have a CHDF 2000, Windows™ 95/98/2000/XP/NT is recommended, but Windows 3.1 will also work. If you have a CHDF 1100 you must use Windows 3.1, Windows 95 will not work with the CHDF1100 instrument.
2. A Pentium I or higher microprocessor running at a speed of 300 MHz or greater.
3. 128 megabytes or more of random access memory (RAM).
4. 240 megabyte or larger hard disk drive.

If your computer does not meet these specifications do not install the software. Upgrade your computer, or find a computer that meets or exceeds the specifications given above.

1.2 *Software Installation*

The installation instructions in this section are for newly installed CHDF 2000 instruments. If you have a CHDF-1100 model instrument, and DOS (Asyst) software version, do not follow these instructions. Go to Appendix 1 of this manual for installation instructions. If you are already using CHDF Windows software (CHDF1100, or 2000), follow the instructions below.

Note: If you have a DOS version of CHDF Software, refer to Appendix 1 for installation instructions.

If you are a CHDF-2000 user (or are currently using CHDF1100 Windows software), to install CHDF software V.3.20, do the following.

1. Place the install disk in your floppy disk drive or CD ROM (the floppy disk and CD ROM contain the same files). Using the Windows File Manager™, copy the software's executable file, NEWCHDF.EXE (or CHDF1100.EXE, or mCHDF110.EXE for older 1100 systems) to any directory on your hard disk. Also, copy all other files and *.DLLs from the floppy disk to this directory. It is recommended that you give the software its own directory, such as "C:\CHDF2000 (or CHDF1100)". You can create a directory from the Windows File Manager.

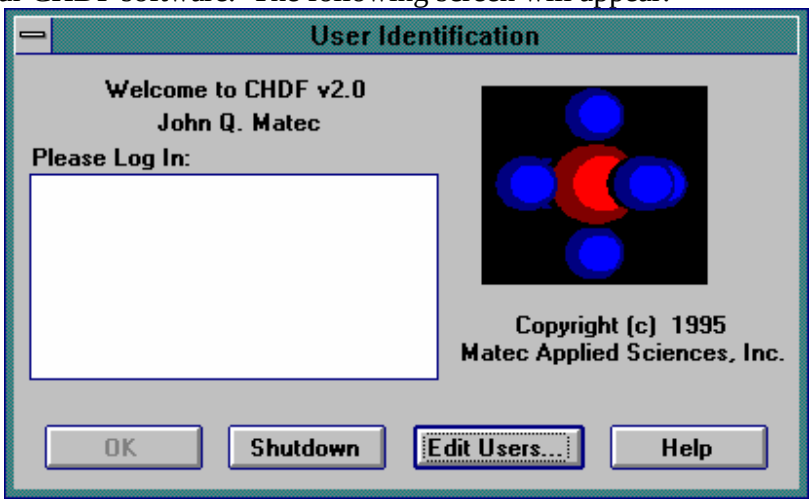
2. Create an icon or shortcut to load NEWCHDF.EXE. Follow the Windows 95/98/NT to create a shortcut on the desktop and skip to step 6.

3. Double click on your newly created icon to make sure it loads your CHDF Software V3.20 properly.

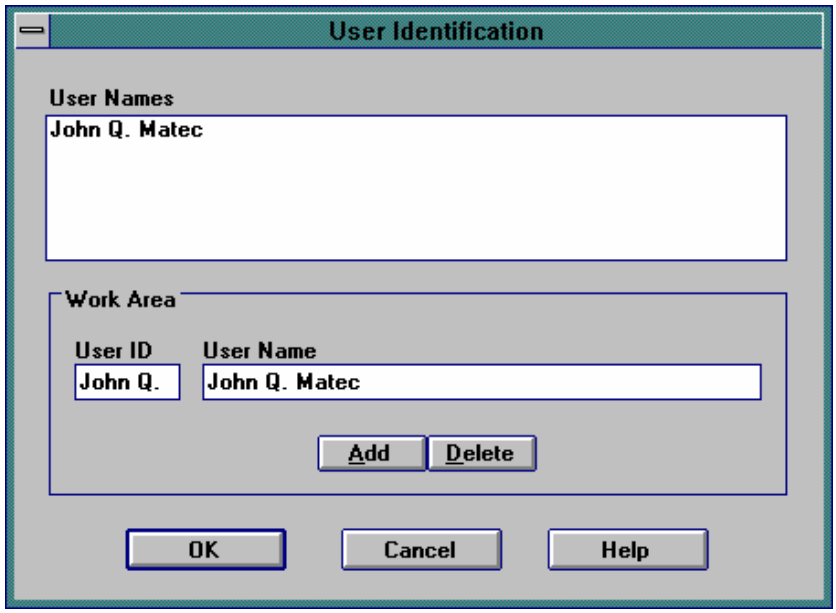
1.3 *Configuring Your System*

Configuring your system provides important information to the software that it will need to run your instrument properly. You must configure your CHDF before running any samples, so it should be the first thing you do after installing your CHDF V.3.20 software. Assuming your instrument is installed and running properly, and your computer is running Windows, do the following:

1. Start your CHDF software. The following screen will appear:

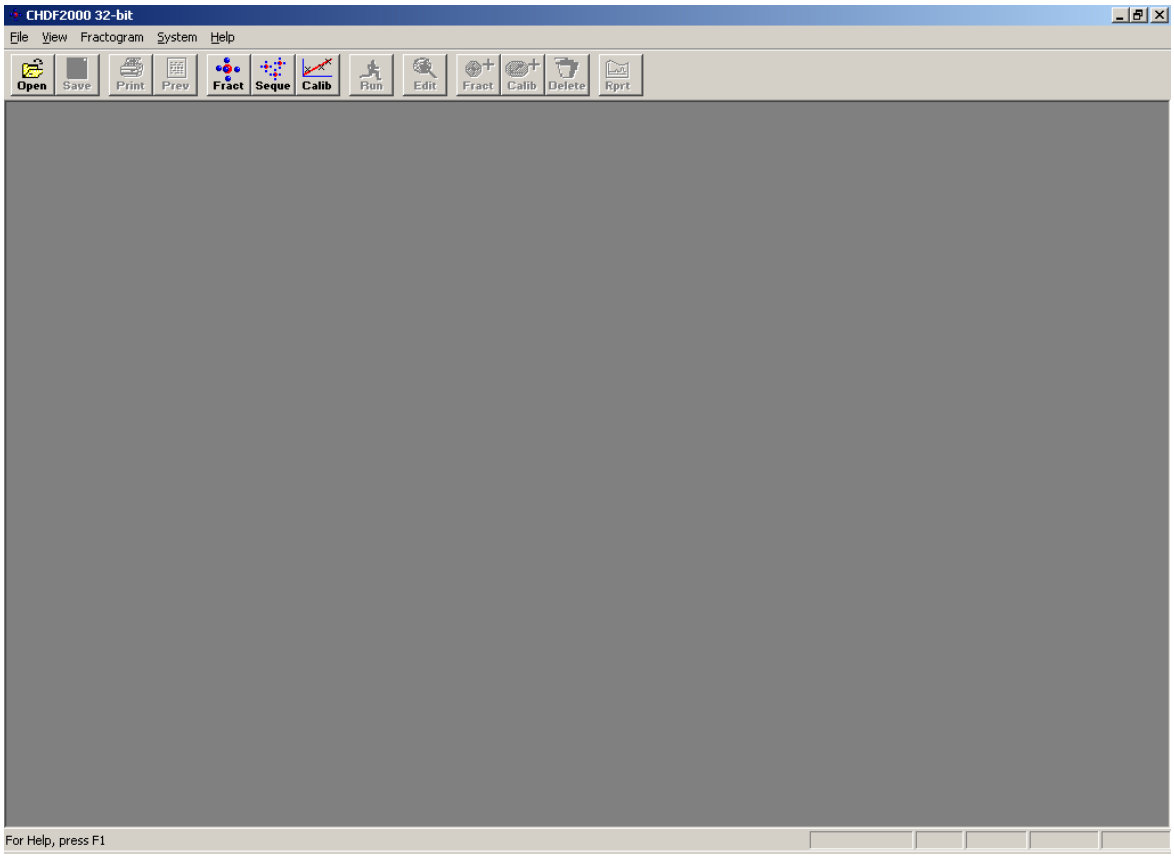


2. If you have used the system before, double click on your name to log on. If this is the first time you have used the system, click on *Edit Users*. The following dialog appears.

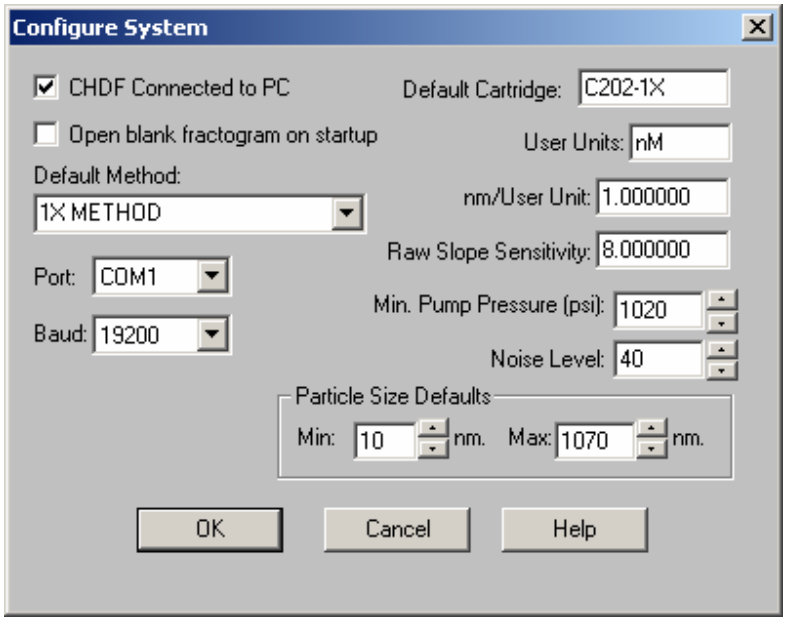


Type in a User Name and User ID for yourself. The User ID is used when analyzing samples to denote who ran the analysis. Click on the *Add* button and your name will appear on the user's list. To remove a name from the users list, highlight it by clicking on it with the mouse, then click the *Delete* button. Click on *OK* to return to the "Welcome" dialog box, then double click on your User Name to launch the software.

3. This is what the main screen of the software looks like.



4. To begin the configuration of your system, click on *System/Configure System;* the following dialog box appears.



Here is a definition of this dialog box's contents:

CHDF Connected to PC - Click in this checkbox so that a "check mark" appears if you have an actual CHDF instrument hooked up to your computer. If you do not have an instrument hooked up, but want to use the software in a stand-alone fashion for data analysis, leave the check box blank.

Open Blank Fractogram on Startup - If this box is checked, an empty window will appear when you start the software that is used for collecting new data (only useful if you are not using an auto-sampler). If this checkbox is left blank, no blank window will appear on software startup.

Default Cartridge - Enter the serial number of the fractionation cartridge installed in your instrument. This information is important for reference purposes.

Default Method - Click on the drop down menu and choose one of the methods that are shipped with the software, or choose a user-created method. A method contains important information on how a fractionation is to be performed (see the reference section of this manual for guidance on how to create a method).

Min. Pump Pressure - The pump will shut off if the system pressure falls below this value. The pressure is measured in units of pounds/square inch.

Noise Level - After a fractogram is collected, there is often times noise in the data. The software truncates the fractogram by eliminating all data points below the noise

value set by this parameter. This is a way of improving the appearance of your data. With a CHDF 2000 instrument a value of 40 is recommended. For a CHDF 1100 a value of 400 is recommended.

User Units - Determines the units to be used to measure particle size. The default setting of nanometers (nm) is recommended.

nm/User Unit - If you use particle size units other than nanometers, you must define how many of these new units correspond to a nanometer so the software can make the units conversion for you. You can also use this to "shift" the calculated particle size. The CHDF software multiplies the particle size by the *nm/User Unit.* A value grater than unity shifts the particle size higher.

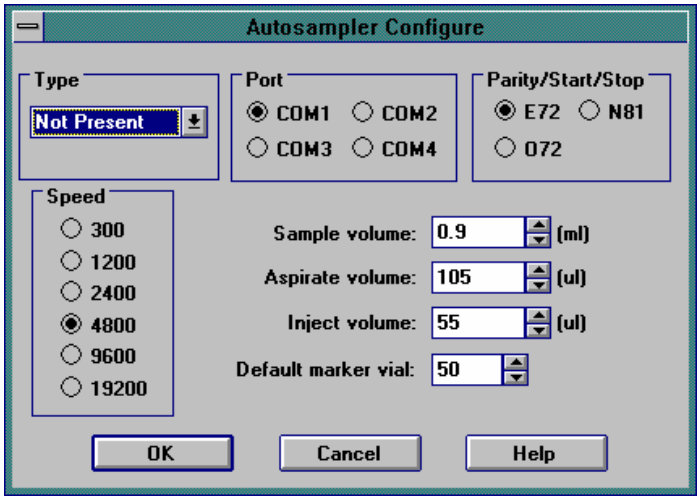
Raw Slope Sensitivity - is used to find the edges of peaks. As this number increases, the number of peaks detected decreases (the PSD and mean particle size are not affected; only the number of peaks listed in the tabular report). A value of 8 works best for most samples.

Port and *Baud* - These two settings determine the communications parameters between your computer and the CHDF 2000. Make sure the CHDF is connected to the specified COM port.

1.4 *Configuring Your Auto Sampler*

If you have an auto sampler hooked up to your instrument, you need to configure it before you can use it. If you do not have an auto sampler, you may skip this section. Assuming your CHDF V.3.20 software is running, and your instrument and auto sampler are installed and running properly, do the following.

1. Click on *System/Configure Autosampler*, The following dialog box will appear.



2. Here are the definitions of the contents of this dialog box:

Type - use the drop down menu to select the type of autosampler you are using, such as Gilson, Alcott, Hitachi or LabPro (used in on-line applications). This field will say "Not Present" if there is not an autosampler attached to your CHDF instrument.

Port/Parity Start Stop/Speed - these are all parameters that determine how your autosampler communicates with your computer. The proper settings depend on your autosampler type as follows.

Autosampler	Com. Port	Parity	Baud Rate
Gilson	Default	Default	Default
Alcott	Select appropriate	N81	4800
Hitachi	Select appropriate	E72	4800

Default means that the settings for the Gilson autosampler are loaded automatically. NOTE: Com1 is usually used for CHDF-2000 communications to the computer.

Sample Volume - The autosampler can automatically dilute samples for you. This parameter determines the total sample volume after dilution (1.9 ml is recommended).

Aspirate Volume - The volume of sample drawn into the autosampler (A/S). 100 µl is recommended if using a 25 µl A/S sample loop.

Inject Volume - The volume of sample injected into the auto sampler. 90 µl is recommended if using a 25 µl A/S sample loop.

Default Marker Vial - The vial in which the Sodium Benzoate marker should be put. This depends on the type of autosampler as follows.

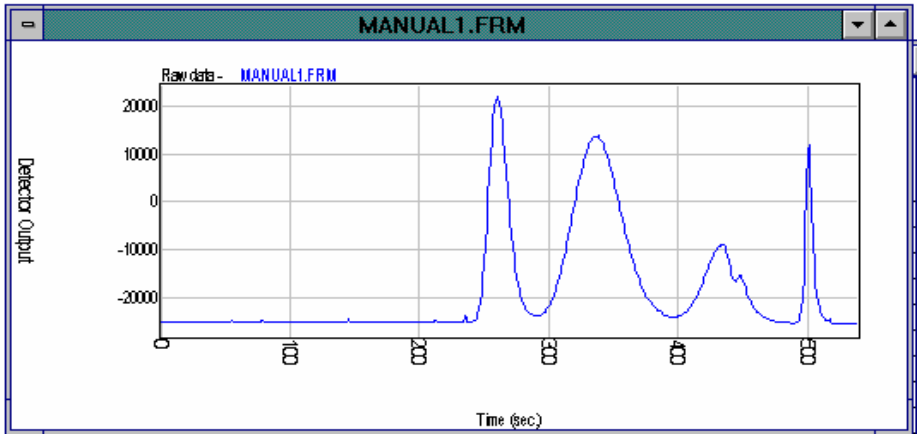
Autosampler	Default Marker Vial
Gilson	72
Alcott	201, 202 or 203 (place marker vial in same number)
Hitachi	50
LabPro	Not used (any value is ok)

1.5 *File Types Used in CHDF V.3.20*

There are several different types of files used with the CHDF, and it is important you understand how they work, and how they are different from each other. The file types are defined as follows.

1.) *Fractogram Files*- are files that contain the raw data and PSD generated by the CHDF instrument. The data in these files are plots of instrument detector response versus time. Each sample injected into your instrument will give rise to a fractogram. Fractograms are saved to disk as *.FRM files.

Here is what a fractogram looks like.



2.) *Sequence Files*- A sequence file contains a series of instructions that determine how an autosampler analyzes a series of samples. If you do not have an autosampler, you will have no need for this type of file. Sequence files are constructed using a sequence setup dialog box as discussed in later chapters. Each line in a sequence file is called a *command line*, and it contains the complete instructions for the analysis of 1 sample. The 5 columns in a command line are as follows.

1.) *Vial Number* - denotes the number of the vial that the sample is in.

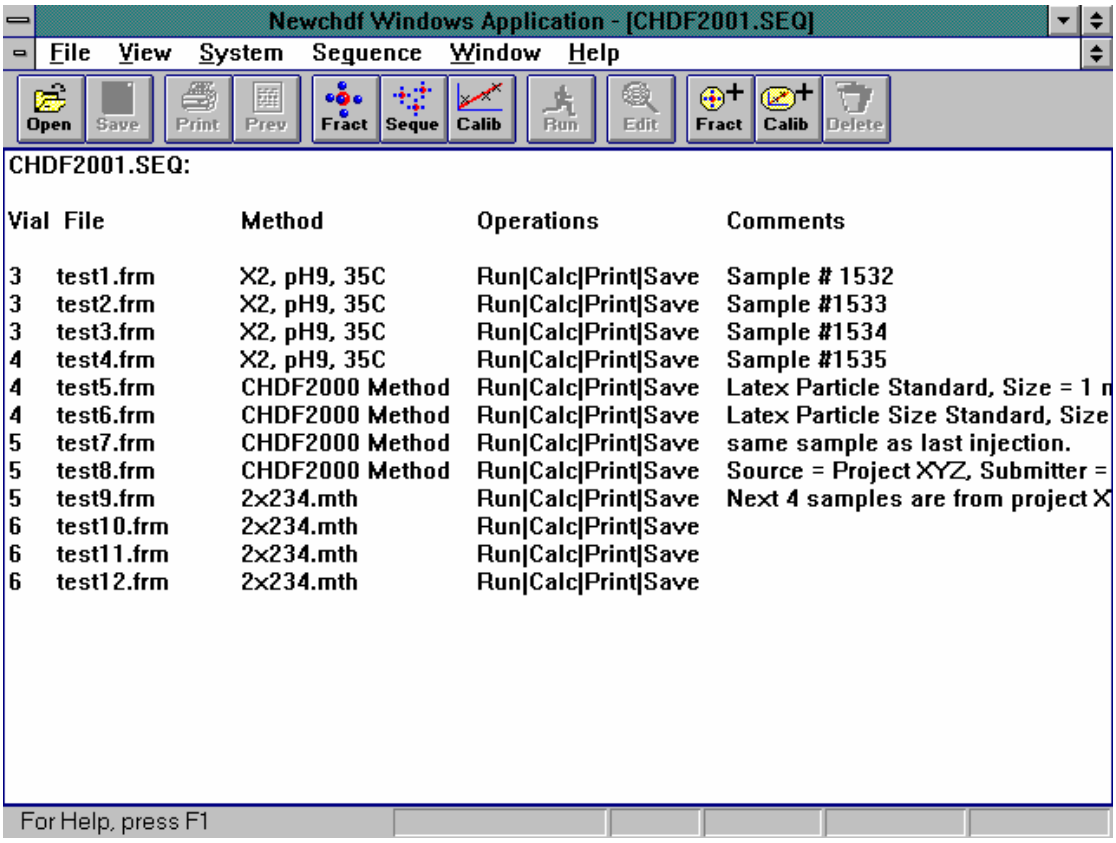
2.) *File* - for the current sample; it is the name of the fractogram file where the data will be stored. Long file names with spaces are allowed.

3.) *Method* - the method to be used when running this sample.

4.) *Operations* - A series of operations that can be performed automatically after the analysis of a sample. These include printing a report, exporting, and saving data to disk.

5.) *Comments* - textual information, up to 250 characters long, about a specific sample.

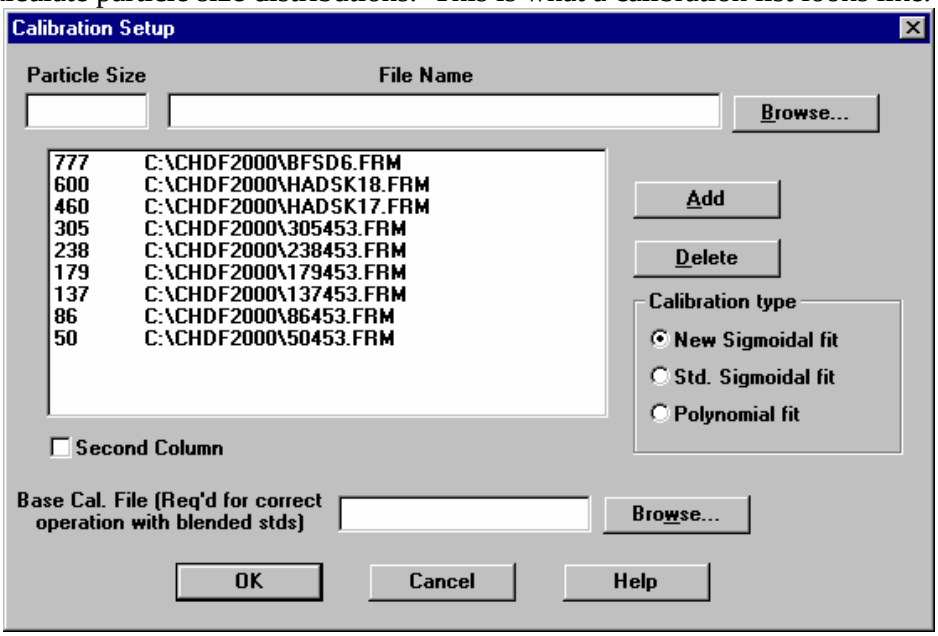
Sequence files are saved to disk as *.SEQ files. This is what a sequence file looks like.



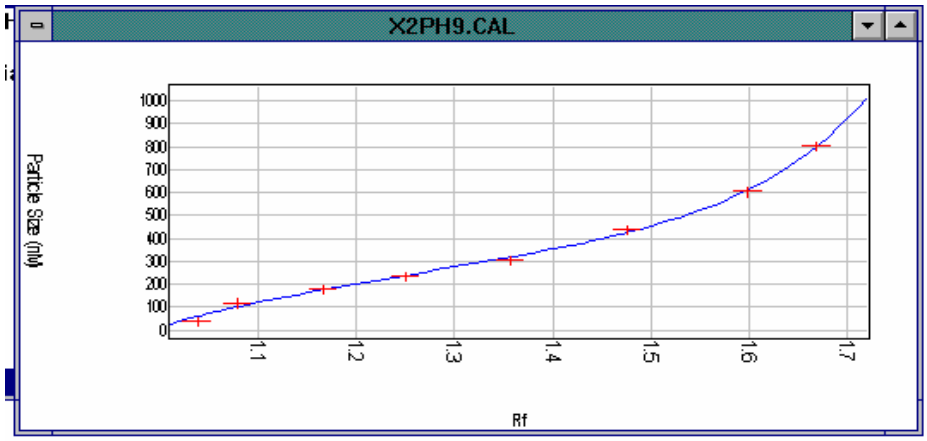
Two operations can be performed if you highlight any command lines (containing existing frm files): click the *Print* button on the toolbar, and the reports for each sample analyzed will be printed; click *Fractogram/Calculate* and these samples are recalculated. The formats for these reports are set by the method used to analyze the samples. A discussion on how to create and edit methods will be contained in a later chapter.

3.) *Calibration Files* - contain a list of fractograms, called the *calibration list*, which is a series of fractograms obtained on standard samples of known particle size. The Rf of each standard is plotted against the known particle size to construct a

calibration curve. Calibrations are applied to fractograms of unknown samples to calculate particle size distributions. This is what a calibration list looks like.



This dialog box is used to construct and edit calibration lists, and is called a "Calibration Setup" dialog box. Clicking *Cancel* produces (view) the calibration graph without re-building the calibration curve. The next figure shows an actual calibration curve, and is a plot of particle size versus Rf. Note that the plot has a sigmoidal shape to it. The "New Sigmoidal" option (recommended) tries to fit more closely the particle size data points below 100 nm than "Std. Sigmoidal Fit". The "Polynomial Fit" consists of two 4th order polynomials spanning the whole data.



4.) *Particle Concentration (or Percent Solids) Files, (PCC)*- contain a list of fractograms, called the *Particle Concentration calibration list*, which is a series of

fractograms obtained on standard samples of known percent solids. The percent solids value of each sample is plotted against the measured values of either sample fractogram peak area or the ratio of sample to marker peak areas. This data is used to construct a particle concentration *calibration curve*. PCC curves are applied to fractograms of unknown samples to calculate percent solids. These curves must be used cautiously. The calculated data assumes that the particle size of unknown samples is similar to that of the PCC calibration samples.

5) *Extinction Cross Section Files (QExt, or *.UVX):* These files can be used to accurately quantify the particle size populations in a sample, i.e. to perform refractive-index corrections to the UV-detector response. These corrections are necessary because the UV signal is a function of particle size, detector wavelength, and particle and liquid (carrier fluid) refractive index. Therefore, the CHDF software must compensate for this in order to calculate the ratios of all the particle size populations in a sample. These files are also useful if you want to run the CHDF UV detector at a wavelength other than 200 or 220 nm.

The CHDF software already contains a built-in extinction cross section correction, specified in the Method. The user can select the particles to be “UV absorbing” or “Non-UV absorbing”. If so, there is no need to use a UVX file. See Method Section.

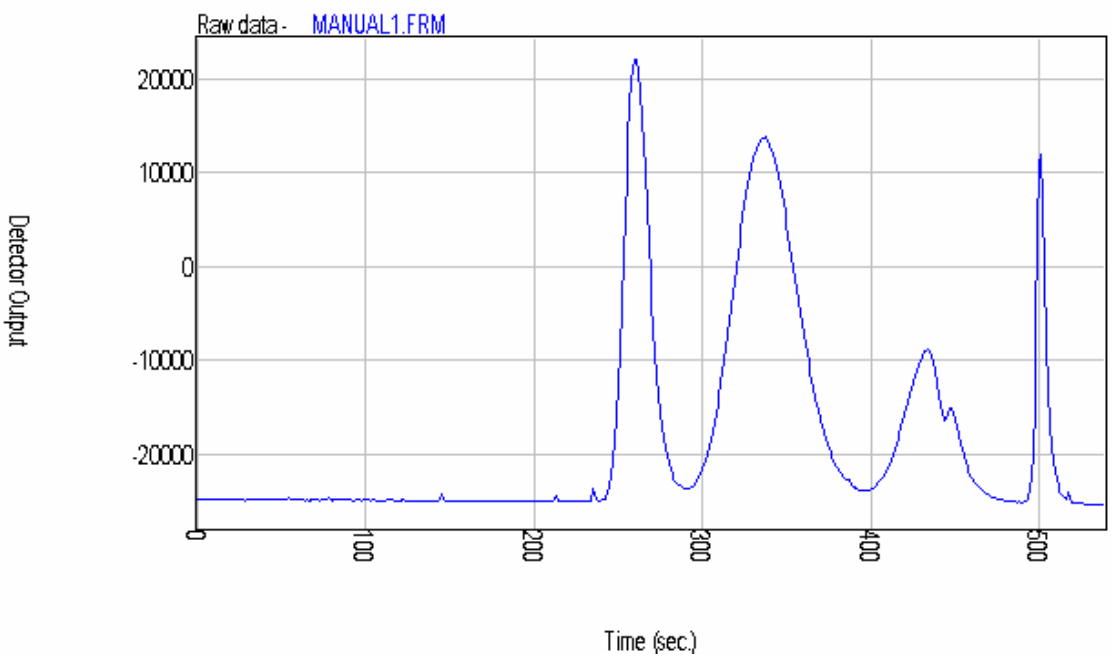
If you want to use a UVX file, you need to create one by selecting *File/New/UVX*; then, enter the particle’s refractive index real (for scattering) and imaginary (for absorption) values, plus the water refractive index and detector wavelength. Save this file, then select it in the Method for use in the PSD computations.

1.6 *How to Manipulate Fractogram Data*

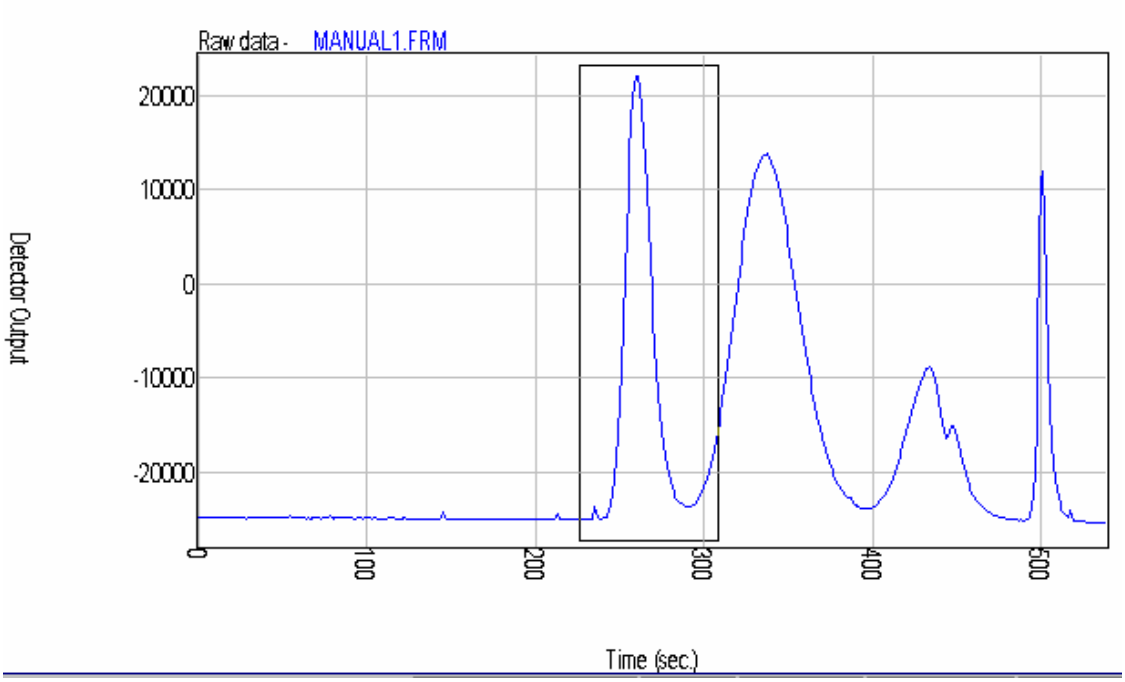
Each of the three different file types used in the CHDF V.3.20 software can be displayed in their own window. These windows can be sized, moved, and manipulated like any other window in any other Windows™ application. The *active* window is the one that is the topmost in the stack of open windows, and it is the data in this window that can be manipulated and plotted. The contents of the toolbar, the menu bar, and menus change depending upon which of the three file types is active.

Fractograms can be displayed graphically, and there are special tools within the software to manipulate fractogram data. Firstly, one can perform a *Box Zoom* using the mouse to enlarge a piece of a fractogram. Here is how to load and zoom a fractogram.

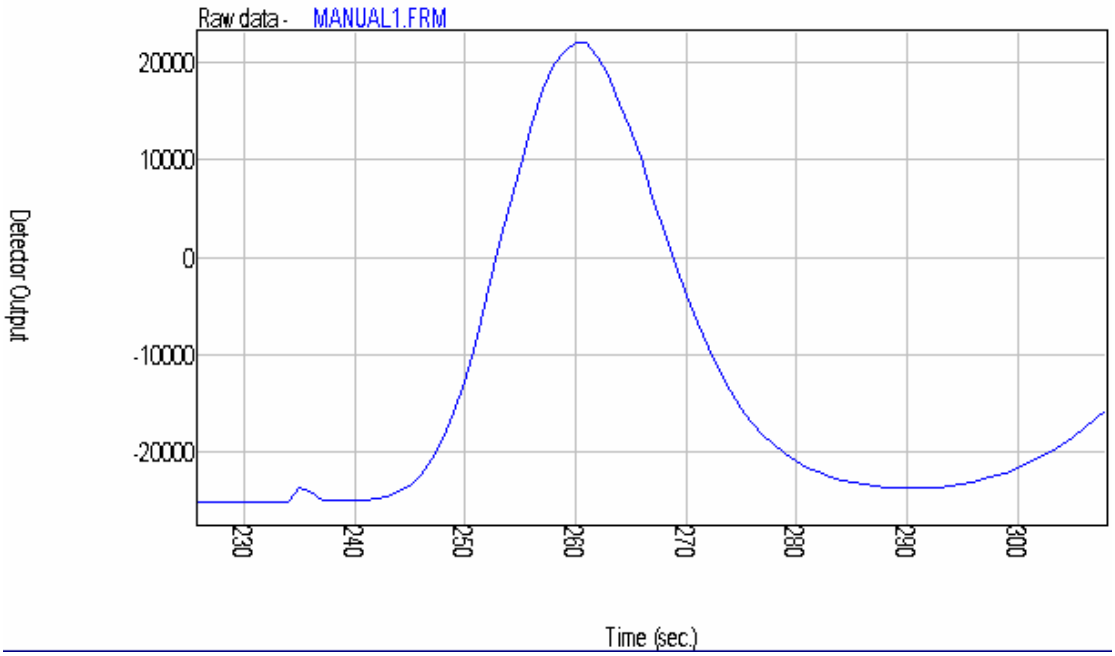
1. Click on *File/Open* (or the toolbar icon), double click on the name of the *.FRM (fractogram) file you want to open. Your fractogram will appear in the active window as such.



2. Click and drag the mouse on the fractogram. A box appears as shown below.



3. Let go of the mouse button; the region of the fractogram within the box will expand to take up the entire active window as such.



4. To autoscale the fractogram and display all of it again, simply perform a right mouse click anywhere on the fractogram.

Chapter 2

Sample Analysis Tutorials

Assuming your CHDF instrument is installed, your CHDF V.3.20 software is installed, and your system is configured, you can begin analyzing samples. This section will give you step-by-step instructions on how to analyze a sample via manual injection, how to use an autosampler, and how to perform a calibration. It will not explain in great detail the fine points of the software, only those things you need to know to run a sample will be included. For a detailed description of all important software functions, see the Software Reference section beginning with Chapter 3.

The first section of this tutorial assumes that you have a sample properly prepared for CHDF analysis, and that it will be injected manually. Analysis using an autosampler will be covered later in this section, as will how to perform a calibration.

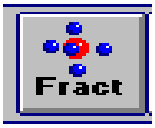
2.1 *Manual Injection Sample Analysis*

1. Turn on the CHDF instrument.

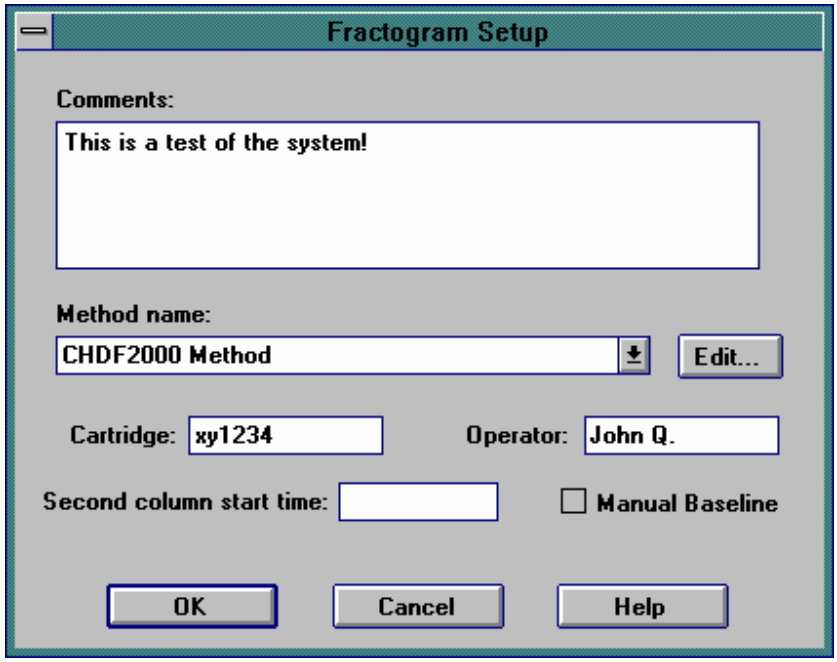
2. Prepare eluant for your analysis. The eluant must be fresh, its conductivity must be suitable, and it must be the same type of eluant used in calibrating the system. Make sure the eluant vessel is plumbed into the instrument. Turn on the pump, and let the system equilibrate for 1/2 hour.

3. Turn on your computer, start Windows, then start the CHDF V.3.20 software.

4. Click the “Fract” button on the toolbar. It looks like this.



5. After clicking on the Fract button the following dialog appears. It prompts you for important information about the analysis.



6. This dialog box's contents are defined as follows.

Comments - This is used to enter information about the analysis, such as a description of the sample, or the time and day the analysis was performed. You can enter up to 5 lines of text, and each line can be up to 50 characters long for a total of 250 characters.

Method Name - Click on this drop-down menu to see the names of the different methods that can be used with your system. Click on the name of the method you want to use. To edit a specific method, click on its name and then click on the *Edit* button (a discussion of how to create and edit methods will be given in a later chapter).

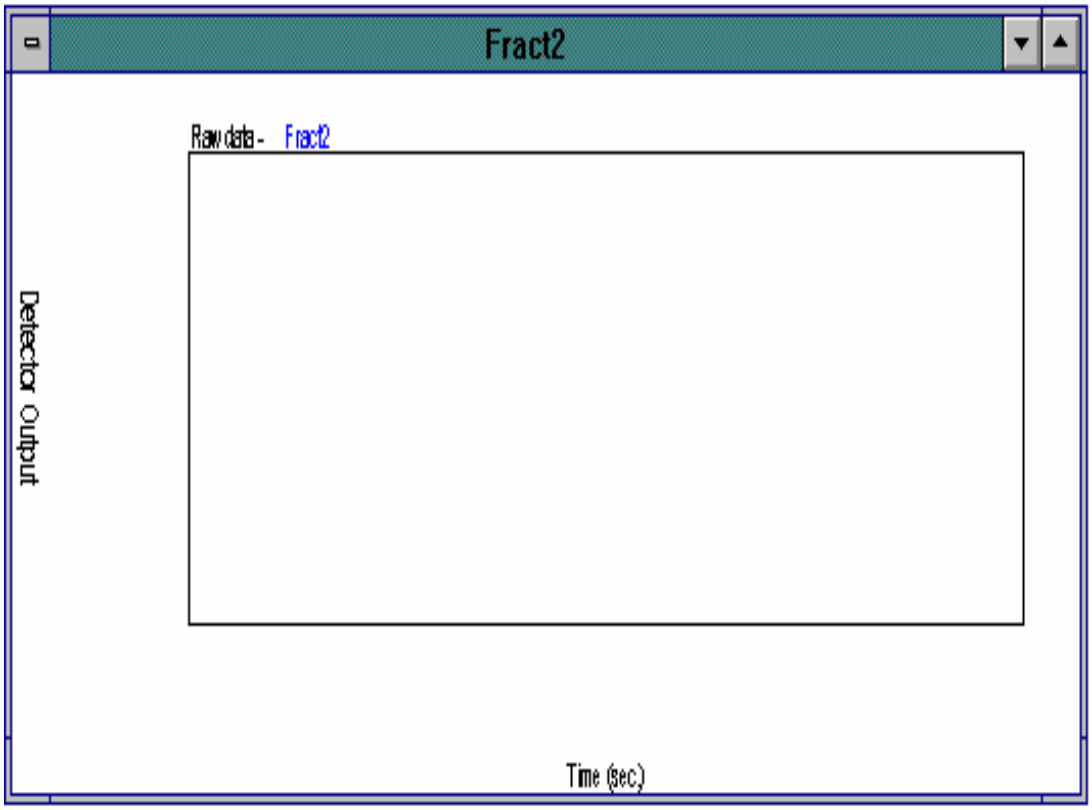
Edit - Click on this button to edit the currently selected method. Details on how to edit methods will be provided in a later chapter.

Cartridge - This is the default serial number for the CHDF cartridge you are using. This number was entered when the system was configured (see Chapter 1). Make sure this number is updated every time a new cartridge is installed.

Operator - The User ID for the person who logged onto this session of using the software. The user ID is set by clicking on *System/Change User ID*. The

User ID can also be changed from the initial screen seen after the software is first loaded.

7. Once done with Fractogram Setup, click on *OK*, and a blank fractogram window will be seen as follows.



The raw fractogram will appear in this window as it is collected.

Click *Run* in order to start the sample analysis. Click again to finish the run. The sample PSD is automatically calculated and a PSD report is shown. See below.

8. Rinse the injector with eluant by inserting your rinse-syringe port cleaner into the injector, and injecting eluant that has passed through an in-line particle filter hooked up to your syringe. Fully flip the handle on the manual injector to LOAD. Rinse the injector a second time. Prepare a syringe containing a properly prepared sample. Such a sample should contain 0.5% to 3% solids. Slide the syringe square-tip (do not use sharp needles) needle fully into the injection port. If using a sample syringe filter, make sure the filter is filled with sample.

9. Flip the injector handle fully to INJECT. The software will detect that a sample has been injected, and will begin accumulating data.

10. Rinse the injector, then turn the injector handle to LOAD. Rinse the injector again. Take ~0.1 ml of the sodium benzoate marker and dispense it into the injector. The marker solution should be passed through an in-line particle filter just like the eluant used to rinse the injector. When refilling the syringe, remove the syringe filter.

11. Smoothly flip the injector handle to INJECT. The marker will now be injected into the CHDF cartridge. Ideally, you should inject the marker about 1 minute after injecting the sample. Typically, it takes about 1 minute to perform step 10. However, if you perform the step faster than this, then make sure there is about 1 minute delay between injecting your sample and marker.

2.2 *Sample Analysis with an Autosampler*

An autosampler is an automated robotic device capable of automatically diluting and injecting samples into your CHDF instrument. If your system is not equipped with an autosampler, you must manually inject samples as discussed above in section 2.1. There are currently three different types of autosampler supported by your CHDF V.3.20 software, made by Hitachi, Gilson, and Alcott. Prior to performing any analyses using your autosampler, you must first configure your autosampler system. This process is described in section 1.4.

Some Practical Advice Before You Get Started

Autosamplers are very powerful sample preparation and injection tools. However, like any other instrument they only operate as well as the information that is given them. Here are some practical pointers to help you prevent disastrous autosampler runs.

1. Make sure that each sample is prepared and sealed properly in a sample vial that is compatible with your autosampler. Make note of what sample is in what spot in the autosampler tray. You will need this information later to construct the sequence file properly.

2. Make sure there is a vial full of marker in the default position for this type of autosampler. The default marker position depends on the manufacturer of your autosampler. This information is given in section 1.4 above. A fractogram obtained without a marker also being run is meaningless. Therefore, make sure there is enough marker in the marker vial for each sample injection that is going to be made. You can select multiple marker vials for a sequence with many samples.

3. If you plan on having the autosampler dilute your samples for you, you need empty vials mounted in the autosampler sample tray for each dilution. You need one empty vial for each sample to be diluted. These empty vials need be next to each other in sequence, e.g. vials 10, 11, and 12 if three different samples are going to be diluted. You must tell the software where the first mixing vial is located, it assumes the rest are located in sequence after the first one.

The commands used to instruct an autosampler in how to analyze a sample are stored in *Sequence Files*. A sequence file contains a series of lines, called *command lines*, and each command line contains the complete instructions for the analysis of 1 sample. Sequence files are saved to disk as *.SEQ files. This is what a sequence file may look like.

CHDF2001.SEQ:

Vial	File	Method	Operations	Comments
3	test1.frm	X2, pH9, 35C	Run|Calc|Print|Save	Sample # 1532
3	test2.frm	X2, pH9, 35C	Run|Calc|Print|Save	Sample #1533
3	test3.frm	X2, pH9, 35C	Run|Calc|Print|Save	Sample #1534
4	test4.frm	X2, pH9, 35C	Run|Calc|Print|Save	Sample #1535
4	test5.frm	CHDF2000 Method	Run|Calc|Print|Save	Latex Particle Standard, Size = 1 n
4	test6.frm	CHDF2000 Method	Run|Calc|Print|Save	Latex Particle Size Standard, Size
5	test7.frm	CHDF2000 Method	Run|Calc|Print|Save	same sample as last injection.
5	test8.frm	CHDF2000 Method	Run|Calc|Print|Save	Source = Project XYZ, Submitter =
5	test9.frm	2x234.mth	Run|Calc|Print|Save	Next 4 samples are from project X
6	test10.frm	2x234.mth	Run|Calc|Print|Save	
6	test11.frm	2x234.mth	Run|Calc|Print|Save	
6	test12.frm	2x234.mth	Run|Calc|Print|Save	

The 5 columns in each command line are...

1.) *Vial Number* - denotes the number of the vial that the sample is in.

2.) *File* - the name of the fractogram file where the data for the current sample is to be stored.

3.) *Method* - the method to be used when running this sample. The method contains many pieces of information on how to analyze a sample, including what calibration curve to use, and what type of Report format to use to report the data.

4.) *Operations* - A series of operations that can be performed automatically after the analysis of a sample. These include printing a report, exporting, and saving data to disk.

5.) *Comments* - textual information, up to 250 characters long, about a specific sample.

A unique feature of sequence files is if you highlight several command lines, and click the *Print* button on the toolbar, the reports for each of these samples will be printed. The formats for these reports are set by the method used to analyze the samples. A discussion on how to create and edit methods will be contained in a later chapter.

Sequence files are either created from scratch, or existing sequence files can be edited. The two processes are slightly different, and will be discussed separately below. You may add samples to an existing sequence while samples are being run.

Creating a New Sequence File
A new sequence file can be created in two different ways.

1. By clicking on the "Sequence" button in the Toolbar.

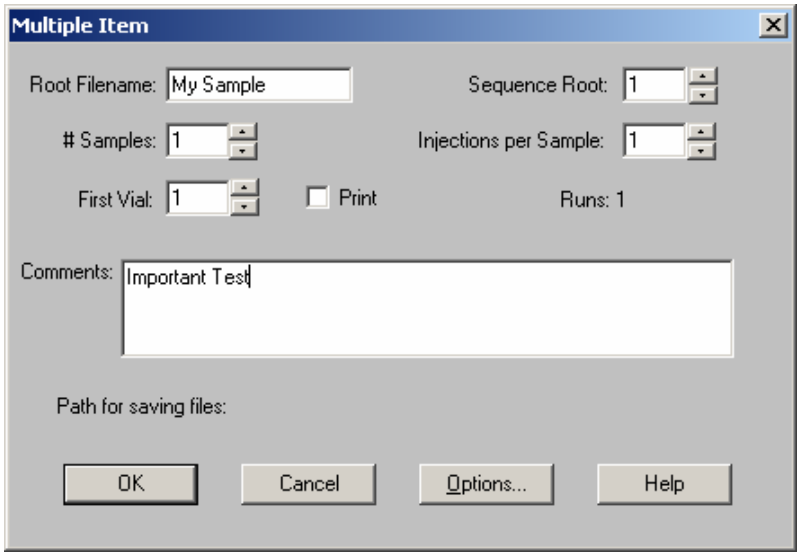
2. By clicking on the *File/New/Sequence*.

You can add a new command line to a sequence file as follows:

1. By clicking on the "Fract+" button in the toolbar while a sequence file is in the active window.

2. By clicking on *Sequence/Add New Fractograms* while a sequence file is in the active window.

In any case, all of these operations bring up the following dialog box.



Root Filename - All the samples run from one line in a sequence file are given the same name called the root filename. Each root name is followed by a number indicating where in the injection order a specific sample was analyzed. Long file names with spaces may be used.

Sequence Root - The number to be appended to the root filename for the first sample in a sequence. Samples run after this in a sequence are given the same root filename and an appropriate number. For example, in the dialog box above the first sample will be called SAMPL1, the second sample will be called SAMPL2 etc.

 # Samples - The total number of vials to be injected using the commands in this sequence.

Injections per Sample - The number of injections to be made from each vial.

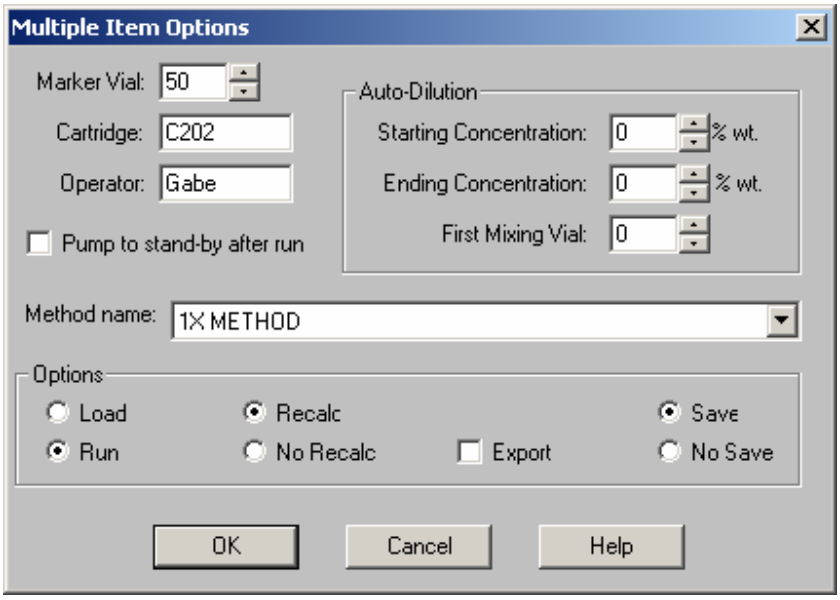
First Vial - the position in the autosampler rack where the first vial to be analyzed is located.

Runs - This is the total number of fractograms to be measured in a particular sequence of samples. This number is calculated automatically by multiplying the # of samples times the injections per sample.

Comments - allows a description of the sequence of over 1,000 characters in length.

Print – Automatically prints the PSD report of each finished sample. Note: the contents of the Report must be specified in the Method/Report Setup. Otherwise, a blank Report is generated.

Options - clicking on this button brings up a dialog box that is used to set the parameters for the injection of an individual sample. Much of the information entered here is displayed in the command lines in a sequence file. Here is what the “Sequence Setup Options” (multiple item options) dialog looks like.



Marker Vial - The number of the vial that contains the Sodium Benzoate marker.

Cartridge and *Operator* - these fields are filled in automatically using information that should have been previously entered in the system configuration and User ID dialog boxes.

Pump to Standby After Run - if checked, this command will turn off the pump after analysis of the last sample in this command line. Generally, the pump should be left on so the instrument stays equilibrated. The only time you should have the pump turned off automatically is if the system is running unattended, and this is the last sample injected.

Auto Dilution - If you desire, you can have the autosampler dilute the concentration of your sample to the proper level for you. The three parameters listed under *Auto Dilution* determine how this sample preparation takes place. *Starting Concentration* is the percent solids of your sample before dilution. *Ending Concentration* is the percent solids you will want to have after dilution. *First Mixing Vial* is the first empty vial in which mixing of the sample will take place. You must have some empty vials available in the autosampler sample rack for this purpose.

Method Name - this field lists the method used to analyze the current sample. It can be changed by clicking on the drop down menu and choosing a new method.

Options - allows a number of actions to take place automatically after the measurement of a fractogram as follows.

Load - load a data file from disk to be printed out as part of a report rather than acquiring data with the CHDF instrument.

Run - Acquire data using the CHDF instrument.

Recalc - calculate a particle size distribution after acquiring the data.

No Recalc - do not calculate a particle size distribution after acquiring data.

Export - automatically export the fractogram file to a format that older versions of the CHDF software can read. These files can also be read by spreadsheets (such as Excel), word processors, and text editors since the file format is "tab delimited".

Save - save the fractogram file to disk once it is accumulated (recommended).

No Save - do not save the fractogram file to disk after it is measured.

Editing an Existing Sequence File

Editing an existing sequence file involves loading an already existing *.SEQ file, then making desired changes to the command lines in it. For example, you can alter the command lines in a sequence file, add a new command line, delete a command line, or place a calibration sample command line into a sequence file.

There are 3 buttons in the toolbar that can be used to edit sequence files. These buttons are enabled when a sequence file is in the active window. The buttons are used to add a new command line to a sequence file, to add a calibration sample to a sequence list, or to delete command lines from a sequence file.



This is the "add fractogram" button. It is used to add a new command line to a sequence file, which will lead to the acquisition of a fractogram. Clicking on this button brings up the same sequence setup dialog box discussed above in the section on creating a new sequence file.



The "add calibration" button rebuilds the calibration curve for the purpose of updating the calibration curve. Calibration standards can be run in a given sequence prior to

Calib+. These standards will replace those in the calibration curve with the same file name once Cal+ is executed. The resulting calibration is applied to further fractograms obtained during following runs. A detailed description of this function is contained in section 4.2.



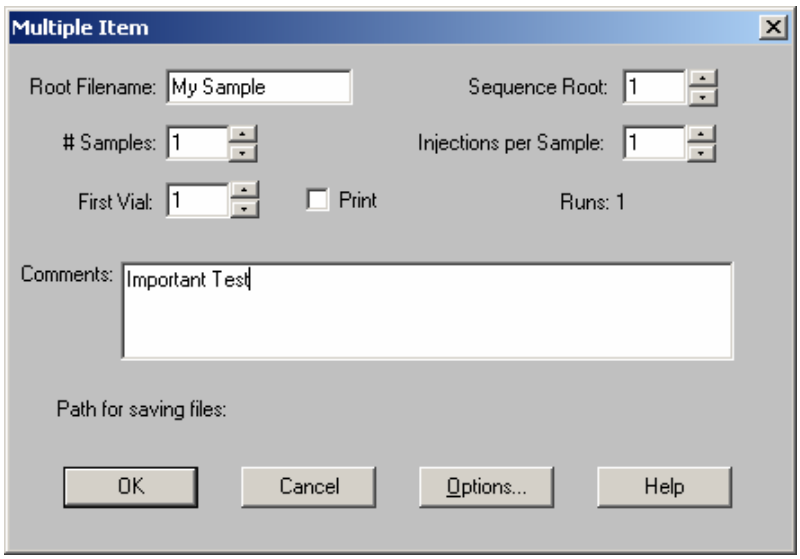
This button will delete a command line in a sequence which has been highlighted by clicking on it with the mouse.

The functions performed by these three toolbar buttons can also be accessed from the Sequence menu, which is seen in the menu bar only when there is a sequence file in the active window. The details of the Sequence menu will be discussed in Chapter 4.

The dialog box for editing command line files is opened in three different ways:

1. By clicking on *View/Setup* when the active window contains a sequence and a command line in the sequence is highlighted.

The Edit Sequence Setup dialog box, which contains the title “multiple item” is illustrated below. This dialog box is similar to the one used to create sequence files discussed above. The differences between creating and editing a sequence will be discussed here.



Filename - lists the name of the fractogram to be created when a specific sample in the list is analyzed.

View - Will display the fractogram listed in *Filename*.

Starting an Autosampler Run

First, your autosampler must be configured properly (see section 1.4), your samples, markers, and mixing vials must be prepared properly and placed in their correct locations in the autosampler tray, and the sequence file must be filled out accurately, and be seen in the active window. Once these conditions are met, an autosampler analysis is begun in one of three ways.

1. Click on the *Run* button in the toolbar. This begins the sequence starting with the first item.

2. Click on *System/Run*. This also starts the sequence at the first item.

3. Highlight an item in the sequence, then click the *Run* button. The sequence will begin running at the highlighted item and continue until the end of the sequence.

An empty fractogram window will appear, the autosampler will prepare and inject your sample, and the data will begin to appear on the computer's screen.

2.3 *Step-by-Step Calibration Instructions*

A calibration must be performed on your system prior to running any unknown samples. The purpose of the calibration is to measure the instrument's response to samples of known particle size, known as *standards*. In CHDF, standards typically consist of a suspension of latex microspheres of known size. The correlation of the instrument response with particle size is obtained by plotting a *calibration curve*, which is a plot of sample Rf versus known particle size. In CHDF such curve can have a sigmoidal or polynomial shape. New sigmoidal, as well as Sigmoidal calibration curves require a minimum of 6 samples. A polynomial calibration curve requires exactly eight standards. After the initial calibration of your instrument, you should re-calibrate your system at least once a week.

To perform a typical calibration, fractograms of standard samples containing particles of specific, known, particle size are obtained. The recommended number of standards is 6 to 8. Next, a *calibration list* is constructed, which are the names of the set of standard fractograms to be used in the calibration. After constructing the calibration list, the calibration curve is calculated automatically. The curve gives us a calibration equation which relates a sample's Rf to particle size for this particular instrument. Finally, unknown samples can be run, and the Rfs of the sample measured. It is then a simple matter for the software of plugging these Rfs into the calibration equation to calculate the particle size distribution of a sample.

To construct a calibration list, a "calibration setup" dialog box must be used. This dialog box can be opened in four different ways.

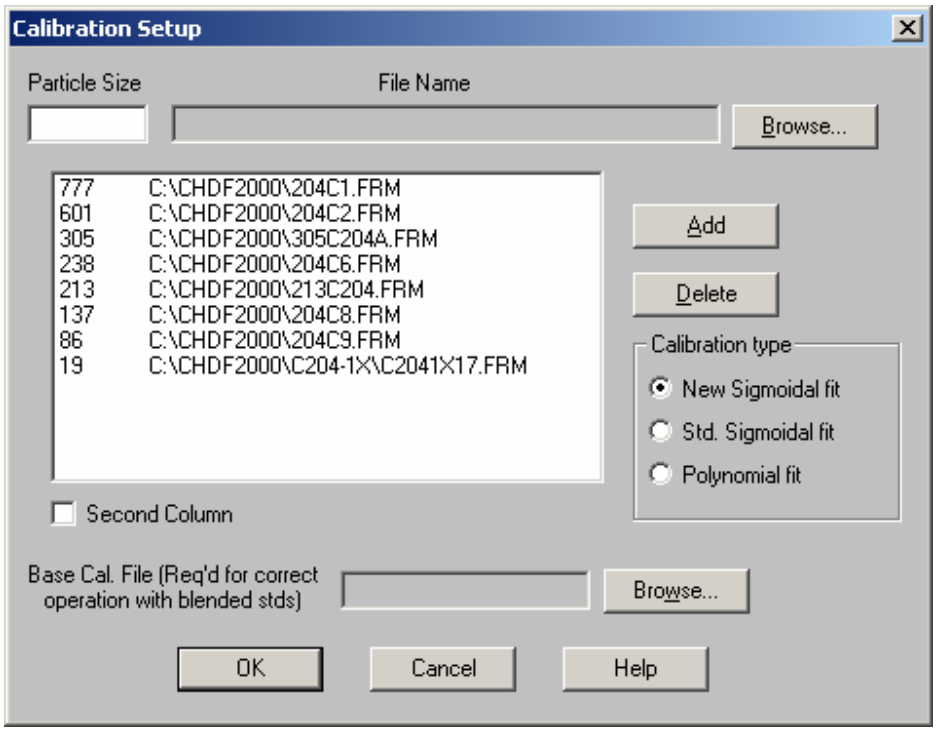
1. By clicking on *View/Setup* when the active window contains a calibration.

2. By clicking on the "Calibration" button in the Toolbar.

3. By clicking on the *Edit* button in the toolbar when the active window contains a calibration.

4. By clicking on *File/New/Calibration*.

The Calibration Setup dialog box is discussed and illustrated below.



Filename - the name of a standard fractogram to be added to a calibration list.

Particle Size - the size of the particles used in generating the standard fractogram defined by *Filename*.

Calibration List - is shown in the center of the dialog box. It contains the names of the standard fractograms to be used in a calibration, along with the particle sizes of the standards.

Browse (upper)- when this button is clicked, it brings up a file selection dialog box that can be used to add new standard fractograms to the calibration.

Add - when clicked, this button adds the standard fractogram in *Filename* to the Calibration List.

Delete - once a standard fractogram name in the *Calibration List* is highlighted by clicking on it, its name can be removed from the calibration by clicking on the *Delete* button.

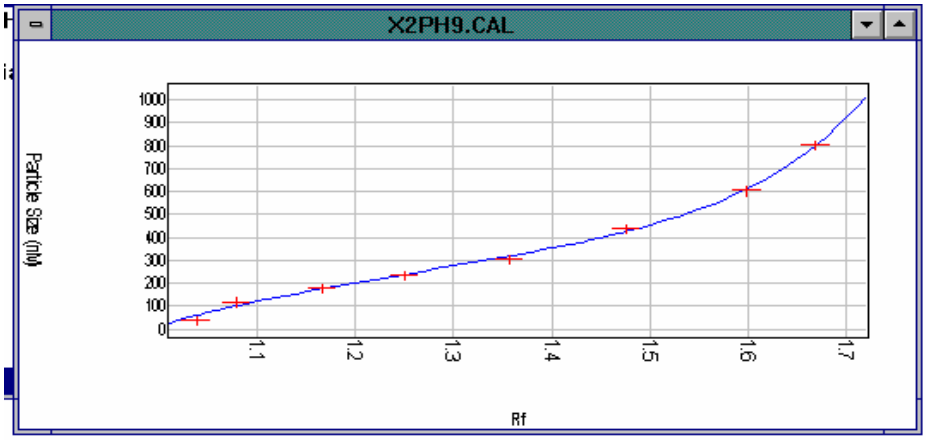
Calibration Type - You have the choice of fitting your calibration data to three different types of functions. The first, New Sigmoidal, is the recommended function for particle sizes below 100 nm. The second, Std. Sigmoidal, was used in the past and is included for back compatibility. If starting a new calibration from scratch, New Sigmoidal should be used instead of Std. Sigmoidal. For either of these functions, a minimum of 6 standards is required. The third type of calibration function is polynomial. This calibration function requires exactly 8 standards. The calibration plot is of particle size vs. Rf (the calibration curve).

Second Column - This box is used to specify the "long-capillary" calibration curve for special "dual-capillary" fractionation cartridges. Leave this unchecked if using regular columns.

Base Cal. File - stands for "base calibration file". This file is to be used when a standard sample contains particles of more than one size.

Browse (lower)- click on this button to load a base calibration file.

Once you click *OK* in the calibration setup dialog box, the calibration curve is calculated automatically and displayed for you. This is an example of what a calibration curve looks like. Clicking Cancel displays the existing curve without re-calculation.



This is what a sigmoid shaped curve looks like. This graph may be zoomed by clicking and dragging on it to draw a box. The region inside the box will expand to fill the window. To autoscale and see the entire graph again, click the right mouse button on the graph.

When performing an analysis of an unknown sample, you must tell the software what calibration file to use in analyzing the results. This is done in method editing, which is discussed in a later chapter.

Chapter 3

Software Reference Guide

Part 1: The Toolbar, File and View Menus

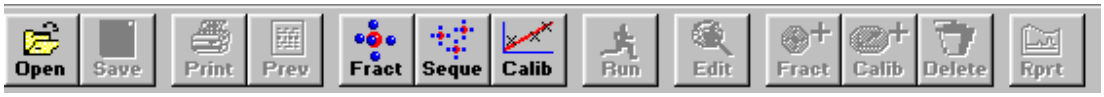
The purpose of this chapter is to cover all important aspects of the software in detail. You will probably find this section to be of the most use after using your CHDF V.3.20 software for a while. Once you are comfortable with the software, this chapter can give you information to make your use of the software easier and more powerful.

This Chapter is organized by menus. Each software menu will be discussed in turn, and the commands available in each menu will be described in order. Be aware that the contents of the menu bar and the menus change depending upon what type of file is active. Therefore, not all the menu choices listed here will always be available at a certain point in using the software.

3.1 *The CHDF V.3.20 Toolbar*

The toolbar runs across the top of the screen, and by clicking on the various buttons on it specific software functions can be performed. Functions that are important and used frequently have been chosen for inclusion in the toolbar. All toolbar functions can also be accessed from menus, but the toolbar is generally a faster and easier way of performing these functions. Certain toolbar buttons are enabled or disabled as appropriate, so you do not have access to all toolbar buttons at any given point during use of the software.

This is what the toolbar looks like:



Individual toolbar buttons will be defined below.



This button is used to open files. It brings up a file selection dialog box as discussed in the section on the *File* menu below.



Is used to save files to disk under the current file's name. Be careful when using this function because it does not provide any overwrite protection.



Pressing this button will create a printout of whatever is in the active window.



This is the print preview button. Pressing it will allow you to preview a plot of your data before it is printed.



This is the fractogram button. When pressed it will bring up a "fractogram setup" dialog box and an open data window. Once the fractogram setup is filled out the, the blank data window is left on the screen. New data will appear in this window once a sample is injected.



This is the sequence button. When it is pressed a "Sequence Setup" dialog box appears, which allows one to program an autosampler for automatic injection of samples.



The calibrate button. When pressed this button brings up a "Calibration Setup" dialog box that is used to determine what standard fractograms to use in creating a calibration curve.



Click on this button when you want to run a sample, or stop the analysis of a sample currently underway. When a run is stopped using this button, it will automatically display a particle size distribution report.



Clicking on this button will display a Setup dialog box for the type of file that is currently active. For example, if there is a fractogram in the active window, a "Fractogram Setup" dialog box will appear showing information on the fractogram.

The next 3 buttons are used to edit the sequence files, which are used to setup and run an autosampler.



This is the “add fractogram” button. It is used to add a new line to a sequence file that will lead to the acquisition of a fractogram



The “add calibration” button allows the addition of “check calibration” samples to an autosampler run. Fractograms generated from these samples can be added to an already existing calibration to update them, then the resulting calibration applied to other fractograms obtained during a run.



This button will delete any item in a sequence list that has already been highlighted using the mouse.

The “Rprt” button displays the PSD report of an active FRM file.

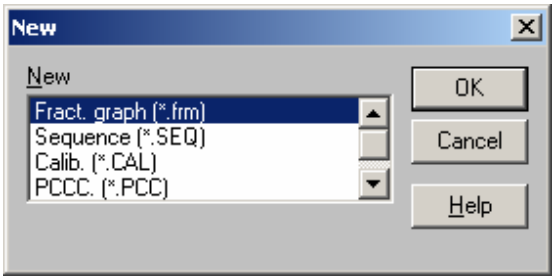
3.2 *The File Menu*

When you click on the file menu, the following commands appear.



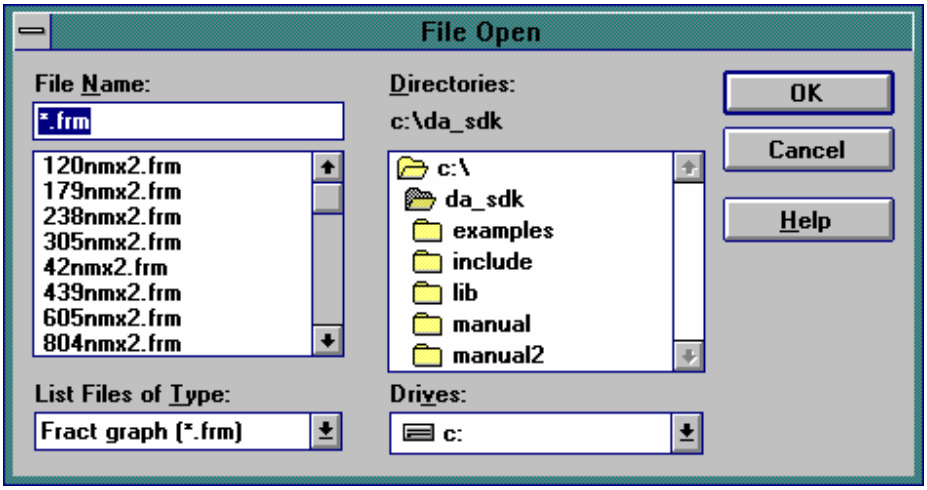
Here is what the commands in the *File* menu do.

File/New - Is used to create a new fractogram, sequence or calibration file. When this choice is clicked the following dialog box appears. Double click on the name of the type of file you wish to create.



The three types of file that can be opened from this dialog box are described in Section 1.5.

File/Open - Is used to open an already existing fractogram, sequence, or calibration. It brings up the following file selection dialog box.

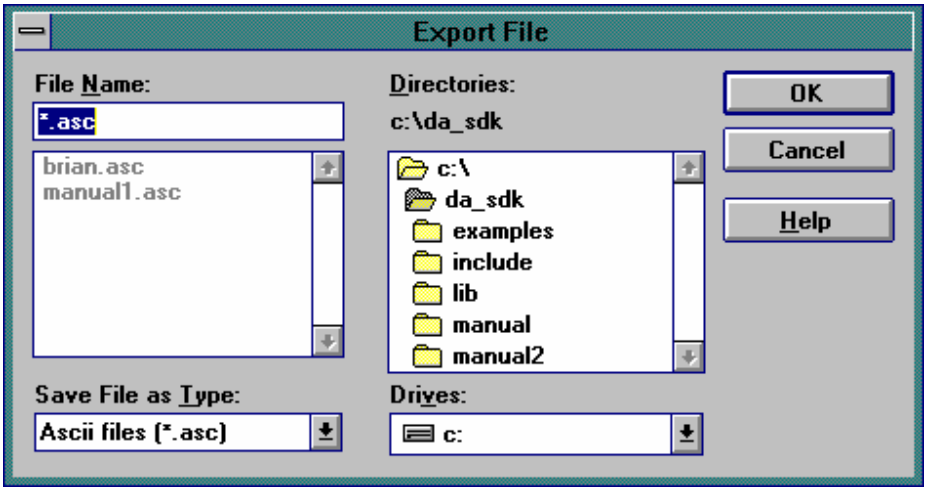


You may choose a directory or disk drive as in any other Windows file selection dialog box. A list of fractogram (*.FRM) files is shown by default. Click on the *List Files of Type* drop down menu to look at a listing of sequence files (*.SEQ) or calibration files (*.CAL). Double click on the name of the file you want to open.

File/Save - will save changes to the current file without an overwrite warning. Only use this choice if you are certain that you want to save the changes made.

File/Save As - Brings up a file selection dialog, similar to those in any other Windows application. This dialog allows you to save a file under a specified name to any directory and drive on your computer.

File/Export - Is used to export V.3.20 fractograms to ASCII format that can be read by previous versions of CHDF software, as well as, spreadsheet programs. It brings up the following dialog.

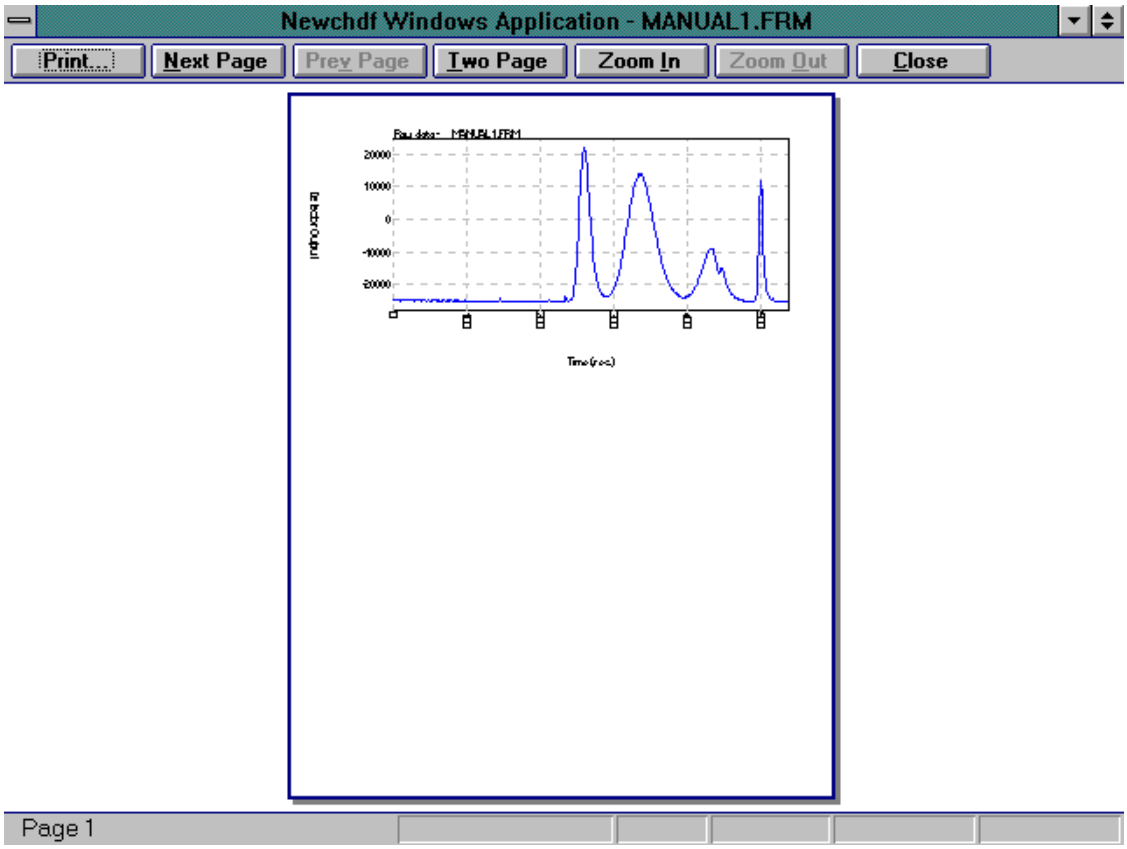


This is simply a file selection dialog box. Create a name for the exported file. Choose the desired directory and disk drive, then click on *OK*. The file will be exported. The file format upon exportation is ASCII tab delimited. Translation: an exported fractogram can be read by Excel, Microsoft Word™, any other spreadsheet, or any other word processor or text editor.

ASCII files are composed of seven columns as follows (from left to right): Particle Size, Number %, Cumulative Number, Weight %, Cumulative Weight, Raw Data, and Run Parameters.

File/Import - Is used to bring fractograms from older versions of the CHDF software into V.3.20. It brings up a dialog almost identical to the one for exporting, which is simply a file selection dialog box. Find the name of the *.ASC file you want to import, double click on its name, and it will be imported.

File/Print Preview - Allows the user to view what a plot of the active window will look like before it is sent to the printer. The buttons in *Print Preview* allow you to zoom in and out on the plot, and see one or two pages of plot at a time. You can also print directly from the *Print Preview*. This is what the *Print Preview* looks like.



File/Print Setup - Brings up a dialog box that is used to configure a printer before printing. The nature of this dialog is printer specific, so no example of one is given here.

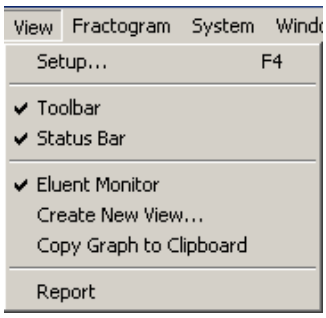
File/Print - Brings up a dialog box allowing you to set parameters affecting printing. This dialog box is printer specific, so no example is given here.

File/Filenames - your CHDF V.3.20 software keeps track of the last four files opened, and lists them at the bottom of the file menu. Clicking on any of these filenames will load said file.

Exit - closes the CHDF software and returns you to the Windows program manager.

3.3 *The View Menu*

The view menu is used to set up fractograms, sequences, and calibrations. This menu also allows you to view and customize the display of important information. It contains the following choices.



All 7 of these commands are displayed when a fractogram is open and active. If a sequence file, calibration file, or no file is active the menu choices are limited to less than these.

View/Setup - Brings up one of three different types of Setup dialog box depending on what type of file is in the window that is active. These setup dialogs will be referred to as "Fractogram Setup", "Sequence Setup", and "Calibration Setup".

3.3.1 *Fractogram Setup*

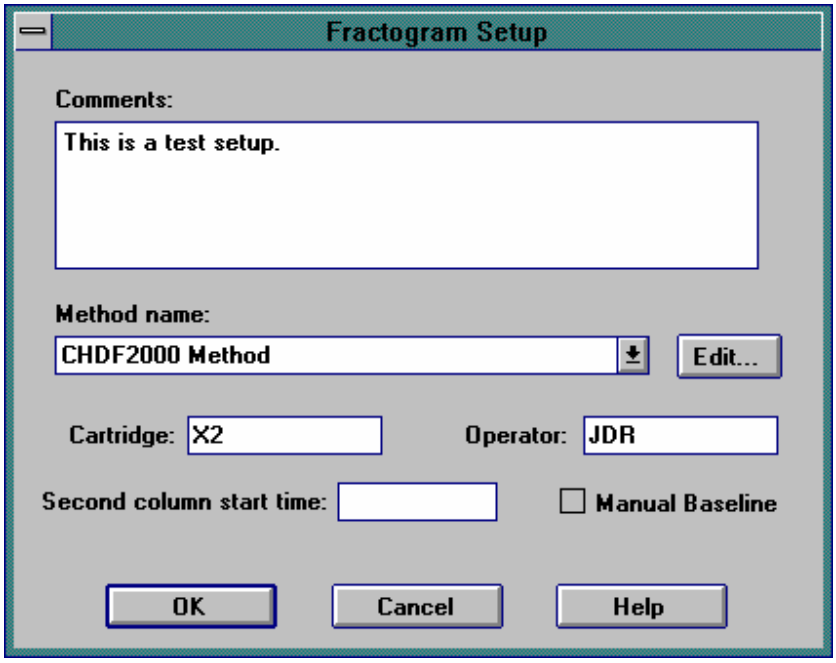
This dialog box is opened in three different ways:

1. By clicking on *View/Setup* when the active window contains a fractogram.

2. By clicking on the "Fractogram" button in the Toolbar.

3. By clicking on the *Edit* button in the toolbar when the active window contains a fractogram.

The Fractogram Setup dialog box is illustrated and discussed below.



Comments - This is used to enter information about the analysis, such as a description of the sample, or the time and day the analysis was performed. You can enter up to 5 lines of text, each line can be up to 50 characters long for a total of 250 characters.

Method Name - Click on this drop-down menu to see the names of the different methods that can be used on your system. Click on the name of the method you want to use. To edit a specific method, click on its name then click on the *Edit* button (a discussion of how to create and edit methods will be given in a later chapter).

Edit - Click on this button to edit the currently selected method. Details on how to edit methods will be provided in a later chapter.

Cartridge - This is the serial number for the CHDF cartridge you are using. This number was entered when the system was configured (see Chapter 1). Make sure this number is updated every time a new cartridge is installed.

Operator - The User ID for the person who logged onto this session of using the software. The user ID is set by clicking on *System/Change User ID*. The User ID can also be changed from the initial screen seen after the software is first loaded.

3.3.2 *Sequence Setup*

Sequence files are either created from scratch, or existing sequence files can be edited. The two processes are slightly different, and will be discussed separately below.

Creating a New Sequence File
A new sequence file can be created in two different ways.

1. By clicking on the "Sequence" button in the Toolbar.

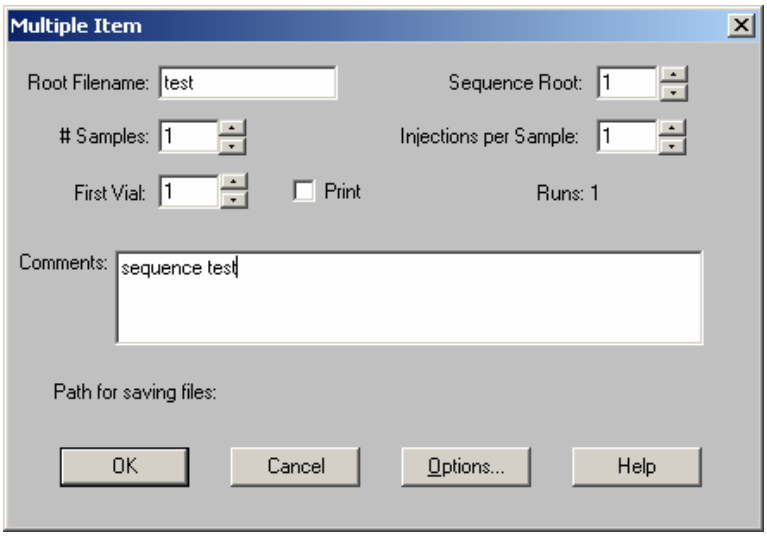
2. By clicking on the *File/New/Sequence*.

You can add a new command line to a sequence file as follows:

1. By clicking on the "Add Fractogram" button in the toolbar while a sequence file is in the active window.

2. By clicking on *Sequence/Add New Fractograms* while a sequence file is in the active window.

In any case, all these operations bring up the following dialog box.



Root Filename - All the samples run from one line in a sequence file are given the same name called the root filename. Each root name is followed by a number indicating where in the injection order a specific sample was analyzed.

Sequence Root - The number to be appended to the root filename for the first sample in a sequence. Samples run after this in a sequence are given the same root filename and an appropriate number. For example, in the dialog box above the first sample will be called SAMPL1, the second sample will be called SAMPL2 etc.

 # Samples - The total number of vials to be injected using the commands in this sequence.

Injections per Sample - The number of injections to be made from each vial.

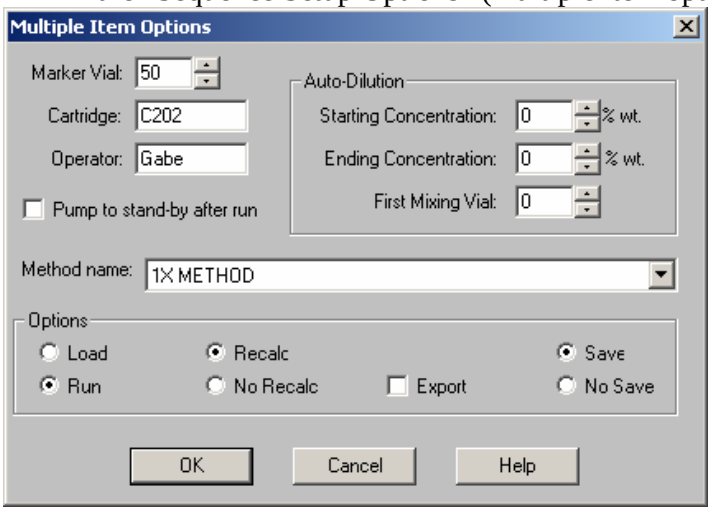
First Vial - the position in the autosampler rack where the first vial to be analyzed is located.

Runs - This is the total number of fractograms to be measured in a particular sequence of samples. This number is calculated automatically by multiplying the # of samples times the injections per sample.

Comments - allows a description of the sequence of over 1,000 characters in length.

Print - Print a report automatically (designed from the method editor) after measuring a fractogram.

Options - clicking on this button brings up a dialog box that is used to set the parameters for the injection of an individual sample. Much of the information entered here is displayed in the command lines in a sequence file. Here is what the "Sequence Setup Options" (multiple item options) dialog looks like.



Marker Vial - The number of the vial that contains the Sodium Benzoate marker.

Cartridge and *Operator* - these fields are filled in automatically using information that should have been previously entered in the system configuration and User ID dialog boxes.

Pump to Standby After Run - if checked, this command will turn off the pump after analysis of the last sample in a sequence. Generally, the pump should be left on so the instrument stays equilibrated. The only time you should have the pump turned off automatically is if the system is running unattended, and this is the last sample injected.

Auto Dilution - If you desire, you can have the autosampler dilute the concentration of your sample to the proper level for you. The three parameters listed under *Auto Dilution* determine how this sample preparation takes place. *Starting Concentration* is the percent solids of your sample before dilution. *Ending Concentration* is the percent solids you will want to have after dilution. *First Mixing Vial* is the first empty vial in which mixing of the sample will take place. You must have some empty vials available in the autosampler sample rack for this purpose.

Method Name - this field lists the method used to analyze the current sample. It can be changed by clicking on the drop down menu and choosing a new method.

Options - allows a number of actions to take place automatically after the measurement of a fractogram as follows.

Load - load a data file from disk to be printed out as part of a report rather than acquiring data with the CHDF instrument.

Run - Acquire data using the CHDF instrument.

Recalc - calculate a particle size distribution after acquiring the data.

No Recalc - do <u>not</u> calculate a particle size distribution after acquiring data.

Export - automatically export the fractogram file to a format that older versions of the CHDF software can read. These files can also be read by spreadsheets (such as Excel), word processors, and text editors since the file format is “tab delimited”.

Save - save the fractogram file to disk once it is accumulated.

No Save - do not save the fractogram file to disk after it is measured.

Editing an Existing Sequence File

Editing an existing sequence file involves loading an already existing *.SEQ file, then making desired changes to the command lines in it. For example, you can alter the command lines in a sequence file, add a new command line, delete a command line, or place a calibration sample command line into a sequence file.

There are 3 buttons in the toolbar that can be used to edit sequence files. These buttons are enabled when a sequence file is in the active window. The buttons are used to add a new command line to a sequence file, to add a calibration sample to a sequence list, or to delete command lines from a sequence file.

This is the "add fractogram" button. It is used to add a new command line to a sequence file, which will lead to the acquisition of a fractogram. Clicking on this button brings up the same sequence setup dialog box discussed above in the section on creating a new sequence file.



The "add calibration" button rebuilds the calibration curve for the purpose of updating the calibration curve. Calibration standards can be run in a given sequence prior to Calib+. These standards will replace those in the calibration curve with the same file name once Cal+ is executed. The resulting calibration is applied to further fractograms obtained during following runs. A detailed description of this function is contained in section 4.2.



This button will delete a command line in a sequence which has been highlighted by clicking on it with the mouse.

The functions performed by these three toolbar buttons can also be accessed from the Sequence menu, which is seen in the menu bar only when there is a sequence file in the active window. The details of the Sequence menu will be discussed in Chapter 4.

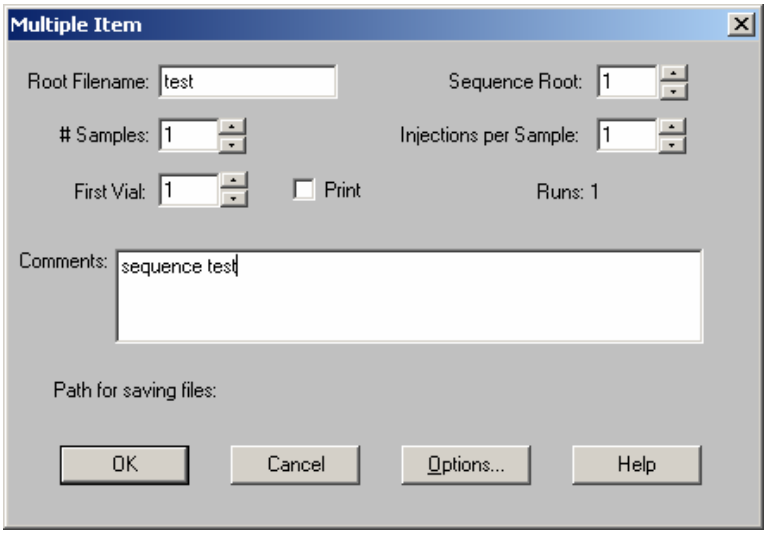
The dialog box for editing a command line files is opened in three different ways:

1. By clicking on *View/Setup* when the active window contains a sequence and a command line in the sequence is highlighted.

2. By double clicking on a command line in an active sequence.

3. By clicking on the *Edit* button in the toolbar when the active window contains a sequence and a command line in the sequence is highlighted.

The Edit Sequence Setup dialog box, which contains the title "multiple item" is illustrated below. This dialog box is similar to the one used to create sequence files discussed above.



Filename - lists the name of the fractogram

3.3.3 *Calibration Setup*

This dialog box is opened in four different ways:

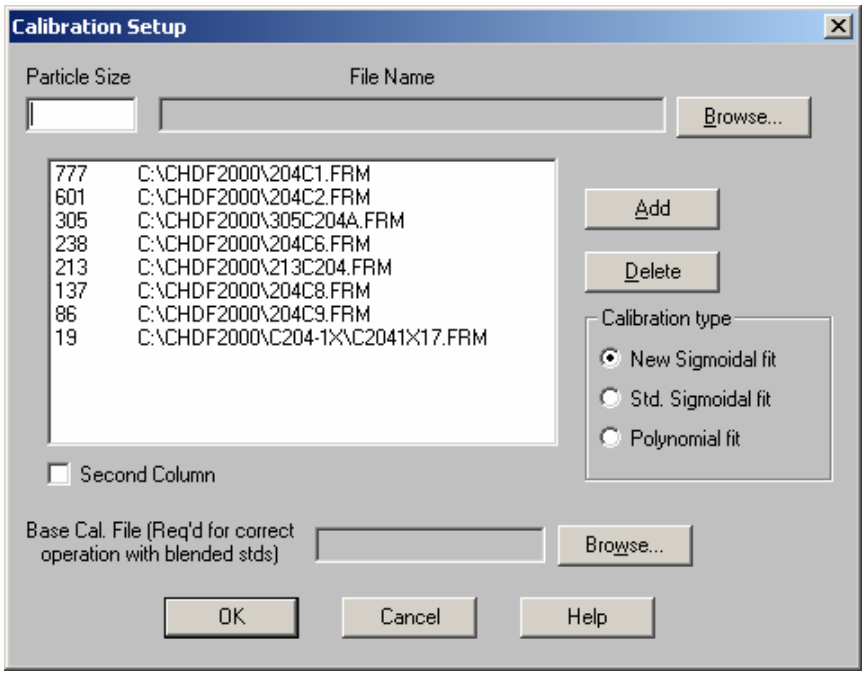
1. By clicking on *View/Setup* when the active window contains a calibration.

2. By clicking on the "Calibration" button in the Toolbar.

3. By clicking on the *Edit* button in the toolbar when the active window contains a calibration.

4. By clicking on *File/New/Calibration*.

The purpose of this dialog box is to create a calibration list, which is a list of standard fractograms to be used in generating a calibration curve. Such a curve is a plot of particle size vs. Rf, and is used to relate measured Rf to particle size for unknown samples. Standard fractogram samples usually consist of monodisperse latex beads with a known particle size. The Calibration Setup dialog box is discussed and illustrated below.



Filename - the name of a standard fractogram to be added to a calibration list.

Particle Size - the size of the particles used in generating the standard fractogram defined by *Filename*.

Calibration List - is shown in the center of the dialog box. It contains the names of the standard fractograms to be used in a calibration, along with the particle sizes of the standards.

Browse - when this button is clicked, it brings up a file selection dialog box that can be used to add new standard fractograms to the calibration.

Add - when clicked, this button adds the standard fractogram in *Filename* to the Calibration List.

Delete - once a standard fractogram name in the *Calibration List* is highlighted by clicking on it, its name can be removed from the calibration by clicking on the *Delete* button.

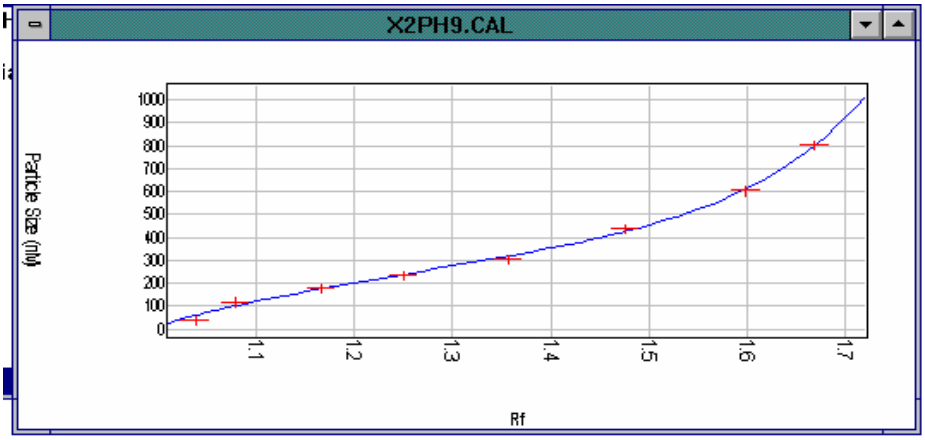
Calibration Type - You have the choice of fitting a sigmoid or a polynomial to the plot of particle size vs. Rf (the calibration curve). The sigmoid is most commonly used. It requires a minimum of 6 calibration standards. The polynomial function requires exactly 8 standards.

Second Column - Used for special "dual capillary" cartridges. The second column is the long capillary which requires its own calibration curve. Leave this checkbox blank if using regular cartridges.

Base Cal. File - stands for "base calibration file". This file is to be used when a calibration standard blend is used in place of a single particle size (for time saving).

Browse - click on this button to load a base calibration file.

Once you click *OK* in the calibration setup dialog box, the calibration curve is calculated automatically and displayed for you. This is an example of what a calibration curve looks like. Clicking *Cancel* only displays the current calibration curve without re-calculating it.



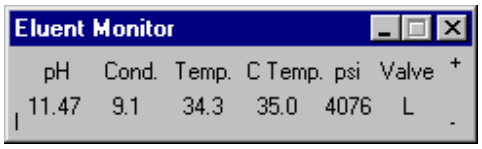
This is what a sigmoid shape curve looks like. This graph may be zoomed by clicking and dragging on it to draw a box. The region inside the box will expand to fill the window. To autoscale and see the entire graph again, click the right mouse button on the graph.

3.3.4 *Other View Menu Commands*

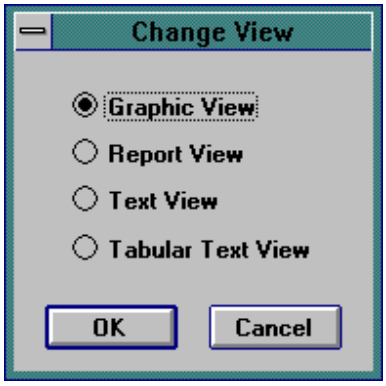
View/Toolbar - a toggle switch that allows viewing/hiding of the CHDF V.3.20 software toolbar.

View Status Bar - a toggle switch that allows viewing/hiding of the CHDF V.3.20 software status bar.

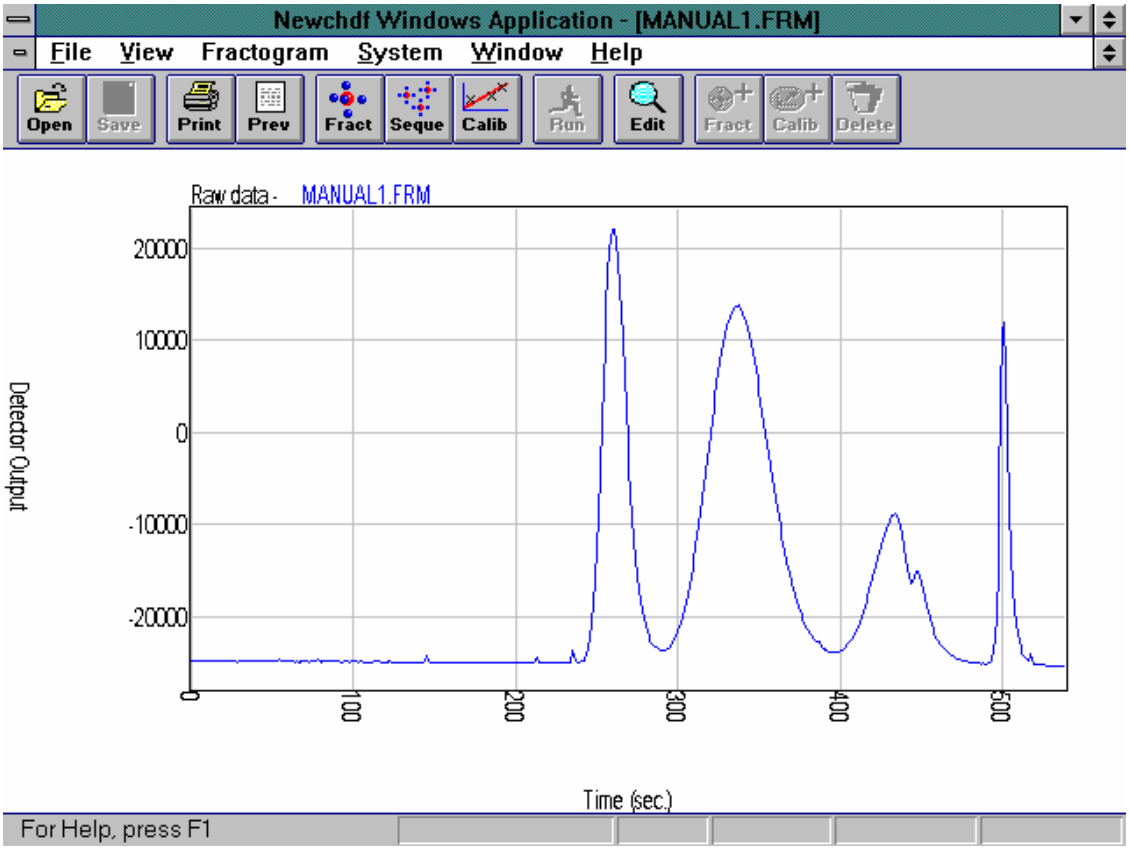
View/Eluent Monitor - a toggle switch that allows viewing/hiding of the CHDF V.3.20 software pump monitor. The pump monitor is a small window that opens to display the pump pressure in pounds/square inch. It looks like this. Note that the normal operating pressures are from 4000 to 5500 psi.



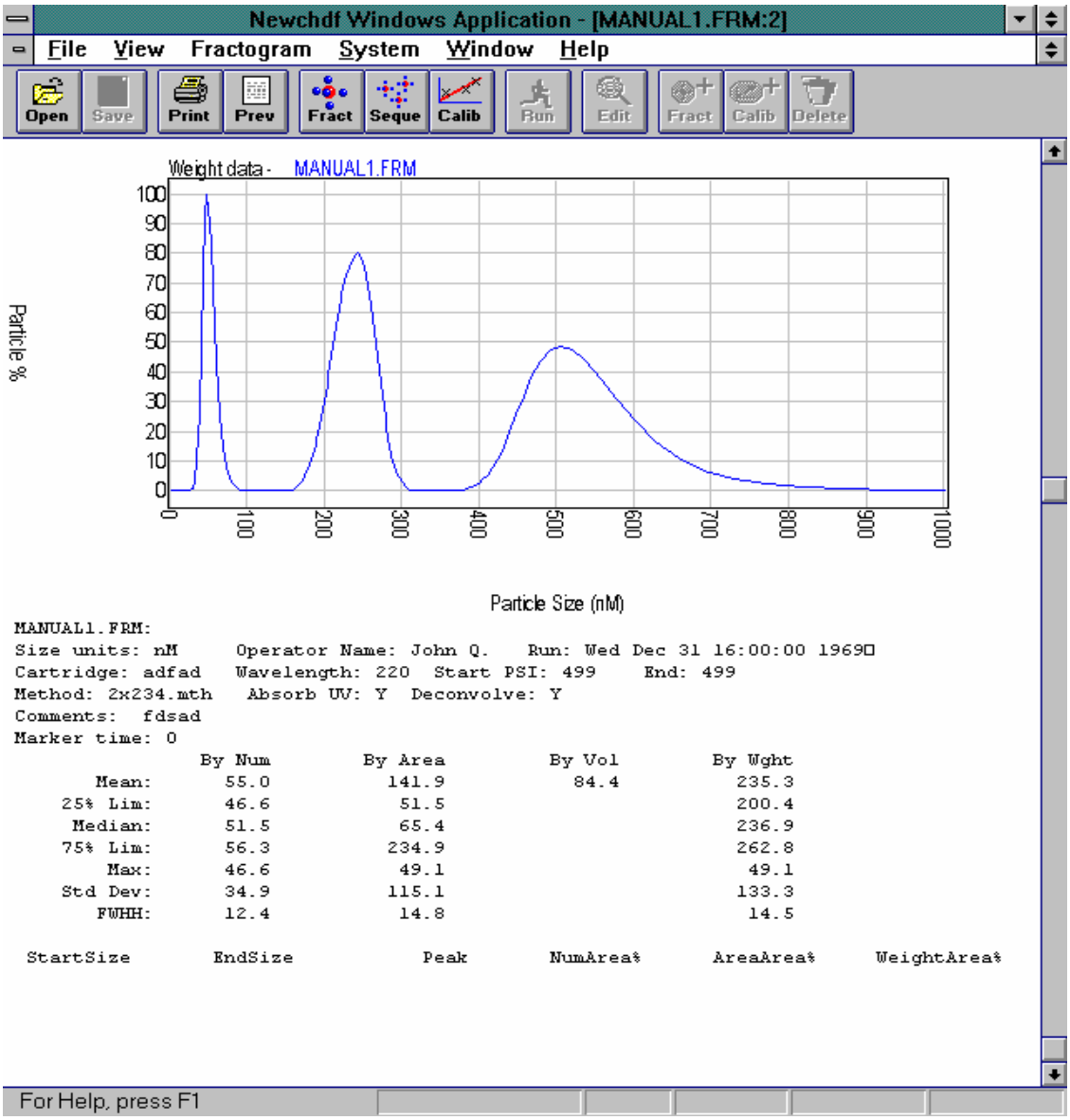
View/Create New View - Is activated only if a fractogram is in the active window. Allows viewing of fractogram data in 4 different formats as seen below. Clicking on *View/Create New View* brings up the following dialog box.



Graphic View - is the standard way of looking at a fractogram, displaying the data graphically as a plot of detector output versus time. Here is what a graphics view looks like.



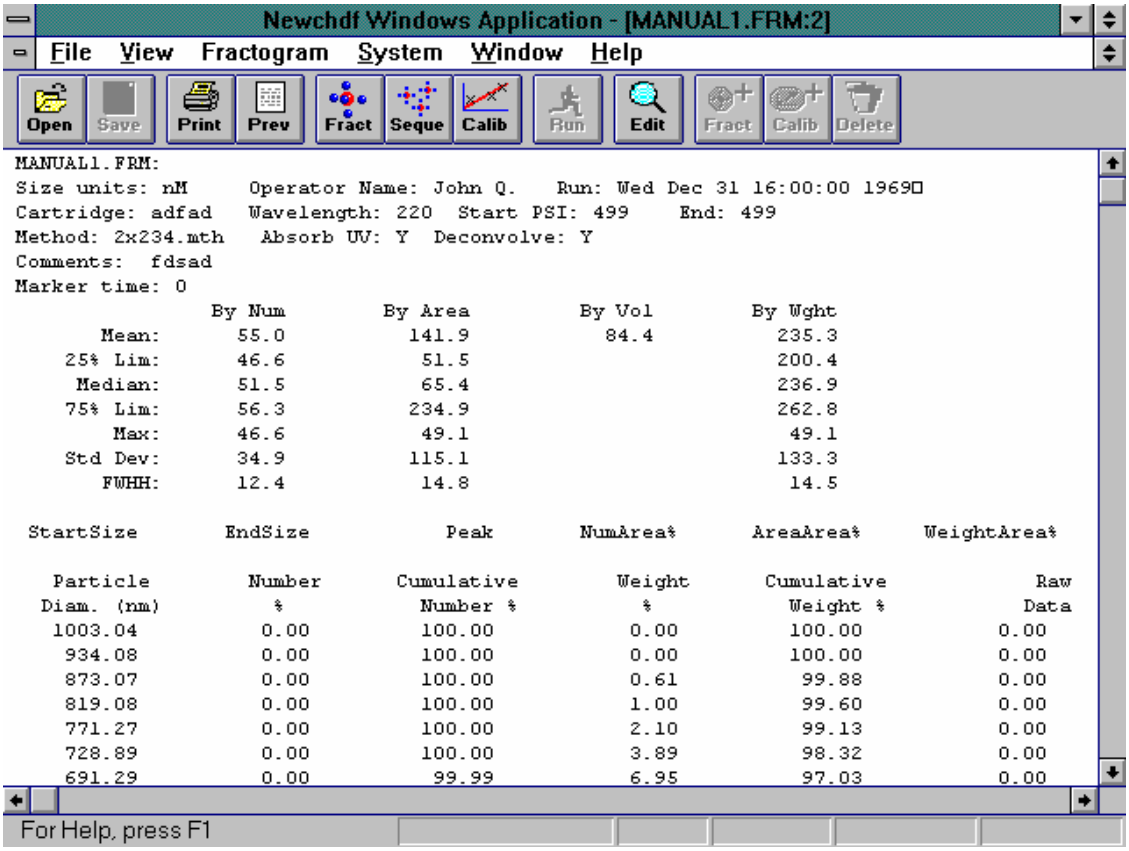
Report View - A view format that allows you to print out fractograms, various plots of particle size distribution, and textual information about the sample analysis. The contents of a report are determined using the method editor. This is what a report might look like.



View/Create New View/Text View - creates a report that consists strictly of the textual information about a view, and is identical to the bottom of the above figure.

Note: If using Text or Tabular View, you should place it a the bottom of the report.

View/Create New View/Tabular Text View - creates a report with the textual information as seen in the above figure, and columns listing all the particle size distribution data for a sample. This is a very long report, and it is not recommended that you print this out unless you need a printed record of all a fractogram's data. This is part of what a tabular text view looks like.



View/Copy Graph to Clipboard – Copies the active window to the Windows clipboard for pasting onto other applications. To copy a full report, first click on an area of the report window outside a graph or text or tabular area.

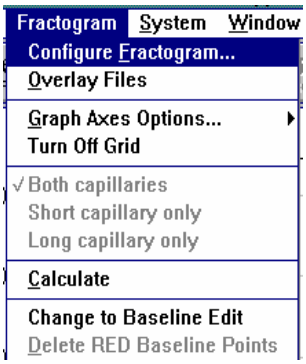
Chapter 4

Software Reference Guide

Part 2: The Fractogram, Sequence and System Menus

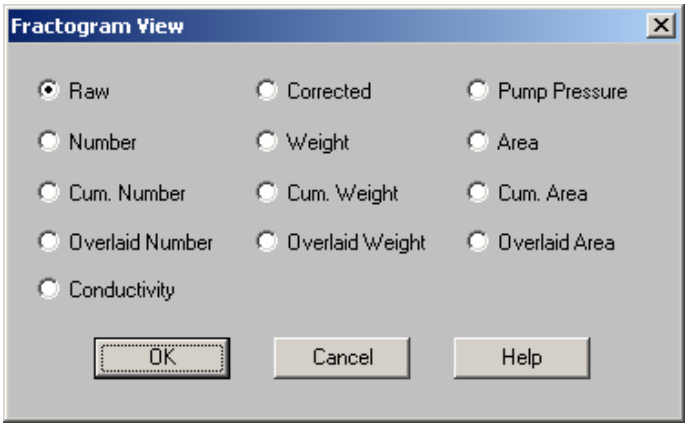
4.1 *The Fractogram Menu*

The purpose of the fractogram menu is to allow you to calculate many different types of particle size distributions from a raw fractogram. This menu also allows you to display fractograms and particle size distributions in different ways, such as overlaying two fractograms or displaying a window with or without grids. The fractogram menu is only seen in the toolbar when there is a fractogram in the active window. This is what the fractogram menu looks like.



4.1.1 *Configuring Fractograms to Create Particle Size Distributions*

Clicking on *Fractogram/Configure* brings up a dialog box that allows you to choose amongst a plethora of different particle size distributions that are calculated from a fractogram. Here are your choices.



The choices in the second row of the dialog box give *differential plots*, meaning that the Y axis values for each data point are not added together, and that each data point is different. Differential plots are *normalized*, which means all Y axis values are divided by the value of the tallest peak in the fractogram. This is why the largest peak in these plots always reads 100%. The percentage values for other data points are calculated relative to the biggest peak in the fractogram. For example, a peak that has a maximum value of 15% is 15% of the height of the tallest peak in the fractogram.

The opposite of a differential plot is a *cumulative plot* which adds together each successive Y axis value, and plots this sum versus particle size. Cumulative plots are also normalized, which is why they reach a maximum at 100%. The third row of choices in the Fractogram View dialog box all give differential plots.

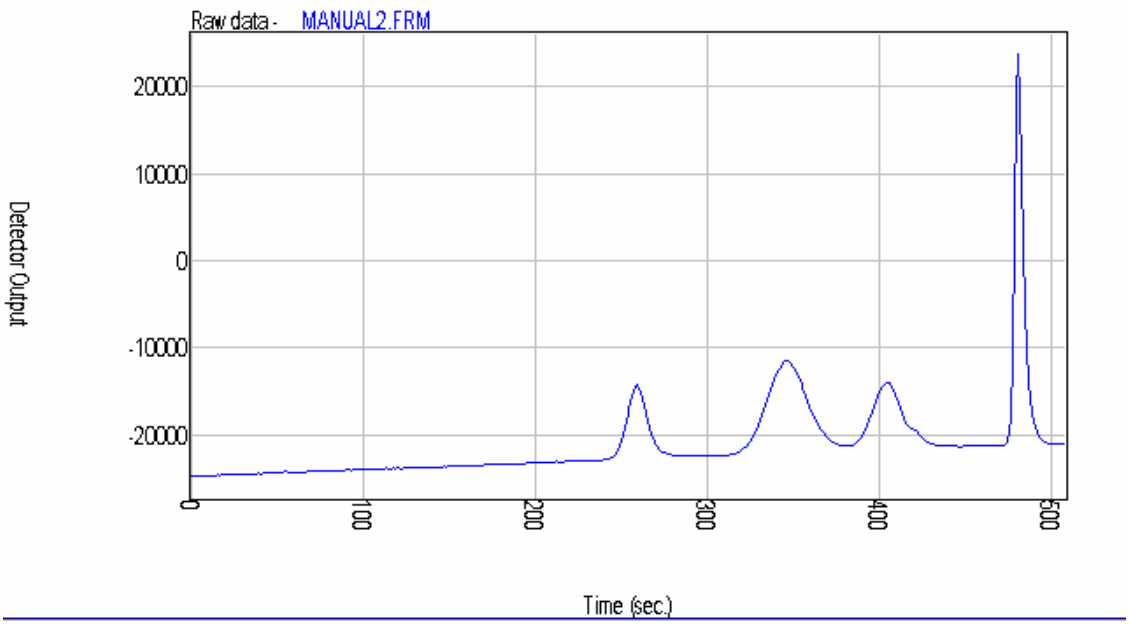
The fourth row of choices in the Fractogram View dialog box give *overlaid plots*, which display the differential and cumulative plots of a specific type of particle size distribution in the same window. For example, an overlay of the differential and cumulative plots of weight weighted particle size can be obtained.

Particle size distributions can be displayed as *number weighted, area weighted, or weight weighted*. A number weighted particle size distribution is simply a plot of normalized percent number of particles vs. particle size. The area weighted plot is calculated by multiplying the number of particles at a given size times the square of the diameter. Then, the normalized percent of particles is plotted vs. size. Weight weighted distributions are calculated by multiplying the number of particles at a given size times the cube of the diameter. Then, the normalized percent of particles is plotted vs. size. Examples of each one these particle size distributions are shown below.

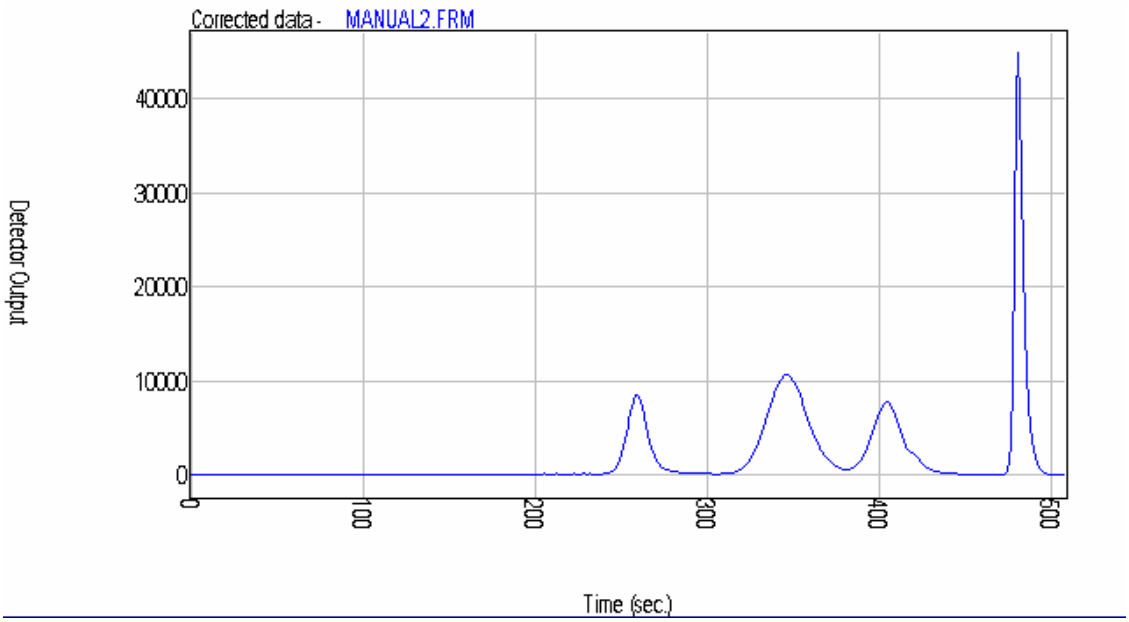
All the plots below show different ways of displaying the same fractogram file. This will give you the opportunity to determine which mode you want to use to present your data.

Following is a description of the particle size distribution plots available in the Fractogram View dialog box.

Raw - shows fractogram data as a plot of detector response vs. time, which is the raw data obtained from the CHDF instrument. This is what a raw fractogram looks like.

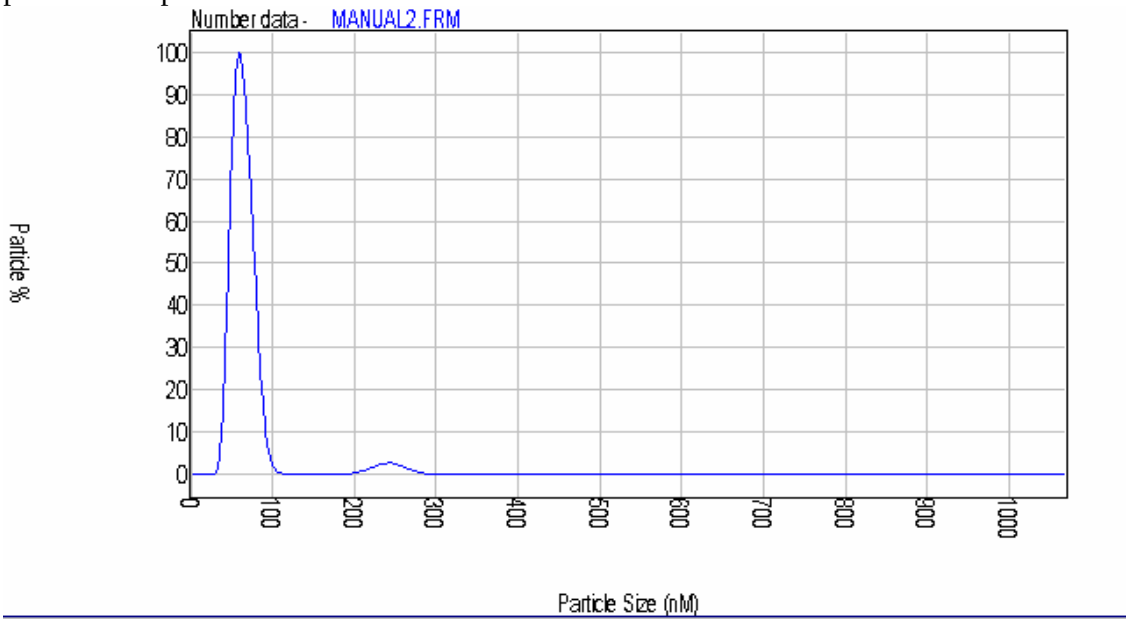


Corrected - takes a raw fractogram and baseline corrects it, and smoothes it by truncating points below a certain value (see "Noise Level" in section 1.3). This is what the above fractogram looks like after it has been corrected (note the flattened baseline, and that all data points are now above zero).

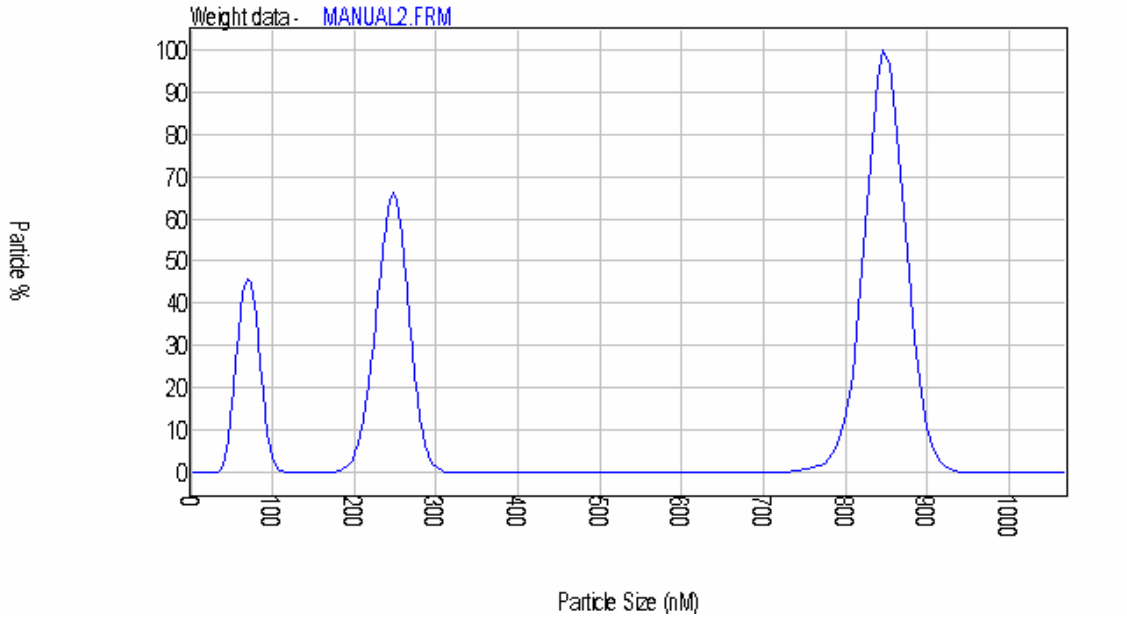


Pump Pressure - plots the pump pressure during the course of the analysis of a sample versus time. This plot can be used to ascertain whether the pump functioned properly during the course of an analysis.

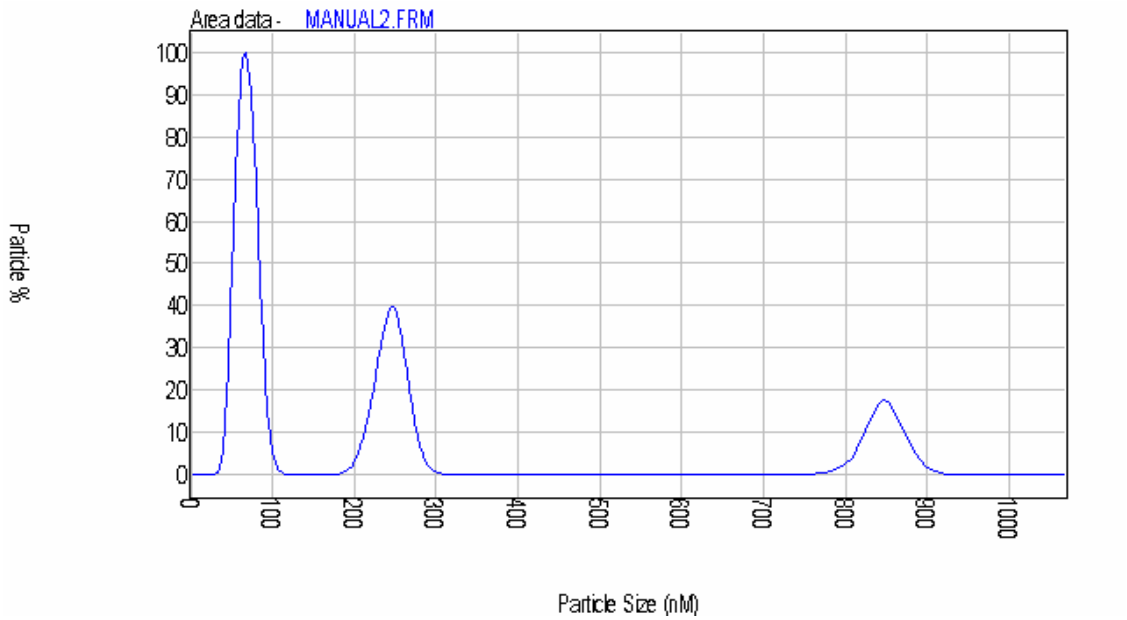
Number - This type of plot is also known as a “number weighted particle size” plot. The plot is of the number of particles of a certain size versus particle size (usually measured in nanometers). This is a differential, normalized plot as explained above. The normalization causes the Y axis units to be in percent. Here is what a number weighted particle size plot looks like.



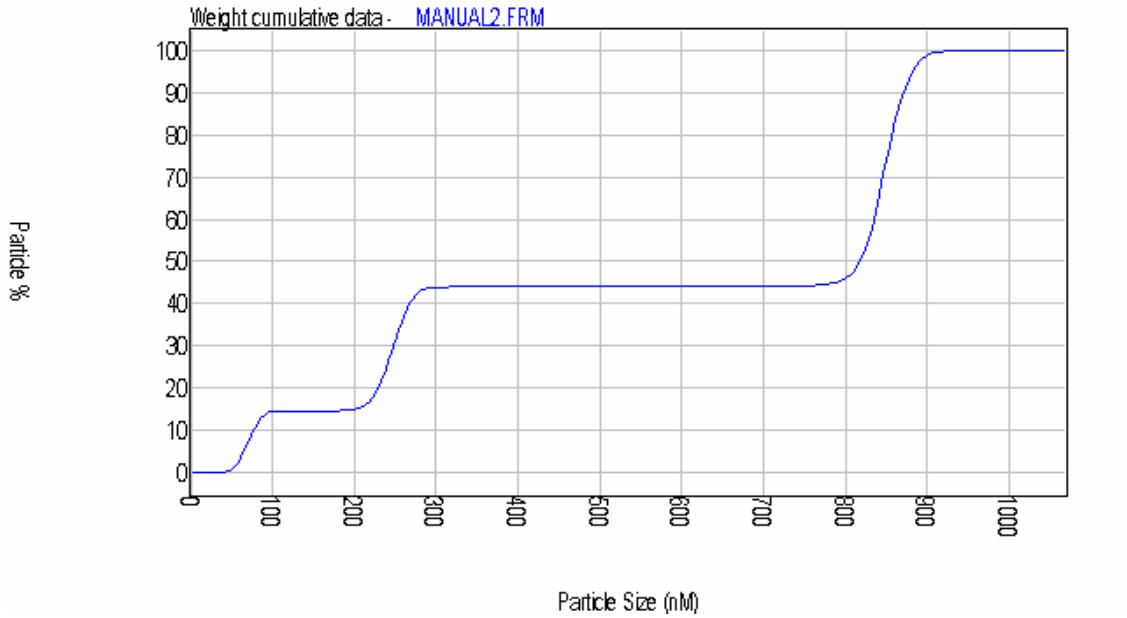
Weight - This type of plot is also known as a "weight weighted particle size" plot. This is a differential, normalized plot as explained above. The normalization causes the Y axis units to be in percent. Here is what a weight weighted particle size plot looks like.



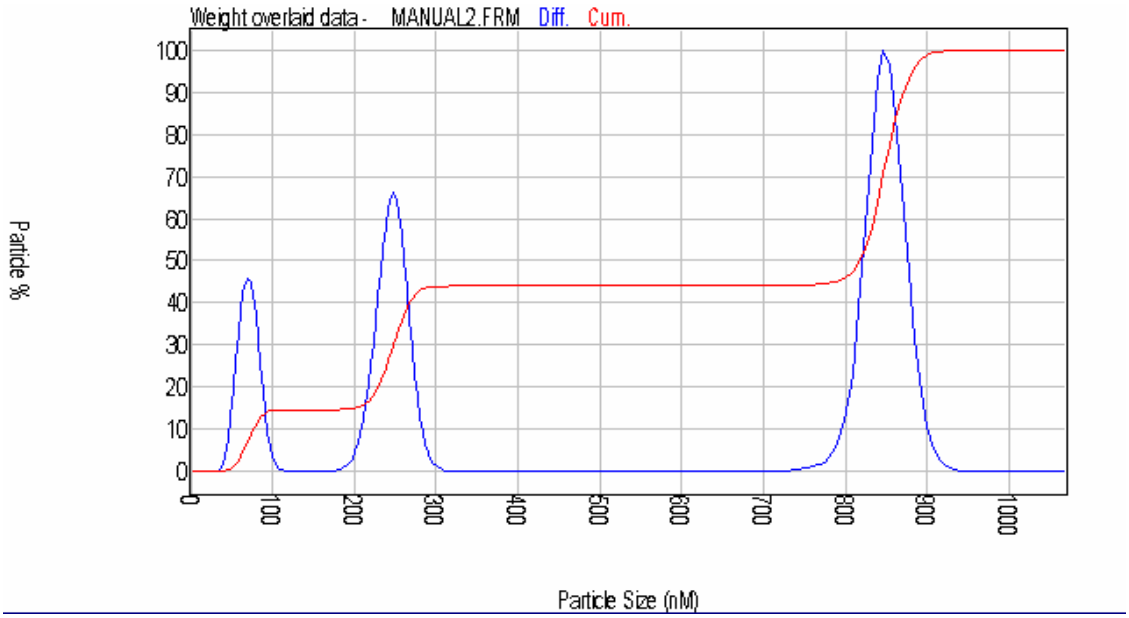
Area - This type of plot is also known as an "area weighted particle size" plot. This is a differential, normalized plot as explained above. The normalization causes the Y axis units to be in percent. Here is what an area weighted particle size plot looks like.



Cum. Number/Cum. Weight/Cum. Area - presents cumulative plots of the number weighted, weight weighted, and area weighted particle sizes. These plots are normalized, so the Y axis units are percentage, and the plot reaches a maximum at 100%. The details of cumulative plots are explained earlier in this section. This is what a weight weighted cumulative plot looks like.



Overlaid Number/Overlaid Weight/Overlaid Area - Displays both the differential and cumulative plots for a specific type of particle size distribution in the same window. For example, the cumulative and differential weight weighted particle size plots are shown below. On your computer screen, the differential plot will be displayed in blue, and the cumulative plot will be displayed in red.

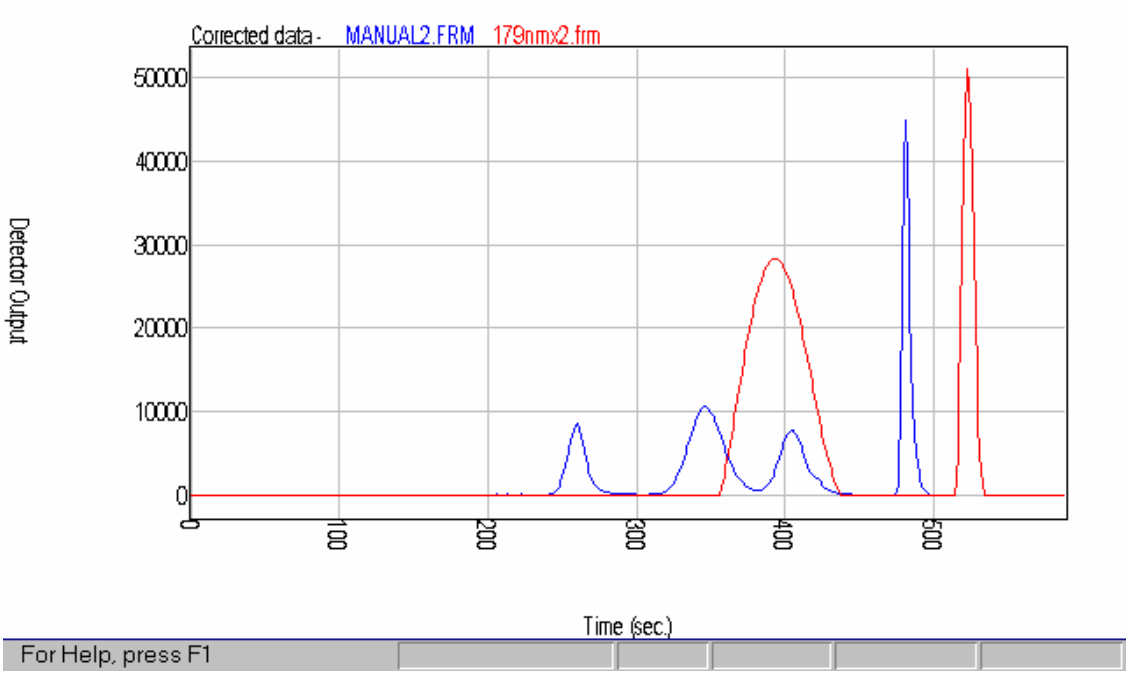


Conductivity – Displays the eluant conductivity (not the sample's). This is measured by the internal eluant conductivity/temperature sensor.

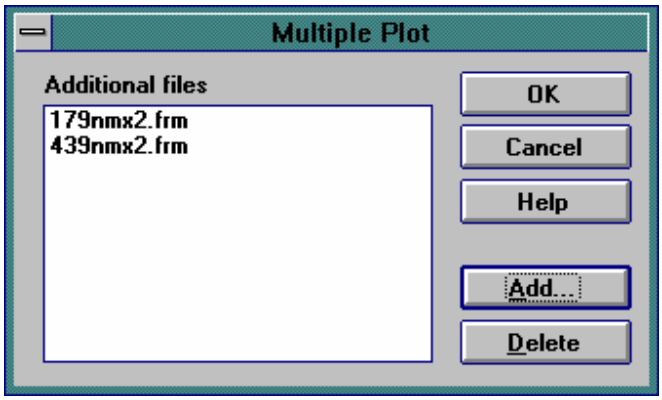
4.1.2 *Overlaying Fractograms*

By clicking on *Fractogram/Overlay Files* multiple fractograms can be displayed on top of each other in the same display window. This mode of viewing data is useful in trying to compare and contrast one fractogram with another. You can view up to five overlaid fractograms. Viewing more than five is possible, but the display becomes very confusing if you exceed five. Each fractogram overlaid in a display window is shown in a different color. The first one is red, the second blue, the third is black, the fourth is green, and the fifth is purple. The name of each fractogram is displayed in the appropriate color at the top of the display window.

This is what two overlaid fractograms look like.



The dialog box that appears when *Fractogram/Overlay Files* is clicked shows a list of files to display in the active window. The files listed in this dialog box are displayed in the active window in addition to the fractogram already in the window. You can add and delete files from this list as desired. Here is what it looks like and how it works.

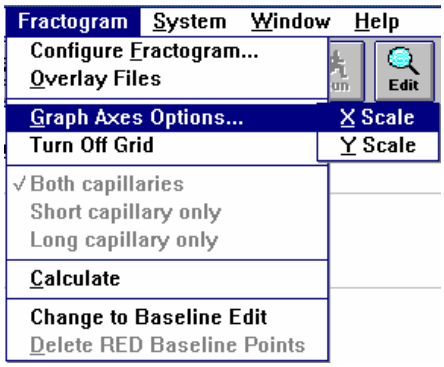


Add - clicking on this button brings up a file selection dialog box, allowing you to pick fractograms from any directory and any hard drive for display. You can only add one fractogram at a time to a list, so creating a list with multiple fractograms means clicking on the *Add* button multiple times.

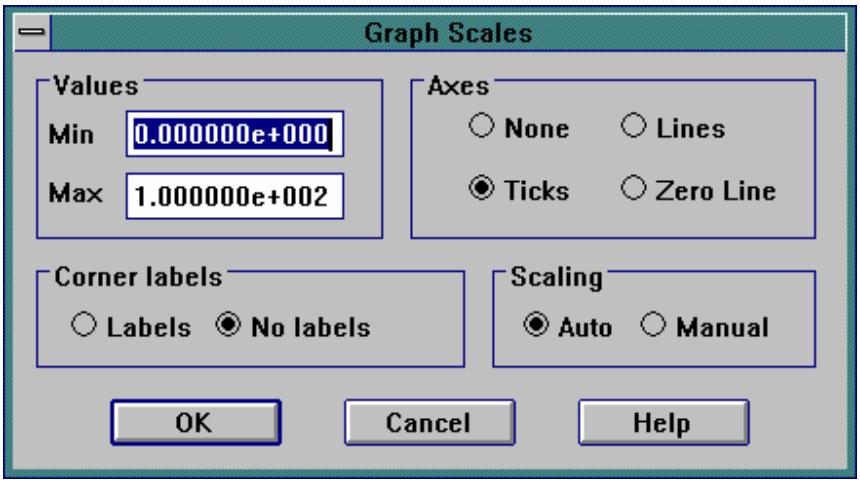
Delete - is used to delete a file from a display list. Simply highlight the file's name by clicking on it, then click the *Delete* button to remove its name from the list.

4.1.3 *Graph Axes Options*

When you click on *Fractogram/Graph Axes Options* a pop-out menu appears as shown below.



Clicking on *X Scale* or *Y Scale* brings up the same dialog box, which is shown below.

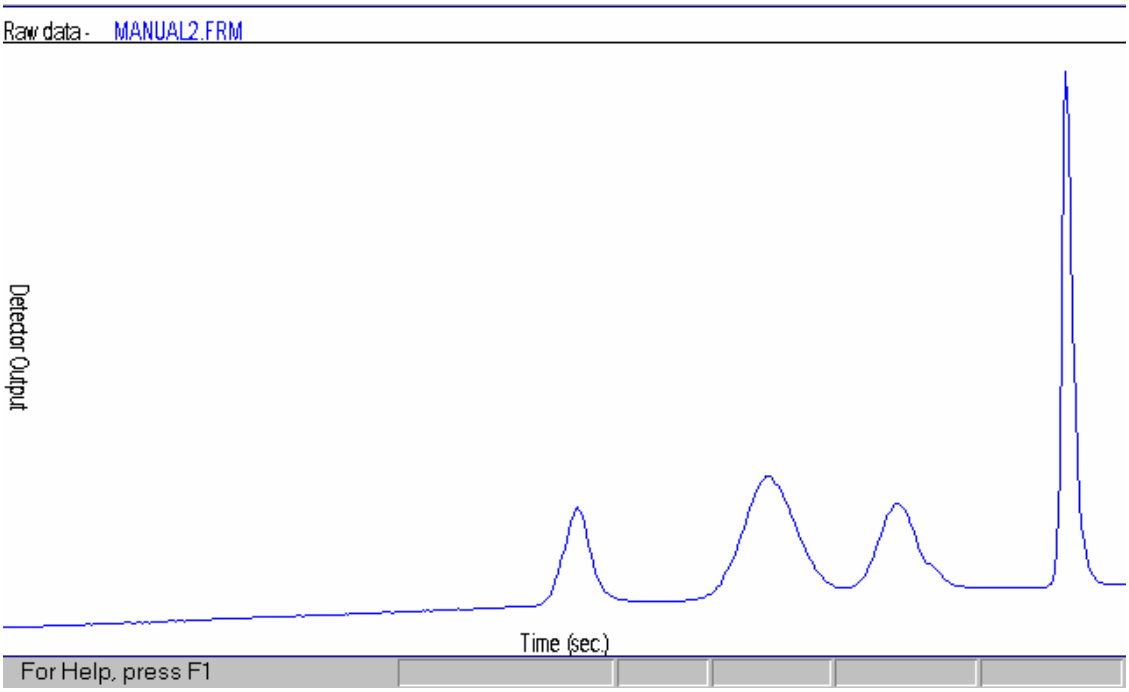


This dialog box is used to determine how the axes in a fractogram display are labeled and presented. The dialog box sets parameters for either the X or Y axis depending on which axis was chosen in the pop-out menu. Only one of these axes is affected by changes made in this dialog box.

Scaling - determines whether the axes display limits are set manually by the user, or if they are set automatically by the software. When set to *Auto* the display limits are set automatically, and the numbers in the *Min Value/Max Value* fields are ignored. If set to *Manual*, the *Min Value/Max Value* fields are used to determine the axis display limits.

Min Value/Max Value - Sets the display limits for an axis. For instance, if you wanted to display data on the X axis from 0 to 100 seconds, type these numbers into the *Min Value/Max Value* fields. These values will only take effect if the *Scaling* is set to "Manual". If this parameter is on "Auto", the min and max values will be ignored, and the display will be scaled automatically. The change in display limits occurs after you press *OK*.

Corner Labels - When this parameter is turned on, the tick marks and labels for a particular axis are labeled with numbers. If this parameter is turned off, the tick marks disappear. The following figure is what the display looks like with the corner labels turned off for both axes.



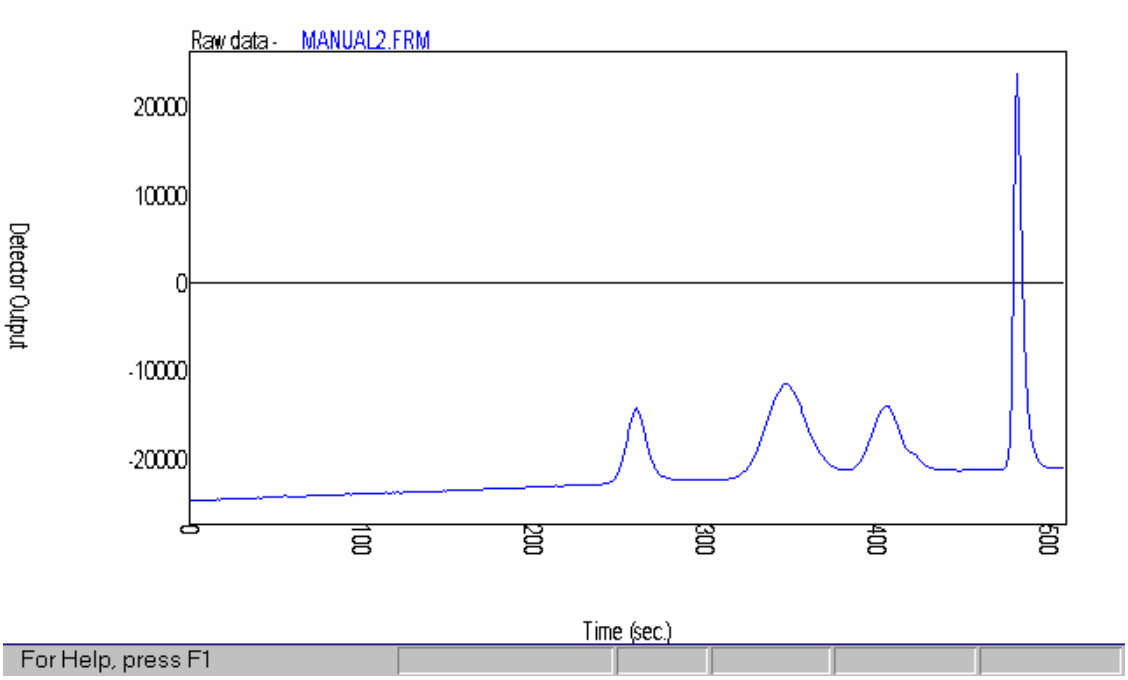
Axes - determines the appearance of the X and Y axes as follows.

None - no tick marks or grid lines are marked on the fractogram display.

Lines - Adds grid lines perpendicular to and passing through the axis being changed. For example, for the X axis evenly spaced lines are drawn on the fractogram parallel to the Y axis.

Ticks - places or removes tick marks from the axis being changed.

Zero Line - Draws a line at the zero point perpendicular to and passing through the axis being changed. For the X axis, this line usually falls on top of the Y axis, so it is difficult to see. Below is an example of a Y axis zero line.



4.1.4 *Other Fractogram Commands*

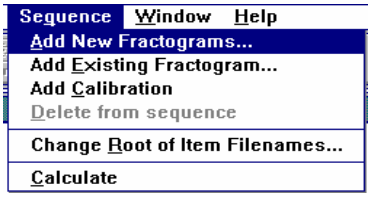
Turn Off Grid/Turn On Grid - a toggle that adds or removes a set of grid lines from the fractogram display. This toggle overrides the *Graph Axes Options/X* (or Y) *Scale/Axes Parameters.* You may need to click on the toggle twice to get grid lines to appear.

Calculate - is used to recalculate fractogram data using a different calibration curve and/or method than was originally used. The method must be set by clicking on *View/Setup* to obtain a fractogram setup dialog box.

Change to Baseline Edit/Delete Baseline Points - this is a function to manually correct a fractogram for baseline slope or curvature. This feature will be enabled in a future version of your CHDF software.

4.2 *The Sequence Menu*

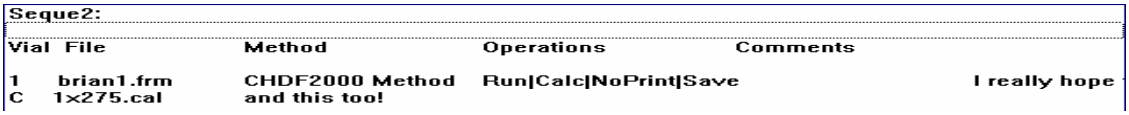
The sequence menu appears in the menu bar only if there is a sequence file displayed in the active window. This menu is used to add, edit, and delete lines from a sequence file. All the functions in this menu can be accessed from other places in the software. For example, the three sequence editing buttons in the toolbar perform the exact same function as *add new fractograms*, *add calibration*, and *Delete* from the sequence menu. The reader is referred to section 3.3.2 of this manual for detailed descriptions on how to create and edit sequence files. Here are the commands available in the sequence menu.



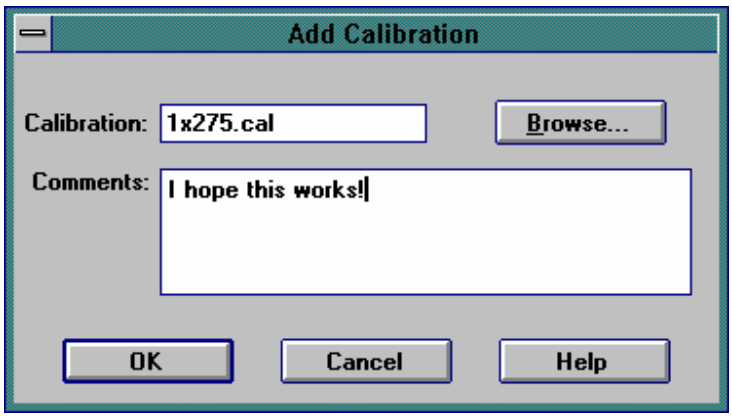
Add New Fractograms - allows creation of a new command line in a sequence file. Brings up the same dialog box as pressing the "Fract. Plus" button on the toolbar (see section 3.3.2).

Add Existing Fractogram - allows an already existing fractogram file to be added to the sequence list so its report can be printed out in a batch along with the other fractograms accumulated using this sequence file. Choosing this command brings up a file selection dialog box, allowing you to choose any fractogram you desire.

Add Calibration - allows the addition of "check calibration" samples to an autosampler run. This command performs the same function as the "Add Calibration" toolbar button. Fractograms generated from check calibration samples replace calibration standards (with the same file name) contained in an already existing calibration. The resulting calibration is applied to further fractograms obtained during subsequent runs. The name of the calibration file comes after that of the check calibration sample in the sequence file list. It is given a vial number of "C" as shown here. Also note that the calibration comment falls in the wrong column.



To add a calibration to a sequence file, click on *Sequence/Add Calibration*, which brings up an add calibration dialog box as follows.



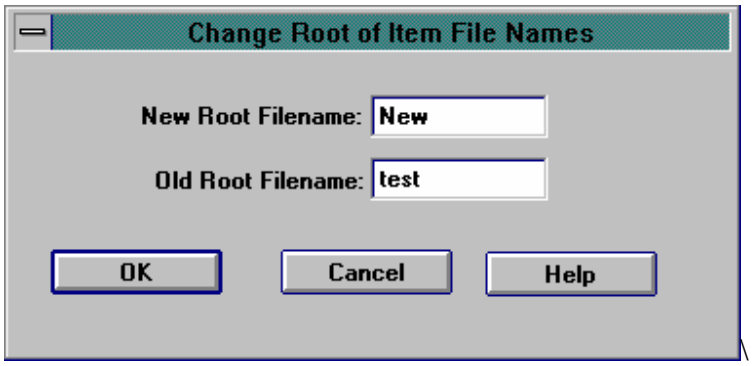
Browse - click on this button to bring up a file selection dialog box, where you choose what calibration to add to the sequence list. The name of the file you select will appear in the *Calibration* field.

Calibration - the name of the calibration file to be added to the sequence file list. It is selected by clicking on the *Browse* button.

Comments - information about the calibration file that will be included in the sequence file list.

Delete From Sequence - this menu choice is only enabled when a command line in a sequence file is highlighted. Clicking on this command will cause the highlighted command line to be deleted from the sequence file. This command performs the same function as the "Delete" button on the toolbar.

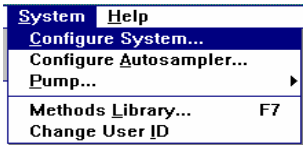
Change Root of Item Filenames - This command is used to change the root name for items in a sequence list. It is useful if you want to use the same sequence to run two sets of samples, and the only thing different between the runs is the rootname of the files. Choosing this command brings up the following dialog box:



First highlight the samples in the sequence you want to rename, then type in the new root name, all the items in the sequence will be updated automatically.

4.4 *The System Menu*

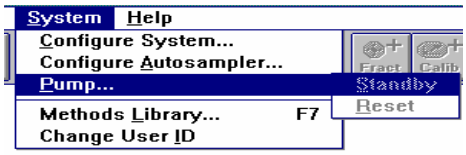
The system menu is used to configure your CHDF instrument and autosampler, to turn the pump on and off, to add or delete user's from the system, and create and edit methods. Here are the commands available in this menu.



Configure System - is used to record important system information about the CHDF instrument. This function is covered in detail in section 1.3.

Configure Autosampler - is used to record important information about the autosampler attached to your CHDF instrument. This function was covered in detail in section 1.4.

Pump - when clicked, brings up the following pop-out menu.



Standby - stops the pump.

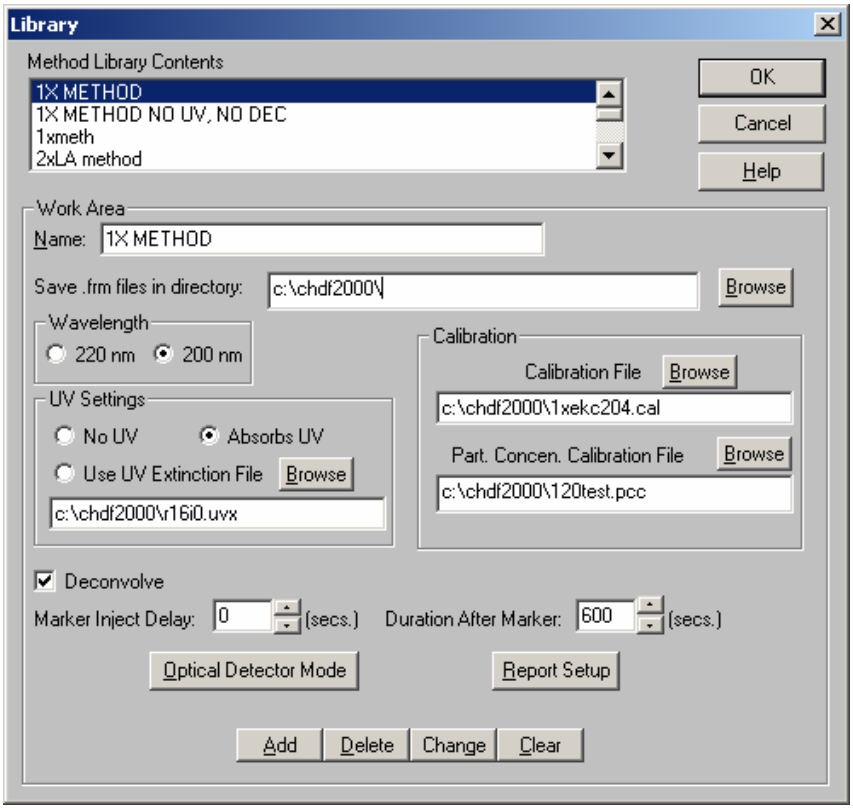
Reset - starts the pump.

Note: If you turn the pump off using the front panel of the instrument, you must turn it back on by clicking on *System/Pump/Reset*.

Change User ID - is used to add or remove user names from the system. This is covered in detail in section 1.3.

4.4.1 *Creating and Editing Methods*

A method contains important information on how to analyze a sample. You must create a method and a calibration for each and every sample that you run on your CHDF instrument. The method includes the calibration file name, therefore, the method links the parameters used to obtain a fractogram with the calibration information needed to calculate the fractogram. All methods created using your CHDF V2.0 software are kept in a file called METHLIB.ARC. This file should be kept in the same directory as the *.EXE file used to run this software package. A new method may be created, or an existing method modified, by clicking on *System/Methods Library*. The following dialog box appears.



Method Library Contents - lists the names of all the existing methods stored in the file METHLIB.ARC. To edit an existing method, scroll through the list and click on its name. The other fields in the dialog box will be filled in automatically with pertinent information about this method.

Work Area - the section of the dialog box where the parameters that are saved into a method file are displayed and changed.

Name - the name of the current method being edited. To edit an existing method, highlight its name in the *Method Library Contents* field, and its name will appear in this field. To create a new method file, put a new name into this field. Then, click on the *Add* button to add this method to the file METHLIB.ARC.

Save .frm files in directory – only useful for auto-sampler operation. Enter folder where frm files will be automatically saved.

Add - Adds a new method to the list of methods stored in METHLIB.ARC. The name given to this new method must be typed into the *Name* field.

Delete - clicking this button will delete a method whose name is highlighted in *Method Library Contents*. Be sure you want to delete a method when pressing this button, since there is no warning telling you the method is about to be deleted.

Change - Automatically saves a changed method under the current method's name.

Clear - sets all the parameters in the *Work Area* to their default values.

Wavelength - Selects the wavelength used in the particle extinction cross section computations. Make sure this wavelength agrees with the wavelength setting made using the firmware on the instrument.

Absorbs UV - activate this parameter if your sample particles absorb UV light at the wavelength being used for detection.

Use UV Extinction File – activate this parameter if you prefer to use your own extinction cross section curve instead of the approximation "UV-Yes or –No". Click Browse to select your UVX file.

Deconvolve - if checked, your fractogram will automatically be deconvolved. This process involves mathematically enhancing the resolution of the data to help pull apart overlapped peaks.

Marker Inject Delay - the amount of time the autosampler should wait after injecting the sample to inject the marker. Ideally, the marker should be injected one minute after the sample. However, practical experience shows that autosamplers take about a minute to

inject the marker anyway. Therefore, if this parameter is left at 0, there should still be a minute delay between sample and marker inject.

Duration After Marker - this parameter applies to manual and autosampler injections. It determines how long the instrument will continue to measure data after the marker has been injected. This should be set for a minimum of 600 seconds, because this is how long it usually takes the marker to pass through the system.

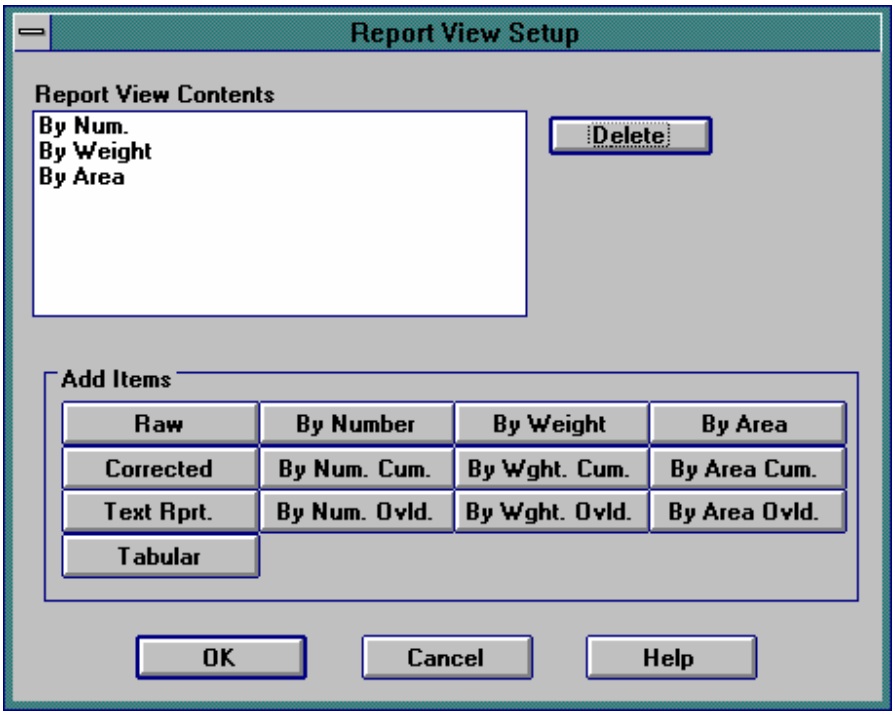
Cartridge/Calibration Files - this tells the software what calibration curve to use when analyzing samples using this method. To pick a calibration file, click on the *Browse* button, a file selection dialog box will appear allowing you to select any calibration file. 2-column is used only for special dual-capillary cartridges.

Optical Detector Mode - brings up a dialog box containing parameters that can be used to detect peaks in a fractogram.

4.4.2 *Generating and Customizing Reports*

The CHDF V.3.20 report editor is loaded by clicking on *System/Methods Library/Report Setup*. A method must be selected before clicking on the *Report Setup* button. A report contains particle size distributions in graphical and tabular formats. You can choose to have some, all, or none of the available particle size distribution plots included in your report. Reports can be printed out automatically after autosampler injection of a sample. The ability to turn automatic report generation on and off is obtained by first having a sequence file in the active window. Then, highlight a command line in the sequence file and click on the toolbar *Edit* button, then *Options* and make sure there is a mark in the *Print* checkbox. More details on sequence file setup is given in Chapter 3.

Clicking on *System/Methods Library/Report Setup* brings up the following dialog box.



Report View Contents - this is a list of particle size distributions that will be plotted in a report. Each one of these items takes up 1/3 of an 8 1/2" by 11" sheet of paper (except for the tabular report, which can take up many pages), so only 3 of these items can fit on one page. Add items to this list by clicking on the different particle size distribution names shown in the dialog box. Delete items in the list by highlighting them and clicking the *Delete* button.

Add Items - Click on any of these buttons to add a specific type of particle size distribution plot to a report. The different types of plots are explained in section 4.1.1.

Appendix 1

Installation Instructions for Existing CHDF-1100 User's

If you have a CHDF-1100 instrument, and are currently using a DOS (Asyst) version of CHDF software, you must follow the installation instructions listed here.

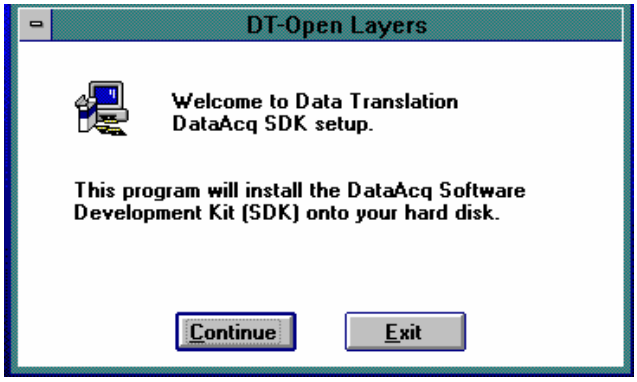
Note: if you own a CHDF-2000 unit, or are already using CHDF Windows software, go to Chapter 1 for installation instructions.

The software will come shipped to you on three floppy disks. Boot up your computer, start Windows 3.1, then follow these instructions.

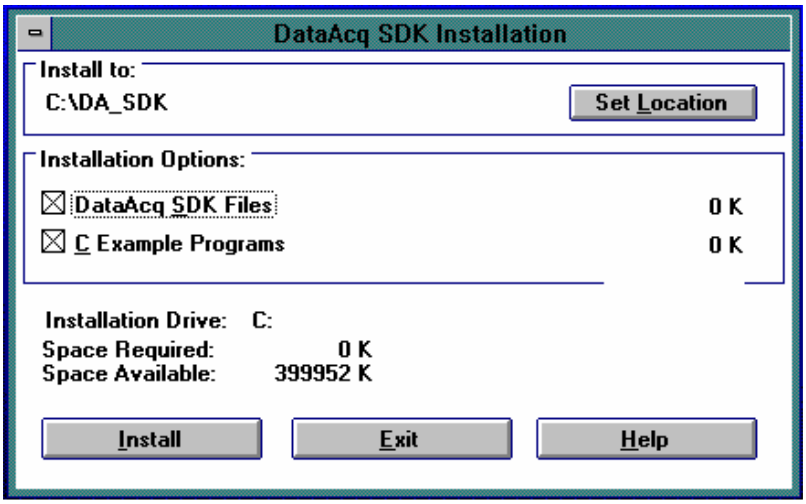
1. Insert Disk 1 ("SDK disk) of the software into the floppy drive of your computer.

2. From the Windows Program Manager, click on *File/Run* and type in the following: **A:setup**.

3. After an "Initializing Setup" message is displayed, the following dialog box appears.

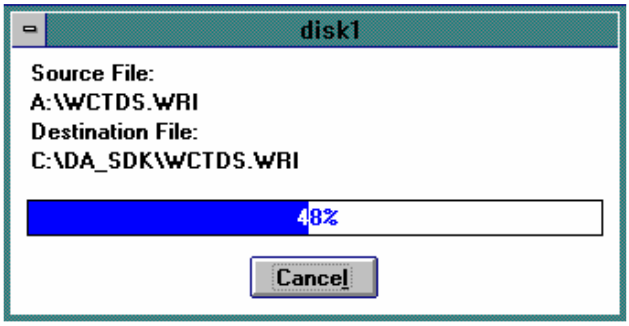


4. Click on *Continue* and the following dialog appears:

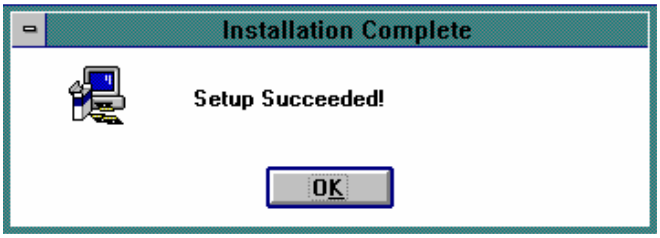


Click on *Install* to install the software.

5. A screen similar to the following will appear during software installation, allowing you keep track of the installation's progress.



6. When the first disk is done installing, it will show the following if the installation is successful.



7. Place the second install disk ("Driver disk) in your floppy disk drive. From the Windows Program Manager, click on *Control/Drivers/Add*. Double click on "Unlisted or Updated driver", type **A:** in the ensuing dialog box to tell Windows where the driver is located, then click on *OK*. When "Add Unlisted DT-Open Layers" appears, click *OK*. When the DT-2801 Installation Window appears, enter the name "CHDF" in the board name field (Note: your software will not work if the board is not named properly). Click on *Add*. When prompted "A DT-2801/5716 was found, Add it?" click on *Yes*. Click *Close* several times until all dialog boxes are closed, and you have returned to the Windows Program Manager.

8. Place the third install disk ("CHDF disk") in your floppy disk drive. Using the Windows File Manager™, copy the software's executable file, CHDF.EXE (mCHDF.EXE for manual operation units) to any directory on your hard disk. It is recommended that you give the software its own directory, such as "C:\CHDF". You can create a directory from the Windows File Manager.

9. Create an icon to load CHDF.EXE using the *File/New* command in the Windows Program Manager. The instructions for this are given in steps 2 through 6 on pages I-3, I-4, and I-5.

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