NBSIR 87-3669

System of Hardware and Software Developed for Size Exclusion Chromatography

B. Dickens and F. L. McCrackin

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U.S. DEPARTMENT OF COMMERCE, C. William Verity, *Secretary*
NATIONAL BUREAU OF STANDARDS, Ernest Ambler, *Director*

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DISCLAIMER

This system of programs has been written to allow size exclusion chromatography (data collection and data analysis) to be performed in the Polymers Division of the National Bureau of Standards using the MS-DOS system of computers. The programs are under development. This "manual" accompanies version 1.00 of the programs. The authors reserve the right to make changes in the programs and in the manual without the necessity to inform users. The authors do not warrant that the programs are correct, and indeed they intend to make corrections and enhancements as the programs evolve.

Users are requested to inform the authors of errors in calculation and cases where the programs do not do what is expected. Within our modest capability to supply copies of disks, the programs will be made available to all who are interested. The programs should not be sold other than by N.T.I.S.

SYNOPSIS OF PROCEDURE

First, run the program SECMENU and select "Enter parameters for data collection" to go to the SETUP program. Information on the identity of the specimens is provided to the system of computer programs in this way and is kept in a small data base on the hard disk. You may create a new data base, or update the existing data base. To create a new data base, use the "Provide new default parameters" route in SETUP. To update the existing data base, use the "Change titles, run times, etc." option in SETUP. Save the new information on the hard disk as a new specimen data base, then exit from the SETUP program.

Get the chromatograph ready to run. When you are ready to collect data, select the option "Begin data collection" in the main menu. Data collection will start when the computer receives a signal, known as a "trigger", from the chromatograph. The data will be graphed on the screen as they are measured. A better plot will be provided after data collection has been completed if you so specified in the "Processing" option in SETUP.

After data have been collected from all the specimens in the chromatograph, the results may be examined further. Some processing may already have taken place automatically if you so specified in SETUP. For this to occur, you must have a sample file on the disk which has already been treated manually in the manner in which you want the computer to treat further files of that type automatically. Select the main menu option "Analyze a chromatogram interactively" to process chromatograms manually (interactively) using the program ANALYZE. This option allows the user to calculate molecular sizes and molecular weights. You may select "Compare two chromatograms" to examine two chromatograms visually and calculate a difference chromatogram using the program COMPARE. In the option "Look at up to 9 chromatograms simultaneously", the chromatograms may be visually compared, cut, and normalized using the program COMPARE9. The Table of Contents lists all the programs in the system.

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INTRODUCTION

The objective of this work was to adapt the technique of size exclusion chromatography to the point where it can be easily and routinely applied to monitor molecular size distributions in materials.

Reliable quality control procedures are needed to help reduce and correct variations which arise in manufacturing processes. Except for a few research type materials made using very special techniques, the component molecules of polymeric materials exist in distributions of sizes. Resin manufacturers typically monitor the viscosity of the resin as a check on the degree of polymerization. Although the viscosity of a liquid with several components is determined by the molecular sizes of the components of the liquid, the viscosity is not an accurate measure of the molecular sizes. In fact, the viscosity of a multi-component liquid can be adjusted by adding more solvent to decrease the viscosity or more high molecular weight material to increase the viscosity, as the case requires.

However, since the relative amounts of the different molecular sizes have a strong bearing on the physical properties of the material, it is important to monitor any changes in molecular size distributions in polymeric and pre-polymeric materials and to include the distribution of molecular size in the specifications. Such distributions of molecular sizes are well measured by size exclusion chromatography. Therefore, we have developed a computerized system which will allow size exclusion chromatography to be applied routinely and which will help to tighten specifications on procurements.

HARDWARE

Computer

The computer is a "turbo" IBM-XT type with 640K memory on the mother board, a 4.77/8MHz switchable clock rate, a 20MB internal hard disk drive, two 360K floppy disk drives, a calendar and clock, two serial ports, a keyboard with separate numerical and cursor control pads, a hard reset button, and the 8088-2 ("turbo") processing unit with 8087-2 math chip. The computer has eight expansion slots and a 135 watt power supply. The screen operates in both text and IBM color graphics adapter modes. The operating system is IBM DOS 3.1 The programs will run under MS-DOS 2.xx and 3.xx. The programs do not require the math chip. A BASIC interpreter such as IBM BASICA or GWBASIC is required for the current version of the SETUP program. The other programs are compiled.

Interface card

The interface card in the computer converts the analog signals from the chromatograph into numbers which are available to the computer. The card we have installed and developed the programs for is the Strawberry Tree ACPC-16-8-T11, which is of the integrating type and measures to 18 bits resolution. We do not mean to imply that this card is the best for the purpose, nor do we intend to compare the various types of cards available. The features on the card we have selected fit our needs. It digitizes at least 2 signals from the chromatograph to a resolution of 18 bits, detects changes in TTL levels to trigger the beginning and end of data collection. The card is supplied with a driver for use with BASIC programs. The card comes with a terminal box to which connections may conveniently be made, and the source code for the card driver is made available by the manufacturer. We are using the card to digitize low level signals (less than 250 mv), at which level it performs acceptably. A few statements in the programs DATCOLL and WATCHGPC must be changed if another card is used.

Printer

The printer is used to provide rapid plots of the results of data collection, for printing of programs during program development, and for communicating the results of analyses to the operator. It is of the IBM graphics printer type.

SOFTWARE

Setting up series of data collections

The process of analyzing specimens using size exclusion chromatography consists of several main elements. The first is to prepare solutions of the specimens in a suitable solvent, typically tetrahydrofuran, and load the vials containing these solutions into the injector, where, in our configuration, up to 16 vials may be sampled in one run. The injection process is driven by a microprocessor in the chromatograph. The computer does not control this microprocessor. It passively accepts the output of the chromatograph to produce files of information which are labeled with the identity of the specimen in the solution and the date and time of data collection. These data files are then treated further in the computer as the operator specifies.

The computer must therefore be in step with the chromatograph, in that it must collect data when the chromatograph is producing data and it must give the appropriate labels to each data file. The program which conveys the labelling information to the computer is called the SETUP

program. To document the identity of the specimen and the conditions under which the chromatogram was measured, the data file collected from the chromatograph for the contents of each vial contains a title, a time to begin collection of closely spaced data points instead of merely monitoring the base line of the chromatogram, a time to end the collection of closely spaced data points, and a time to end the run, which is usually when the base line has had time to become steady again. This information is entered by the operator in a fill in the form type of screen. The Mark-Houwink coefficients for this specimen to relate log hydrodynamic volume to molecular weight may also be supplied on this screen if data treatment to this extent is required. A similar type of screen allows the operator to enter other specimen-specific information such as the number of injections from this vial, the flow rate, the sensitivities (a sort of scale factor) and the scale factors used in the chromatograph.

Another screen allows the operator to specify the type of columns and solvent used in the chromatograph, and the name of the operator.

For quality control, it is necessary to compare a chromatogram with a standard chromatogram. The correspondence between the newly collected chromatogram and the standard chromatogram is made through a variable which specifies the class of the specimen, e.g., a varnish of a particular manufacturer, and which is entered into the SETUP program either directly by the operator or by selection from a table supplied by the program.

We envisage that a wide variety of specimens will be used. Some will require only matching of chromatograms, mainly because they are mixtures of materials which cannot be completely separated or identified, and for which calculations of molecular weight distributions are meaningless. Others will be specimens of well-known molecular weight which will be used to calibrate the columns. Some will be specimens of a homogeneous material for which the molecular weight distribution is to be measured. The type and extent of post data collection processing is controlled by the "processing" variable in the SETUP program which is selected from a table displayed by the program.

During the setup of the data collection process, the operator should print out the complete set of instructions to the computer to verify that he has entered all the pertinent information correctly, and then must save the results in the form of a file on the hard disk which will be read by the data collection program.

Data collection

The process of collecting data representing a chromatogram begins when the computer receives an electrical pulse from the chromatograph. Once the run has started, the data collection program monitors the base line signal from the chromatogram until the time as specified by the operator in the SETUP program is reached. Data are then collected more frequently until the important part of the information has been acquired, at which point the base line is again monitored at a reduced rate. As the data are being measured, they are written onto the hard disk. If data collection terminates normally, the data collection program updates the file written by the SETUP program to reflect the fact that data from that specimen has been obtained. If data collection terminated abnormally, the file from SETUP is not updated. In this case, the next time the data collection program is run, it will assume that the data are a repeat of the run which ended abnormally. The reason for this method of operation is that the data are processed immediately after data collection so that the operator does not have to wait for up to 20 hours before seeing the results. After data processing has finished, the data collection program resumes by measuring the chromatogram of the next specimen.

Plotting of data

One of the choices in the SETUP program is for the chromatogram to be plotted out on the printer immediately after data collection. If this is requested, the plotting program reads in the file written by the data collection program and scales the results to fill the plot area.

Data analysis

Analysis of the chromatogram may be carried out interactively with the operator directing the flow of analysis from the computer keyboard, or automatically. Analysis is carried out in the automatic mode immediately after data collection to an extent which depends on what the operator specified during setup. Automatic analysis may also be applied to a series of chromatograms. The general sequence in all cases is to determine the levels of the base line before and after the part of the chromatogram which characterizes the specimen, apply a correction so that the overall base line is effectively zero, and cut out from the entire chromatogram that part which pertains to the specimen. The "cut" chromatogram may then be written to a file.

Up to this point, all chromatographic information is in terms of elution volume. If the operator so specified, and if a calibration file is available to the program, the chromatogram is converted to terms of log hydrodynamic volume, which effectively removes dependence on the particular set of columns used, and, if the calibration is sufficiently recent, on the age of the columns. The chromatogram in terms of log hydrodynamic volume may be written to a file for later use.

If Mark-Houwink coefficients were supplied at setup time, and if the operator so requested, the chromatogram is converted into the differential molecular weight distribution of the specimen, and various averages characterizing this molecular weight distribution are calculated. The molecular weight distribution may be written to a file.

These files are not very long. Some 30 uncut chromatograms will fit on a 360K floppy disk. About 90 cut chromatograms or log hydrodynamic volume files will fit on a similar disk. If files of all three kinds are kept together, which is probably not the most efficient arrangement, the files for about 18 specimens will fit on one disk, which is about one day's worth since the sample carrousel on the size exclusion chromatograph has room for a maximum of 16 specimens. These considerations are important only in archiving the data. The available storage on the hard disk in the computer corresponds to about 45 floppy disks, which is enough for about 10 weeks of data.

Comparison of chromatograms

When the chromatogram is to be compared with a standard chromatogram for that class of specimen, the standard chromatogram is found from the "class" specified at setup time. The two chromatograms are matched in a way also specified at setup time. This does not usually present any difficulty, because once the procedure has been worked out, the way in which to match the chromatograms is well-known. Entering this information at setup time allows the operator to collect data on various types of specimens in an given run. The comparison may be carried out in terms of elution volume or log hydrodynamic volume, as specified.

Unrecognized shifts in the calibration of the columns will affect the raw data, which are in terms of elution time of the components of the specimen from the chromatographic columns. Use of log hydrodynamic volume allows the operator to correct for such changes in column calibration and to compare specimens taken at considerably different times, and also to compare specimens taken with different sets of columns. Log hydrodynamic volume is therefore is a more constant quantity to use to specify materials which will be analyzed at the factory and in the receiving laboratory.

Methods of characterizing the difference between the two chromatograms are currently under development. In our initial approach, we provide an overall mismatch index, and a list of the areas, heights, and positions of the main peaks in the difference chromatogram. This difference chromatogram is taken between the chromatogram of the specimen and the standard chromatogram for that class of specimens. Such indices will with experience lead to a quantitative means of deciding whether to accept or reject a particular material during procurement.

Calibration of the columns

In many of the uses of size exclusion chromatography, the elution volumes over which the specimen emerges from the columns and enters the detector must be related to molecular size. The size of polymer molecules in a solvent depends on the polymer-solvent interactions as well as the length of the polymer molecules. The relationship between molecular weight and molecular size in a solvent is given for a given range of molecular weight by the Mark-Houwink coefficients, which are available in table form for many common polymer-solvent pairs.

The procedure of calibration is to find the elution volumes at which polymer specimens of known narrow molecular weight range elute, and to use the log hydrodynamic volumes calculated from the Mark-Houwink coefficients and the molecular weights of these polymers to relate log hydrodynamic volume and elution volume, often in terms of a polynomial.

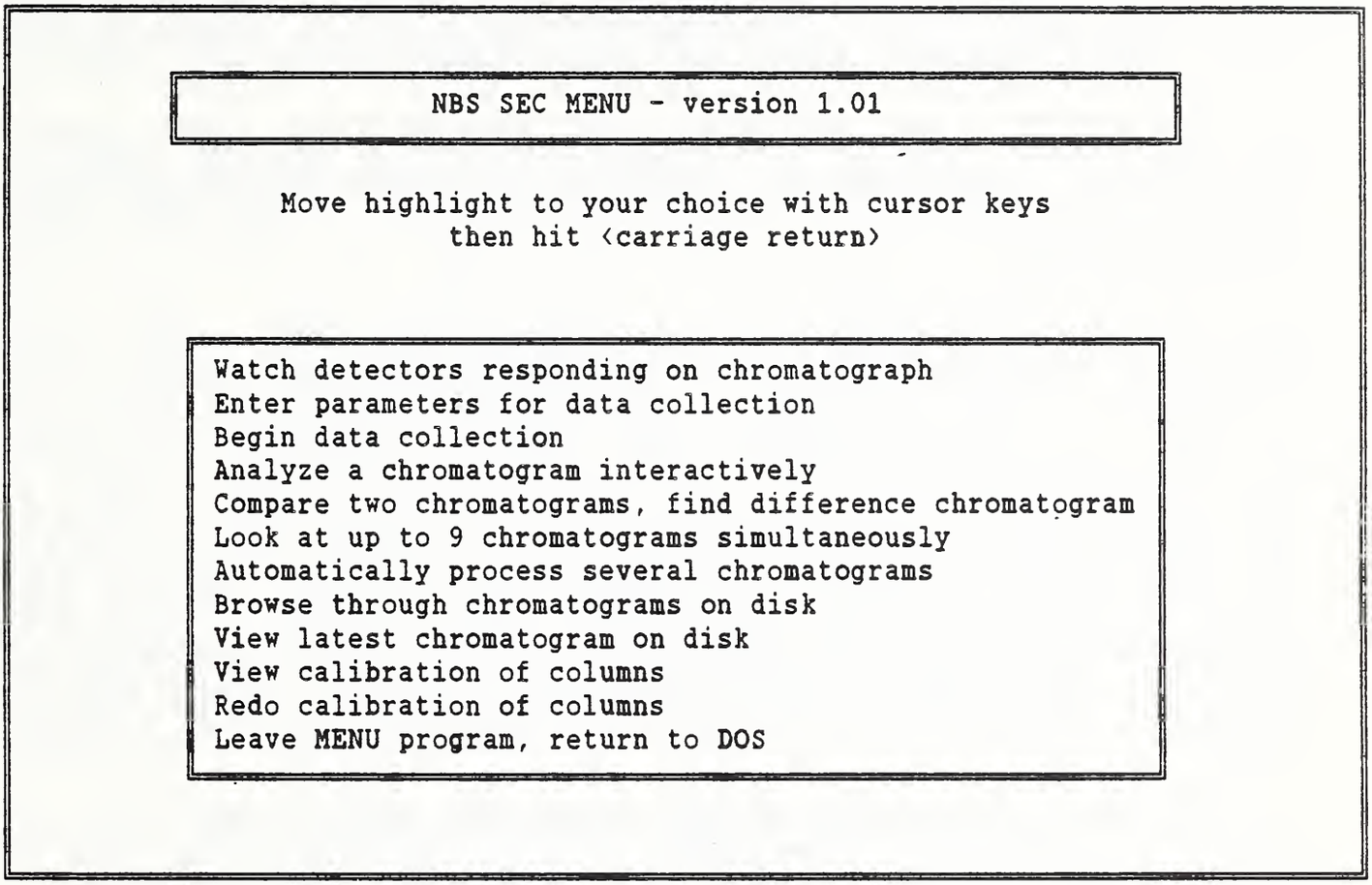
One of the options in the setup program is to automatically find the elution volume of the highest peak in a chromatogram. When enough such data have been measured to cover the calibration range needed for the specimens to be analyzed, a least squares refinement program is used to compute the appropriate polynomial. An appropriate least squares procedure has been provided in the program CALFIT.

Molecular weight distribution averages

The calibration relates elution volume to log hydrodynamic volume. The Mark-Houwink relationship relates log hydrodynamic volume to log molecular weight. Thus, with the parameters in these two relationships available to it, the program is able to calculate the distribution of molecular weights in a homogeneous specimen. The distribution is then summarized in terms of various averages which reflect the position of the distribution and its width in terms of molecular weight. A graph of the distribution is also provided.

DETAILED DESCRIPTION OF PROGRAMS

SECMENU - main menu for SEC programs



This representation of the computer screen shows the main menu in the SECMENU program, which is typically run from the AUTOEXEC.BAT file. To invoke an option in the program, move the cursor to a line in the rectangle of options and hit the {Return} key. On the screen, the line selected by the cursor is in inverse video (backlit).

SETUP program to record specimen identification and run conditions

SETUP (interactive)

The SETUP program is written in interpreted BASIC because it consists of fill-in-the-form types of data entry in which cursor movement is important. The program is started by selecting the option "Enter parameters for data collection" in the main menu.

The first screen gives the main options (see SETUP screen 1). The cursor is moved to the appropriate line and the {Return} is pressed. The usual first step is to read in the data base file from the disk, which is the first line in the option list (SETUP screen 2).

If only small changes to the existing data base need to be made, the operator should go to the screen to change titles, run times, etc. (SETUP screen 3). The old information is shown in yellow on a black background, and the new information is shown in blue. It is important to note that the new information is only on the screen and is not available to the program unless the operator wishes to transfer it to the program. The operator may transfer the information currently in the form on the screen into the program by moving the cursor down to the next to the bottom line and pressing {Return}. The screen is then read (SETUP screen 4)and re-displayed in yellow on black. Moving the cursor to the bottom line and pressing {Return} returns to the main menu. Any information still in blue will be lost at this point.

Similar screen forms allow the operator to change all the remaining quantities associated with collecting data for a chromatogram. Titles, number of injections from each vial, sensitivities, scale factors, flow rates, and injection volumes are changed as shown in SETUP screen 5.

If a new series of runs are to be set up, it is probably more worthwhile to use the default value route to enter the appropriate information into the program. Default values which will later be given to all specimens may be entered in the appropriate form (SETUP screens 6 and 7). New information (in blue) may be transferred to the program by pressing {Return} when the cursor is on the next to the bottom line. After this operation, the screen will be written entirely in yellow on black. Note that partial titles can be set up, with the operator later entering the rest of the title in the appropriate form, such as "change titles, run times, etc.". The default values may be transferred to each specimen by pressing {Return} on the appropriate line in the main menu (SETUP screen 8).

So far, we have covered the method of providing data on the chromatographic conditions, such as run times, and other pertinent information, such as run titles and Mark-Houwink parameters. The operator should also choose the class into which

the specimen falls so that later data processing can automatically find 1) the desired places at which the background in the chromatogram should be estimated, and 2) the limits of the relevant part of the chromatogram in terms of elution volume. For this automatic processing to be possible, a chromatogram representing this class must be processed interactively using the program ANALYZE. Also, the class name, the name of the representative chromatogram, and the name of the calibration file to use must be available in a file called STANDARD.CHR

The class of a specimen may be selected in SETUP from the table of possibilities presented after selecting "Choose class" in the main menu. This table is read in by the program from file SETUP.CLA when the program is first started. Up to twenty classes are allowed, and several versions of the SETUP.CLA files may be kept to give a wide choice. The limitation of twenty in any one run of the SETUP program arises because there is not room on the screen for more classes. We have not found it necessary to provide multiple screens of choices. SETUP screens 9 through 13 show the sequence of choosing the specimen class.

The operator should also choose the processing required after the chromatogram has been measured. A simple example is "Plot data". The options should be chosen from the table of possibilities provided after selecting "Choose processing to occur after data collection" in the main menu. As in the case of the selection of the class of the specimen, up to twenty types of treatment are allowed. Each line on the screen in this section encompasses all previous choices. The list of treatments is read by the program SETUP from file SETUP.PRO when the program is first started. SETUP screens 14 through 17 show the sequence for choosing the processing to occur after data collection.

The documentation of the data file includes the type of solvent (mobile phase) used, the columns used, and the operator. This information is entered into the program as in SETUP screen 18.

After the necessary information has been provided to the program, the operator should print out the data base and verify that all data have been given correctly. This is done by pressing return on the third line of the main menu.

The specimen details must be saved on the disk so that the data collection program can refer to them and include them with the appropriate data file. This is done by hitting {return} with the cursor on line 2 of the main menu. The screen then displays a message that the information is being saved (SETUP screen 19).

The help screen is reached from the main menu, and is shown in SETUP screen 20.

When the End program option is chosen in the main menu, the program displays SETUP screen 21 if no further changes have been made (or if no possibility of making changes has occurred) since the data base was last saved. Otherwise, a reminder is provided (SETUP screen 22). To give the user a second chance, both y (or Y) and {Return} must be given before the program stops and the information in the computer's memory is lost. The information must be on the disk in order to survive so it can be used later.

SETUP screen 1 - Main menu

```
                Main menu for setting up SEC run data base

 Read specimen details in from data base on disk
 Write specimen details out to data base on disk
 Print out specimen details in data base
 Change titles, run times and Mark-Houwink parameters
 Change titles, # injectns, sensitivities, scales, inj vols, flows
 Choose class for automatic matching of chromatograms
 Choose processing to occur after data collection
 Change mobile phase descriptor, column description and operator name
 Provide new default values
 Assign default values to all vials
 End program (Save the specimen details by writing them out to the data base!)
 HELP!

 Move cursor to a line and hit RETURN
```

This representation of the computer screen shows the main menu in the SETUP program. To invoke an option in the program, move the cursor to a line and hit the {Return} key.

SETUP screen 2 - Reading in specimen details

```
                Main menu for setting up SEC run data base

 Read specimen details in from data base on disk
 Write specimen details out to data base on disk
 Print out specimen details in data base
 Change titles, run times and Mark-Houwink parameters
 Change titles, # injectns, sensitivities, scales, inj vols, flows
 Choose class for automatic matching of chromatograms
 Choose processing to occur after data collection
 Change mobile phase descriptor, column description and operator name
 Provide new default values
 Assign default values to all vials
 End program (Save the specimen details by writing them out to the data base!)
 HELP!

 Move cursor to a line and hit RETURN




                      Reading in specimen details
```

This SETUP screen shows that the specimen written to the disk in the previous use of the program is now being read into the program.

SETUP screen 3 - Changing titles and run times

Change titles, plot scale, Mark-Houwink parameters, and run times

vial	title		ymax	ymin	k	a	begin	end	stop
1	ink specimen 1		.03	-.02	0E-6	0	10	45	50
2	ink specimen 2		.03	-.02	0E-6	0	10	45	50
3	ink specimen 3		.03	-.02	0E-6	0	10	45	50
4	ink specimen 4		.03	-.02	0E-6	0	10	45	50
5	A1 Sicpa S-4 45.8mg		.03	-.02	0E-6	0	10	45	50
6	A2 Fountain 44.3mg		.03	-.02	0E-6	0	10	45	50
7	A3 Prewipe 43.7mg		.03	-.02	0E-6	0	10	45	50
8	A4 Reconstituted 36.7mg		.03	-.02	0E-6	0	10	45	50
9	B1 Sicpa S-4 42.8mg	dil	.03	-.02	0E-6	0	10	45	50
10	B2 Fountain 42.6mg	dil	.03	-.02	0E-6	0	10	45	50
11	B3 Prewipe 35.0mg	dil	.03	-.02	0E-6	0	10	45	50
12	B4 Reconstituted 42.8mg	dil	.03	-.02	0E-6	0	10	45	50
13	B4-A Reconstituted 40.3mg	dil	.03	-.02	0E-6	0	10	45	50
14	8/27/2A Bk-3795-21-IRD 45.3mg	dil	.03	-.02	0E-6	0	10	45	50
15	8/27/2 Bk-3795-21-IRD 39.3mg	dil	.03	-.02	0E-6	0	10	45	50
16	8/27/3 Bk-3795-21-IRD 44.1mg	dil	.03	-.02	0E-6	0	10	45	50

Move cursor with arrows, type in new values, type over don't backspace
Hit RETURN on this line to read the values on the screen
Hit RETURN on this line to return to main menu
!!! No collection of data if total run time (in stop column) = 0 !!!

The screen in which the titles, plot maxima and plot minima, Mark-Houwink parameters, and run times are changed. Each quantity is displayed in a colored box on the screen to show how long the entry on the screen may be. The plot maxima and minima are necessary because the data collection program does not know what the range of the data will be but must set up the plot frame before any data are collected.

SETUP screen 4 - Reading changes in titles

Change titles, plot scale, Mark-Houwink parameters, and run times

vial	title		ymax	ymin	k	a	begin	end	stop
1	ink specimen 1		.03	-.02	0E-6	0	10	45	50
2	ink specimen 2		.03	-.02	0E-6	0	10	45	50
3	ink specimen 3		.03	-.02	0E-6	0	10	45	50
4	ink specimen 4		.03	-.02	0E-6	0	10	45	50
5	A1 Sicpa S-4 45.8mg		.03	-.02	0E-6	0	10	45	50
6	A2 Fountain 44.3mg		.03	-.02	0E-6	0	10	45	50
7	A3 Prewipe 43.7mg		.03	-.02	0E-6	0	10	45	50
8	A4 Reconstituted 36.7mg		.03	-.02	0E-6	0	10	45	50
9	B1 Sicpa S-4 42.8mg	dil	.03	-.02	0E-6	0	10	45	50
10	B2 Fountain 42.6mg	dil	.03	-.02	0E-6	0	10	45	50
11	B3 Prewipe 35.0mg	dil	.03	-.02	0E-6	0	10	45	50
12	B4 Reconstituted 42.8mg	dil	.03	-.02	0E-6	0	10	45	50
13	B4-A Reconstituted 40.3mg	dil	.03	-.02	0E-6	0	10	45	50
14	8/27/2A Bk-3795-21-IRD 45.3mg	dil	.03	-.02	0E-6	0	10	45	50
15	8/27/2 Bk-3795-21-IRD 39.3mg	dil	.03	-.02	0E-6	0	10	45	50
16	8/27/3 Bk-3795-21-IRD 44.1mg	dil	.03	-.02	0E-6	0	10	45	50

Move cursor with arrows, type in new values, type over don't backspace
Hit RETURN on this line to read the values on the screen
Hit RETURN on this line to return to main menu Reading the screen
!!! No collection of data if total run time (in stop column) = 0 !!!

The "Reading the screen" line in the lower right hand corner shows that the operator has hit {return} on the "Read the screen" line. The screen is yellow on black when first printed. Changes are shown in blue. When the screen has been read, all the text is in yellow. Changes may be made and read as long as the form is on the screen. Hitting {Return} on the next to the bottom line returns to the main menu.

SETUP screen 5 - Changing injection volumes, etc.

Change titles, # injectns, sensitivity, scale, inj vol, flow

vial	title		# injects	flow	sensit	scale	injvol
1	ink specimen 1		1	1	-256	99	400
2	ink specimen 2		1	1	-256	99	400
3	ink specimen 3		1	1	-256	99	400
4	ink specimen 4		1	1	-256	99	400
5	A1 Sicpa S-4 45.8mg		1	1	-256	99	400
6	A2 Fountain 44.3mg		1	1	-256	99	400
7	A3 Prewipe 43.7mg		1	1	-256	99	400
8	A4 Reconstituted 36.7mg		1	1	-256	99	400
9	B1 Sicpa S-4 42.8mg	dil	1	1	-256	99	400
10	B2 Fountain 42.6mg	dil	1	1	-256	99	400
11	B3 Prewipe 35.0mg	dil	1	1	-256	99	400
12	B4 Reconstituted 42.8mg	dil	1	1	-256	99	400
13	B4-A Reconstituted 40.3mg	dil	1	1	-256	99	400
14	8/27/2A Bk-3795-21-IRD 45.3mg	dil	1	1	-256	99	400
15	8/27/2 Bk-3795-21-IRD 39.3mg	dil	1	1	-256	99	400
16	8/27/3 Bk-3795-21-IRD 44.1mg	dil	1	1	-256	99	400

Move cursor with arrows, type in new values, type over don't backspace
Hit RETURN on this line to read the values on the screen
Hit RETURN on this line to return to main menu
!!! Always keep the same injection volume for the same class !!!

The SETUP screen in which the number of injections, flow rate, sensitivity, scale, injection volume may be changed. Typical values for these variables are shown. Changes are made and read as in the previous screen.

SETUP screen 6 - Using default values (1)

```
Current default values
Mark-Houwink K                                          0E-6
Mark-Houwink a                                          0
Time after which to begin collecting data (mins)        0
Time at which to stop collecting data (mins)            0
Total time of data collection (mins)                    0
Sensitivity                                             0
Scale Factor                                            0
Injection Volume                                        0
Number of Injections                                    0
Flow Rate                                               0
Ymax and Ymin in plot on screen                         0          0


title is:
class is:         Not assigned
processing is:    Not assigned


Move cursor with arrows, type in new values, type over don't backspace

Hit RETURN on this line to read the values on the screen
Hit RETURN on this line to return to main menu
```

The SETUP screen in which default values may be given. This is the form as first written by the program if the data base was not read from the disk. If the data base was read from the disk, the old default values will be available and are written into the form by the program.

SETUP screen 7 - Using default values (2)

```
Current default values
Mark-Hcuwink K                                          .861e-4
Mark-Houwink a                                          .74
Time after which to begin collecting data (mins)        10
Time at which to stop collecting data (mins)            45
Total time of data collection (mins)                    50
Sensitivity                                             -256
Scale Factor                                            99
Injection Volume                                        400
Number of Injections                                    1
Flow Rate                                               1
Ymax and Ymin in plot on screen                         .05        -0.02


title is:         Black ink test run #
class is:         Black ink
processing is:


Move cursor with arrows, type in new values, type over don't backspace

Hit RETURN on this line to read the values on the screen
Hit RETURN on this line to return to main menu
```

Typical default values in the SETUP default value screen. Note the partial title, which will be augmented in the titles screen by typing in the specimen numbers.

SETUP screen 8 - Using default values (3)

```
                Main menu for setting up SEC run data base

Read specimen details in from data base on disk
Write specimen details out to data base on disk
Print out specimen details in data base
Change titles, run times and Mark-Houwink parameters
Change titles, # injectns, sensitivities, scales, inj vols, flows
Choose class for automatic matching of chromatograms
Choose processing to occur after data collection
Change mobile phase descriptor, column description and operator name
Provide new default values
Assign default values to all vials
End program (Save the specimen details by writing them out to the data base!)
HELP!

Move cursor to a line and hit RETURN


                    Use the default titles? Y or N?

  Remember to check the descriptors for mobile phase, columns and operator
                        starting from the main menu
```

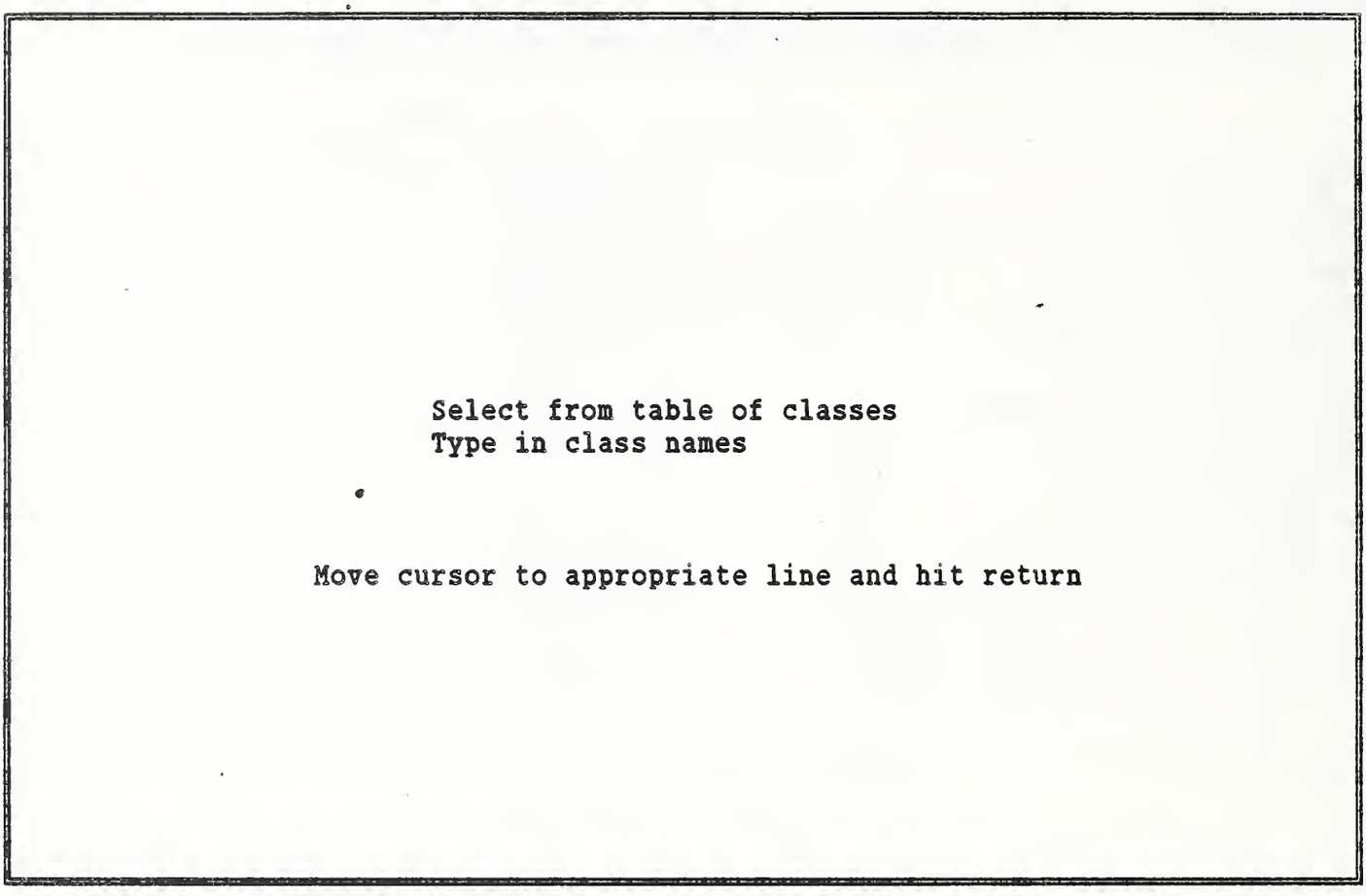
On return to the main menu from the default screen, the operator has the choice of using the title in the default screen or keeping the titles already in the titles screen. The reminder to check the mobile phase, columns and operator identification is provided because the default route does not automatically cover specifying these details.

SETUP screen 9 - Choosing the specimen class (1)

```
     Choose class for specimens for automatic matching of chromatograms

            Titles                                        Class

  ink specimen 1                                  Not assigned
  ink specimen 2                                  Not assigned
  ink specimen 3                                  Not assigned
  ink specimen 4                                  Not assigned
  A1 Sicpa S-4 45.8mg                             Not assigned
  A2 Fountain 44.3mg                              Not assigned
  A3 Prewipe 43.7mg                               Not assigned
  A4 Reconstituted 36.7mg                         Not assigned
  B1 Sicpa S-4 42.8mg          dil                Not assigned
  B2 Fountain 42.6mg           dil                Not assigned
  B3 Prewipe 35.0mg            dil                Not assigned
  B4 Reconstituted 42.8mg      dil                Not assigned
  B4-A Reconstituted 40.3mg    dil                Not assigned
  8/27/2A Bk-3795-21-IRD 45.3mgdil                Not assigned
  8/27/2 Bk-3795-21-IRD 39.3mg dil                Not assigned
  8/27/3 Bk-3795-21-IRD 44.1mg dil                Not assigned


                          change classes? y or n?
                        n(=no) returns to main menu
```

The SETUP screen showing the class specified for each specimen. Answering Y or y to the classes generates the next screen. Any other answer returns to the main menu.

SETUP screen 10 - Choosing the specimen class (2)



Usually, the operator will choose to select from a table of classes. If not, the program will generate a form like the one in the previous screen in which the classes may be typed and then read by moving the cursor to the appropriate line and pressing {Return}.

SETUP screen 11 - Choosing the specimen class (3)

```
 1  Standard
 2  Bodied tung oil
 3  Bodied tung oil - Degen
 4  Bodied tung oil - Superior
 5  Bodied tung oil - Standard
 6  Solvar varnish
 7  Solvar varnish - Lawter
 8  Solvar varnish - Sun-Ray
 9  Solvar varnish - Superior
 10 Gloss HS
 11 Gloss HS - Sun-Ray
 12 Gloss HS - Superior                  Enter number of class at ? mark
 13 Gloss HS - Standard                    Return moves to next vial
 14 Aquawipe - Sicpa
 15 THF
 16 E-beam resin component
 17 Green ink - BEP
 18 Green ink - Sicpa
 19 Black ink - BEP BK62/3A
 20 Black ink - Sicpa
 21 Not assigned
vial title                          class     number of class in above list
 1  ink specimen 1                  Not assigned                          ?
```

This SETUP screen in which the specimen class is chosen is generated by answering y to the question about selecting the class from a table. The appropriate number from the table in the screen is typed in the lower right hand corner of the screen at the question mark, and {return} is pressed to move on to the next specimen, as shown in the next screen.

SETUP screen 12 - Choosing the specimen class (4)

```
 1  Standard
 2  Bodied tung oil
 3  Bodied tung oil - Degen
 4  Bodied tung oil - Superior
 5  Bodied tung oil - Standard
 6  Solvar varnish
 7  Solvar varnish - Lawter
 8  Solvar varnish - Sun-Ray
 9  Solvar varnish - Superior
 10 Gloss HS
 11 Gloss HS - Sun-Ray
 12 Gloss HS - Superior                  Enter number of class at ? mark
 13 Gloss HS - Standard                    Return moves to next vial
 14 Aquawipe - Sicpa
 15 THF
 16 E-beam resin component
 17 Green ink - BEP
 18 Green ink - Sicpa
 19 Black ink - BEP BK62/3A
 20 Black ink - Sicpa
 21 Not assigned
vial title                          class     number of class in above list
 2  ink specimen 2                  Not assigned                        ? 17
```

Selection of class 17, Green ink - BEP, for the specimen in vial 2.

SETUP screen 13 - Choosing the specimen class (5)

Choose class for specimens for automatic matching of chromatograms

Titles		Class
ink specimen 1		Green ink - BEP
ink specimen 2		Green ink - BEP
ink specimen 3		Black ink - BEP BK62/3A
ink specimen 4		Black ink - BEP BK62/3A
A1 Sicpa S-4 45.8mg		Green ink - Sicpa
A2 Fountain 44.3mg		Black ink - Sicpa
A3 Prewipe 43.7mg		Aquawipe - Sicpa
A4 Reconstituted 36.7mg		Green ink - Sicpa
B1 Sicpa S-4 42.8mg	dil	Green ink - Sicpa
B2 Fountain 42.6mg	dil	Black ink - Sicpa
B3 Prewipe 35.0mg	dil	Aquawipe - Sicpa
B4 Reconstituted 42.8mg	dil	Green ink - BEP
B4-A Reconstituted 40.3mg	dil	Green ink - BEP
8/27/2A Bk-3795-21-IRD 45.3mg	dil	Black ink - BEP BK62/3A
8/27/2 Bk-3795-21-IRD 39.3mg	dil	Black ink - BEP BK62/3A
8/27/3 Bk-3795-21-IRD 44.1mg	dil	Black ink - BEP BK62/3A

change classes? y or n?
n(=no) returns to main menu

After classes have been chosen for all specimens, the program generates this screen, which shows the choices which have been made. The operator may choose again by answering y to the question.

SETUP screen 14 - Choosing processing (1)

```
          Choose processing for specimens after data collection

            Titles                                Data processing

  ink specimen 1                                  Not assigned
  ink specimen 2                                  Not assigned
  ink specimen 3                                  Not assigned
  ink specimen 4                                  Not assigned
  A1 Sicpa S-4 45.8mg                             Not assigned
  A2 Fountain 44.3mg                              Not assigned
  A3 Prewipe 43.7mg                               Not assigned
  A4 Reconstituted 36.7mg                         Not assigned
  B1 Sicpa S-4 42.8mg          dil                Not assigned
  B2 Fountain 42.6mg           dil                Not assigned
  B3 Prewipe 35.0mg            dil                Not assigned
  B4 Reconstituted 42.8mg      dil                Not assigned
  B4-A Reconstituted 40.3mg    dil                Not assigned
  8/27/2A Bk-3795-21-IRD 45.3mgdil                Not assigned
  8/27/2 Bk-3795-21-IRD 39.3mg dil                Not assigned
  8/27/3 Bk-3795-21-IRD 44.1mg dil                Not assigned

                        change processing? y or n?
                        n(=no) returns to main menu
```

A series of screens similar to the screens in which the classes are chosen is used to choose the processing to occur after data collection. This screen shows the initial choices, which are the choices already in the program.

SETUP screen 15 - Choosing processing (2)

```
 1  Not assigned
 2  Collect data
 3  Plot data
 4  Find elution time of main peak
 5  Subtract background
 6  Compare with class standard
 7
 8
 9
10
11
12
13
14                                   Enter number of processing at ? mark
15                                        Return moves to next vial
16
17
18
19
20

vial title                           treatment   # of treatment in above list
 1  ink specimen 1                   Not assigned                        ?
```

Answering y or Y to the question in the previous screen results in this screen, which allows the operator to specify the processing from the table. Each choice includes all the previous choices. For example, "Find Elution time of main peak" includes collect data(!) and plot data.

SETUP screen 16 - Choosing processing (3)

```
          Choose processing for specimens after data collection

            Titles                                  Data processing

  ink specimen 1                                    Plot data
  ink specimen 2                                    Plot data
  ink specimen 3                                    Plot data
  ink specimen 4                                    Plot data
  A1 Sicpa S-4 45.8mg                               Plot data
  A2 Fountain 44.3mg                                Plot data
  A3 Prewipe 43.7mg                                 Plot data
  A4 Reconstituted 36.7mg                           Plot data
  B1 Sicpa S-4 42.8mg           dil                 Plot data
  B2 Fountain 42.6mg            dil                 Plot data
  B3 Prewipe 35.0mg             dil                 Plot data
  B4 Reconstituted 42.8mg       dil                 Plot data
  B4-A Reconstituted 40.3mg     dil                 Plot data
  8/27/2A Bk-3795-21-IRD 45.3mgdil                  Plot data
  8/27/2 Bk-3795-21-IRD 39.3mg dil                  Plot data
  8/27/3 Bk-3795-21-IRD 44.1mg dil                  Plot data


                          change processing? y or n?
                          n(=no) returns to main menu
```

This SETUP screen shows the choices typically made when mixtures of materials are being studied, i.e., the data are merely to be plotted without further processing.

SETUP screen 17 - Choosing processing (4)

```
          Choose processing for specimens after data collection

            Titles                                  Data processing

  ink specimen 1                                Compare with class standard
  ink specimen 2                                Compare with class standard
  ink specimen 3                                Compare with class standard
  ink specimen 4                                Compare with class standard
  A1 Sicpa S-4 45.8mg                           Compare with class standard
  A2 Fountain 44.3mg                            Compare with class standard
  A3 Prewipe 43.7mg                             Compare with class standard
  A4 Reconstituted 36.7mg                       Compare with class standard
  B1 Sicpa S-4 42.8mg           dil             Compare with class standard
  B2 Fountain 42.6mg            dil             Compare with class standard
  B3 Prewipe 35.0mg             dil             Compare with class standard
  B4 Reconstituted 42.8mg       dil             Compare with class standard
  B4-A Reconstituted 40.3mg     dil             Compare with class standard
  8/27/2A Bk-3795-21-IRD 45.3mgdil              Compare with class standard
  8/27/2 Bk-3795-21-IRD 39.3mg dil              Compare with class standard
  8/27/3 Bk-3795-21-IRD 44.1mg dil              Compare with class standard


                        change processing? y or n?
                        n(=no) returns to main menu
```

This screen shows the choice which could reasonably be made if a series of single component specimens were being examined and the operator wanted the program to calculate the molecular weight averages and log molecular weight distribution for each specimen.

SETUP screen 18 - Choosing column label etc.

```
        Change descriptors for mobile phase, columns and operator



mobile phase is:    THF
columns are:        Columns go here
operator is:        B. Dickens & L.Low









  Move cursor with arrows, type in new values, type over don't backspace

  Hit RETURN on this line to read the values on the screen
  Hit RETURN on this line to return to main menu
```

In a sequence which is typical for SETUP, the operator may identify the mobile phase, the columns, and the operator.

SETUP screen 19 - Saving specimen details in data base on disk

```
                Main menu for setting up SEC run data base

Read specimen details in from data base on disk
Write specimen details out to data base on disk
Print out specimen details in data base
Change titles, run times and Mark-Houwink parameters
Change titles, # injectns, sensitivities, scales, inj vols, flows
Choose class for automatic matching of chromatograms
Choose processing to occur after data collection
Change mobile phase descriptor, column description and operator name
Provide new default values
Assign default values to all vials
End program (Save the specimen details by writing them out to the data base!)
HELP!

Move cursor to a line and hit RETURN




                Writing specimen details to data base
```

The specimen details must be written to the disk before the SETUP program is ended if they are to be available to the data collection program. However, there may be cases when the SETUP program is being demonstrated rather than being used to set up a series of runs. Therefore, the data file is not automatically written to disk. To save the data, the operator hits {Return} on the second line of the menu. The screen then shows that the data are being written to disk. The DO.BAT file containing a series of DOS instructions for data collection and further processing is generated and written to disk at the same time. Data collection is started by typing DO at the DOS prompt or by selecting the "Collect data" option in the main menu of program SECMENU.

SETUP screen 20 - Help screen

HELP INFORMATION

This program allow you to put into the computer the information to label and hence identify the chromatograms as they are taken and to give the data collection program the information it needs to collect data. Each chromatogram is processed immediately after it has been measured. You may process it again later if you wish.
First, read the previous data file from the disk, using the first option in the list. You can alter it as you see fit, using most of the other options. Generally, you will update the titles, and maybe the run times and other parameters. The data collection program will only collect data for those specimens which have a non-zero run time. Hence, to include a vial or remove a vial from the list, you change its run time. The data collection program takes a reading about once a minute up to the beginning of data collection proper, when the specimen is expected to emerge from the columns. After the specimen has eluted, leave a few minutes for the data collection to continue, and a few more for the data collection to collect a point a minute to allow it and you to establish the background again. When you are done, print out your data, and check them. Then save the data on the disk, using the second option, so the later programs can read them. After running this program, you start data collection by typing DO at the DOS prompt.

Press RETURN when ready

This SETUP help screen is generated by hitting {Return} on the bottom line of the main menu.

SETUP screen 21 - Leaving SETUP after saving data base on disk

```
Read specimen details in from data base on disk
Write specimen details out to data base on disk
Print out specimen details in data base
Change titles, run times and Mark-Houwink parameters
Change titles, # injectns, sensitivities, scales, inj vols, flows
Choose class for automatic matching of chromatograms
Choose processing to occur after data collection
Change mobile phase descriptor, column description and operator name
Provide new default values
Assign default values to all vials
End program (Save the specimen details by writing them out to the data base!)
HELP!

Move cursor to a line and hit RETURN



        If you are ready to end the program, press y and then return
                        otherwise press return
```

If the specimen details have been written out to disk and no further chance for editing (such as going to a section of the program which allows the titles to be altered) has occurred, the program will give this message to allow the operator to terminate the program.

SETUP screen 22 - Leaving SETUP after not saving data base

```
Read specimen details in from data base on disk
Write specimen details out to data base on disk
Print out specimen details in data base
Change titles, run times and Mark-Houwink parameters
Change titles, # injectns, sensitivities, scales, inj vols, flows
Choose class for automatic matching of chromatograms
Choose processing to occur after data collection
Change mobile phase descriptor, column description and operator name
Provide new default values
Assign default values to all vials
End program (Save the specimen details by writing them out to the data base!)
HELP!

Move cursor to a line and hit RETURN




     You have not saved the most recent version of the data on the disk
           If you are ready to end the program, press y and then return
                         otherwise press return
```

In this case, the operator invoked at least one of the editing screens after the data were saved to disk and then wanted to end the program without saving the data to disk. The program warns the operator that this has occurred.

Data collection and automatic processing programs

DATCOLL (automatic)

Data collection is begun by selecting "Begin data collection" in the main menu or by typing DO at the DOS prompt. The program SETUP must have been run first to generate a file DO.BAT which contains the list of programs to be run to accomplish what the operator specified. Execution of the group of data collection and automatic processing programs is terminated by pressing {Control} and {Break} simultaneously (hold down {Control} and then press {Break}) to set a error signal in the operating system. Then, if data collection is underway, press {Escape}. Most of the "automatic" programs can be terminated by pressing the {Escape} key. Otherwise, wait until the program which is running has finished. Only the data collection program and the plotting program run for more than a few seconds.

Before data collection begins, the specimen details are read from file SETUP.DAT, which was prepared by the SETUP program. Much of this information is written at the beginning of each data file so that it is available to later programs. As the data collection is successfully completed (i.e., without abnormal interruptions), the file SETUP.DAT is re-written to the disk to reflect the fact that data have apparently been collected successfully for this specimen. If the data collection program is stopped and re-started, it will not recollect data for runs which have been marked as being successfully completed.

RUN.NBR (file containing the latest run number)

The data files are named beginning with SEC, for size exclusion chromatography, and then a number, known as the run number, and end with an extension of RAW for unprocessed data, or other extensions such as CUT, HYD, MWD, and DIF. As data are collected, the run number is increased by one for successive specimens. The automatic programs look in the file RUN.NBR to find out the name of the latest data file so they can read it in and process the data.

These automatic programs may also be used to process many data files at once. The file names of the data files to be treated should be put into the file RUN.NBR, using a text editor, or using the DOS piping command (e.g., DIR SEC9?.RAW > RUN.NBR to put all raw data files with run numbers between 90 and 99 inclusive in the file RUN.NBR) and then editing the resultant RUN.NBR with a text editor to give the needed file names.

The name of the automatic program should be typed at the DOS prompt >. Then the program will process each of the files named in RUN.NBR. When data collection is begun again, a program checks to see that RUN.NBR exists, and makes sure that it contains the latest run number.

AUTOPLOT (automatic)

The program AUTOPLOT reads in the contents of the file RUN.NBR and generates from it a file name beginning with SEC and ending with RAW. It then attempts to read in the contents of that file, which is usually the latest chromatogram, and plots it, first on the screen, and then, using a screen dump routine, on the printer.

AUTOPICK (automatic)

The program AUTOPICK reads the latest run number from the file RUN.NBR and finds the elution volume of the largest peak in the chromatogram between the limits given in the file AUTOPICK.LMT If the program can not find the file AUTOPICK.LMT, it finds the largest peak in the entire chromatogram. This is often not desirable, because the solvent injection peak is sometimes larger than the peak from the specimen.

BACKGRND (automatic)

This program determines the background level of the signal in the chromatogram and subtracts it from the data. Any number of chromatograms may be processed. The program uses two files in addition to the chromatogram files. The calibration of the columns must be in file CALIBR.DAT and the file names or run numbers of the chromatograms must be in file RUN.NBR with one file per line. If the extensions of the files are RAW, only the run numbers need be given in file RUN.NBR.

The program uses four positions in the chromatogram to determine points on the background line. These four positions are 1) the beginning and 2) the end of the chromatogram, and 3) the beginning and 4) the end of the calibration range (in elution volume) of the columns. Positions 3 and 4 are obtained from the calibration file. Ten points at each position are averaged to give an estimate of the background level and the standard deviation is calculated. If the standard deviation is less than 0.0004, the average is provisionally taken as a point on the background line, otherwise it is discarded.

The surviving average background levels are then fitted to a straight line and the deviation of every level from the line determined. If the largest deviation is less than 0.0004, the line so determined is taken as the background line. Otherwise, the level with the largest deviation is eliminated and the process repeated. If the number of levels on the background line becomes less than two, a comment is printed and the background is not subtracted from the chromatogram. Otherwise,the background line is then subtracted from the chromatogram and the program continues with the next file in RUN.NBR or ends if it has read all the information in file RUN.NBR.

The results of the calculations are written to the screen and to the printer.

If this program fails to find a background line in a chromatogram, the chromatogram should be examined using the program ANALYZE to determine the background interactively. It may be necessary to reject the chromatogram.

AUTOGP (automatic)

This program reads a data file from the file RUN.NBR and the classes, standard chromatogram files, and corresponding calibration files (for the columns) from the file STANDARD.CHR It then finds the standard chromatogram file corresponding to the class of the data file, and performs the same analysis on the data file as was performed on the standard chromatogram file.

The files needed are RUN.NBR, STANDARD.CHR, the calibration file, the file containing the standard chromatogram, and the data file. A report is provided on the printer and on the file AUTOGP.OUT The program is a combination of BACKGRND and ANALYZE and runs automatically using run number information read from file RUN.NBR. It is placed in the file DO.BAT by the SETUP program as needed to fulfill the requirements for data collection and processing the operator made when filling out the forms in the SETUP program.

Examples of these files are given below:

RUN.NBR:

SEC925.RAW
SEC204.RAW
SEC930.RAW

Note that run numbers (such as 925) can also be used if the file name begins with SEC and ends with RAW.

STANDARD.CHR:

```
Bodied tung oil - Superior,sec207.hyd,clonecal.1
Bodied tung oil - Degen,sec207.hyd,clonecal.1
Not assigned,cutchr.std,clonecal.3
Black ink - Sicpa,sec423.cut,clonecal.1
Branched epoxy,cutchr.std,clonecal.3
PSD/PVME irradiated,pvme.hyd,clonecal.3
```

The lines contain the class name, the file containing the standard chromatogram, and the calibration file, separated by commas.

CALIBRATION FILE:

```
B DICKENS Calibration of Shodex cols 804 & 802 NOV 1986
    20.370      35.355
   1
    27.022      3.244
   -0.12144020E+00    -0.40413329E+00    -0.85624680E-02
    0.35396945E-02     0.48640074E-03    -0.86872809E-04
```

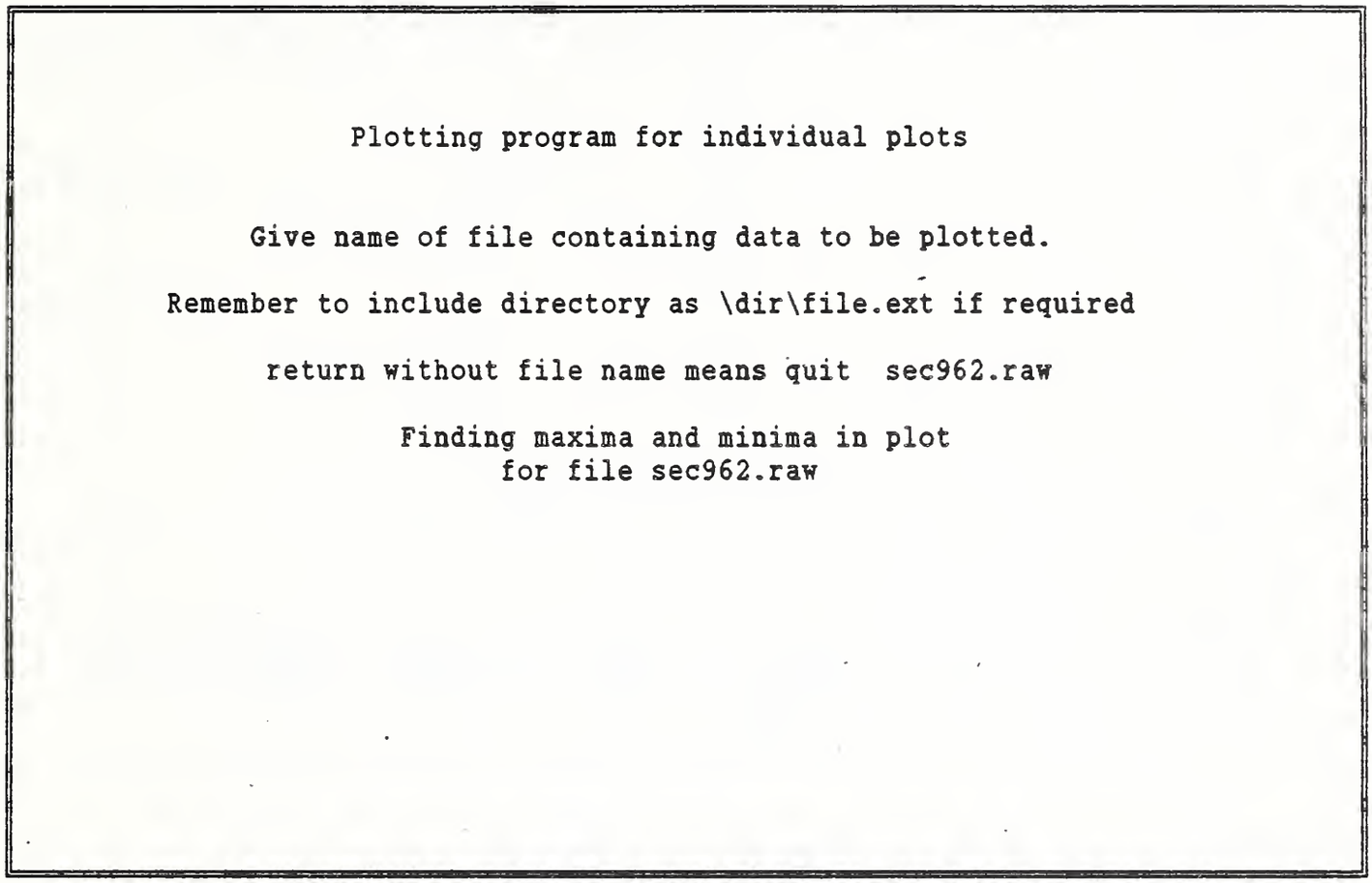
The calibration file contains a title, the range of elution volumes over which the calibration is valid, the type of calibration (1=polynomial), the points in elution volume and log hydrodynamic volume about which the polynomial is fitted, and the coefficients for a polynomial of up to fifth degree.

Utility programs to plot, list titles

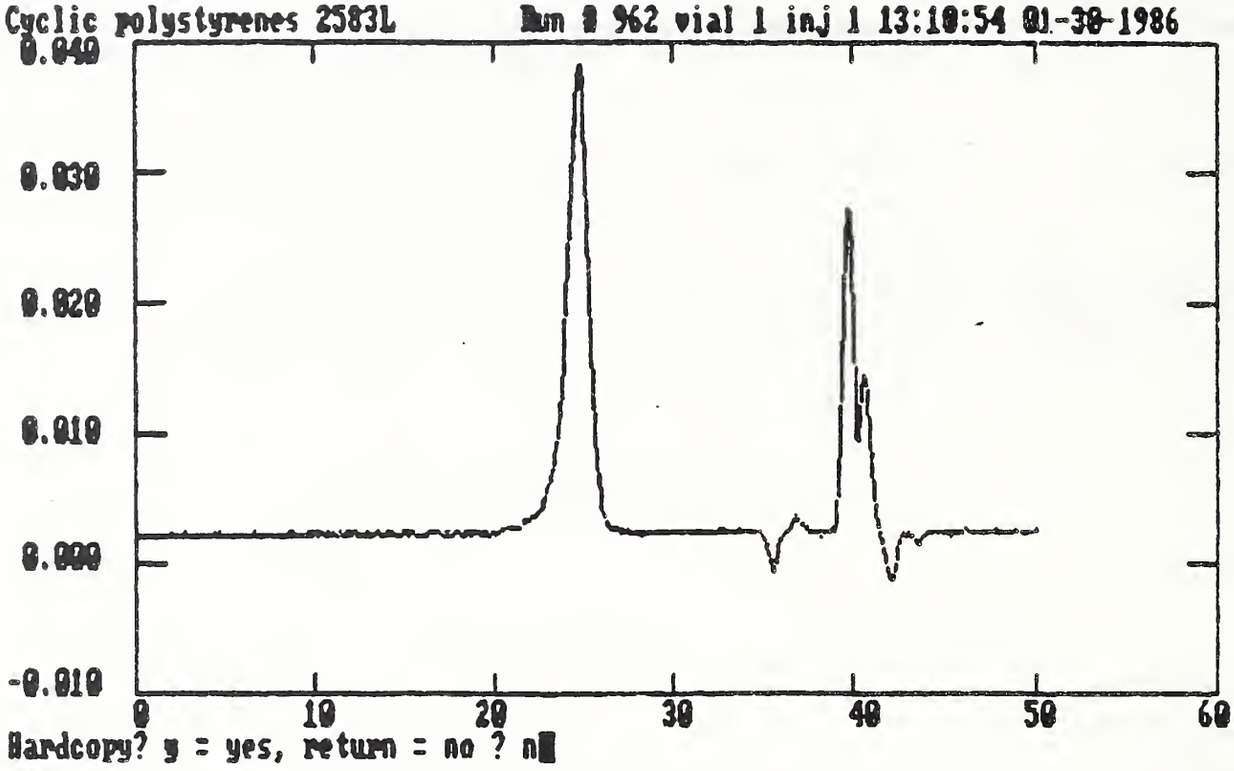
PLOTSEC (interactive)

The program PLOTSEC is a interactive version of the AUTOPLOT program, except that the operator must specify the file name which the program will try to find and plot the contents of. Any type of chromatography data file can be plotted. By responding appropriately to a question from the program, the operator can plot the file on the line printer.

PLOTSEC screen - Menu for plotting all types of SEC files



The PLOTSEC program will plot all types of SEC data files on the computer screen and will optionally also plot them on the line printer.



PLOTSEC plot - typical example

The plot generated with PLOTSEC is in high resolution (for the color graphics adapter). Answering y to the question at the bottom of the screen transfers the plot to the line printer. The x axis is labelled correctly with elution volume, log hydrodynamic volume or log molecular weight before the plot is made.

TITLES (interactive)

The program TITLES gives a list on the printer of the titles, classes and sizes of all the RAW data files available on the current directory, a specified directory, or a floppy disk.

TITLES screen - Menu for selecting range of RAW files

```
Print a list of titles of RAW data files on disk

Where are the files ?
A: for left hand side floppy disk drive:
B: for right hand side floppy disk drive:
Directory, e.g. \gpcdata\, (Return = current directory)  \gpcdata\

From run number (return = from smallest) 950
to run number (return = to largest) 1012

                        PRINTING LIST OF TITLES
```

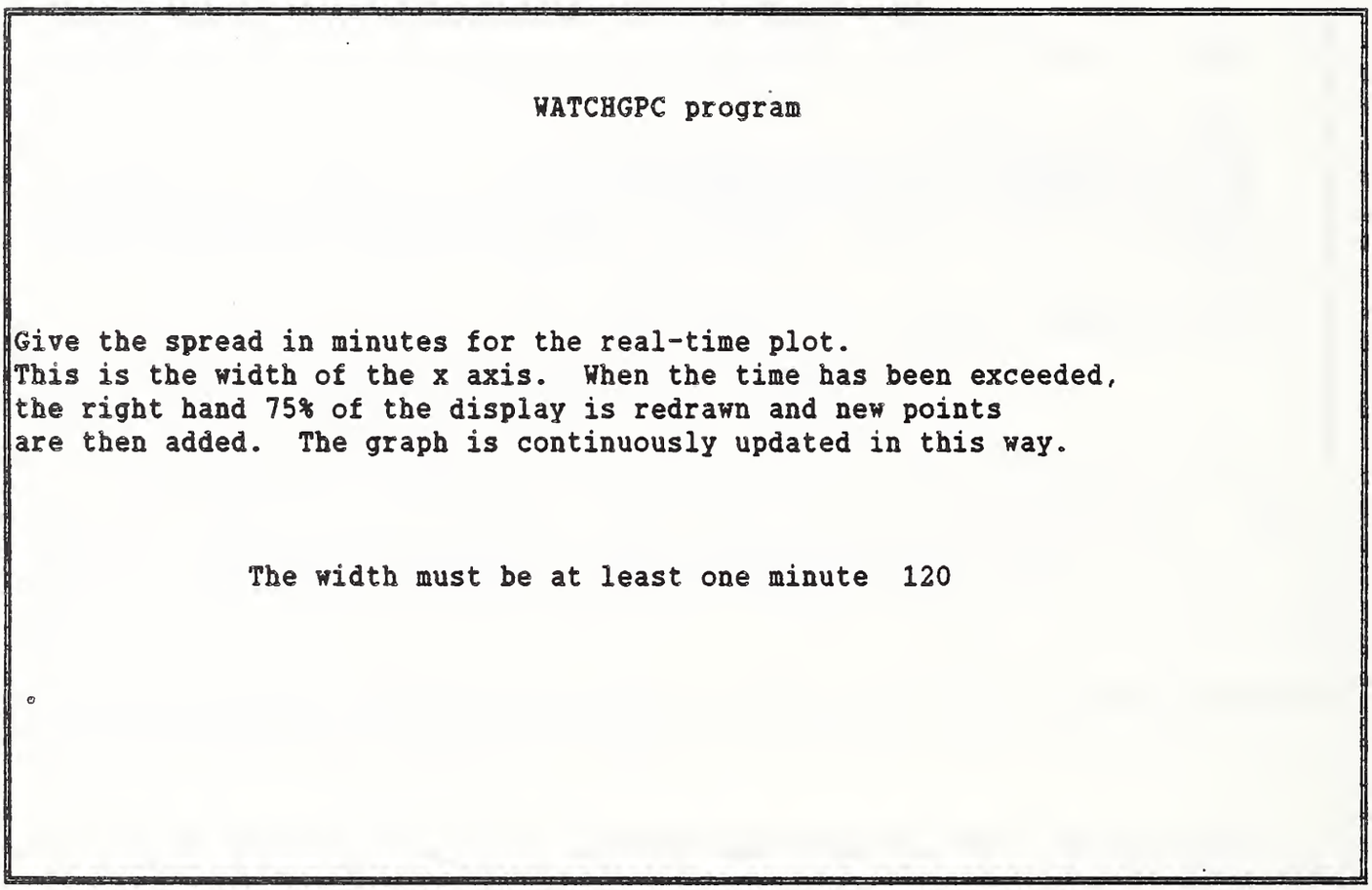
This screen shows that the TITLES program has read files \GPCDATA\SEC950.raw to \GPCDATA\SEC1012.RAW from the disk and is generating a list of the file names, titles, class, and size in Kbytes for each file.

Program to monitor steadiness of SEC sensors

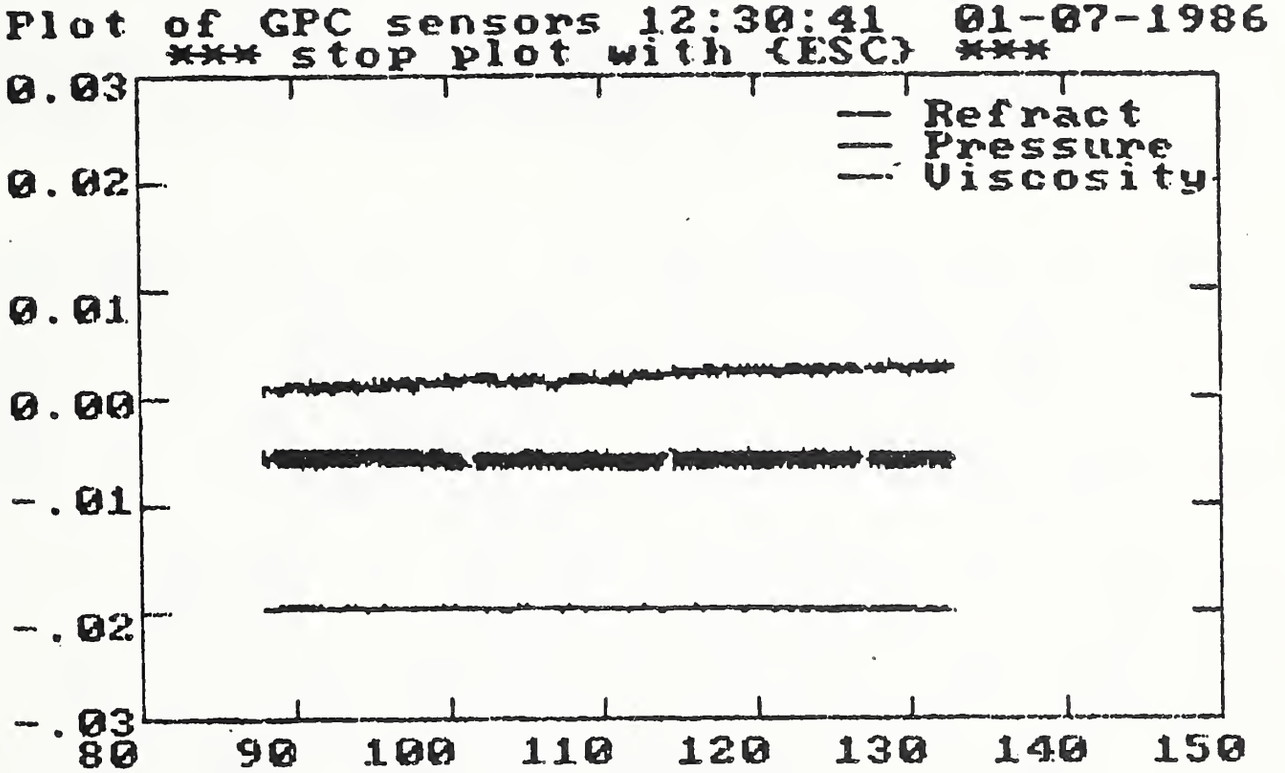
WATCHGPC (interactive)

To obtain good chromatograms, it is important to allow the chromatograph to stabilize before collecting data. The program WATCHGPC allows the operator to observe the stability of the detector by making a real-time plot of the detector response on the screen. Sudden changes often occur after about an hour if the chromatograph has just been started after a period of inactivity.

WATCHGPC screen - Menu to choose time over which to display data



The sensors in the chromatograph may be monitored continuously using the WATCHGPC program. This screen shows the choice of the time window to be displayed. The window is moved to cover the most recent 75% of the display when the trace reaches the right hand side of the screen.



WATCHGPC plot - Typical display

This screen shows the behavior of three sensors in the size exclusion chromatograph over the period of an hour. The sensors have been followed for about 130 minutes. On the screen, the three lines are in different colors. The bottom line in this plot is from the pressure sensor, the middle line is from a prototype viscosity detector which has not yet been implemented in the software, and the top line is from the refractive index detector. The program runs until the {Escape} key is pressed.

Program to analyze a chromatogram interactively

ANALYZE (interactive)

This program allows the operator to read and analyze a chromatogram interactively. It allows the background to be subtracted from the chromatogram, a section to be cut from the chromatogram, elution volumes in the chromatogram to be converted to log hydrodynamic volumes, molecular weight averages and the molecular weight distribution to be computed, and the analyzed chromatograms to be stored in files.

The program first asks for the name of the file to be analyzed. If the file is a raw file, i.e. has an extension RAW, only the number of the file need be entered, otherwise the full name must be entered. After the file has been read into the program, it may be plotted by pressing one of the function keys F1 to F6. F1 displays the full chromatogram, and F2 to F6 each display a different section of the chromatogram with the elution volume scale expanded. F2 and F3 display the left and right halves, respectively, of the chromatogram and F4, F5 and F6 display the left, middle and right thirds, respectively, of the chromatogram. The chromatogram may also be expanded to show greater detail in the vertical direction by pressing the Up Cursor key one or more times then pressing F1 to re-display the chromatogram. The lower part of the chromatogram will then be expanded but the upper part of the chromatogram will not be displayed. Pressing the Page Up key one or more times followed by F1 will display the upper parts of the chromatogram. The Down Cursor and the Page Down key reverse the effect of the Up Cursor and Page Up keys, respectively.

An arrow is displayed on the chromatogram. This arrow is used as a pointer to specify where to define the base line used to subtract the background from the chromatogram and where to specify the limits used to cut a section from the chromatogram. The Right and Left Cursor keys move the arrow one point to the right or left. The Shift Cursor keys move the arrow 10 points, the Control Cursor keys move it 100 points. The Home key moves the arrow to the left of the chromatogram and the End key moves the arrow to the right or end of the chromatogram.

To modify the chromatogram so that there is zero signal where none of the components of the specimen is eluting, the background level in the chromatogram must be determined and subtracted from the chromatogram. The procedure is as follows. First, move the arrow to a point near the beginning of the chromatogram where the signal in the chromatogram is due only to the background, and press F7. That point and 7 points to the left of the point are averaged to determine a point on the background line. Then move the arrow to a point near the end of the chromatogram and press F7 again. That point and 7 points to its right are averaged to determine a second point on the background line and the line is drawn. Examine the line and then press F8

for a menu. Press 1 to correct for background by subtracting the baseline from the chromatogram or press 2 to redetermine the background. Then press F1 to F6 to re-display the chromatogram.

If the background does not depend on the elution volume (i.e., it is level but non-zero), the background may be subtracted by moving the arrow to a point of the chromatogram before the elution of the sample where the height of the chromatogram is only due to the background and pressing F7, then pressing F8. A horizontal base line will then be subtracted from the chromatogram.

The measured chromatogram generally contains a wider range of elution volumes than those corresponding to the sample. Depending on the columns used, some 20 or 30 minutes elapse before even the largest molecules in the specimen elute from the columns. We routinely measure the chromatogram until all the material has eluted from the columns and measure for about another 10 minutes beyond that point to be able to monitor the background level at the end of the chromatogram. Before the chromatogram is analyzed further, the section of the chromatogram corresponding to elution volumes at which the sample is eluted must be isolated from the rest of the chromatogram. This is accomplished by moving the arrow to the beginning of the section to be cut from the chromatogram and pressing F9. Then the arrow is moved to the end of the section and F9 is pressed again. Then F10 is pressed and a menu appears that allows the section cut from the chromatogram to be displayed or stored. Other options are also provided. After F10 has been pressed once to cut the chromatogram, this menu must be obtained by pressing M or m.

The elution volumes should generally be changed to log hydrodynamic volumes. Two chromatograms in terms of elution volume may be compared only if they were obtained before the calibration of column changed. However, chromatograms versus log hydrodynamic volumes may be compared even if the calibration of the columns has changed, or if they were obtained using different columns. In order to change elution volume to log hydrodynamic volume, press M and then 4. A file containing the calibration of the columns must be available. The current version of the calibration function is kept in a file called CALIBR.DAT but another calibration file may also be used. See the section CALIBRATION for a description of a calibration file.

The molecular weight averages and molecular weight distribution may also be calculated. This requires Mark-Houwink parameters, which are provided in the RAW file via the SETUP program.

The section cut from the chromatogram may be stored as a file for further processing by pressing M and then 3. The file will have the same name of the raw file but the extension will be HYD if the elution volume has been changed to hydrodynamic volume

and CUT if the chromatogram is still in terms of elution volume. If the log differential molecular weight distribution has been calculated, it will be stored in a file with extension MWD.

To end the analysis of this particular chromatogram, press M. Pressing 7 will allow the operator to analyze another chromatogram and pressing 8 will exit the program.

ANALYZE screen 1 - Start of program

```
- The ANALYZE program allows the user to analyze a raw chromatogram.
- The program reads in a RAW chromatogram.
- The user decides where to determine the background.
- The program then determines the background at these points and
  subtracts the background.
- The user then decides where to cut the chromatogram in order to
  analyze the central part.  The program cuts the chromatogram
  and analyzes it.
- The background is subtracted, and the chromatogram is analyzed.


Enter file name or run number for RAW chromatogram (quit = stop):
```

This representation of the computer screen shows the initial display for the ANALYZE program, which is used to correct for background, cut out the relevant part of the chromatogram, and calculate averages and distributions based on log hydrodynamic volume and log molecular weight. The operator must type the name of the data file to be read into the program at the colon prompt.

ANALYZE screen 2 - Program can not find data file on disk

```
 - The ANALYZE program allows the user to analyze a raw chromatogram.
 - The program reads in a RAW chromatogram.
 - The user decides where to determine the background.
 - The program then determines the background at these points and
   subtracts the background.
 - The user then decides where to cut the chromatogram in order to
   analyze the central part.  The program cuts the chromatogram
   and analyzes it.
 - The background is subtracted, and the chromatogram is analyzed.

Enter file name or run number for RAW chromatogram (quit = stop):  sec962.raw

        Can not find file sec961.raw
```

If the operator types the name of a file which can not be found in the current directory, the program notes this fact and allows the operator to retry, as shown here. No listing of the files in the current directory can be obtained from inside ANALYZE. To find where a file is, or what the real name is, the operator would have to type quit at the prompt to exit the program and then use the DOS DIR command or a directory listing program.

ANALYZE screen 3 - Successful read of data file from disk

```
- The ANALYZE program allows the user to analyze a raw chromatogram.
- The program reads in a RAW chromatogram.
- The user decides where to determine the background.
- The program then determines the background at these points and
  subtracts the background.
- The user then decides where to cut the chromatogram in order to
  analyze the central part.  The program cuts the chromatogram
  and analyzes it.
- The background is subtracted, and the chromatogram is analyzed.

Enter file name or run number for RAW chromatogram (quit = stop):  sec962.raw

                        Reading file sec962.raw

                     The title of the input file is:

 Cyclic polystyrenes 2583L        Run # 962 vial 1 inj 1 13:10:54 01-30-1986

                              ** READING **
```

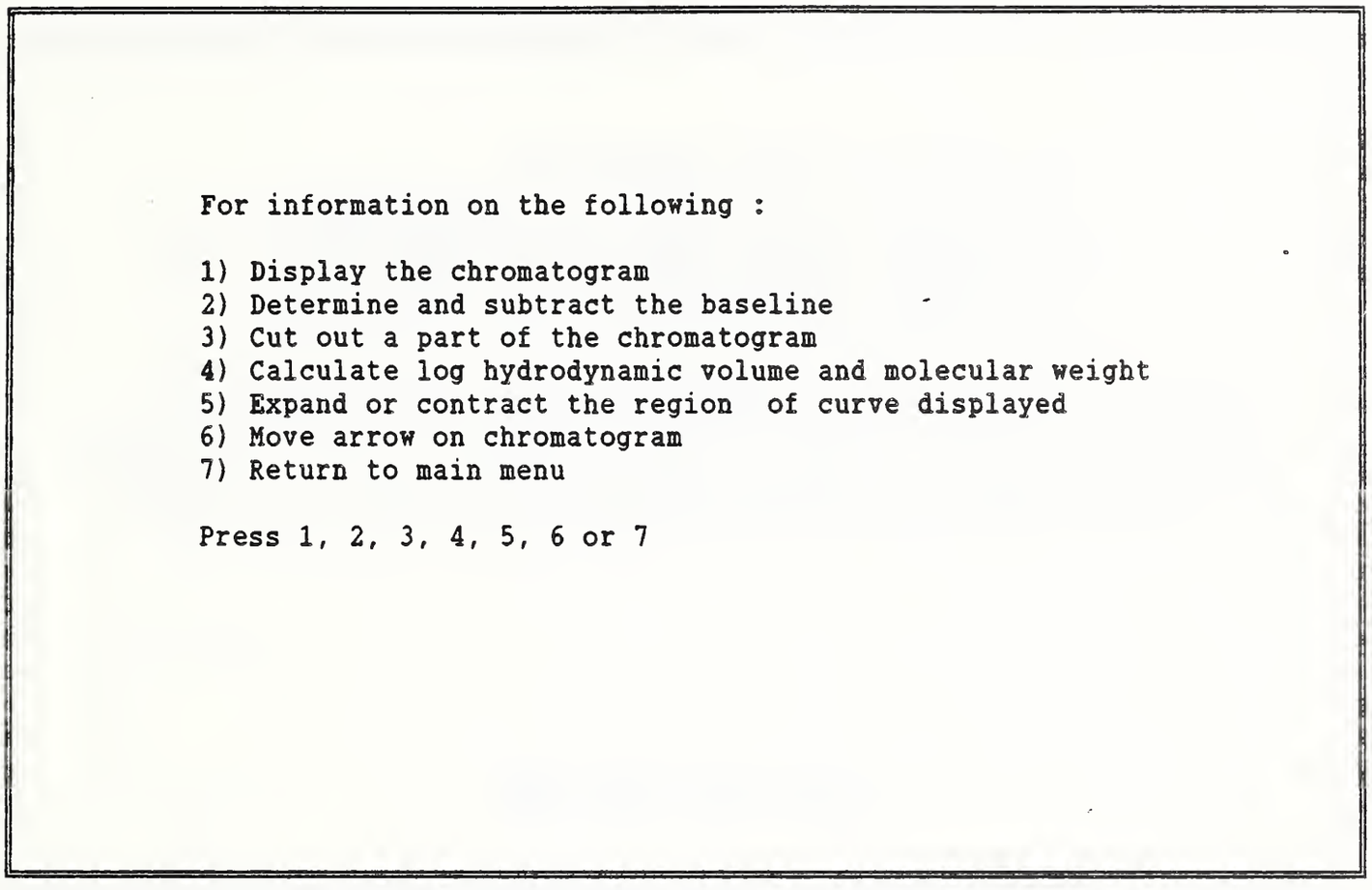
The program has now found a file, and is reading the contents into the computer.

ANALYZE screen 4 - Function key instructions after reading data file

```
        Press a function key

    - F1 displays the full chromatogram.
    - F2 displays the left half of the chromatogram.
    - F3 displays the right half of the chromatogram.
    - F4 displays the left third of the chromatogram.
    - F5 displays the middle third of the chromatogram.
    - F6 displays the right third of the chromatogram.
    - F7 marks a place to pass the baseline through.
    - F8 subtracts the baseline from the chromatogram - mark
      1 or 2 places with F7 before pressing F8.
    - F9 marks where to cut - usually the middle part between
      2 cuts is kept.
    - F10 cuts out the middle part and keeps it - the rest is
      discarded - mark 2 places with F9 before pressing F10.
      - Escape prints this menu.
      - M goes to the menu for further processing after
        the chromatogram has been cut by pressing F10.

Hit `H' for more help.
```

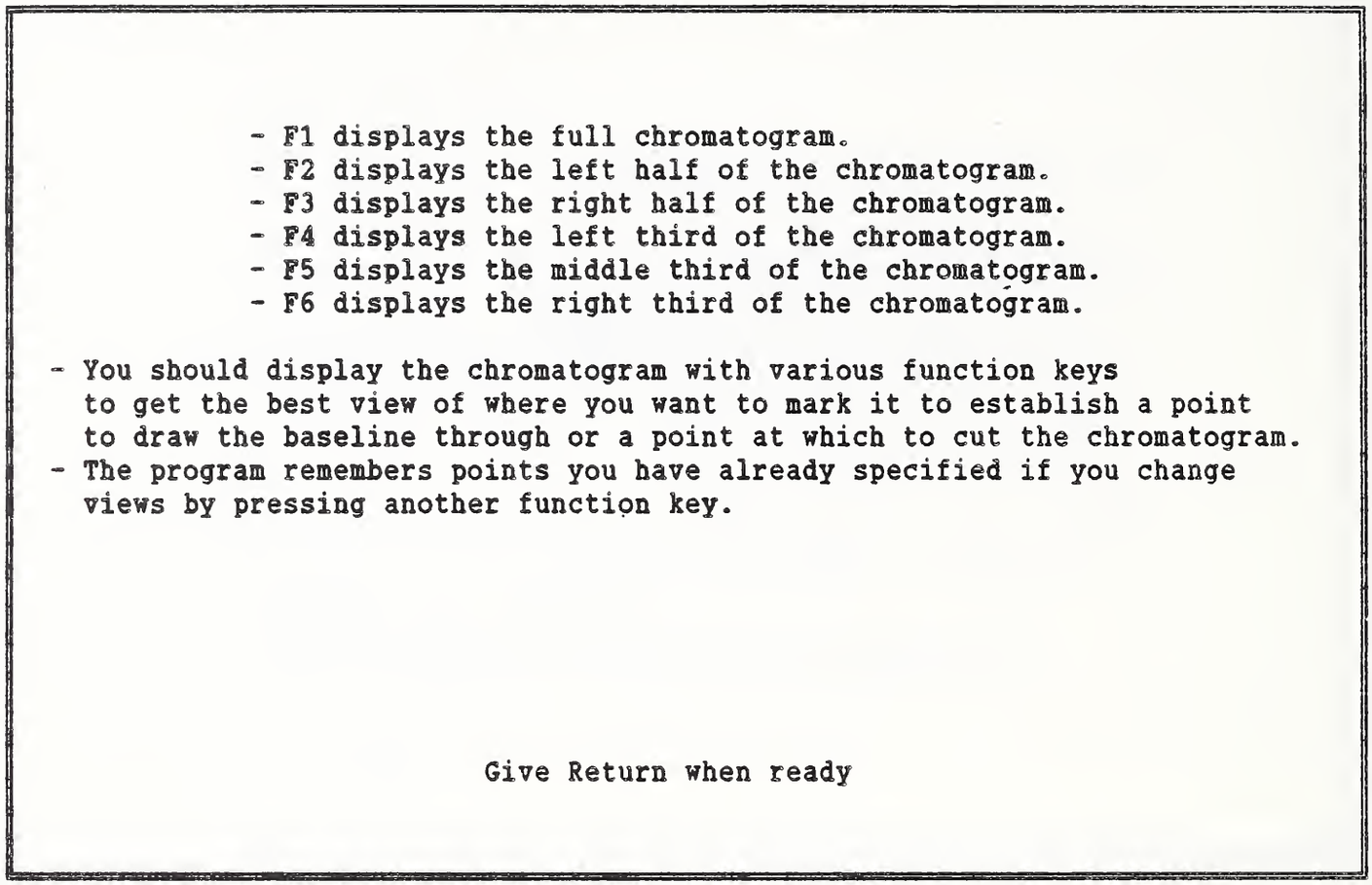
After a file has been read successfully, control is transferred to this menu. The typical sequence is to press F1 to plot the chromatogram, then use the arrow and F7 to select places near the beginning and end of the chromatogram to use to estimate the level of the background. Background correction is done by pressing F8. The part of the chromatogram containing information on the specimen is then cut out using the sequence: arrow movement, F9, arrow movement, F9, F10. All chromatograms can be saved as a "CUT" chromatogram, and all can be transformed to log hydrodynamic volume if the columns in the chromatograph have been calibrated. Chromatograms of specimens for which Mark-Houwink parameters are available may be transformed to log molecular weight.

ANALYZE screen 5 - After pressing H or h: Menu for help screens



Pressing H or h at the main menu leads to several help screens which explain the possibilities of the ANALYZE program. The following screens show the help screens for options 1 through 7 in the menu displayed here.

ANALYZE screen 6 - Help screen for functions keys F1 to F6



This screen is generated following the choice of option 1 in the H help screen menu.

ANALYZE screen 7 - Help screen for background correction

- Move the arrow on the chromatogram, using the cursor keys, to a place to pass the background line through, then press F7.

- To choose a second place to pass the background through, move the arrow to the second place on the chromatogram and press F7 again. The background line will then be drawn so you can see whether or not it is suitable.

- Press F8 to lead to a menu where you have a choice of using this background or editing it.

Give Return when ready

This screen is generated following the choice of option 2 in the H help screen menu.

ANALYZE screen 8 - Help screen for cutting the chromatogram

- The 'meaningful part' may be cut out of the entire chromatogram by moving the arrow to the beginning of the part to be cut out, and pressing F9.
- Then move the arrow to the end of the part to be cut out and press F9 again.
- Pressing F10 cuts the chromatogram and displays a menu for further processing.
- Shift with the cursor keys moves the arrow 10 points.
- Ctrl with the cursor keys moves the arrow 100 points.
- Up cursor expands the graph vertically and Down cursor contracts the graph.

Give Return when ready

This screen is generated following the choice of option 3 in the H help screen menu.

ANALYZE screen 9 - Help screen for further processing of cut chromatograms

- After the chromatogram has been cut, the hydrodynamic volume distribution may be calculated.

- If Mark-Houwink parameters were provided at setup time, the molecular weight distribution and molecular weight averages may also be calculated.

- The resulting distributions may be written to files on the disk.

Give Return when ready

This screen is generated following the choice of option 4 in the H help screen menu.

ANALYZE screen 10 - Help screen for expanding or contracting the plot

A section of the chromatogram may be shown expanded
by moving the arrow to that part of the chromatogram and
pressing the up cursor key. Then keys F1 to F6 will display
the section of the chromatogram magnified by a factor of 2
in the Y direction. The process may be repeated to further
magnify the chromatogram.

The process may be reversed by pressing the down cursor key.

The Page Up and Page Down keys move thew viewing area
up and down the chromatogram.

Give Return when ready

This screen is generated following the choice of option 5 in the H help screen menu.

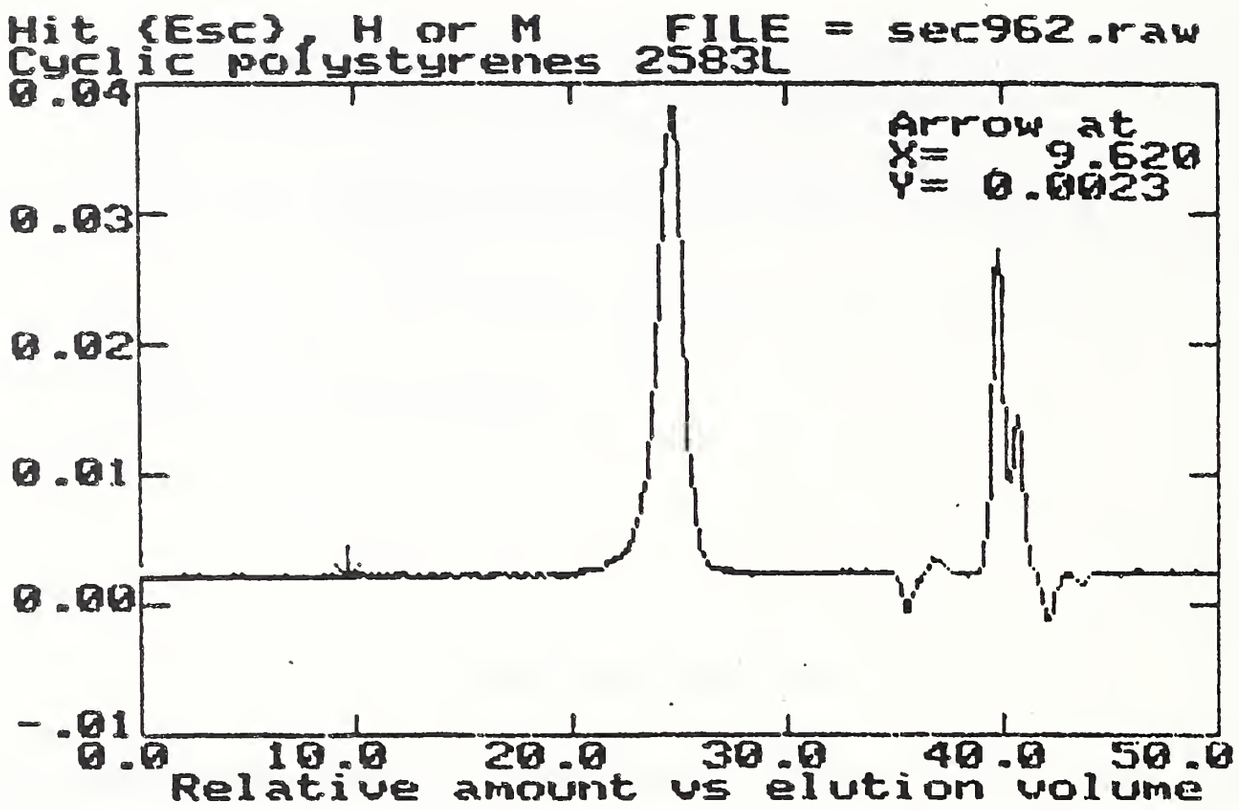
ANALYZE screen 11 - Help screen for moving the arrow on the plots

The arrow on the chromatogram may be moved one point to the
by the right and left cursor keys. It may be moved 10 points to
the right or left by shift cursor keys.

It may also be moved to the left of the chromatogram
by the Home key and to the right of the chromatogram
by the End key.

Give Return when ready

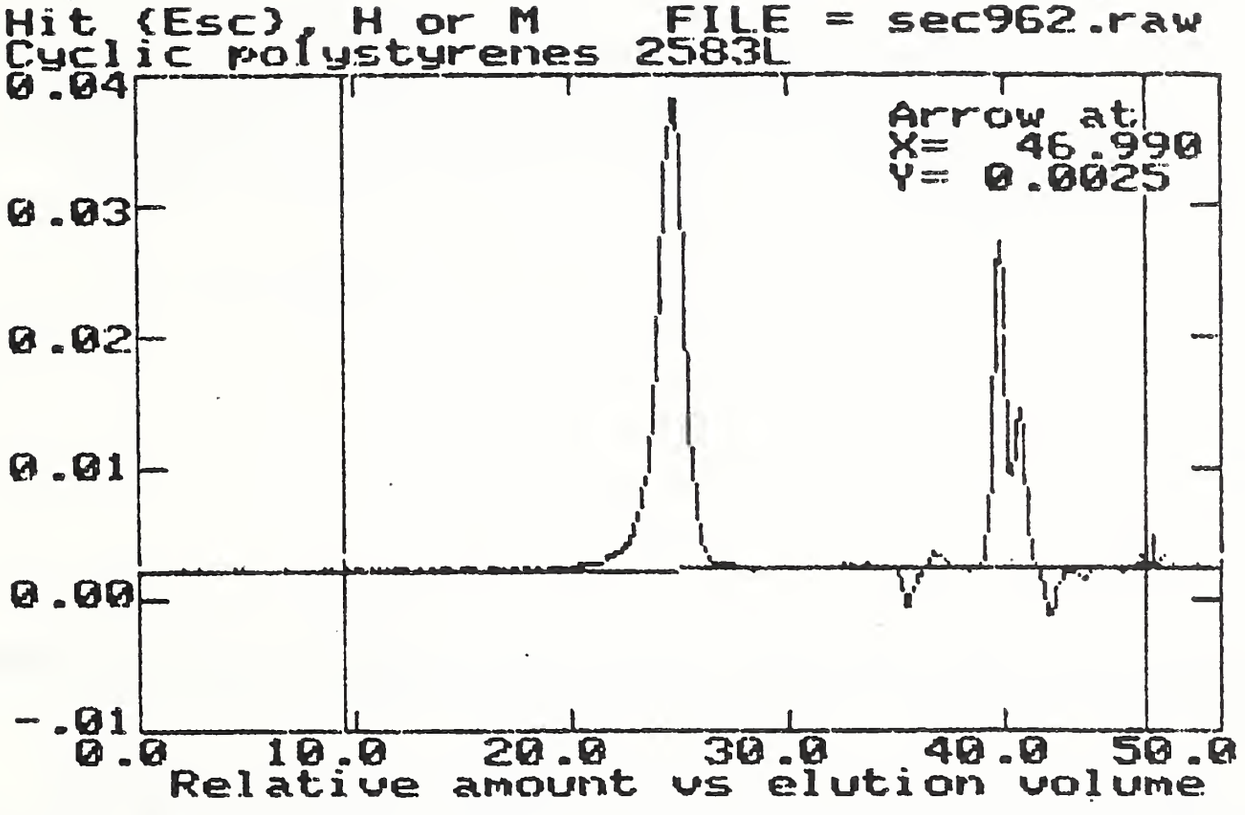
This screen is generated following the choice of option 6 in the H help screen menu.



ANALYZE plot 1 - Chromatogram as input from disk

The display shown here was generated by pressing F1 at the main menu. The operator may proceed to another menu by pressing {Esc} to go to the main menu, H or h to go to the help screens, or M or m to go to the analysis menu. The analysis menu controls output of the cut chromatogram, and transformation from elution volume into log hydrodynamic volume and log molecular weight. It also is the route to restarting the analysis on this chromatogram or reading in another chromatogram, and to ending the program.

The arrow shows where the chromatogram will be marked for background estimation and for cutting. The coordinates of the arrow are displayed. Note that the background of the chromatogram is not at zero on the vertical axis. Background correction involves estimating the heights in the chromatogram near the beginning and end of the chromatogram, and drawing a line which will be subtracted from the vertical coordinates of the chromatogram so that regions of the chromatogram which contain no contribution from the specimen will be zero.



ANALYZE plot 2 - Determining background (with F7)

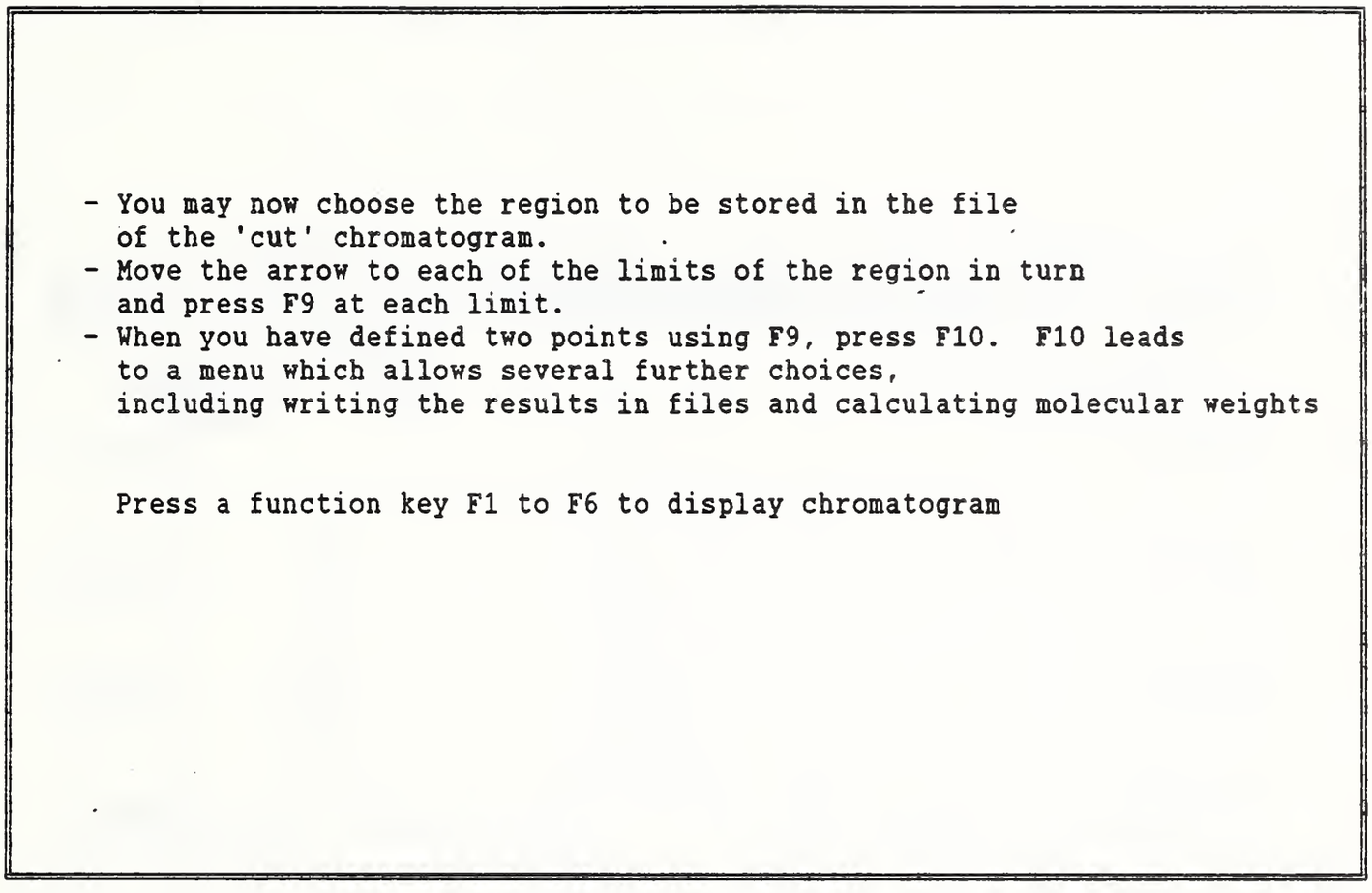
Here, two places at which the background is to be estimated have been chosen by moving the arrow and pressing F7. After the second press of F7, the background line is drawn. Before pressing F8, the operator must verify that this line is reasonable in that it seems to apply to the whole chromatogram.

ANALYZE screen 12 - Menu for background correction, after F8

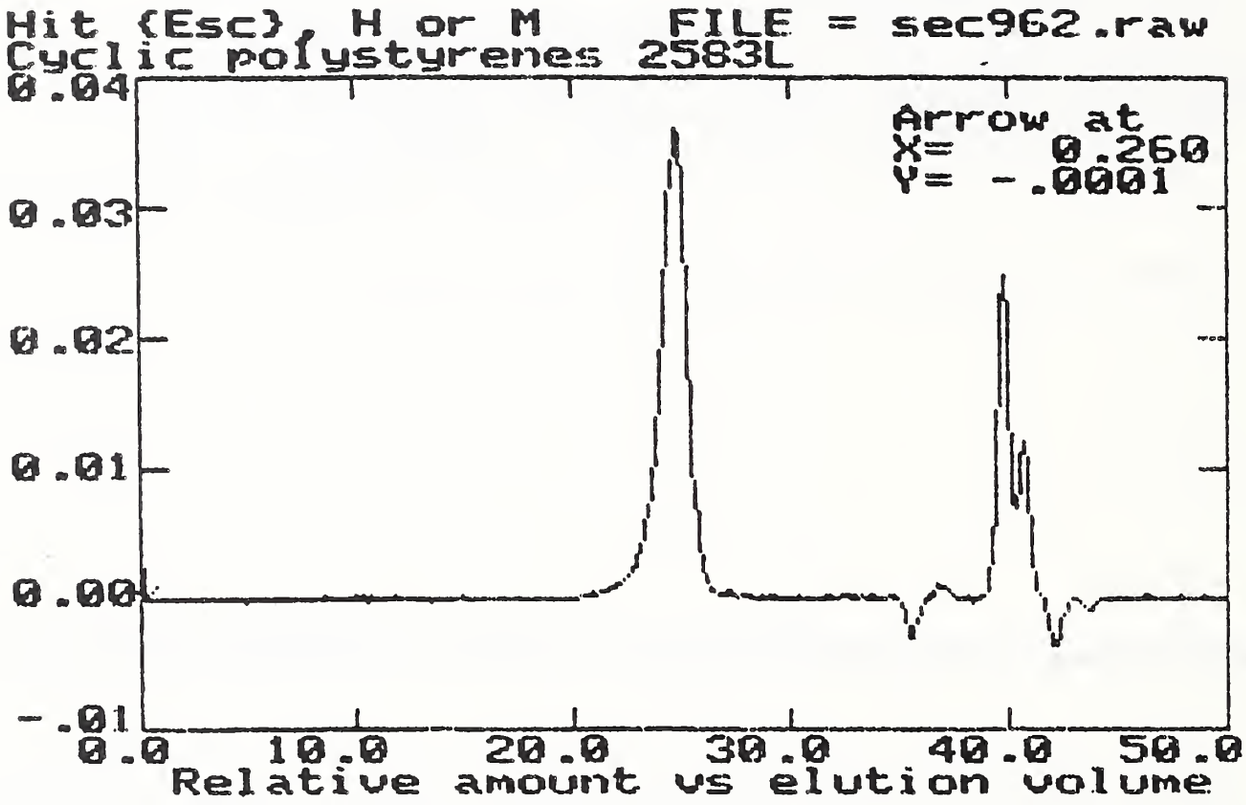
```
   Background points are at elution volumes of  17.74  and  31.75

Do you want to:

        1)  Correct for this background.
        2)  Redetermine background.
        3)  Store part of chromatogram without correcting for background.
        4)  Exit program.

            Press 1, 2, 3, or 4.
```

This screen is provided by the program after F8 has been pressed. Option 1 leads to the next menu, which explains that the next step is to cut the chromatogram.

ANALYZE screen 13 - After F8 and 1 (background has been subtracted)

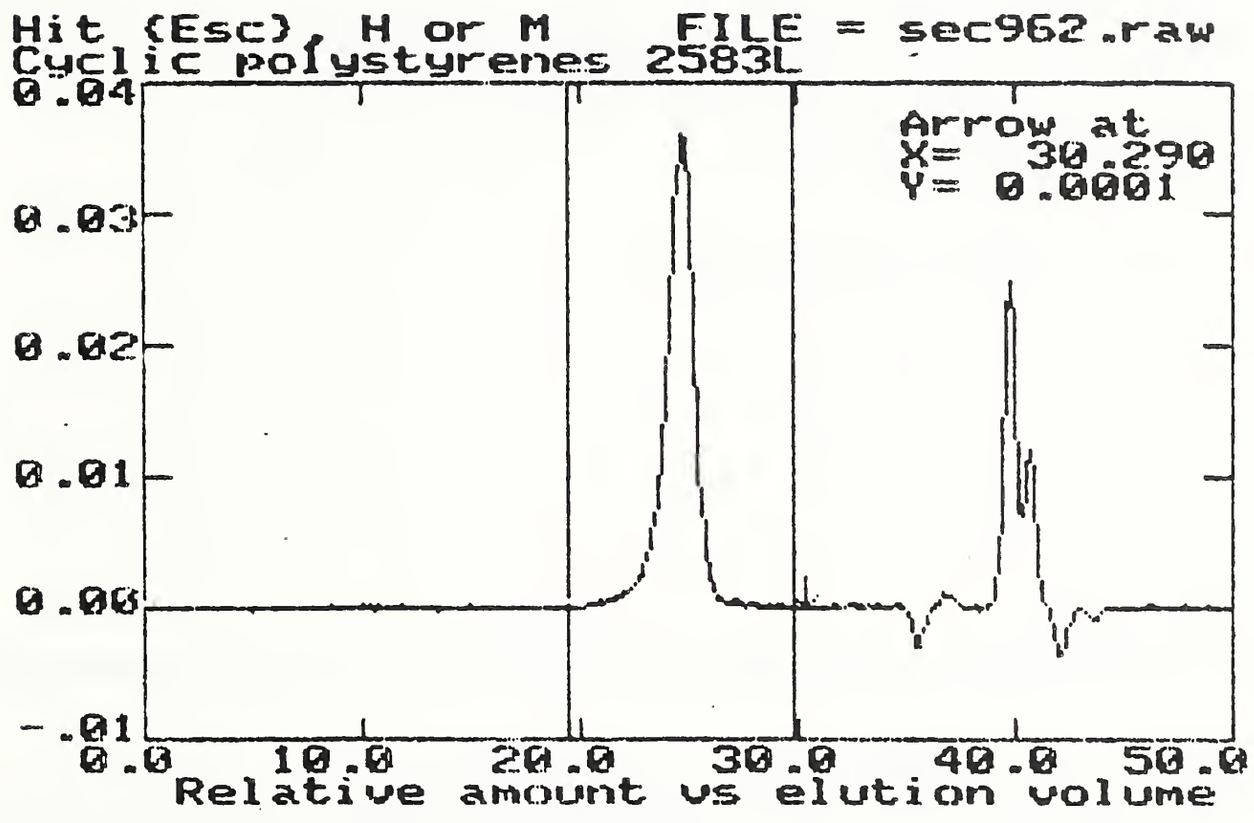


This screen follows option 1 in the previous menu, which followed pressing F8 to subtract the background in the chromatogram. The next step is to use the F9, F9, F10 sequence to cut out the part of the chromatogram which pertains to the specimen.



ANALYZE plot 3 - Chromatogram with background removed (F8)

The non-zero background has now been removed from the chromatogram so that the level at the beginning and end is zero. The next step is to cut out the part relevant to the specimen, which is the peak at about 25 milliliters in elution volume.



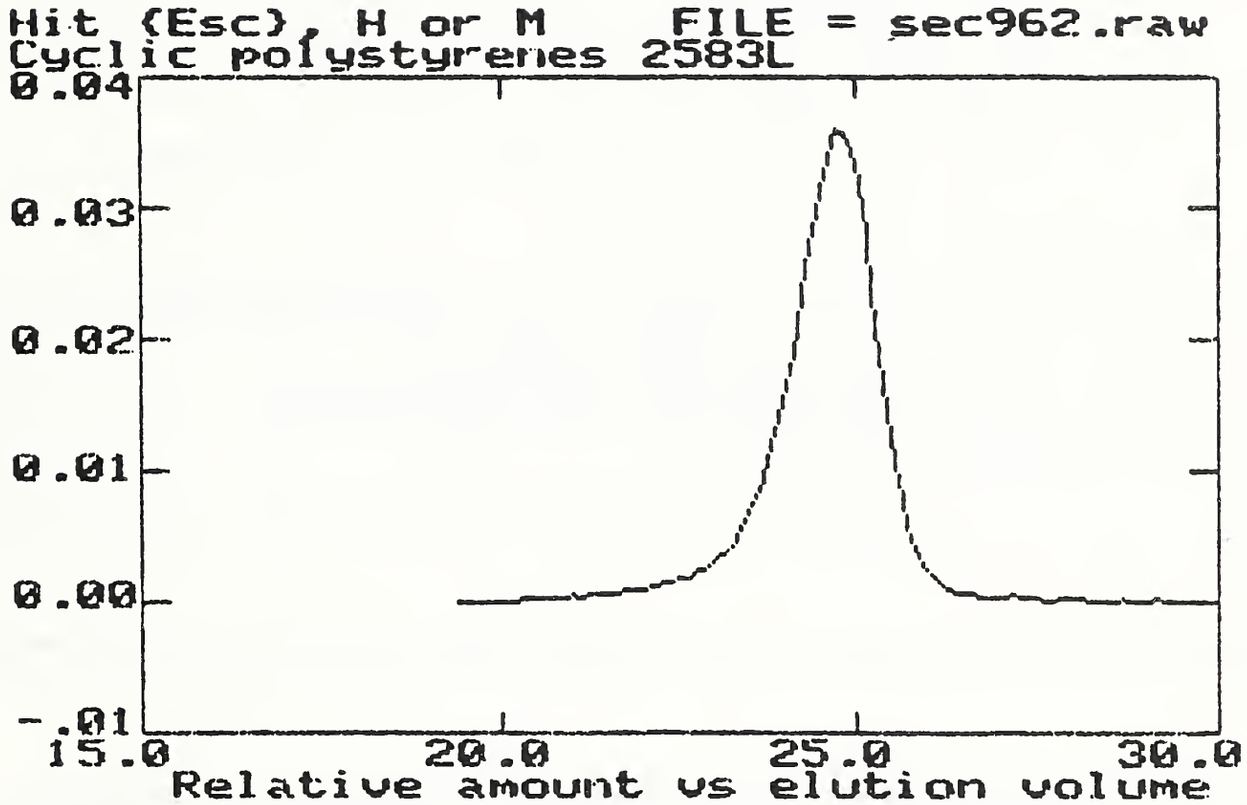
ANALYZE plot 4 - Delineating relevant part of chromatogram (with F9)

After the effect of non-zero background has been removed from the chromatogram using the F7, F7, F8 sequence, the part of the chromatogram pertaining to the specimen may be cut out. This screen shows limits chosen by moving the arrow and pressing F9, once for each limit. The next step is to press F10.

ANALYZE screen 14 - After pressing F10 to cut chromatogram (or M or m)

```
  Limits at elution or hydrodynamic volumes of  19.88  and  26.06

1)  Redetermine limits to cut chromatogram.
2)  Look at graph.
3)  Store clipped chromatogram versus elution or hydrodynamic volume in a file
4)  Change elution volume to log hydrodynamic volume.
5)  Compute and print molecular weight averages.
6)  Compute differential log molecular weight distribution.
7)  Examine another chromatogram.
8)  Exit from program.

Give a number between 1 and 8 inclusive
```

This is the analysis menu. The operator may carry out operations such as in options 4, 5 and 6, and view the results by pressing option 2. Option 3 allows the data to be stored in a file. The extension of the file depends on whether the chromatogram is in terms of elution volume (extension is CUT as in SEC999.CUT) or log hydrodynamic volume (extension is HYD as in SEC999.HYD). Chromatograms which are in terms of log molecular weight are saved to a file on the disk at the end of option 6.



ANALYZE plot 5 - "CUT" chromatogram (with F10 then 2)

The results of clipping the chromatogram with the F9, F9, F10 sequence and plotting the result with choice 2 from the analysis menu are shown here. The analysis menu is obtained by pressing M or m (for "m"enu).

ANALYZE screen 15 - Normalizing and storing cut chromatogram (M and 3)

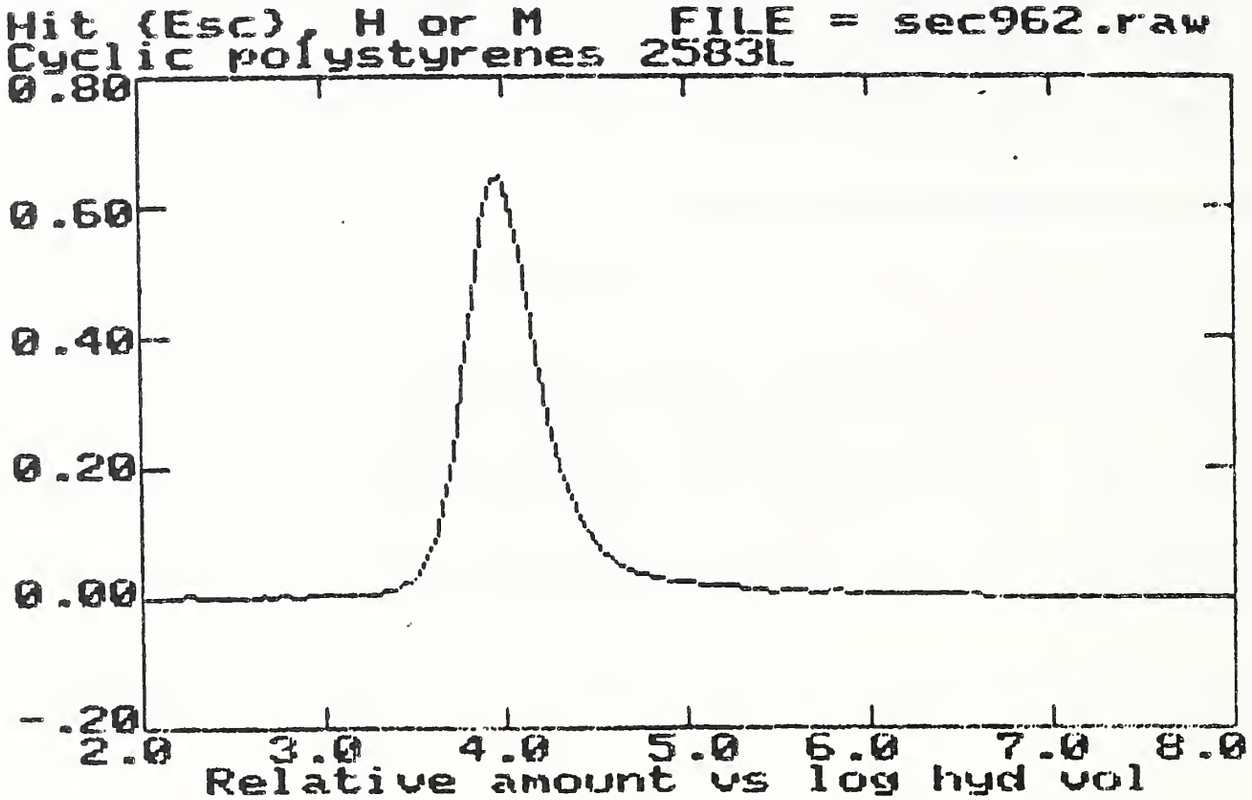
```
 Give a number between 1 and 8 inclusive
  You may normalize chromatogram before it is stored

  1) Normalize chromatogram to unit area
  2) Normalize chromatogram so the highest peak is 1.
  3) Multiply heights of chromatogram by a given scale factor
  4) Do not normalize chromatogram

     Give a number between 1 and 4 inclusive: 1
Chromatogram is being normalized to unit area and stored

         The clipped chromatogram has been saved in file sec924.CUT

 1)  Redetermine limits to cut chromatogram.
 2)  Look at graph.
 3)  Store clipped chromatogram versus elution or hydrodynamic volume in a file
 4)  Change elution volume to log hydrodynamic volume.
 5)  Compute and print molecular weight averages.
 6)  Compute differential log molecular weight distribution.
 7)  Examine another chromatogram.
 8)  Exit from program.

 Give a number between 1 and 8 inclusive
```

This screen shows the activity following pressing 3 in the analysis menu. The chromatogram was in terms of elution volume and hence the program has saved it as a CUT file. The operator chose to normalize the chromatogram.

ANALYZE screen 16 - Elution volume to log hydrodynamic volume (M and 4)

```
  Limits at elution or hydrodynamic volumes of  19.88  and  26.06

 1)  Redetermine limits to cut chromatogram.
 2)  Look at graph.
 3)  Store clipped chromatogram versus elution or hydrodynamic volume in a file
 4)  Change elution volume to log hydrodynamic volume.
 5)  Compute and print molecular weight averages.
 6)  Compute differential log molecular weight distribution.
 7)  Examine another chromatogram.
 8)  Exit from program.

 Give a number between 1 and 8 inclusive

Do you want to use calibration in file CALIBR.DAT ? (Enter Y or N)

Title of calibration is :
B DICKENS Calibration of Shodex cols 804 & 802 NOV 1986
Elution volume   19.88 is outside the calibration range
Elution volume   19.96 is outside the calibration range
Elution volume   20.05 is outside the calibration range
Elution volume   20.13 is outside the calibration range
Elution volume   20.21 is outside the calibration range
Elution volume   20.29 is outside the calibration range
```

This screen shows the program flow following pressing 4 in the analysis menu. To convert from elution volume to log hydrodynamic volume, the program needs to have the calibration of the chromatographic columns. The latest calibration is kept in the file CALIBR.DAT, which is read in automatically on answering Y or y to the question. The program indicates when elution volumes are being transformed which are beyond the calibrated range of elution volumes. This is a dangerous sign, since the calibration is not reliable outside the limits where it was constrained to follow the data points which were measured to find the calibration.



ANALYZE plot 6 - Distribution of log hydrodynamic volumes (M and 2)

The results of clipping the chromatogram with the F9, F9, F10 sequence and plotting the result with choice 2 from the analysis menu are shown here. The analysis menu is obtained by pressing M or m (for "m"enu).

ANALYZE screen 17 - Reading "CUT" file

```
- The ANALYZE program allows the user to analyze a raw chromatogram.
- The program reads in a RAW chromatogram.
- The user decides where to determine the background.
- The program then determines the background at these points and
  subtracts the background.
- The user then decides where to cut the chromatogram in order to
  analyze the central part.  The program cuts the chromatogram
  and analyzes it.
- The background is subtracted, and the chromatogram is analyzed.

Enter file name or run number for RAW chromatogram (quit = stop):  sec962.cut

                        Reading file sec962.cut

                      The title of the input file is:

 Cyclic polystyrenes 2583L        Run # 962 vial 1 inj 1 13:10:54 01-30-1986

                              ** READING **
```

The previous analysis sequence demonstrated how to go from a RAW chromatogram to a chromatogram in terms of elution volume. The chromatogram was stored in the form of a CUT chromatogram (i.e., in terms of elution volume, although it could equally well have been stored in terms of log hydrodynamic volume). This screen shows that the stored CUT file can later be read into the ANALYZE program and the analysis can be continued. In this case, there is no need to correct for the background in the chromatogram. The chromatogram can be re-cut if desired.

ANALYZE screen 18 - Main menu, obtained after reading cut file

```
          Press a function key

        - F1 displays the full chromatogram.
        - F2 displays the left half of the chromatogram.
        - F3 displays the right half of the chromatogram.
        - F4 displays the left third of the chromatogram.
        - F5 displays the middle third of the chromatogram.
        - F6 displays the right third of the chromatogram.
        - F7 marks a place to pass the baseline through.
        - F8 subtracts the baseline from the chromatogram - mark
          1 or 2 places with F7 before pressing F8.
        - F9 marks where to cut - usually the middle part between
          2 cuts is kept.
        - F10 cuts out the middle part and keeps it - the rest is
          discarded - mark 2 places with F9 before pressing F10.
          - Escape prints this menu.
          - M goes to the menu for further processing after
            the chromatogram has been cut by pressing F10.


 Hit `H' for more help.
```

This is the main menu, which gives information on how to carry out plotting and further analysis.

ANALYZE screen 19 - After F10 or M

```
    Limits at elution volumes of  19.41  and  29.59

1)  Redetermine limits to cut chromatogram.
2)  Look at graph.
3)  Store clipped chromatogram versus elution or hydrodynamic volume in a file
4)  Change elution volume to log hydrodynamic volume.
5)  Compute and print molecular weight averages.
6)  Compute differential log molecular weight distribution.
7)  Examine another chromatogram.
8)  Exit from program.

Give a number between 1 and 8 inclusive:
```

The analysis menu is obtained after pressing M or m in the main menu. This would be the normal route to take. A wise step would be to plot the input chromatogram with option 2, then press M to return to this analysis menu for further processing.

ANALYZE screen 20 - After M and 4

```
 7)  Examine another chromatogram.
 8)  Exit from program.

 Give a number between 1 and 8 inclusive:  4


Do you want to use calibration in file CALIBR.DAT ? (Enter Y or N) n

What is the name of the calibration file? clonecal.3

Title of calibration is :

B DICKENS Calibration of Shodex cols 804 & 802 NOV 1986

Elution volume   19.41 is outside the calibration range
Elution volume   19.51 is outside the calibration range
Elution volume   19.61 is outside the calibration range
Elution volume   19.71 is outside the calibration range
Elution volume   19.81 is outside the calibration range
Elution volume   19.90 is outside the calibration range
Elution volume   20.00 is outside the calibration range
Elution volume   20.10 is outside the calibration range
Elution volume   20.20 is outside the calibration range
Elution volume   20.30 is outside the calibration range
```

This screen shows the sequence generated by pressing option 4, the transformation of elution volume to log hydrodynamic volume.

ANALYZE screen 21 - After M and 4 part 1

```
 Give a number between 1 and 8 inclusive:  4

Do you want to use calibration in file CALIBR.DAT ? (Enter Y or N) n

What is the name of the calibration file? clonecal.3

Title of calibration is :

B DICKENS Calibration of Shodex cols 804 & 802 NOV 1986

Elution volume   19.41 is outside the calibration range
Elution volume   19.51 is outside the calibration range
Elution volume   19.61 is outside the calibration range
Elution volume   19.71 is outside the calibration range
Elution volume   19.81 is outside the calibration range
Elution volume   19.90 is outside the calibration range
Elution volume   20.00 is outside the calibration range
Elution volume   20.10 is outside the calibration range
Elution volume   20.20 is outside the calibration range
Elution volume   20.30 is outside the calibration range

          Elution volume has been changed to log hydrodynamic volume
```

This screen shows that the transformation of elution to log hydrodynamic volume has been completed. It also shows where the elution volumes were outside the calibrated range of the columns. This is a warning to the operator that the results in those regions may not be trustworthy.

ANALYZE screen 22 - After M and 4 part 2

```
Elution volume   19.61 is outside the calibration range
Elution volume   19.71 is outside the calibration range
Elution volume   19.81 is outside the calibration range
Elution volume   19.90 is outside the calibration range
Elution volume   20.00 is outside the calibration range
Elution volume   20.10 is outside the calibration range
Elution volume   20.20 is outside the calibration range
Elution volume   20.30 is outside the calibration range

          Elution volume has been changed to log hydrodynamic volume


     Limits at log hydrodynamic volumes of  2.099675  and  7.994616

 1)  Redetermine limits to cut chromatogram.
 2)  Look at graph.
 3)  Store clipped chromatogram versus elution or hydrodynamic volume in a file
 4)  Change elution volume to log hydrodynamic volume.
 5)  Compute and print molecular weight averages.
 6)  Compute differential log molecular weight distribution.
 7)  Examine another chromatogram.
 8)  Exit from program.

 Give a number between 1 and 8 inclusive:
```

At the conclusion of option 4, averages based on log hydrodynamic volume are written to the printer, and the limits of the chromatogram are presented in terms of log hydrodynamic volume rather than elution volume.

ANALYZE screen 23 - After M and 5

```
5)  Compute and print molecular weight averages.
6)  Compute differential log molecular weight distribution.
7)  Examine another chromatogram.
8)  Exit from program.

Give a number between 1 and 8 inclusive:  5


                Computing molecular weight averages

      Limits of log molecular weight values are  3.542915  and  6.930812

                   Area of chromatogram =  .9983551

                          Mn =  41909.16
                          Mw =  54068.64
                          Mz =  87022.96

                     Polydispersity =  1.290139

                   Calculated viscosity =  .2629267

                          Press any key
```

Option 5 in the analysis menu transforms log hydrodynamic volume (or elution volume) into log molecular weight, providing that Mark-Houwink parameters were given at SETUP time. These parameters are available to the program through the data file which contains the chromatogram. The option provides estimates of the molecular weight averages, the polydispersity, and the intrinsic viscosity.

ANALYZE screen 24 - After M and 5

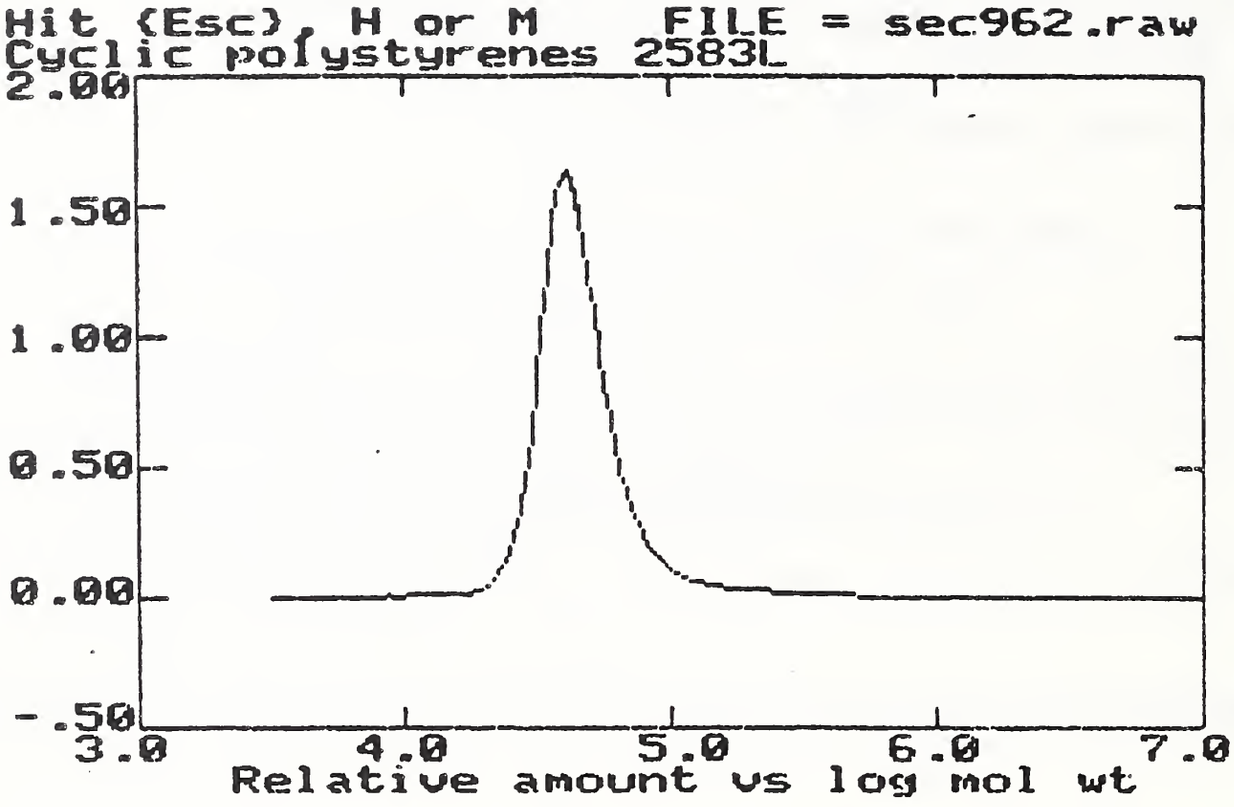
```
                    Mn =  41909.16
                    Mw =  54068.64
                    Mz =  87022.96

                Polydispersity =  1.290139

              Calculated viscosity =  .2629267

                    Press any key


    Limits at log molecular weight values of  3.542915  and  6.930812

1)  Redetermine limits to cut chromatogram.
2)  Look at graph.
3)  Store clipped chromatogram versus elution or hydrodynamic volume in a file
4)  Change elution volume to log hydrodynamic volume.
5)  Compute and print molecular weight averages.
6)  Compute differential log molecular weight distribution.
7)  Examine another chromatogram.
8)  Exit from program.

Give a number between 1 and 8 inclusive:
```

After a key is pressed at the end of option 5, the analysis menu reappears. The limits of the analyzed range of the chromatogram are now given in terms of log molecular weight.

ANALYZE screen 25 - After M and 6

```
 2)  Look at graph.
 3)  Store clipped chromatogram versus elution or hydrodynamic volume in a file
 4)  Change elution volume to log hydrodynamic volume.
 5)  Compute and print molecular weight averages.
 6)  Compute differential log molecular weight distribution.
 7)  Examine another chromatogram.
 8)  Exit from program.

 Give a number between 1 and 8 inclusive:  6


            Calculating differential molecular weight distribution

                  Normalizing molecular weight distribution

    Differential log molecular weight distribution has been calculated.

Do you want to store the distribution in a file (Enter Y or N) : y

            Storing log differential molecular weight distribution

Differential log molecular weight distribution is stored in sec962.MWD
```

Option 6 in the analysis menu calculates the log molecular weight distribution and normalizes it. This means that the program makes the total area under the curve of the log molecular weight equal to one so the results are independent of the amount of material injected and of the refractive index difference between the material and the solvent. Hence, different specimens can be compared meaningfully.



ANALYZE plot 7 - Log molecular weight plot (M then 6 then 2)

Pressing 2 after option 6 has been carried out generates a plot of the log molecular weight distribution, an example of which is shown here.

ANALYZE screen 26 - After M and 6 without going through 4 or 5

```
        Elution volume has been changed to log hydrodynamic volume


Title of data file is :
Cyclic polystyrenes 2583L        Run # 962 vial 1 inj 1 13:10:54 01-30-1986

Title of calibration file is :
B DICKENS Calibration of Shodex cols 804 & 802 NOV 1986

        Limits of log molecular weight values are  3.542915  and  6.930812

                    Area of chromatogram =  .9983551

                            Mn =  41909.16
                            Mw =  54068.64
                            Mz =  87022.96

                        Polydispersity =  1.290139

                      Calculated viscosity =  .2629267

                            Press any key
```

The next three screens show the sequence generated when option 6 is chosen even though the chromatogram is in terms of elution volume. The program automatically generates options 4 and 5.

ANALYZE screen 27 - After M and 6

```
                    Mn =  41909.16
                    Mw =  54068.64
                    Mz =  87022.96

              Polydispersity =  1.290139

            Calculated viscosity =  .2629267

                    Press any key

          Normalizing molecular weight distribution

    Differential log molecular weight distribution has been calculated.

Do you want to store the distribution in a file (Enter Y or N) : y

          Storing log differential molecular weight distribution

Differential log molecular weight distribution is stored in sec962.MWD


                    Press any key
```

Part two of the sequence generated when 6 is pressed immediately after going to the analysis menu for the first time, when the chromatogram is in terms of elution volume.

ANALYZE screen 28 - After M and 6 and "Press any key"

```
    Limits at log molecular weight values of  3.542915  and  6.930812

1)  Redetermine limits to cut chromatogram.
2)  Look at graph.
3)  Store clipped chromatogram versus elution or hydrodynamic volume in a file
4)  Change elution volume to log hydrodynamic volume.
5)  Compute and print molecular weight averages.
6)  Compute differential log molecular weight distribution.
7)  Examine another chromatogram.
8)  Exit from program.

Give a number between 1 and 8 inclusive:
```

Control has now been returned to the analysis menu. The operator can go to another chromatogram or end the program.

Program to compare two chromatograms

COMPARE (interactive)

This program interactively makes a detailed comparison of two chromatograms. The heights in the chromatograms may be multiplied by a factor or made to match at a given elution volume. The chromatograms may be subtracted from one another, when the difference chromatogram is displayed. It may be stored in a file for later processing.

The program uses a file called STANDARD.CHR . A chromatogram may be designated to be a member of a particular class, such as "polystyrene". The class of each chromatogram is stored in the file of the chromatogram. A standard chromatogram for each class may be chosen and all chromatograms of the class may be compared with the standard. The file STANDARD.CHR contains on each line the name of the class, the name of the file containing the corresponding standard chromatogram, and the name of the appropriate calibration file.

The program first reads the file STANDARD.CHR and then requests the file name of a chromatogram to read. If the extension of the file is HYD, only the run number need be given, otherwise the full name of the file must be typed. The chromatogram of the file is read and the name of the second file is requested. A file or run number or {Return} may be given. If {Return} is pressed, the standard chromatogram corresponding to the class of the first chromatogram is read, otherwise the requested file is read.

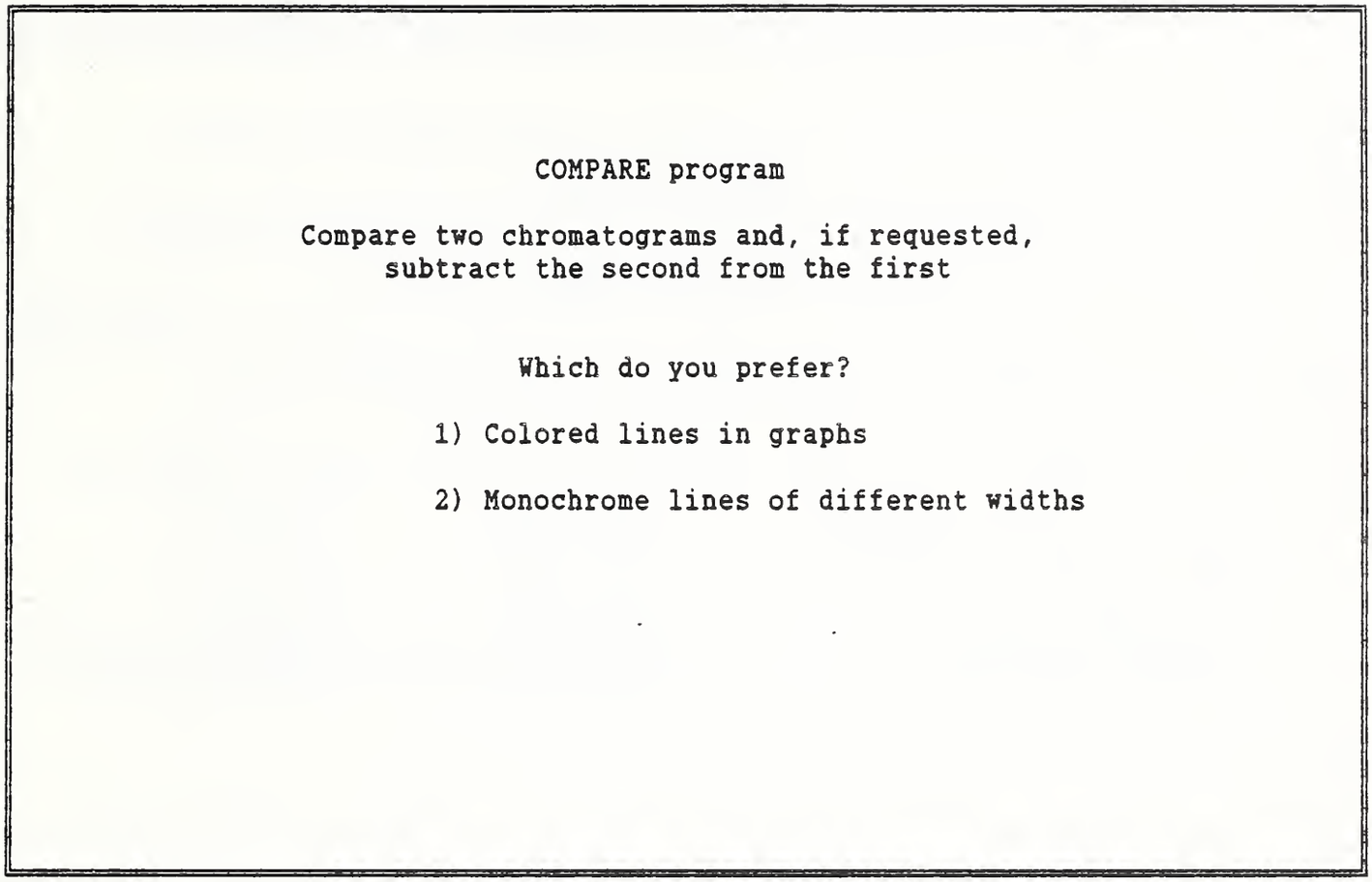
The chromatograms are displayed by pressing one of function keys F1 to F6. The chromatograms may be normalized to unit area by pressing key F7. A curve may be scaled with respect to the other curve by pressing F8 and giving the curve number and factor to scale the curve by. Curve 1 may also to scaled to match curve 2 at a specified point without having to input the factor. The arrow on the curve is moved to the point at which the curves are to be matched and F8 and then 0,0 {Return} are pressed.

Key F9 subtracts the chromatograms and calculates the standard deviation of the difference chromatogram. The difference chromatogram may be displaced by key F1.

Key F10 gives the menu.

The Escape key is used to exit the program or to restart the program after choosing whether or not to store the difference chromatogram.

COMPARE screen 1 - Starting menu (1)



The starting menu of the COMPARE program. If you have a colored graphics adapter screen, select option 1. The following screen representations are necessarily in black and white, so option 2 was chosen.

COMPARE screen 2 - Starting menu (2)

```
                        COMPARE program

              Compare two chromatograms and, if requested,
                   subtract the second from the first  -

          The classes and associated files in file STANDARD.CHR are:

           Class                    Standard chromatogram    Calibration

1  Bodied tung oil - Superior            sec207.hyd           clonecal.1
2  Bodied tung oil - Degen               sec207.hyd           clonecal.1
3  Not assigned                          cutchr.std           clonecal.3
4  Black ink - Sicpa                     sec423.cut           clonecal.1
5  Branched epoxy                        cutchr.std           clonecal.3
6  PSD/PVME irradiated                   pvme.hyd             clonecal.3
```

The second screen provided by the program shows a listing of the standard chromatograms and associated calibration files which the program reads from the file STANDARD.CHR. The first chromatogram to be input can then easily be compared with the standard chromatogram if required.

COMPARE screen 3 - Reading the data files

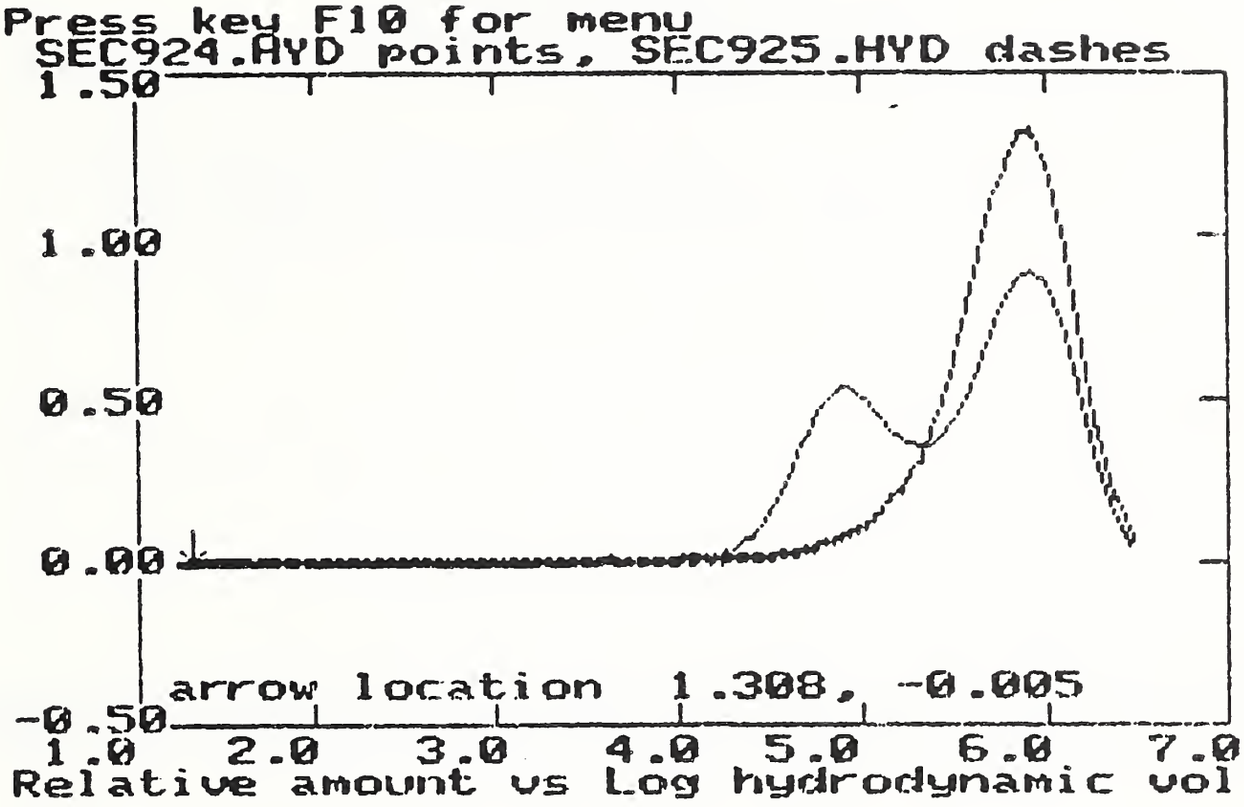
```
     - The COMPARE program allows the user to compare two chromatograms.

     - The first chromatogram may be normalized, expanded, or contracted
       to match the second chromatogram which can be a standard chromatogram.

     - The second chromatogram may be subtracted from the first
       chromatogram and the difference chromatogram may be stored in a file.

Run number is converted to HYD type of file
Enter file name or run number for first chromatogram (quit=stop) : 924

                          Reading file SEC924.HYD

 0 Mrad whole polymer              Run # 924 vial 1 inj 1 18:31:49 01-12-1986



Enter file name or run number for second chromatogram:
or RETURN for the corresponding standard chromatogram (quit=stop):? 925

                          Reading file SEC925.HYD

 0 Mrad methanol insoluble         Run # 925 vial 2 inj 1 19:27:50 01-12-1986
```

In this example, two chromatograms were read into the program. If the chromatograms are in terms of log hydrodynamic volume, i.e., they are HYD files, the operator need only specify the run number.

COMPARE screen 4 - Plotting menu

```
------------------ Display Selection Menu ------------------

- F1 displays the full chromatograms.
- F2 displays the left half of the chromatograms.
- F3 displays the right half of the chromatograms.
- F4 displays the left third of the chromatograms.
- F5 displays the middle third of the chromatograms.
- F6 displays the right third of the chromatograms.
- F7 normalizes the chromatograms to unit area.
- F8 matches chromatogram 1 to chromatogram 2 at the arrow.
  Move the arrow with the cursor keys or use shift/ctrl with
  the cursor keys.
  *****   WARNING Press F8 only when graph is displayed   *****
- F9 subtracts chromatogram 2 from chromatogram 1 and
  calculates the standard deviation of the difference.
- F10 displays this menu

Esc gives the option of storing the difference chromatogram,
    reading in more chromatograms, or quitting the program
Up Cursor expands graph vertically and Down Cursor contracts graph.

    Press one of function keys F1 to F6 to display the chromatograms.
```

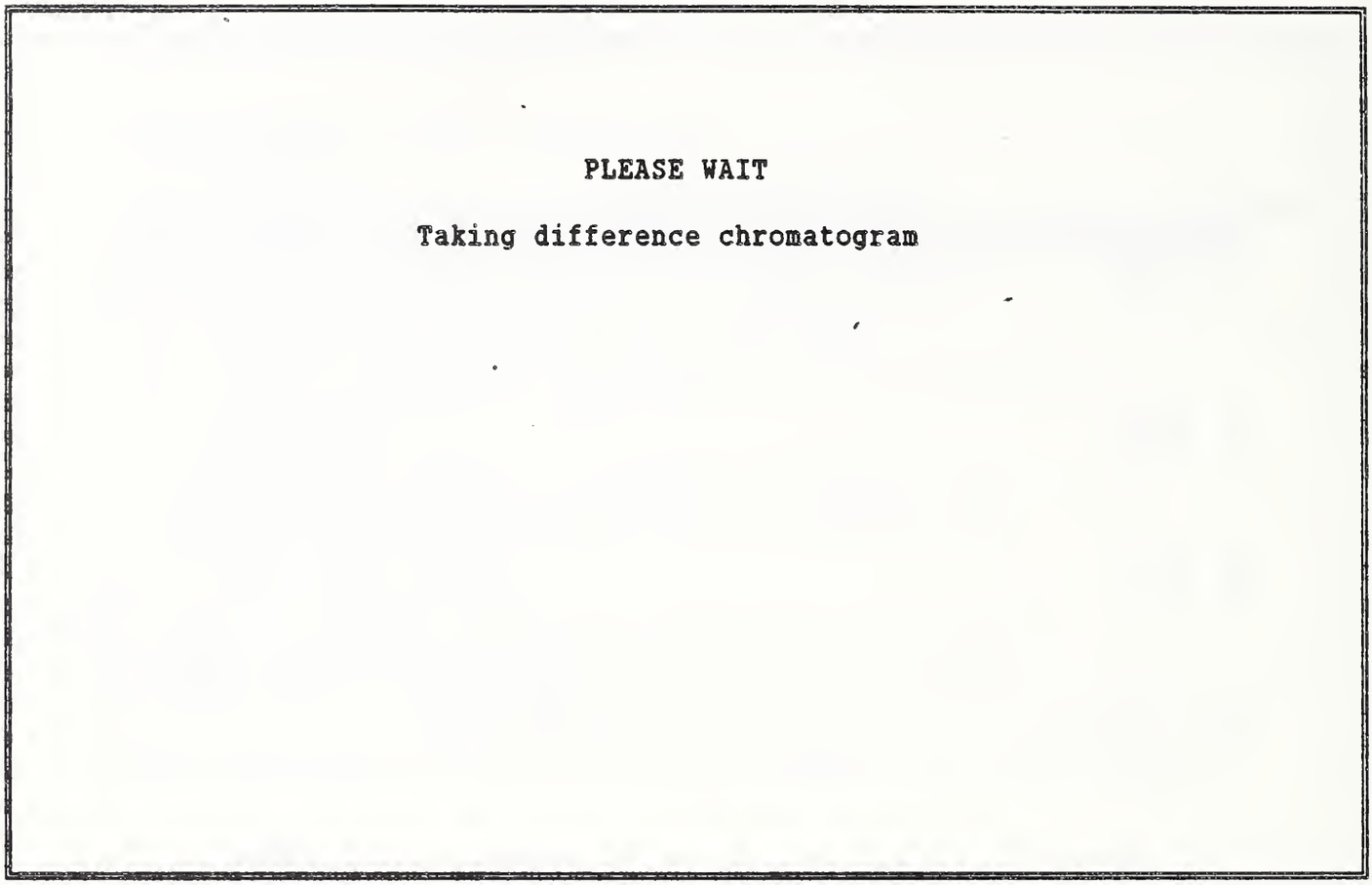
This screen explains the plotting options and how to match the two chromatograms. They may be matched at a point specified by the arrow on the plot (the plot must be on the screen at the time F8 is pressed) or the chromatograms can be scaled by giving the curve number (1 or 2 for first curve read in or second curve read in) and the multiplication factor after pressing F8. The factor always operates on the curve as read in and is not cumulative. F7 normalizes the two chromatograms. This means that the area under each curve is made equal to one, which is a good general way to compare two chromatograms. If the comparison is between two materials one of which has less of one component than the other, it would be more appropriate to move the arrow to a peak in some region of the chromatogram which corresponds to a component which is present in identical amounts in the two cases, and press F8 then 0,0 {Return} to make the two chromatograms of equal height at that point. The 0,0 means apply no factor to the curves.



COMPARE plot 1 - "Clipped" chromatograms as input

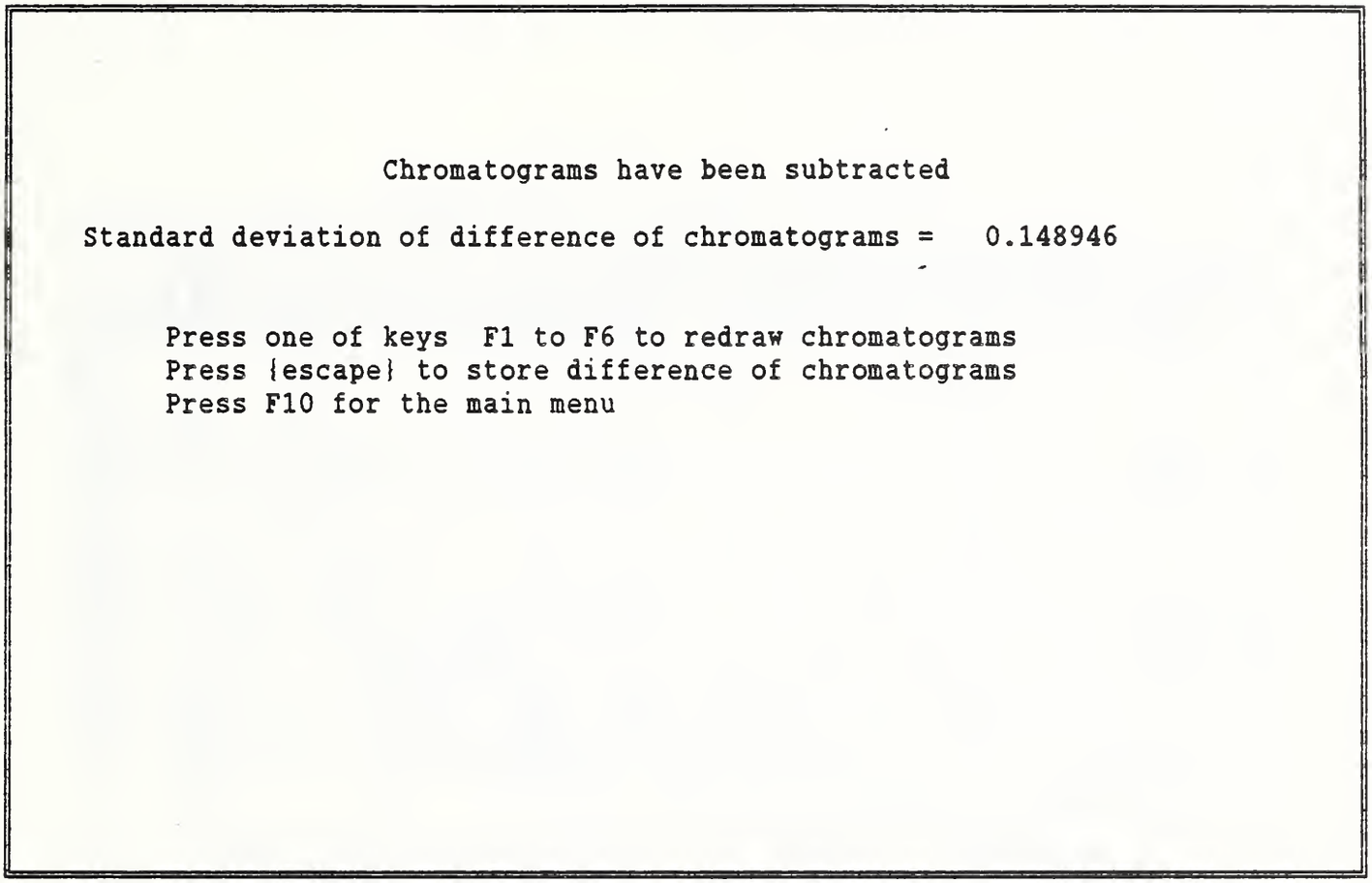
The two chromatograms read into the program in the example are displayed here. The label at the bottom of the plot shows that they are in terms of log hydrodynamic volume. The label at the top of the plot shows that SEC924.HYD is the lighter line, and SEC925.HYD is the heavier line.

COMPARE screen 5 - Taking difference chromatogram

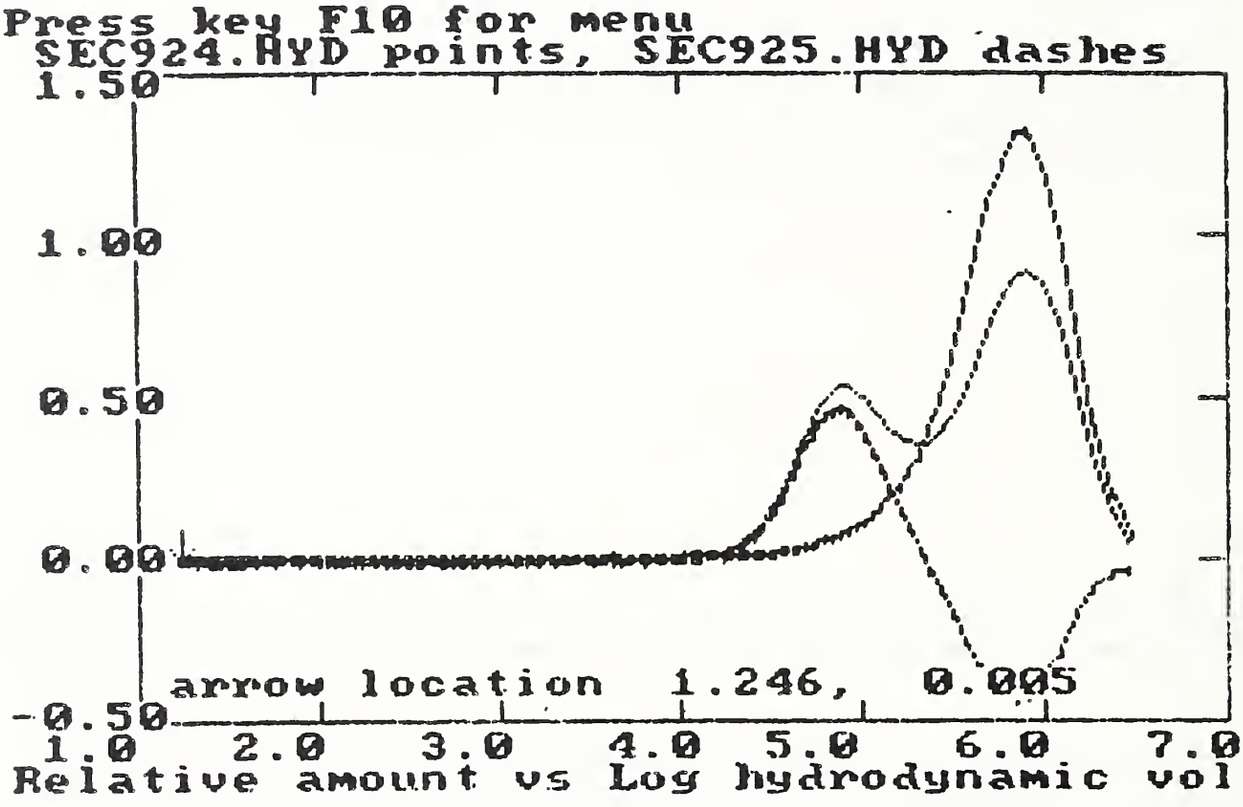


Pressing F9 takes the difference between the two original chromatograms and generates this message on the screen.

COMPARE screen 6 - After taking difference chromatogram

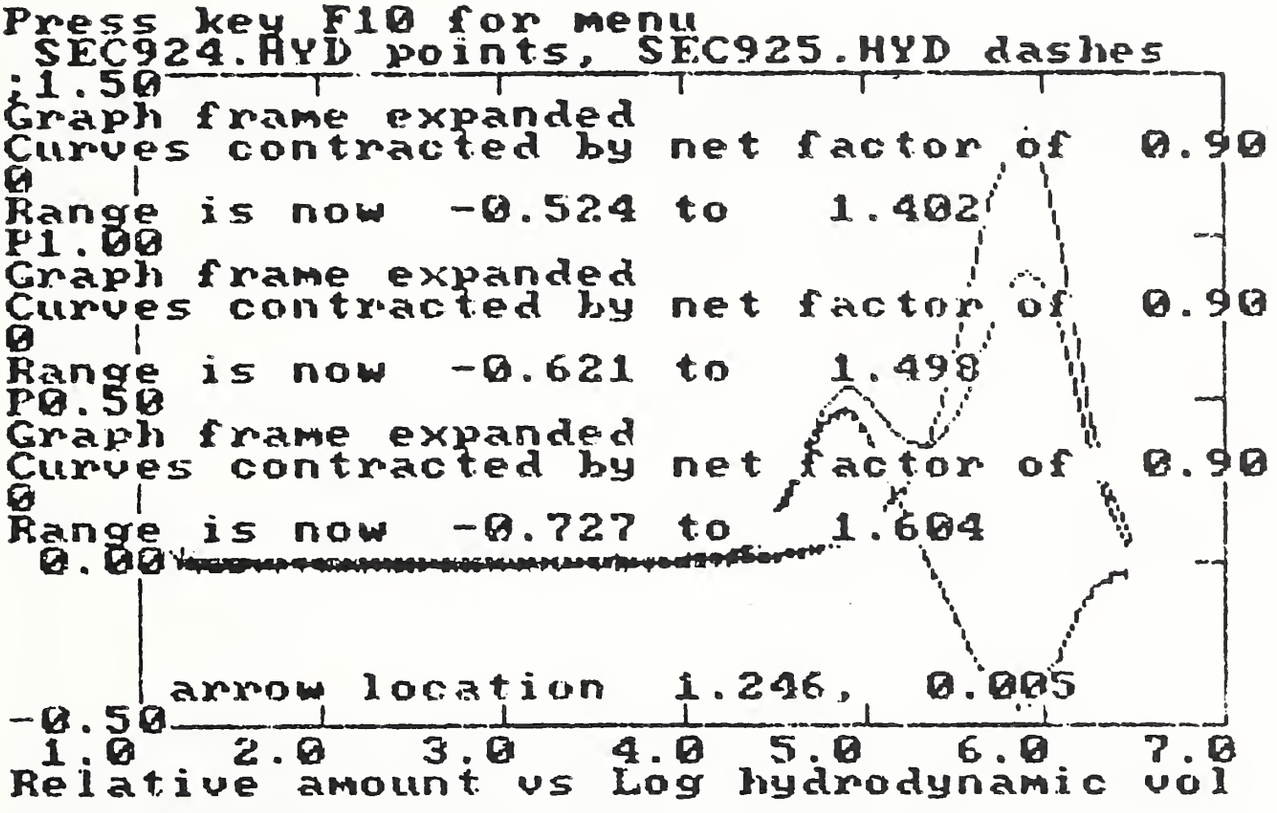


In subtracting the two chromatograms, the program calculates the difference in terms of a standard deviation, which is displayed on the screen as in this example.



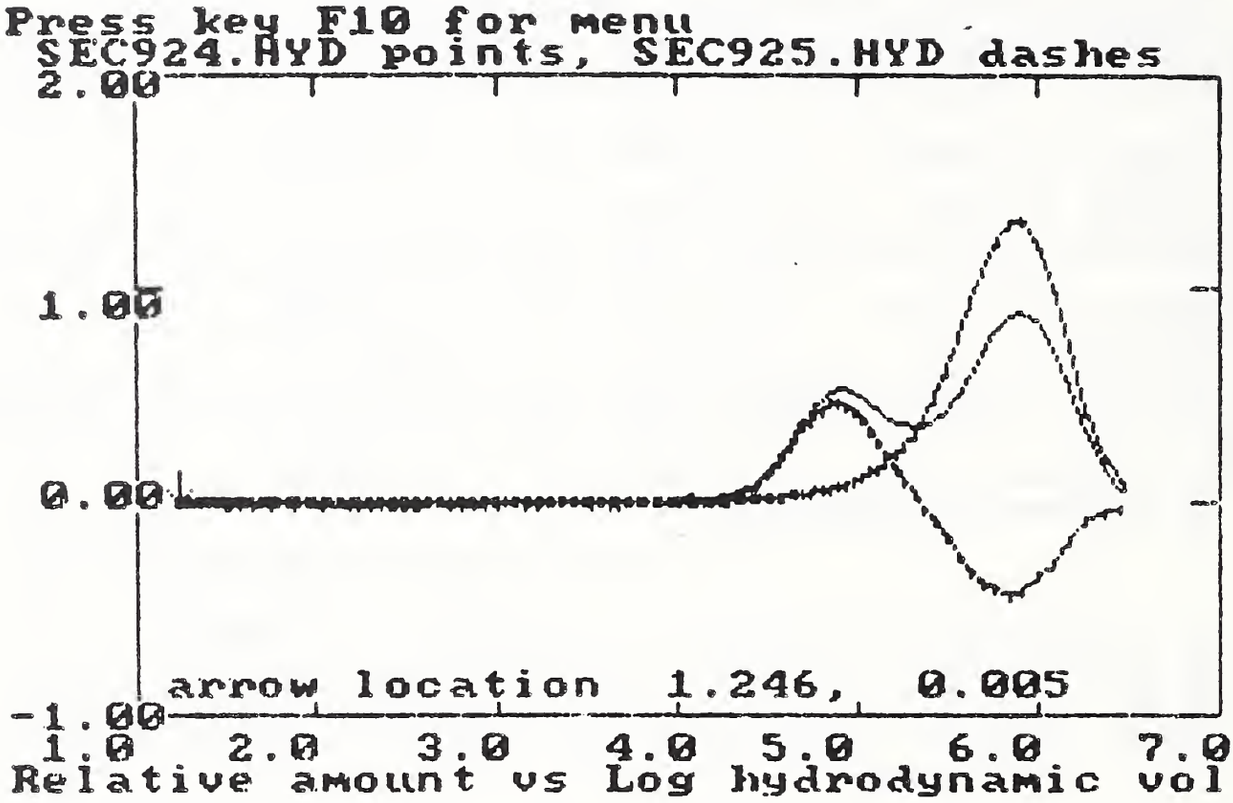
COMPARE plot 2 - Difference chromatogram (after F1)

Re-plotting the chromatograms with F1 generates a plot which also contains the difference chromatogram. The plot frame may be displaced down with the Page Down key, and up with the Page Up key. The curves may be expanded using the Up Cursor key or contracted by using the Down Cursor key. The cursor keys actually change the size of the graph frame. Since the size of the graph frame determines the scale on the screen, the net result is to expand the curves when the frame is shrunk and the plot is automatically rescaled, and to contract the curves when the frame is expanded and the plot is rescaled.



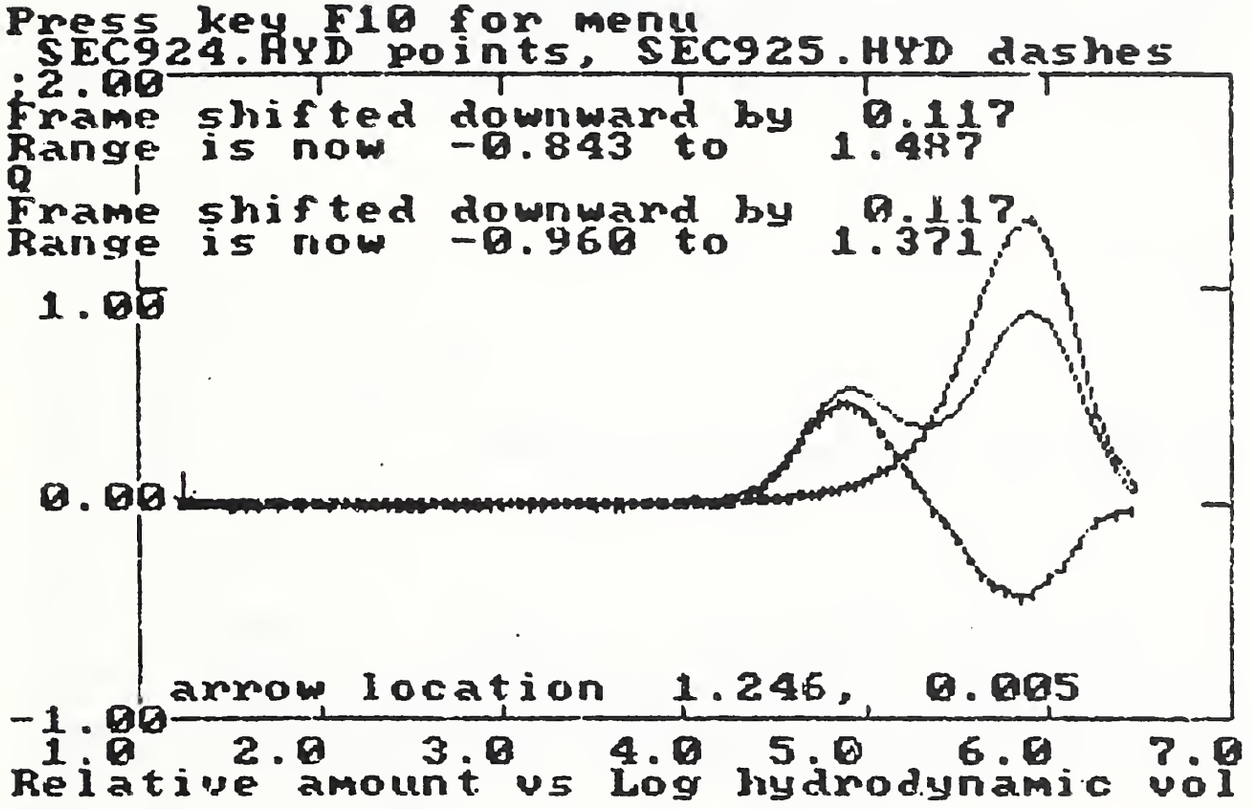
COMPARE plot 3 - Expanding graph frame with Up Cursor key

Pressing the Up Cursor key expands the graph frame and shows the new limits on the screen, as shown here. The frame size changes about 10% with each press. In this case, the operator would compare the limits generated after each press with the limits in the plot as shown and estimate when the frame has been expanded enough to make the lowest curve clear the legend. At that point, the plot should be re-drawn with F1.



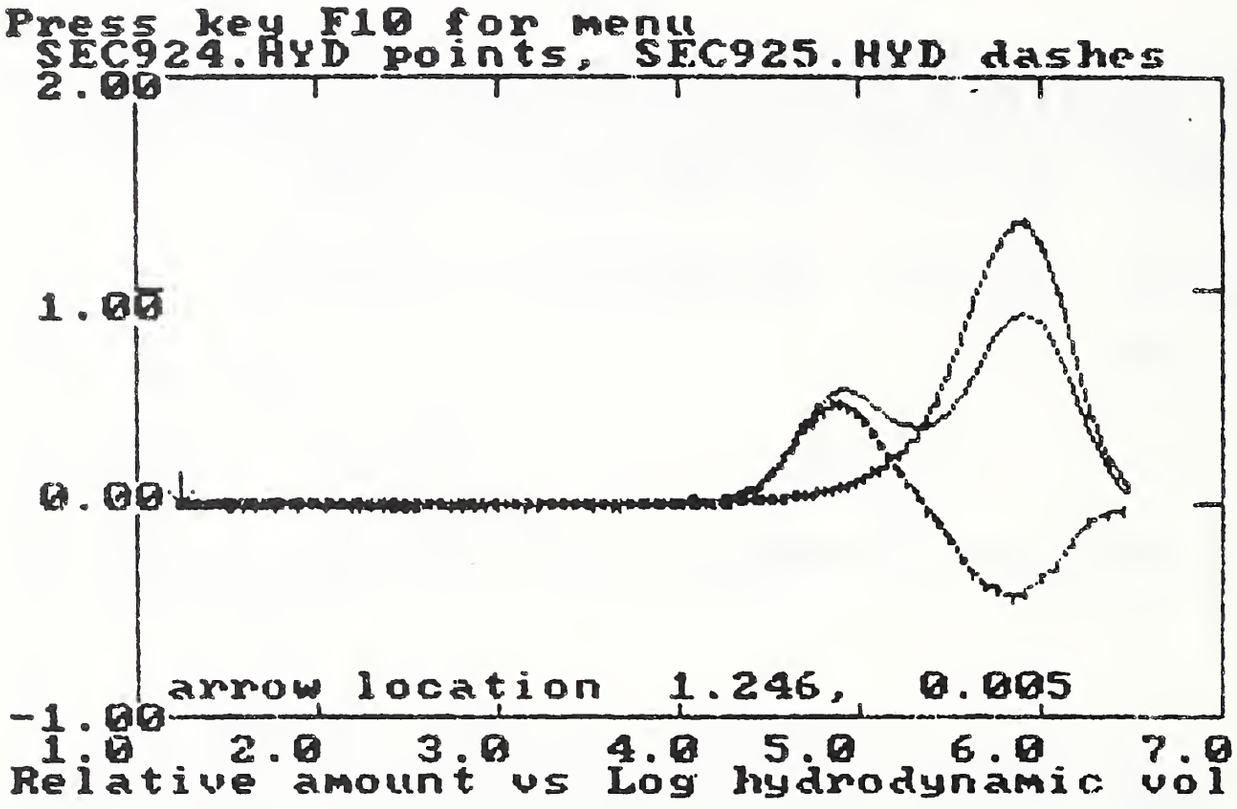
COMPARE plot 4 - Replot with F1

This is the result of pressing F1 to re-draw the previous screen.



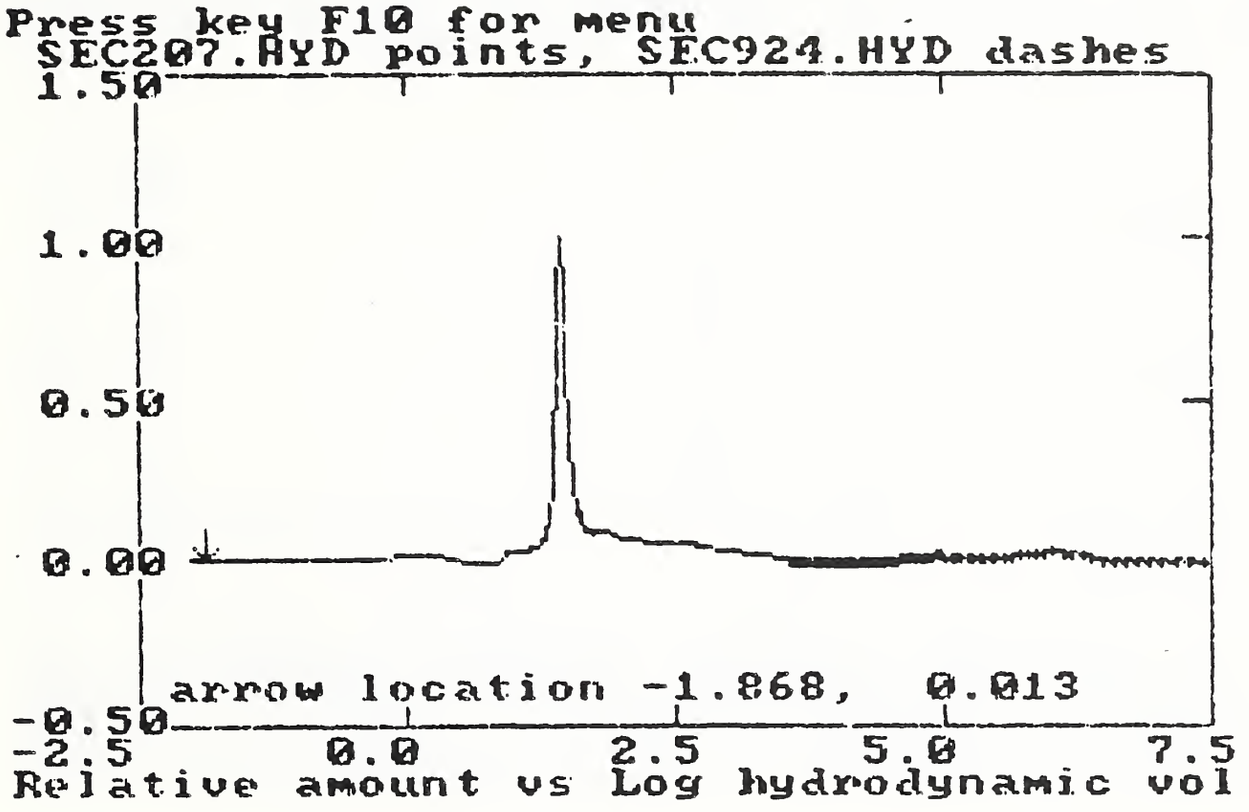
COMPARE plot 5 - Move graph frame down with Page Down key

The graph frame may moved up and down with the Page Up and Page down keys. This screen shows that the Page Down key has been pressed twice. Shifts are about 10% of the y axis with each press.



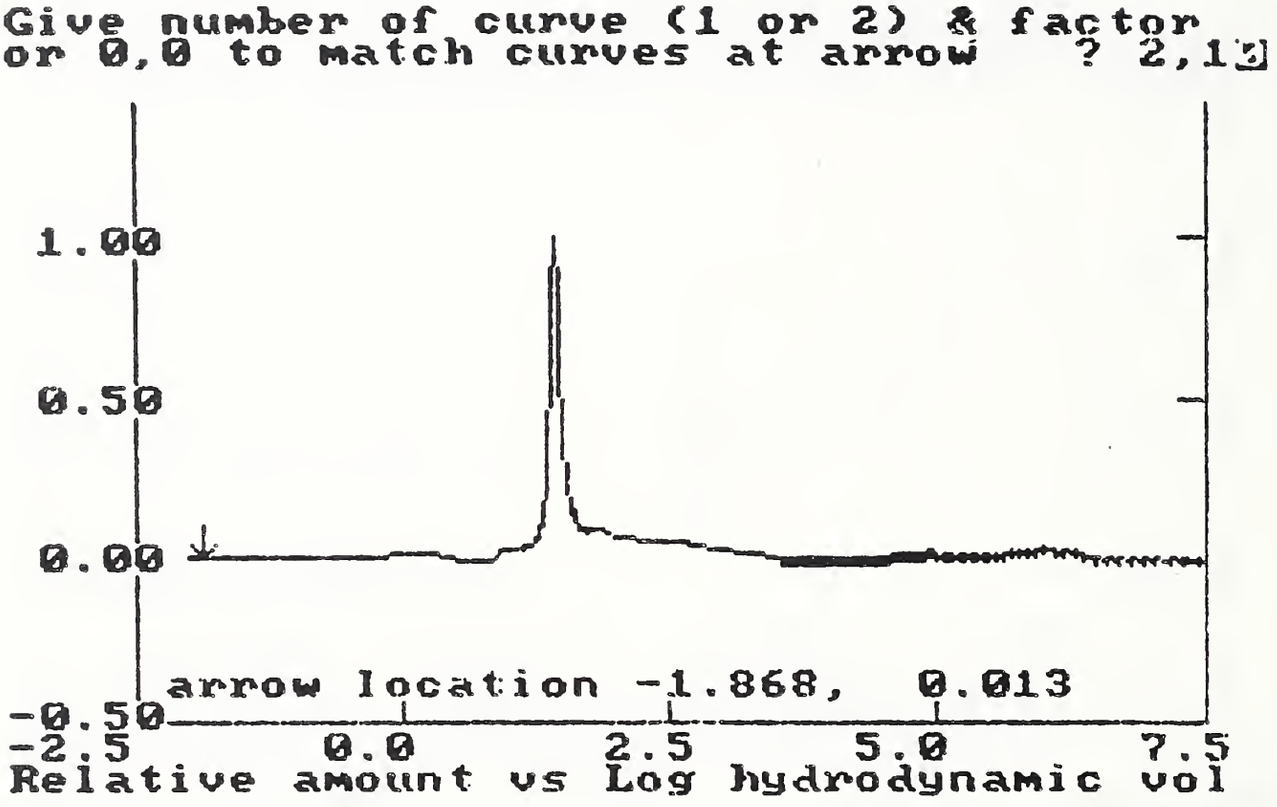
COMPARE plot 6 - Replot with F1

This screen shows the plot re-drawn with F1 after the frame was shifted as in the previous screen.



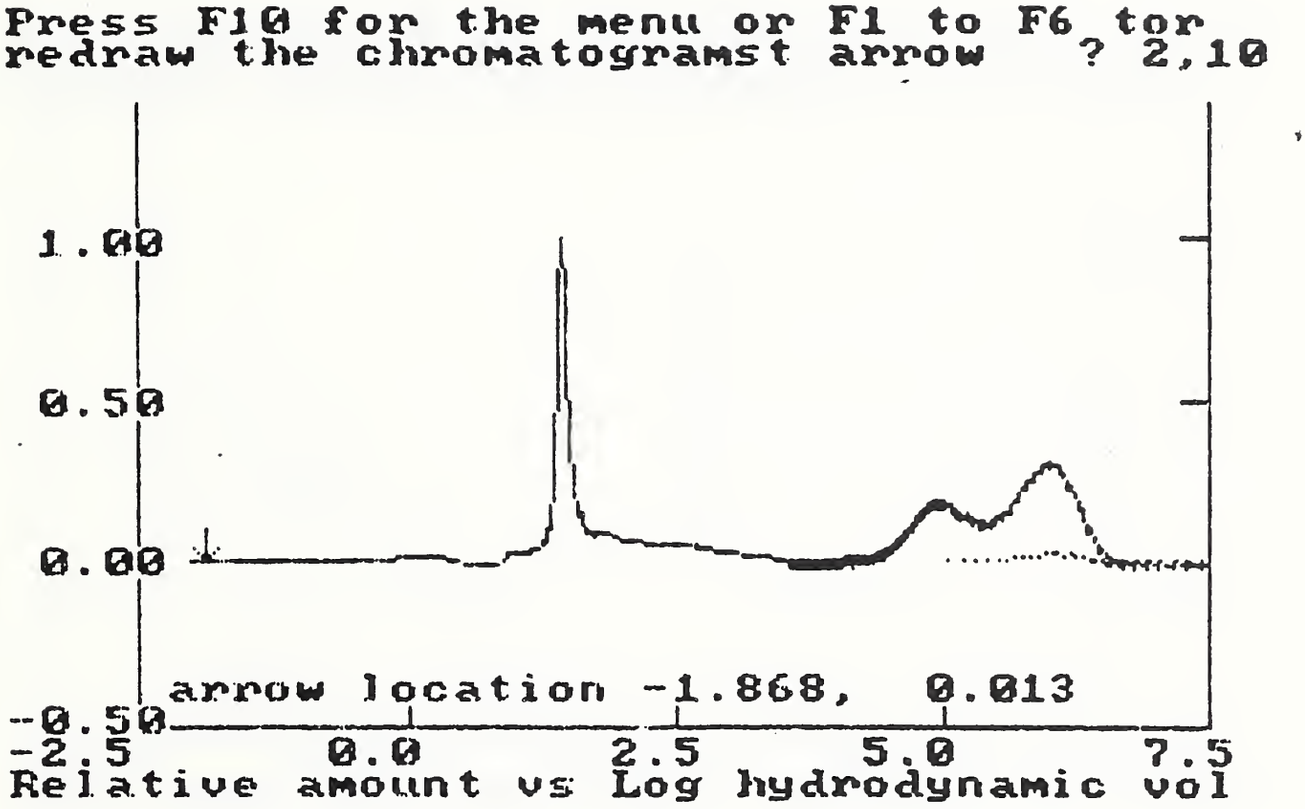
COMPARE plot 7 - Scale two chromatograms - as read in

These two chromatograms are obviously on different scales - because the refractive index of the material in the right hand chromatogram is nearer that of the solvent than the refractive index of the material in the left hand chromatogram is.



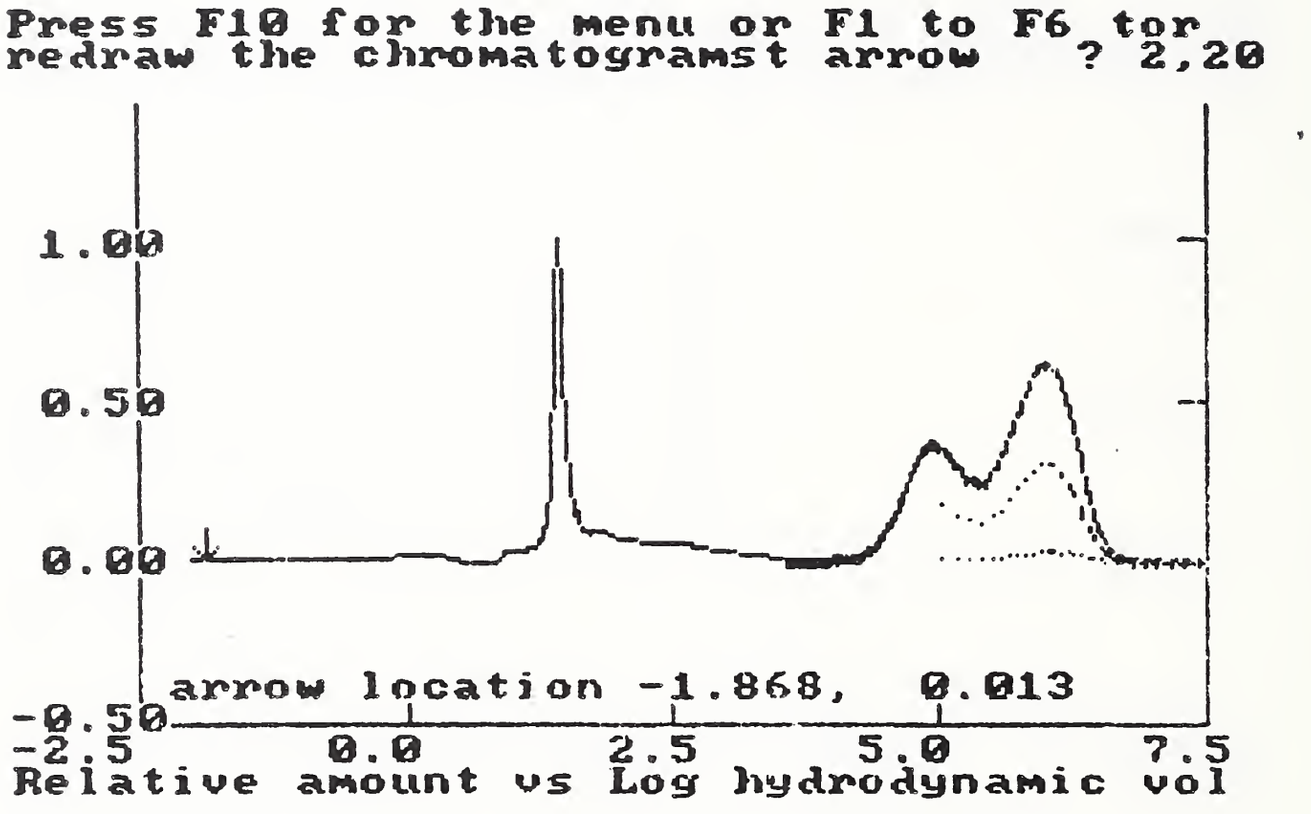
COMPARE plot 8 - Multiply second curve by factor of 10 (using F8)

Pressing F8 with the graph displayed on the screen allows the operator to specify a curve number (here 2) and a scale factor (here 10).



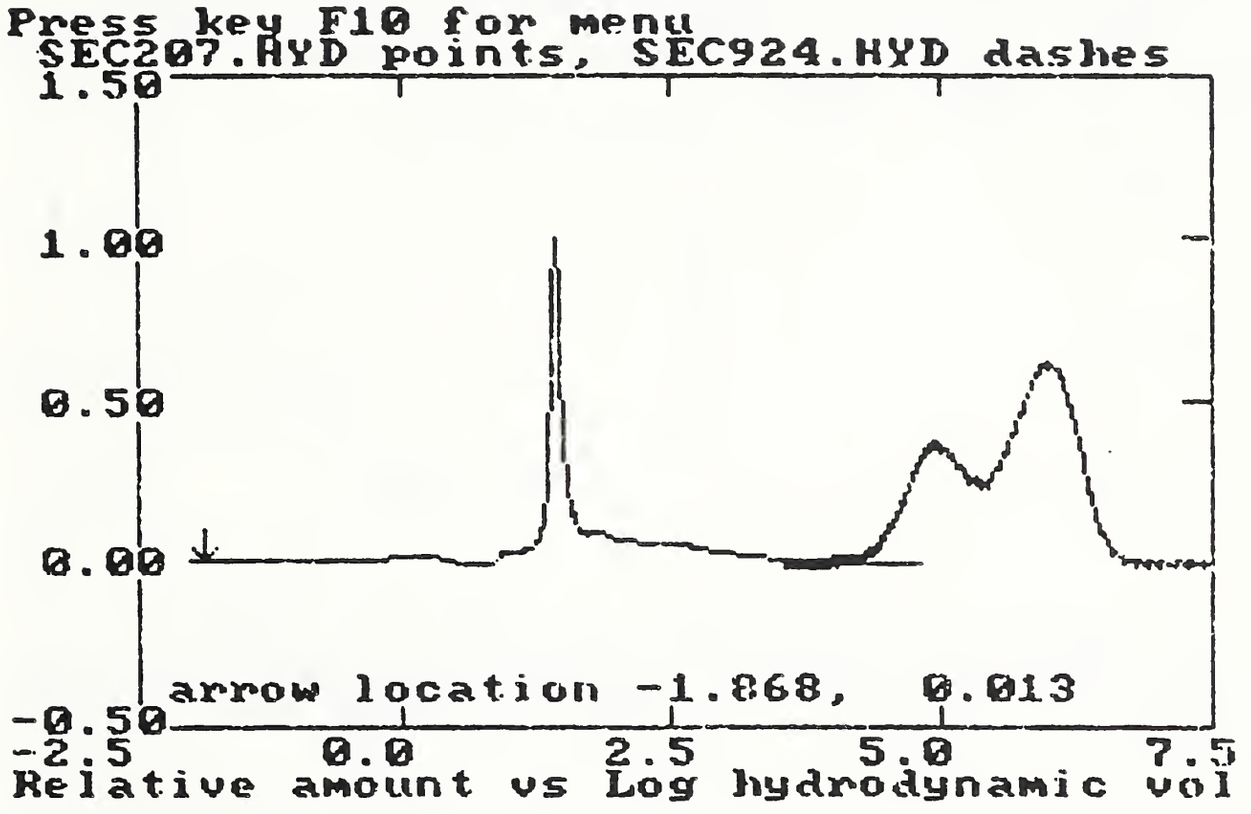
COMPARE plot 9 - Result of replotting previous plot with F1

As soon as the {Return} key is pressed, the program applies the scale factor and re-draws the curve.



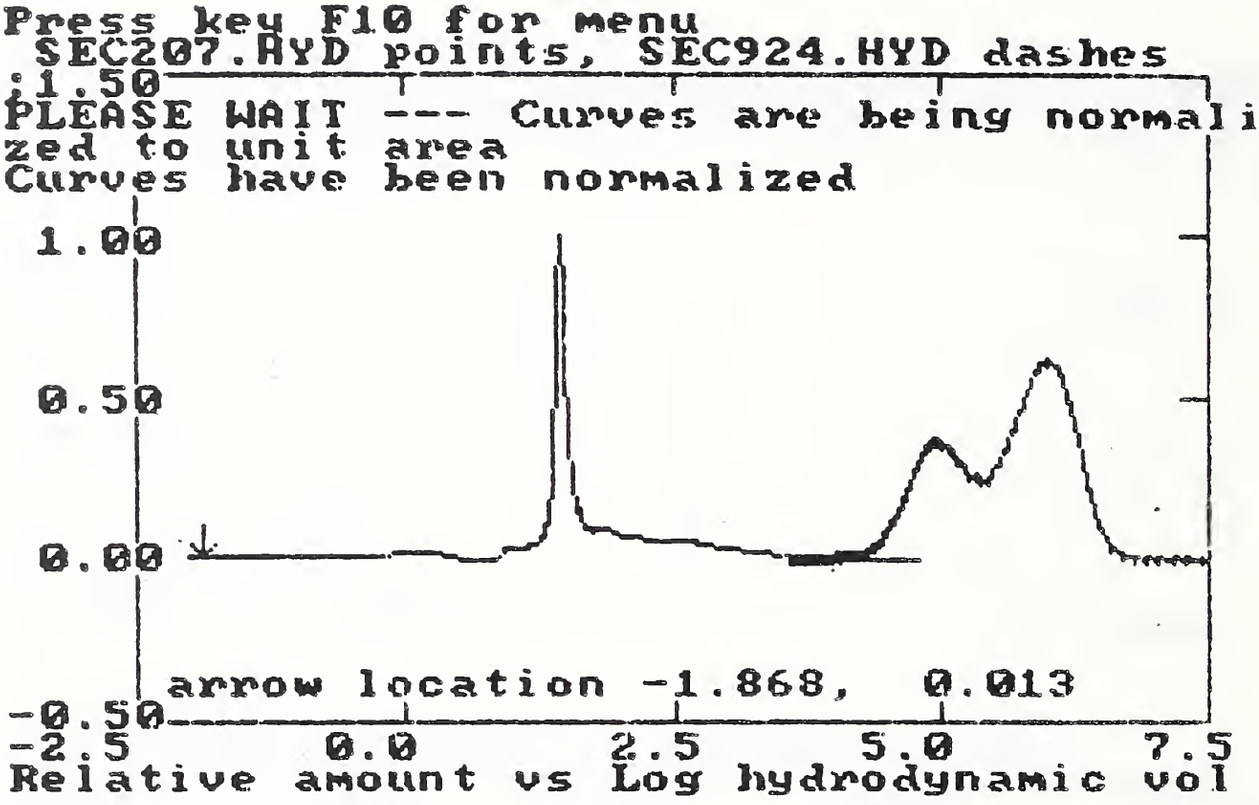
COMPARE plot 10 - Multiply by bigger factor

This is the result of specifying a scale factor of 20 for the second curve. The curves are numbered in the order they were read into the program.



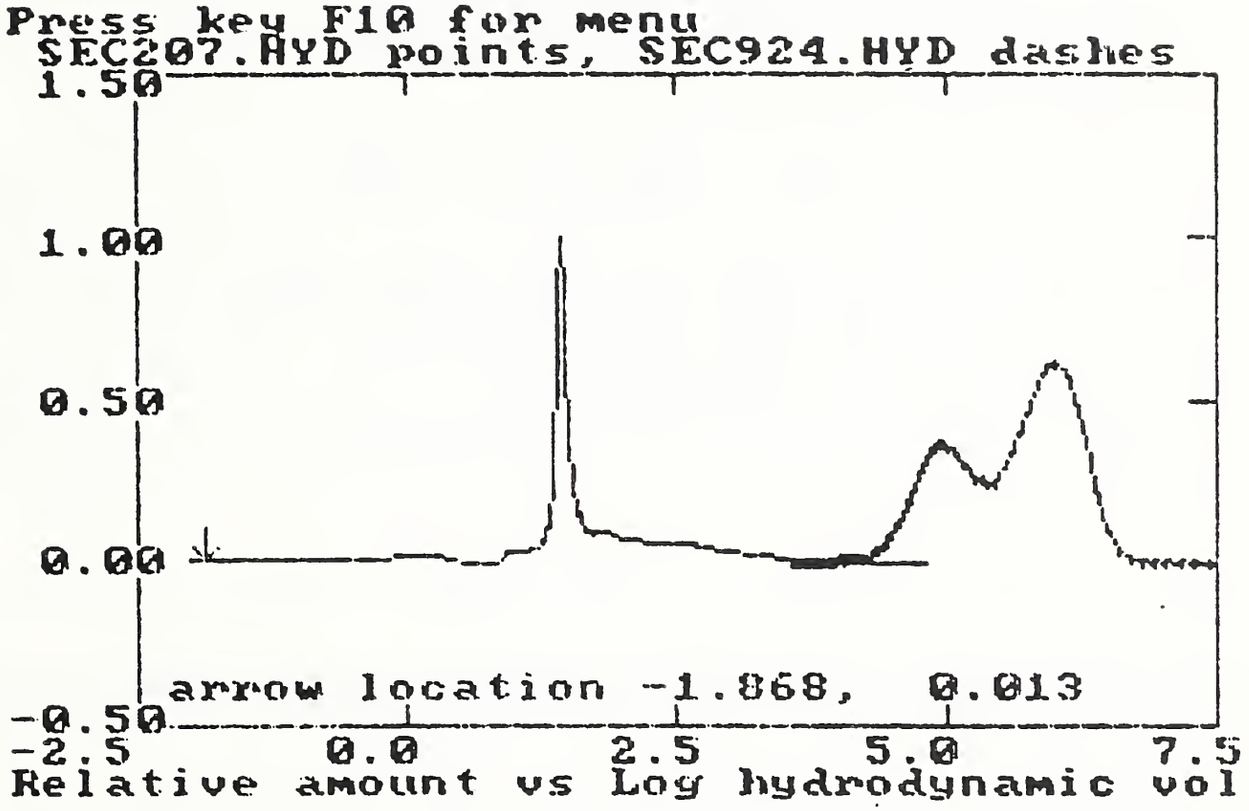
COMPARE plot 11 - Result of replotting previous plot with F1

This screen shows the result of re-plotting the graph by pressing F1.



COMPARE plot 12 - Normalize curves to unit area with F7

The two curves may be normalized (total area under the curve made equal to 1) by pressing F7.



COMPARE plot 13 - Automatic replot after F7

The F7 option for normalizing the curves finishes by re-plotting the curves, as shown here.

COMPARE screen 7 - Writing difference chromatogram to disk file

```
Redo plot with frame in original place ? (Y or N ?) n

Store difference chromatogram (Y or N) ? : y

Give the file name to store the difference chromatogram in : delta45.dif

Chromatogram is being written to disk

Redisplay chromatograms, enter New chromatograms or Exit program?

Press R, N or E :
```

Pressing {esc} allows the operator to store the difference chromatogram, and to continue the analysis, read in more chromatograms, or end the program.

Program to compare up to 9 chromatograms

COMPARE9 (interactive)

This program displays up to 9 chromatograms simultaneously. In the display on the screen, the chromatograms may be displaced so they do not overlap. A section may be cut from the chromatograms. The displayed cut of the displaced chromatograms may be stored in a file to be plotted by a plotting program such as the NBS Polymer Division program GRAPH to produce publication-quality graphs on a plotter.

The desired chromatograms are first read from files. If the files have the extension RAW, only the run numbers need be entered, otherwise the full names of the files are entered. A {Return} with no file name or run number is given after the desired chromatograms have been read into the program. The full range of the chromatograms may then be displayed by function key F1 or sections of the full range may be displayed by one of function keys F2 to F6. The chromatograms may be expanded in the vertical direction by keys Up and Down Cursor and Page Up and Page Down as explained in the section on the program ANALYZE. Key F7 normalizes the chromatograms to unit area and key F8 displays the menu.

Key S allows a displacement of the curves to be given in units of the vertical axis so that the curves will not overlap.

An arrow is drawn above the first curve. Key A allows the arrow to be moved to a different curve, which the operator gives the number of at this point. The arrow may be removed by giving a curve number of 0 (zero). The arrow is used to indicate where to cut a section from the chromatograms. The Right and Left Cursor keys move the arrow one point to the right or left, respectively. The Shift Right and Shift Left Cursor keys move the arrow 10 points and the Ctrl Right and Ctrl Left Cursor keys move the arrow 100 point to the right or left. The Home key moves the arrow to the beginning of the curve and the End key moves the arrow to the end of the curve. To cut a section from the chromatograms, move the arrow to one end of the section and press F9 to make the arrow position define the end of the range. Then move the arrow to the other end of the section and press F9 again to define the other end of the range. Then press F10 to cut out and retain the part of the chromatogram between the limits.

The Escape key allows the operator to exit the program, and provides the option to store the chromatograms to be stored in files on the disk. If the chromatograms are stored in a file, the file will be of the form to be plotted by program GRAPH to give a publication quality-graph.

COMPARE9 screen 1 - Reading in the data files

```
 - The COMPARE program allows the user to compare up to 9 chromatograms.
 - The chromatograms may be normalized or the ends cut from the chromatograms.
 - The chromatograms as displayed may be stored in a file in a form
   suitable to be plotted by program GRAPH after Esc is pressed.

                        Default type of file is RAW

                    Press Return after last chromatogram
        Enter file name or run number for chromatogram : ? 996

                        Now reading file SEC996.RAW
HEM-10 Plm Trans =  8d  %I= 1    Run # 996 vial 1 inj 1 00:33:04 02-13-1986

                    Press Return after last chromatogram
        Enter file name or run number for chromatogram : ? 997

                        Now reading file SEC997.RAW
HEM-10 Plm Trans =  9d  %I= 1    Run # 997 vial 2 inj 1 01:29:06 02-13-1986
```

The initial menu of the COMPARE9 program allows the operator to specify the run numbers or file names of the chromatograms to be read into the program, as shown here.

COMPARE9 screen 2 - End of reading the data

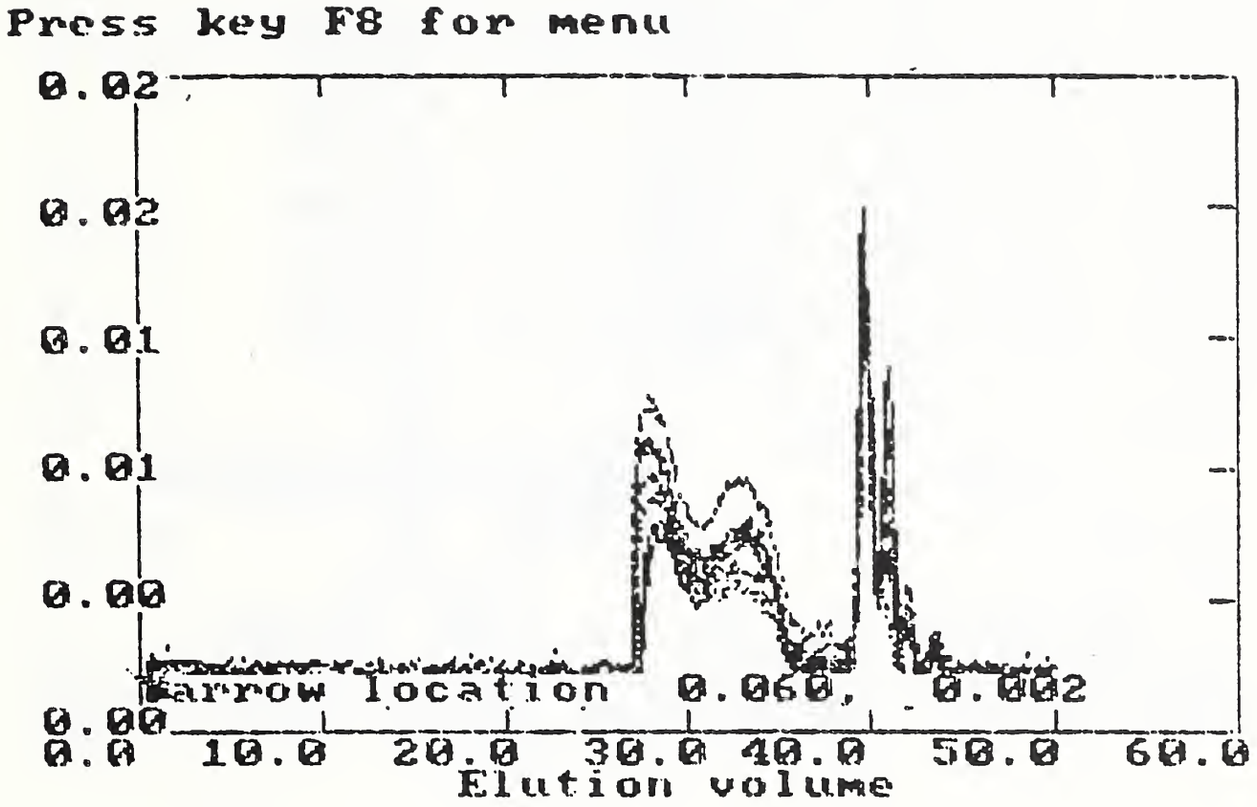
```
                    Press Return after last chromatogram
       Enter file name or run number for chromatogram : ? 1000

                         Now reading file SEC1000.RAW
HEM-10 Plm Trans = 10d  %I= 1.5  Run # 1000 vial 5 inj 1 04:17:11 02-13-1986

                    Press Return after last chromatogram
       Enter file name or run number for chromatogram : ? 1001

                         Now reading file SEC1001.RAW
HEM-10 Plm Trans = 10d  %I= 2.0  Run # 1001 vial 6 inj 1 05:13:13 02-13-1986

                    Press Return after last chromatogram
       Enter file name or run number for chromatogram : ? 1002

                         Now reading file SEC1002.RAW
HEM-10 Plm Trans = 10d  %I= 3.0  Run # 1002 vial 7 inj 1 06:09:15 02-13-1986

                    Press Return after last chromatogram
       Enter file name or run number for chromatogram : ?


                              Finding plot limits
```

After the last chromatogram has been specified, the operator presses {Return} with no other entry, as shown here. The program then searches for the limits to use in drawing the frame round the plot and generates the menu shown in the following screen.

COMPARE9 screen 3 - Plotting menu

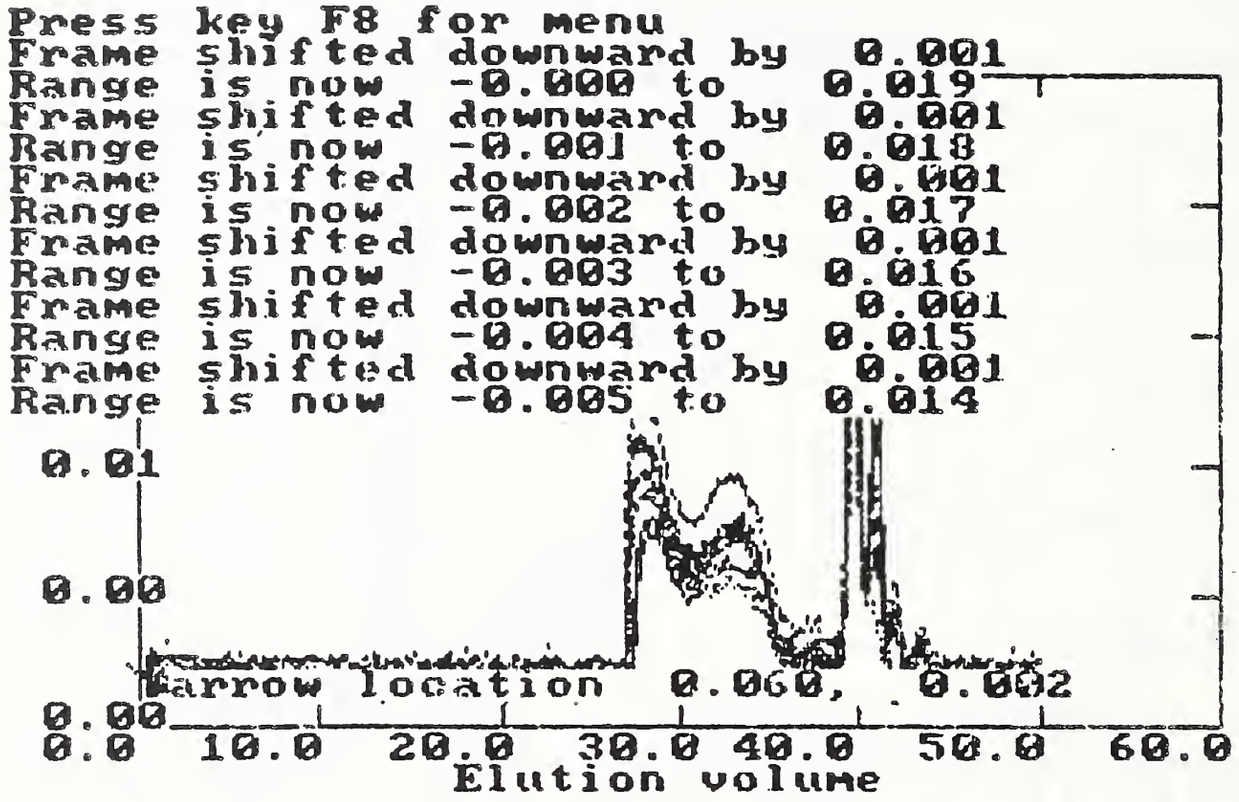
```
------------------ Display Selection Menu ------------------

- F1 displays the full chromatograms.
- F2 displays the left half of the chromatograms.
- F3 displays the right half of the chromatograms.
- F4 displays the left third of the chromatograms.
- F5 displays the middle third of the chromatograms.
- F6 displays the right third of the chromatograms.
- F7 normalizes the chromatograms to unit area.
- F8 displays this menu
 -F9 gives limits to cut chromatograms. To cut chromatograms,
  move arrow to lower limit and press F9, then move arrow to upper
  limit and press F9 again, then press F10.
- F10 gives the option to cut chromatograms,discarding points at
  points at low and high elution volumes.

Esc gives the option of storing the chromatogram,
    reading in more chromatograms, or quitting the program
Up Cursor expands graph vertically and Down Cursor contracts graph.
Page Up and Page Down raises or lowers region of graph shown.
S shifts the chromatograms vertically.
A moves the arrow to a chromatogram.
    Press one of function keys F1 to F6 to display the chromatograms.
```

Pressing F1 from this menu is the first step. This displays the chromatograms.



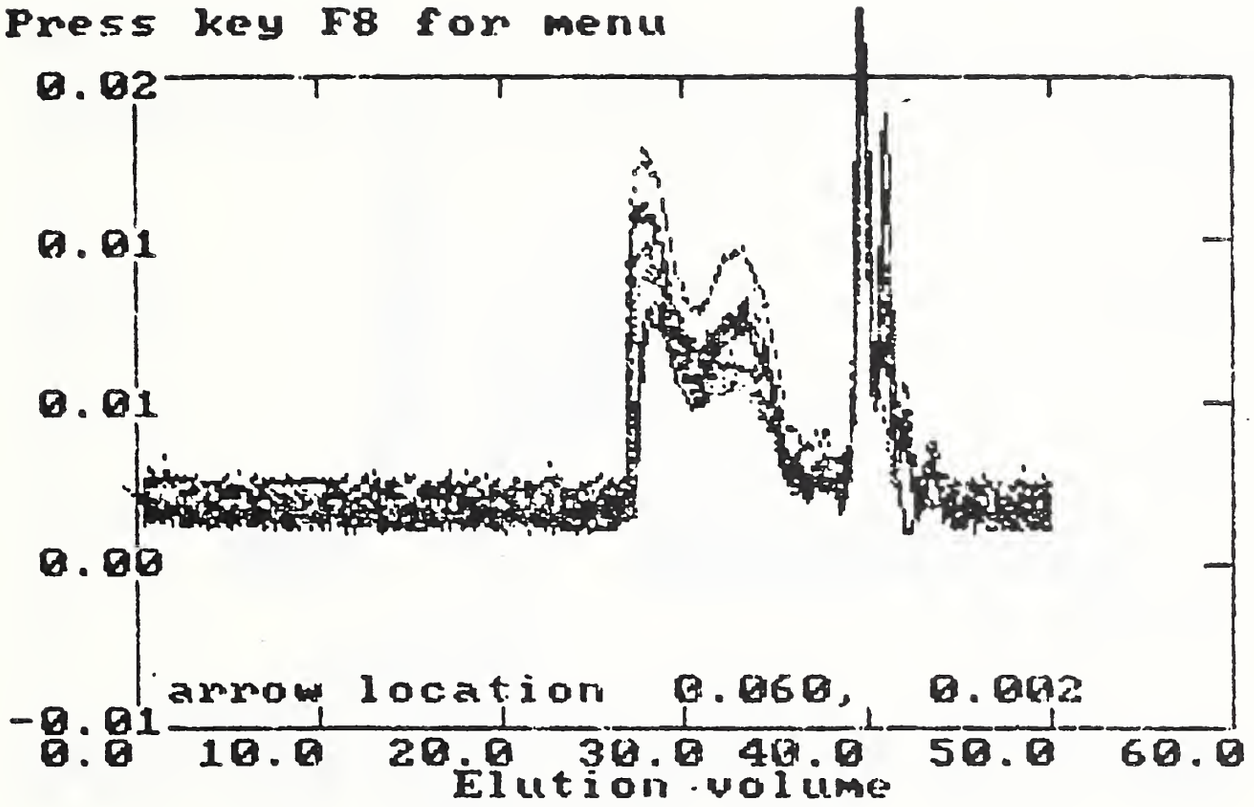
COMPARE9 plot 1 - 7 "raw" chromatograms as input, no vertical shift

The chromatograms are displayed plotted on top of one another. They may be cut all together with the arrow movement, F9, arrow movement, F9, F10 sequence.



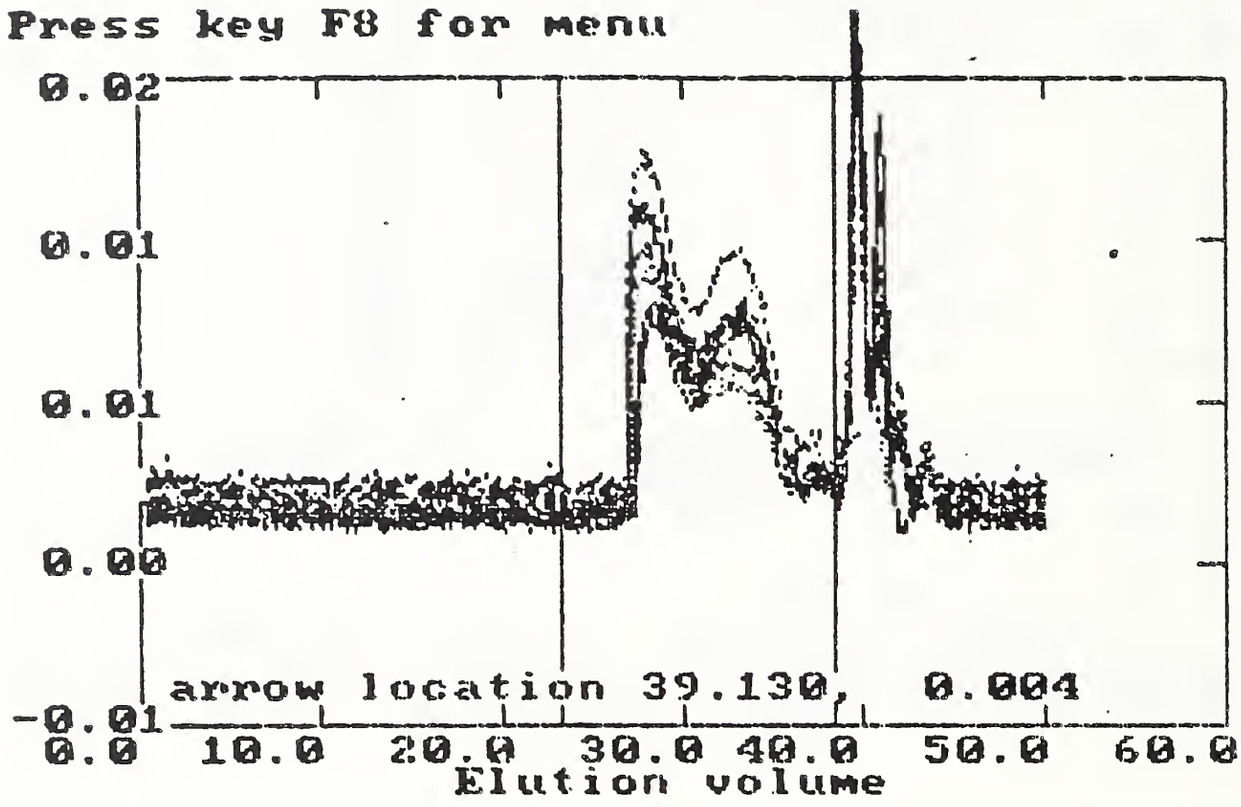
COMPARE9 plot 2 - Move graph frame down with Page Down key

This plot shows that the operator decided to shift the frame down a little by pressing the Page Down key.



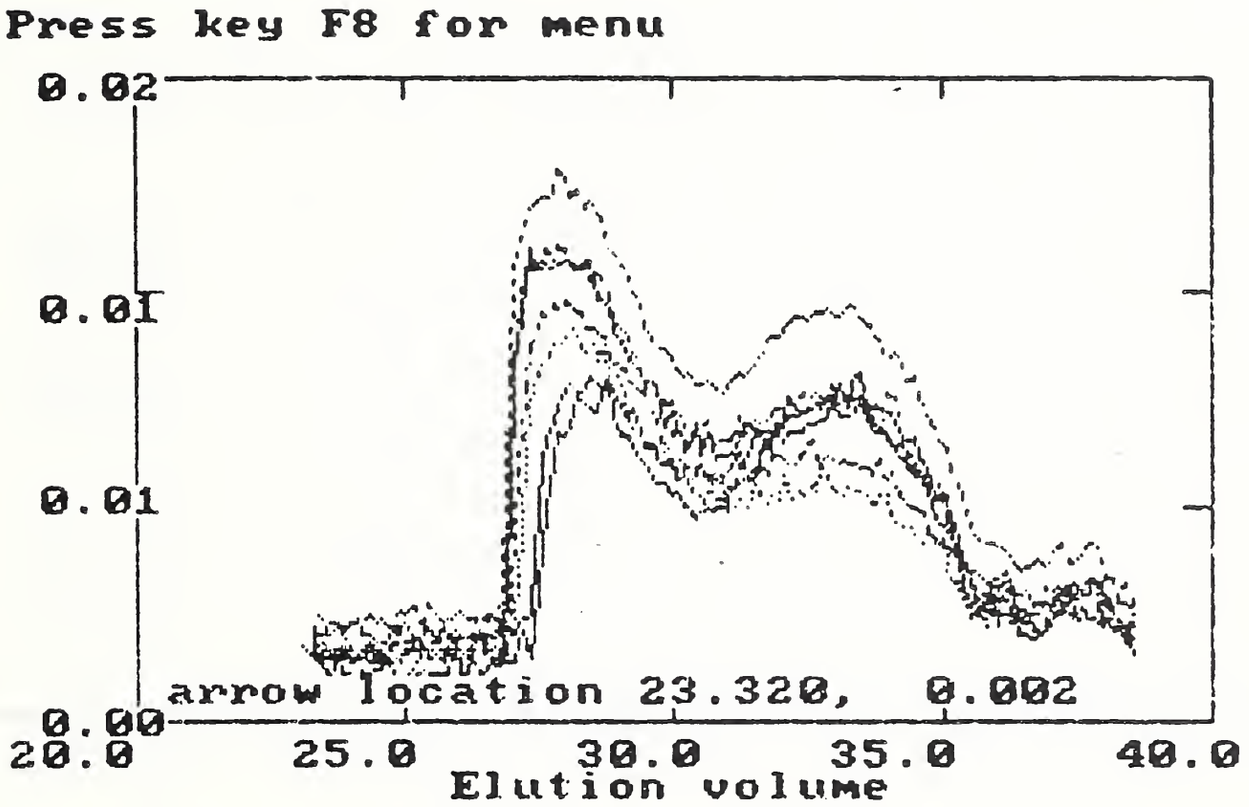
COMPARE9 plot 3 - Replot with F1

The plot has been re-drawn by pressing F10. Now enough detail can be seen to cut the chromatograms easily.



COMPARE9 plot 4 - Region to cut out selected with F9

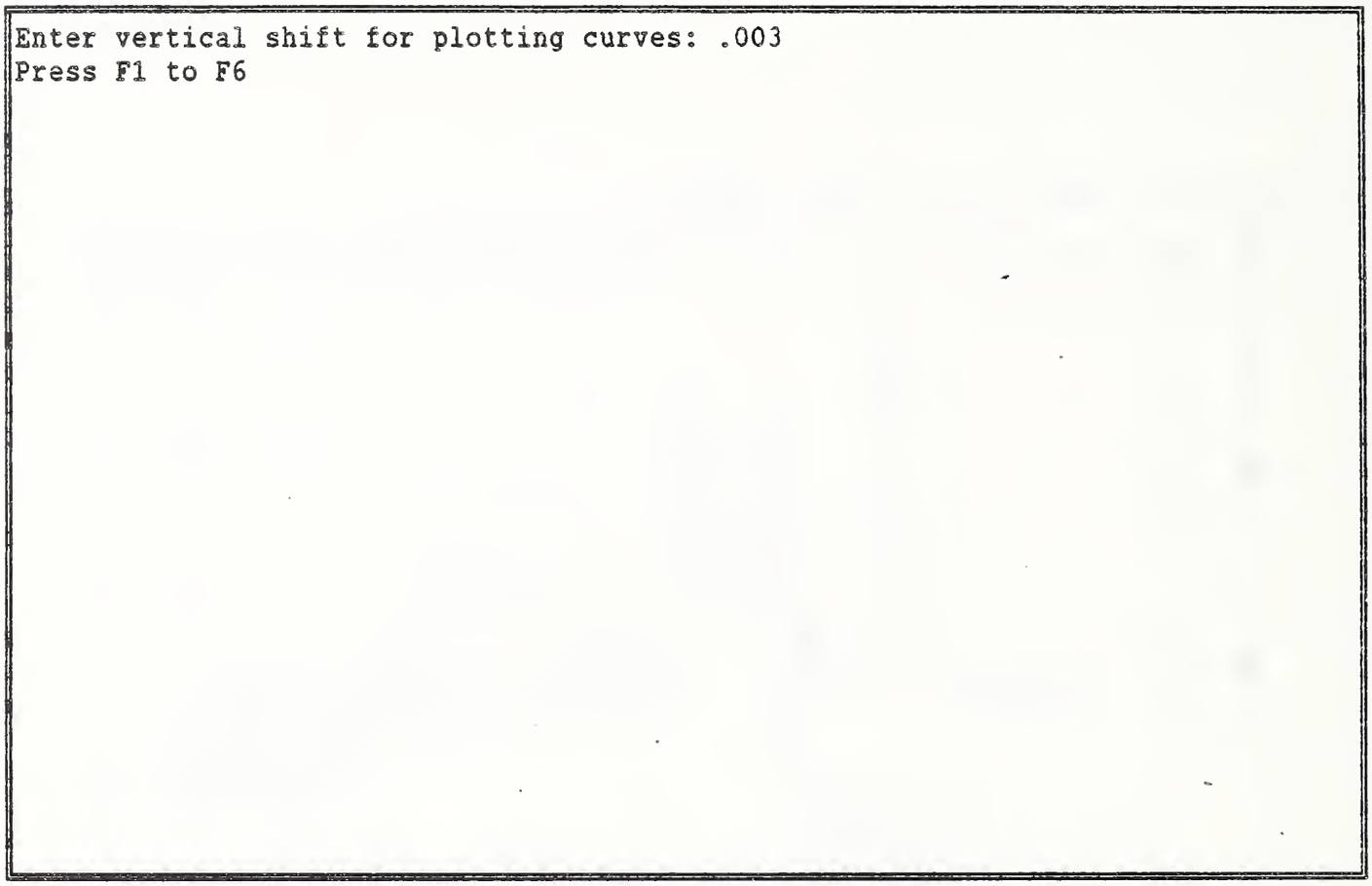
The limits at which to cut the chromatograms have been defined using the arrow, F9 key, arrow, F9 key sequence.



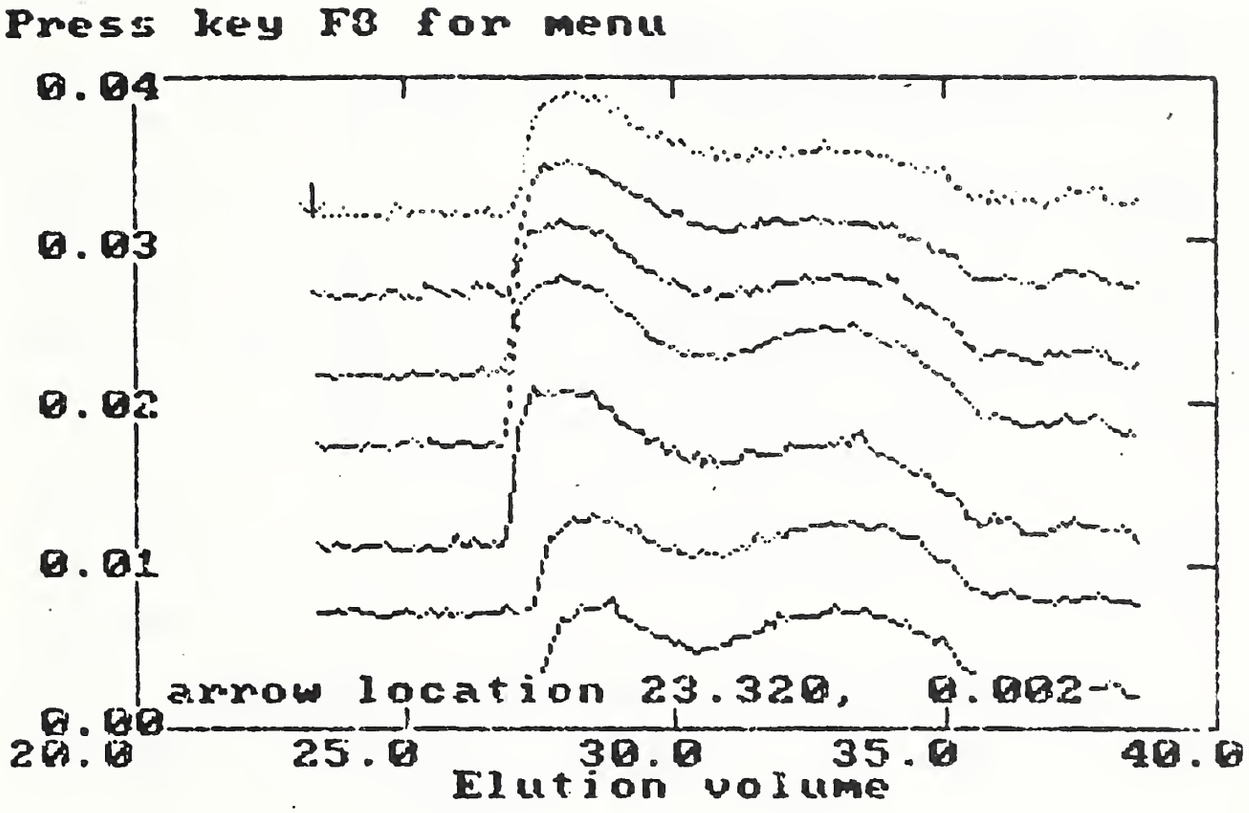
COMPARE9 plot 5 - Chromatograms cut with F10, replotted

The chromatograms have been cut by pressing F10, which then re-plots the cut chromatograms.

COMPARE9 screen 4 - Vertically displacing the curves

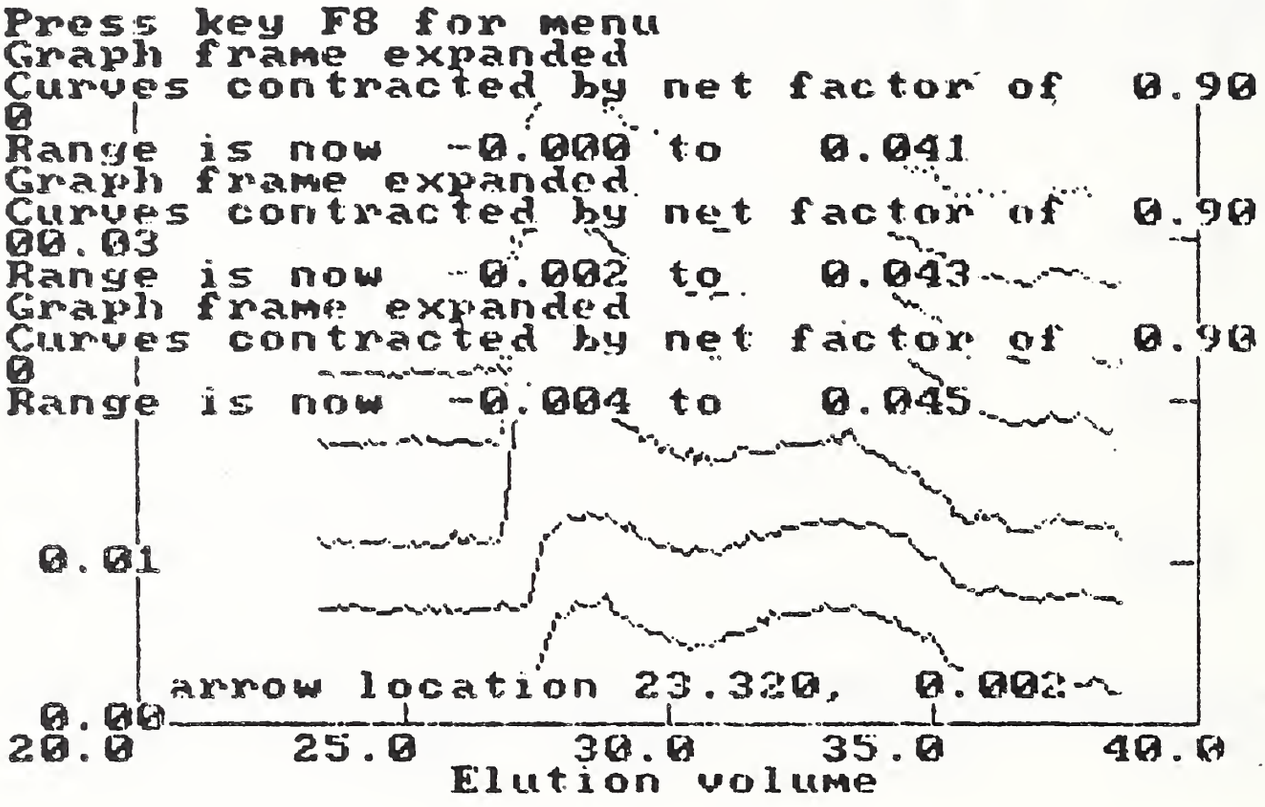


By pressing the S key, the operator is able to shift the curves vertically by a specified amount.



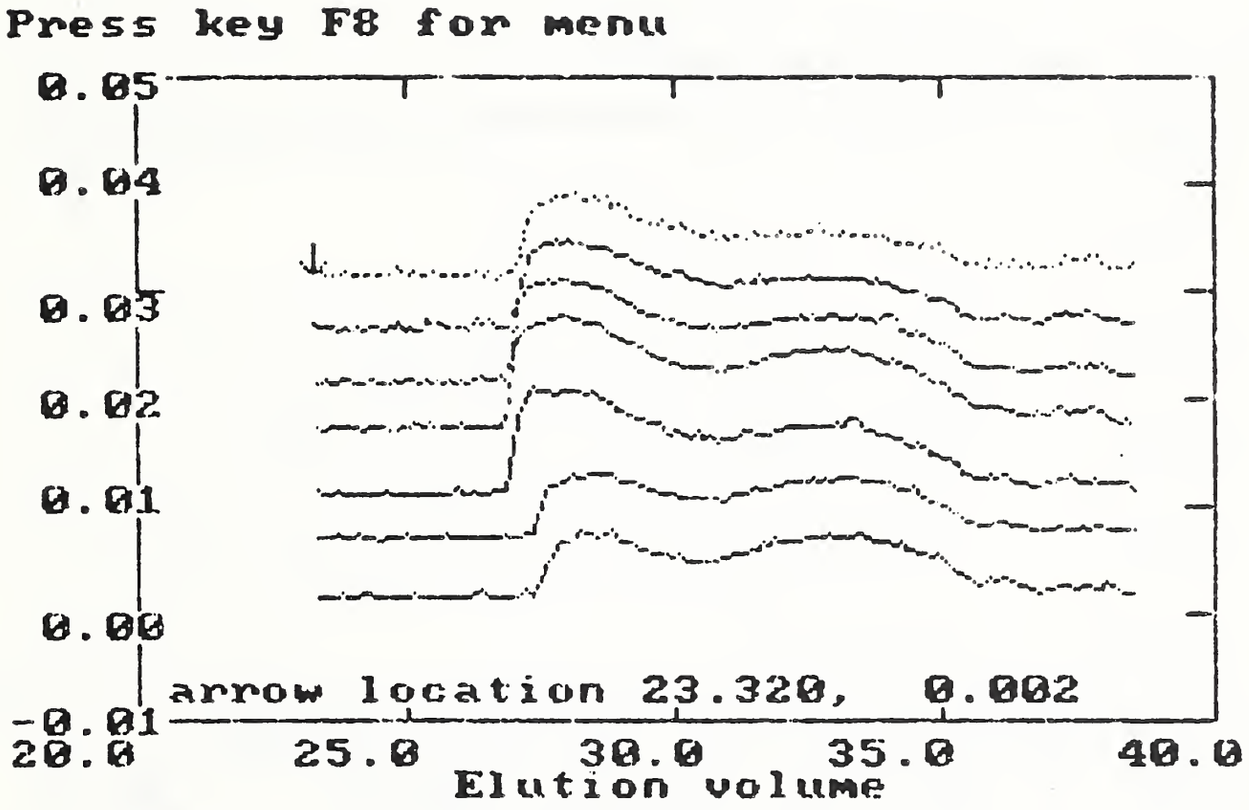
COMPARE9 plot 6 - Replot shifted curves (arrow on top curve with A)

The shifted curves may then be re-plotted, and if desired, re-shifted.



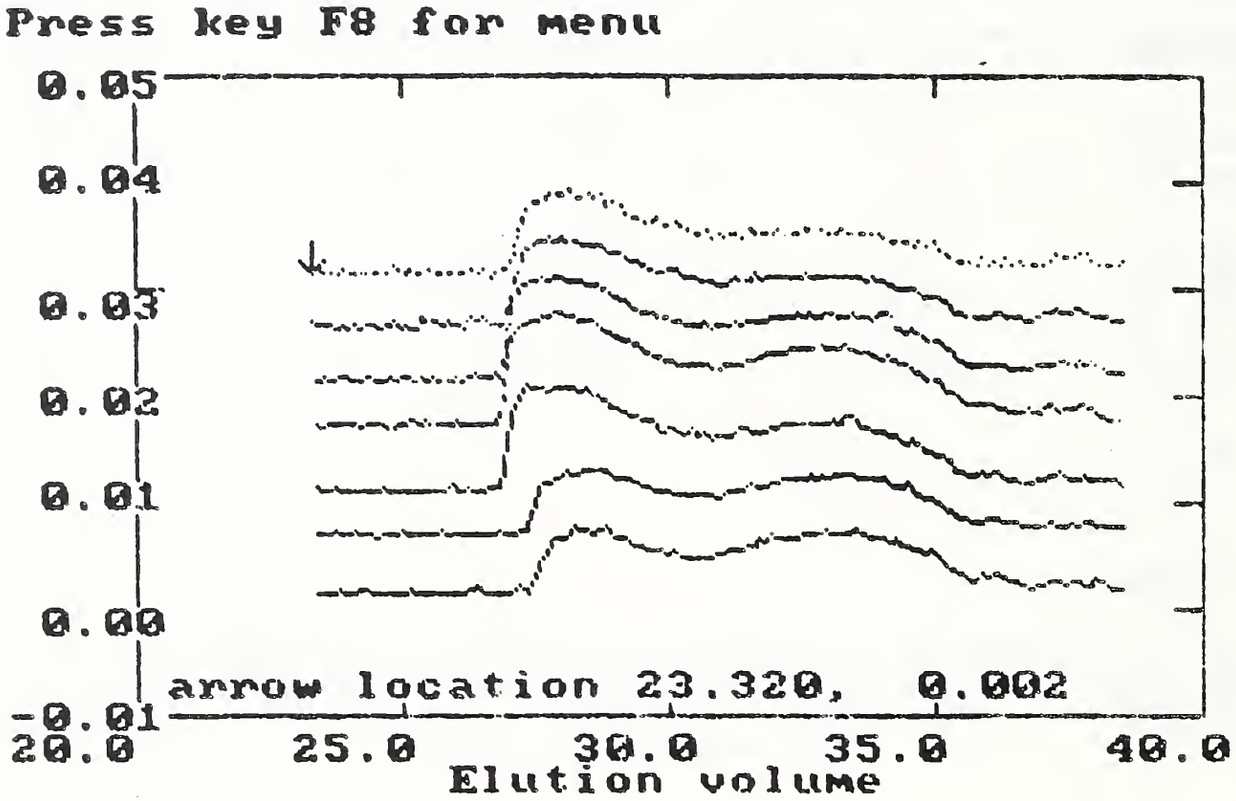
COMPARE9 plot 7 - Expand graph frame with Page Up key

The operator has decided to contract the curves as plotted by expanding the graph frame down using the Up Cursor key, which has been pressed three times.



COMPARE9 plot 8 - Replot with F1

The curves have now been re-plotted using F1.



COMPARE9 plot 9 - Normalize to unit areas with F7

This is the result of normalizing the curves by pressing F7.

COMPARE9 screen 5 - Save plot for future hardcopy graph on plotter

```
        Replot with the original scales and a shift of 0 ? n

    Do you want to plot the chromatograms later on a plotter (Y or N) ? y

 Give file name to store chromatograms for later plotting
  by program GRAPH : comp9.grp

                        Writing GRAPH file

 Give title of graph comparison of 7 mixtures

            Examine New chromatograms or Exit program?

                        Press N or E
```

Several options for ending the program are available when {Escape} is pressed. One is a recovery if the scaling and frame movement has got out of hand. A second is the option of saving the plot for later plotting on a plotter by a graphics plotter program. Finally, the operator can choose to read in more chromatograms or exit from the program.

Programs to calculate and display calibration of columns

Calibration

The calibration of a set of chromatographic columns used in size exclusion chromatography consists of a relationship of the elution volume of the chromatograph to log hydrodynamic volumes of polymer solutes in a particular solvent. Points on the calibration are obtained from chromatograms of standard samples with narrow molecular weight distributions and known molecular weights in the size exclusion chromatograph. The elution volume is determined at the position of the peak in each chromatogram and the log hydrodynamic volume is computed from the molecular weight and Mark-Houwink parameters of each sample so that a curve relating elution volume and log hydrodynamic volume is obtained.

The program AUTOPICK may be used to determine the elution volumes at the peaks of the chromatograms.

The calibration may be expressed in two ways. For type 1 calibration, the calibrating points are fitted to a polynomial by program CALFIT. If not used with care, the polynomial approach gives wrong values, in extreme cases even between the calibrating points. Also, the polynomial often gives wrong values outside the range of calibrating points, where the polynomial function is not constrained. This severity of this effect may sometimes be reduced by eliminating the squared term from the calibration. We usually use the polynomial method of representing the calibration of the columns.

For type 2 calibration, the calibrating points are fitted by a series of connected straight line segments. This avoids the spurious maxima and minima of the type 1 (polynomial) calibration. However, molecular weight distributions computed using type 2 calibration have spurious peaks due to the discontinuous slope at the connection of the straight lines.

The programs described in this manual require a file containing the calibration of the columns used. The first line of the file is a title for the calibration. The next line contain the lower and upper values of elution volume for which the calibration is valid. The next line contains the type of calibration (1 or 2). For type 1 calibration, the next line contains the central point around which the polynomial was filled, the next line contains the constant, linear, and quadratic coefficients, and the next line contains the next three coefficients. A polynomial of up to fifth degree may be used. For type 2 calibration,these lines are replaced with a line giving the number of points the connected lines are drawn through. The calibration points of elution volume, log hydrodynamic volume are then given one point to a line.

An example of a type 1 calibration:

```
J. PHILLIPS & B DICKENS CALIBRATION OF WATERS COLS April 9 1986
32.422    50.282
1
37.463   3.243
-0.26428      -0.3503     0.9349E-02
 0.7332E-03  0.16782E-03  -0.1752E-04
```

An example of a type 2 calibration:

```
H.Wagner Calibration of Shodex columns Bimodal 8/86
10.2 18.6
2
4
10    10.45
10.55 9.776
17.43 4.394
18.94 0
```

CALFIT (interactive)

The CALFIT program provides a polynomial of up to fifth degree to represent the calibration of the columns. The program reads a file called CALDAT.DAT containing the Mark-Houwink parameters of the pure narrow molecular weight standard polymers. This file also contains the elution volumes for the peak in the chromatograms for each of the narrow molecular weight standards, and the weighted average molecular weight, $(M_n M_w)^{0.5}$, for each standard. An example is given below. The program writes an output file CALFIT.DAT which is the calibration file needed by the other programs.

Example of CALDAT.DAT, the input file to the CALFIT program:

The first line is the title. The next two lines are the symbol (S) and Mark-Houwink parameters for polystyrene. Two similar lines for poly (methyl methacrylate) follow. The "E" means that the input of Mark-Houwink parameters has ended. Similar lines give the polymer symbol, the molecular weight, and the elution volume of the peak for each of the narrow molecular weight standards. This section also ends with "E" (for "end").

```
B DICKENS Calibration of Shodex cols 804 & 802 NOV 1986
S
0.861E-4 0.74
M
1.04E-4 .697
E
S
510 34.405
S
519 34.402
```

```
S
1811 31.249
S
5330 28.791
M
7670 28.176
S
600 34.0134
S
6200 28.504
S
726 33.637
S
1.574E3 31.594
S
2E3 30.665
S
8.3E5 20.534
S
1.27E6 20.3697
S
6200 28.494
S
7607 28.215
S
9995 27.474
S
44300 24.419
S
52409 24.365
M
63900 24.137
S
73300 23.476
S
97200 23.126
S
109980 22.819
M
120000 22.900
M
284160 21.695
M
480300 21.060
E
```

Example of the output file CALFIT.DAT:
One of the two last quantities on the second line must be chosen by the operator, as explained in the text

```
B DICKENS Calibration of Shodex cols 804 & 802 NOV 1986
    20.370    34.405
   1
    27.022     3.244
   -0.12144020E+00   -0.40413329E+00   -0.85624680E-02
    0.35396945E-02    0.48640074E-03   -0.86872809E-04
    0.00000000E+00    0.00000000E+00    0.00000000E+00
    0.00000000E+00    0.00000000E+00
```

PLOTCAL (interactive)

The program PLOTCAL allows the operator to examine the form of the calibration function on the screen of the computer, and to see how the molecular weights of polystyrene and poly (methyl methacrylate) vary across the calibration range. Parameters for a third calibration standard may be given, and parameters for a polymer being studied provide useful information on how well the various molecular weight ranges are being resolved by the chromatographic columns. The behavior of the calibration function beyond the calibrated range may be seen by giving appropriate limits to the PLOTCAL program.

PLOTCAL screen 1 - Reading in the calibration file

```
The PLOTCAL program allows the user to plot the calibration
function of the GPC.  The program reads in a calibration function
from a file which you specify.  You can follow the change in MW
of standards used to generate the calibration data.  You can add
your own standard or specimen and follow changes in its molecular
weight with elution volume by giving its Mark-Houwink parameters
and moving the arrow on the graph.

 Do you want to use the calibration in file CALIBR.DAT ? (Press Y or N)
 Give the name of the calibration file clonecal.3

 Title of calibration file is :
B DICKENS Calibration of Shodex cols 804 & 802 NOV 1986

 Limits are  20.37  to  35.355
 Give limits to calculate calibration over 18,38
```

This is the main menu of the program which plots the calibration of the columns. The name of the calibration file is provided to the program, and the limits over which to display the calculated calibration curve are given.

PLOTCAL screen 2 - Specifying Mark-Houwink parameters for polymer

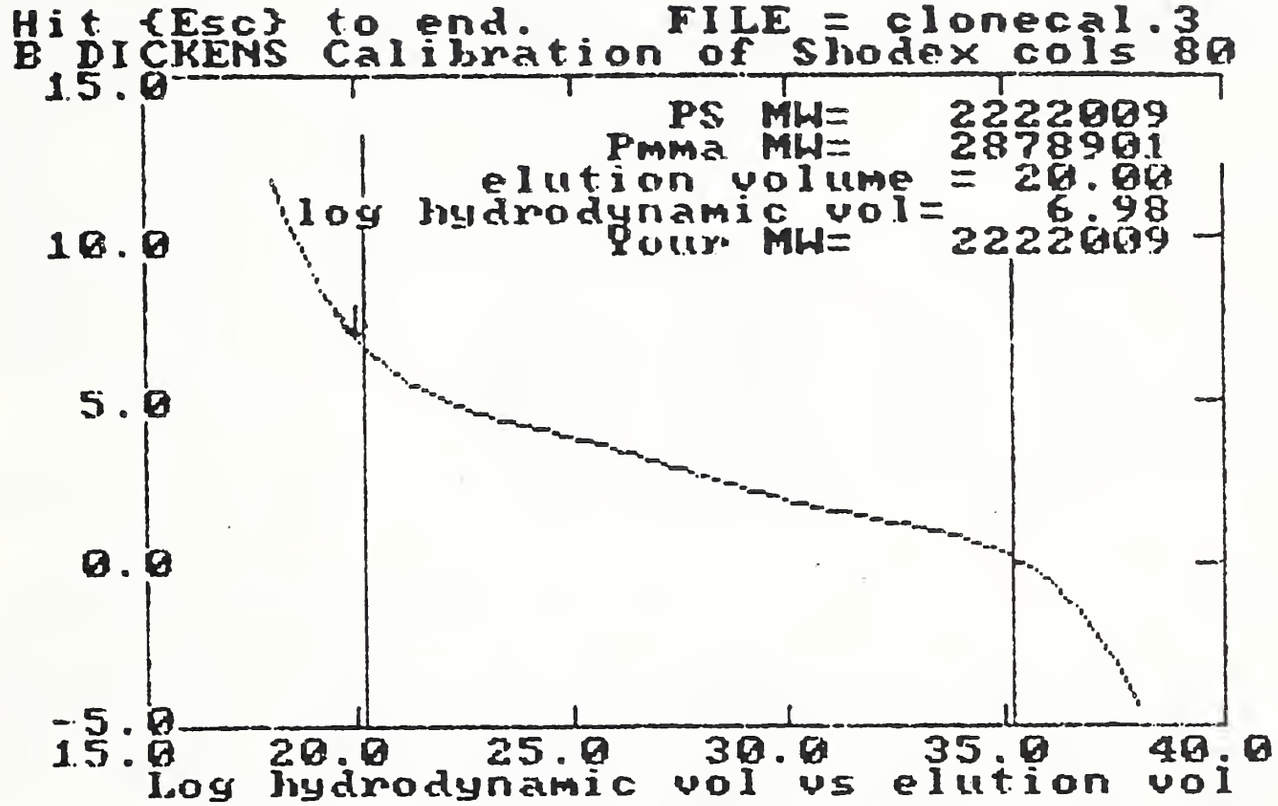
```
  Elution volume 19.80 is outside the calibration range
  Elution volume 20.00 is outside the calibration range
  Elution volume 20.20 is outside the calibration range
  Elution volume 35.40 is outside the calibration range
  Elution volume 35.60 is outside the calibration range
  Elution volume 35.80 is outside the calibration range
  Elution volume 36.00 is outside the calibration range
  Elution volume 36.20 is outside the calibration range
  Elution volume 36.40 is outside the calibration range
  Elution volume 36.60 is outside the calibration range
  Elution volume 36.80 is outside the calibration range
  Elution volume 37.00 is outside the calibration range
  Elution volume 37.20 is outside the calibration range
  Elution volume 37.40 is outside the calibration range
  Elution volume 37.60 is outside the calibration range
  Elution volume 37.80 is outside the calibration range
  Elution volume 38.00 is outside the calibration range


Do you want to follow changes in MW in your own polymer? y or n?
Give the Mark-Houwink k and a for the polymer ? .0000861,.74
Your Mark-Houwink parameters are k= 0.0000861, a=0.7400
Is this ok? y or n ?
```

The program warns which parts of the specified range are beyond the limits covered by the calibration function read in from the calibration file. The operator may give Mark-Houwink parameters for a material of interest and see which part of the molecular weight range for that material is covered by the calibration.

PLOTCAL screen 3 - menu for plotting

```
Press a function key

- F1 displays the full calibration.
- F2 displays the left half of the calibration.
- F3 displays the right half of the calibration.
- F4 displays the left third of the calibration.
- F5 displays the middle third of the calibration.
- F6 displays the right third of the calibration.
- You may move an arrow along the calibration line on the plot.
- The corresponding molecular weights of PS and PMMA standards
  are given in the top right hand corner of the plot.
- The coordinates of the arrow in elution volume and log
  hydrodynamic volume are also displayed.
- If you gave the Mark-Houwink parameters for your
  own polymer, its molecular weight is also displayed
  in the upper right hand corner
```

The plot is generated from this menu. Usually, F1 is pressed.



PLOTCAL plot - Typical calibration plot

The PLOTCAL program shows the relationship between log hydrodynamic volume and elution volume as specified in the calibration file for the chromatographic columns. The molecular weights of polystyrene and polymethyl methacrylate eluting at the position of the arrow are shown in the screen plot, as are the corresponding elution volume and log hydrodynamic volume. If the user gave Mark-Houwink parameters in an earlier screen menu, the molecular weight of that polymer is also shown.

CONTENTS OF DISKS

DISK 1

```
 Volume in drive A is NBS SEC V 1

ANALYZE  EXE   119726  12-27-87   7:08p
AUTOGP   EXE    72890  12-27-87   7:08p
AUTOPICK EXE    52864  12-27-87   7:08p
AUTOPLOT EXE    61262  12-27-87   7:14p
BACKGRND EXE    53530  12-27-87   7:09p
        5 File(s)         0 bytes free
```

DISK 2

```
 Volume in drive A is NBS SEC V 2

CALFIT   EXE   125244  12-27-87  10:34p
CHKRUNBR EXE    45212  12-27-87   7:09p
COMPARE  EXE   101312  12-27-87   7:10p
COMPARE9 EXE    80752  12-27-87   7:10p
        4 File(s)      8192 bytes free
```

DISK 3

```
 Volume in drive A is NBS SEC V 3

PLOTCAL  EXE    69824  12-27-87   7:11p
PLOTSEC  EXE    60974  12-27-87   7:15p
PRT      EXE    39828  12-27-87   7:11p
SECMENU  EXE    48990  12-27-87   7:42p
TITLES   EXE    47004  12-27-87   7:11p
        5 File(s)     94208 bytes free
```

DISK 4

```
 Volume in drive A is NBS SEC V 4

TITLESEC EXE   110654  12-27-87   7:37p
DATCOLL  EXE    68414  12-27-87   7:10p Recompile these two
WATCHGPC EXE    59064  12-27-87   7:12p programs using the
DATCOLL  CMP    18169   5-07-87   3:58p commands for your A/D
WATCHGPC CMP    13051   5-20-87  12:23p data collection card.
ANALYZE  HP1      629   5-04-87   7:22p
ANALYZE  HP2      535   5-04-87   7:22p
ANALYZE  HP3      522   5-04-87   7:22p
ANALYZE  HP4      323   5-04-87   7:23p
```

```
ANALYZE  HP5       511   5-04-87   7:23p
ANALYZE  HP6       304   5-04-87   7:23p
SETUP    BAS     23117   3-09-87  12:30a BASICA is needed to run
SETUP    CLA       722   7-08-86   9:47a the SETUP progam.
SETUP    DAT      2780   1-06-87   4:21p
SETUP    HLP      1390   8-24-86  10:55p
SETUP    PRO       695   8-25-86  10:30a
STANDARD CHR       338   2-20-87   8:39a
AUTOPICK LMT         7   1-31-87   3:29p
CALDAT   DAT       756  11-10-86   3:24p
CALIBR   DAT       335   2-02-87  12:10p
CLONECAL 1         332   1-24-87  12:55p
CLONECAL 3         331  11-17-86   4:47p
DO       BAT       667   2-23-87   7:44p
PVME     HYD      4242   1-22-87   9:40p
RUN      NBR         9   2-24-87   7:25a
SEC207   HYD      3518   8-08-86   9:33a
SEC924   HYD      3364   1-24-87  12:36p
SEC924   RAW     12905   1-14-86   3:02p
SEC925   RAW     12905   1-14-86   3:03p
CALFIT   DAT       226  12-27-87  10:35p
       30 File(s)       5120 bytes free
```

DISK 5

```
 Volume in drive A is NBS SEC V 5

SEC1203  RAW     14743   6-01-87   3:46p
SEC1216  RAW     14744   6-02-87   8:14a
SEC1217  RAW     14743   6-02-87   7:44p
SEC1387  RAW     12357  10-28-87  12:05p
        4 File(s)     303104 bytes free
```

NBS-114A (REV. 2-8C)

U.S. DEPT. OF COMM. BIBLIOGRAPHIC DATA SHEET (See instructions)	1. PUBLICATION OR REPORT NO.	2. Performing Organ. Report No.	3. Publication Date

4. TITLE AND SUBTITLE
System of Hardware and Software Developed for Size Exclusion Chromatography

5. AUTHOR(S)
B. Dickens and F. L. McCrackin

6. PERFORMING ORGANIZATION (If joint or other than NBS, see instructions)

NATIONAL BUREAU OF STANDARDS
DEPARTMENT OF COMMERCE
WASHINGTON, D.C. 20234

7. Contract/Grant No.

8. Type of Report & Period Covered

9. SPONSORING ORGANIZATION NAME AND COMPLETE ADDRESS (Street, City, State, ZIP)

10. SUPPLEMENTARY NOTES

☐ Document describes a computer program; SF-185, FIPS Software Summary, is attached.

11. ABSTRACT (A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here)

Abstract

A series of computer programs to carry out data collection and processing for size exclusion chromatography has been written in BASIC for an IBM XT type computer. This manual documents version 1.0, which uses a single detector. The detector is assumed to measure the concentration of the eluting species. Some provision is provided for quality control by comparing a measured chromatograms with the standard chromatogram for its class immediately after the chromatogram has been measured. There is no limit on the number of classes allowed. The measured chromatogram is automatically processed in the same way as the standard chromatogram was processed.

12. KEY WORDS (Six to twelve entries; alphabetical order; capitalize only proper names; and separate key words by semicolons)
Computer programs; gel permeation chromatography; molecular size; molecular weight determination; quality control; size exclusion chromatography

13. AVAILABILITY

☐ Unlimited

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14. NO. OF PRINTED PAGES

15. Price