

The Use of a Microcomputer with a Gel Permeation Chromatograph

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Synopsis

A description is given of the interfacing of an Apple II Plus "home" microcomputer to a Waters Model 200 Gel Permeation Chromatograph fitted with ultraviolet absorbance and differential refractive index detectors. Hardware design and the software required for data collection and data reduction are described. Flow charts are provided for the various data collection, analysis, and display programs used. Emphasis is placed on the flexibility that such a system offers to the computer programmer and the experimentalist.

INTRODUCTION

Gel permeation chromatography (GPC) is a powerful technique used for determining the molecular weight averages and the molecular weight distribution (MWD) of a polymer. The experimental measurements are rapid, and the calculations involved are relatively simple. However, these calculations are often lengthy, time-consuming, and prone to error if done manually. In this paper we show how a gel permeation chromatograph may be combined with a microcomputer to reduce calculation time and improve accuracy, resolution, and experimental flexibility.

In a gel permeation chromatograph a differential refractive index (RI) detector is normally used either alone or in conjunction with one or more of the following detectors connected in series along the eluant path: (a) an ultraviolet absorbance (UV) detector,^{1,2} (b) an on-line automatic intrinsic viscometer,³⁻⁵ and (c) an automatic densimeter.⁶ Other detectors such as infrared spectrometers have been used⁷ for measuring specific properties of polymer solutions.

The method of calculating the molecular weight averages from a chromatogram consists of dividing the chromatogram into slices and measuring the area of each slice. As each of these slices represents a particular molecular weight fraction, its area will be proportional to the weight fraction of that species in the injected solution. A calibration curve correlating the elution volume with log molecular weight of polystyrene standards of narrow molecular weight distribution and known molecular weights is used to calculate the number- and weight-average molecular weights.⁸⁻¹⁰ Thus, analysis of a gel permeation chromatogram for the computation of the different molecular weight averages and polydispersity may easily take a couple of hours using manual techniques. Additional analysis of the chromatogram to determine the concentration of polymer mixtures using a planimeter¹¹ can be equally laborious.

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Earlier attempts toward reducing the time for these calculations came in the form of computer programs to treat the data.¹² Here the division of the chromatogram into segments, the measurement of heights (or areas) of segments and the data entry into the computer were done by hand. Thus, analysis was still a tedious process. The next step in the automation of GPC analysis involved automatic monitoring and real time analysis using a microprocessor as was done in the analysis of gas chromatograms.¹³ Commercial manufacturers like Hewlett-Packard Co. also developed very elaborate systems to achieve an automatic system in this way.^{14,15} Such an automated system is faster and more accurate than other conventional methods of data analysis, but is also much more expensive. Another drawback is that the data are analyzed as sampled. Since all analyses are part of the sampling program, the program must be written in a language that allows extremely fast execution. Such assembler languages are difficult to program in and prone to error. Also, the same curve cannot be subjected to different types of analysis since the individual data points are not preserved. Thus, although very accurate, these systems tend to be very expensive and not very flexible.

The final method of automation consists of using a microcomputer to sample the data and save them on a disk or tape for later analysis using high-level language routines (i.e., BASIC or PASCAL). This method allows a single, very small, fast program to be written for data collection. Changes in the number and type of detectors will not in general result in significant changes in the routines. The entire analysis is done in a higher level language (such as BASIC) which can be easily mastered. Thus, flexibility is provided for at the analysis level. This paper describes both the hardware and software of an automated GPC facility that exploits the advantages of such a system by using an Apple II Plus Microcomputer for data collection and data analysis in conjunction with a Waters Model 200 Gel Permeation Chromatograph outfitted with both refractive index (RI) and ultraviolet (UV) detectors.

HARDWARE DESCRIPTION

Reasons for using an Apple II Plus Microcomputer for monitoring the GPC detectors are that:

(i) It has room for a reasonable number of readily available expansion boards such as analog-to-digital (A/D) converters, serial interfaces, real time clocks, and floppy disk controllers.

(ii) The game connector interface can be used for controlling and monitoring external hardware.

(iii) It supports full floating point arithmetic.

(iv) It supports a high level language (BASIC).

The full configuration of the microcomputer presently being used consists of:

(i) Apple II Plus Microcomputer with 48K bytes of memory.

(ii) Two 12-bit California Computer Systems (CCS) A/D converters (one for each of the RI and UV detectors).

(iii) A Mountain Hardware real time clock for measuring sample intervals accurately.

(iv) Two 5.25-in. floppy disk drives (one for the program master disk and one for data collection).

(v) An Integral Data System model 440 printer for printing results and for use in program development.

The detector outputs to be monitored were from a Waters Associates Model 440 Ultraviolet (UV) Detector, a Waters Associates Series R-400 Differential Refractive Index (RI) Detector, and a 5-mL elution volume marker. These detectors were connected to the microcomputer through a signal modifying interface unit, as shown in Figure 1. It consists of the following three sections.

RI Detector

This detector puts out a maximum signal of 100 mV, which is characterized by both a large 60 Hz ac ripple and a general wandering of $\pm 0.2\%$. An interface was constructed with a gain of 40 to give a signal into the microcomputer's A/D converter of between 0 and +4 V. A $0.47\text{-}\mu\text{F}$ capacitor was placed in the feedback circuitry of the amplifier to reduce the ac ripple. The effect of the signal wandering is reduced by having the computer software average several measurements for each one recorded.

UV Detector

This detector puts out a pure dc signal of between 0 and -10 V. An interface circuit was placed to invert the signal and alter it to 4 V full scale for the A/D converter of the microcomputer.

Elution Volume Detector

The elution volume marker gives an output pulse of 120 V ac with approximately 2 s duration for every 5 mL of solution. This pulse occurs at intervals of approximately 5 min. Circuitry was used to reduce the 120-V pulse to a 5-V

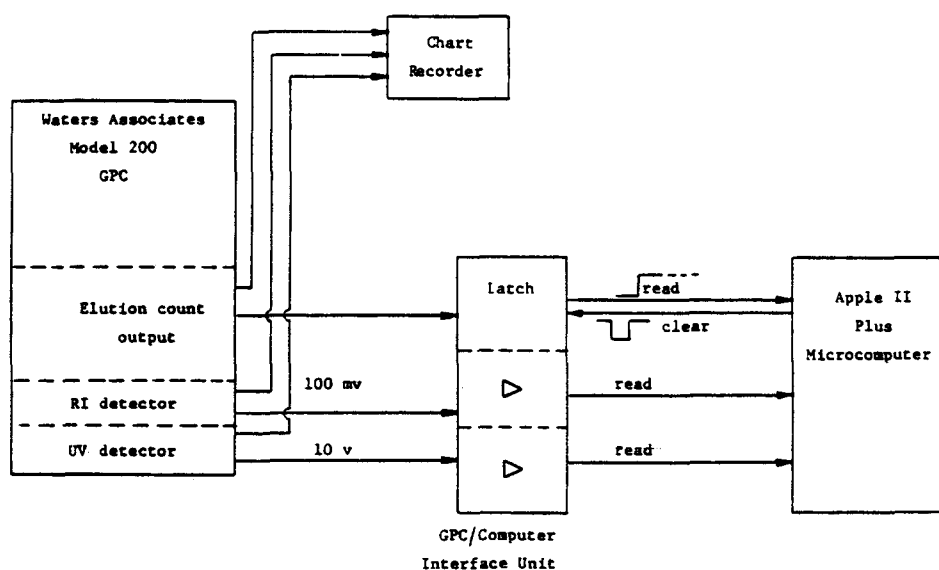


Fig. 1. Layout of GPC monitoring equipment.

latched signal that can be put directly to the game connector of the Apple microcomputer. The latching is necessary so that the pulse is not missed should it fall completely within a measurement interval. Clearing of the latch is controlled by software in the microcomputer which activates the strobe line of the game control port. Double counting is eliminated by checking whether the latch remains 0 after being reset. If not, it is again reset without recording a new count, as shown in Figure 2.

The UV and RI sections of the interface unit each have a general protection circuit to provide protection to the A/D converters in the microcomputer by limiting output voltages from the interface unit to approximately 4 V. Detailed descriptions of the circuitry are available from the authors.

SOFTWARE DESCRIPTION

The hardware connecting the Apple microcomputer to the Waters Gel Permeation Chromatograph was kept to a minimum in order that costs and the potential for failure be reduced. Instead, most of the data collection and control are done by means of software. The basic software routines of "WAT-CHROM," developed specifically for the gel permeation chromatograph-microcomputer system designed and developed in our laboratory, were written entirely in the high-level language known as APPLESOFT BASIC (Trademark of Apple Computers Inc.). Although not as fast as comparable assembler programs would be, this language allows for easy modifications and is sufficiently fast for use with a GPC using a 5-mL syphon. The software programs fall into the categories of data collection, output of raw data for examination purposes, and data reduction.

Data Collection Routines

Data collecting software consists of those routines used for initializing the GPC equipment as well as the routines used in the data collection. Routines presently existing for this function are:

(a) A program which monitors the various detectors and displays their current values. This routine called "MONITOR A/D" is used for setting the proper offset on the detectors to keep the baseline values always positive.

(b) A second program for initializing the Gel Permeation Chromatograph/Microcomputer interface. This routine prepares the interface for data collection by clearing all registers in the microcomputer.

(c) The data collection program itself. This program, called "COLLECT DATA," collects data points on a continual basis from both detectors and from the elution volume marker. These points are then averaged over some time interval specified by the experimenter. The resulting averages are stored in a disk file which also has been specified by the experimenter. Present data collection intervals can vary from 2 s to any time interval. For the GPC equipment in our laboratory, 10 s (approximately 30 points between two elution counts) was found to be optimum. This interval allowed averaging of a reasonable number of intermediate points (more than five) to provide some low pass filtering, while still giving a more than adequate number of data points for each curve.

The flow charts shown in Figures 2, 3, and 4 show the details of the data col-

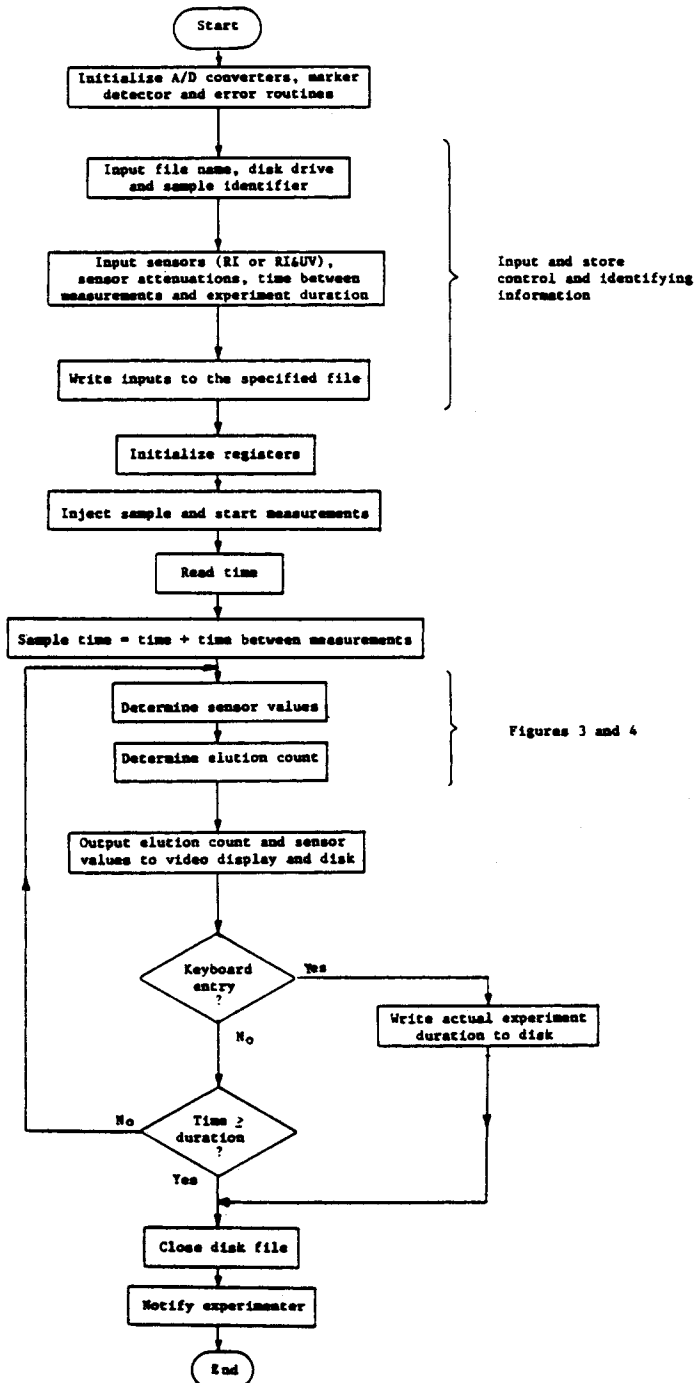


Fig. 2. Flow chart for "determine elution count" block.

lection program. This "COLLECT DATA" program is activated by answering questions, as shown in Figure 5. The actual data are not processed at the time of data collection, but are preserved on a data disk for later examination and reduction. Thus, the same data can be processed using a variety of analysis routines. Disk space is kept to a minimum by converting the sensor values to integer and by not storing times. These times can be recreated in the output and analysis routines. A sample of a data output program is shown in Figure 6.

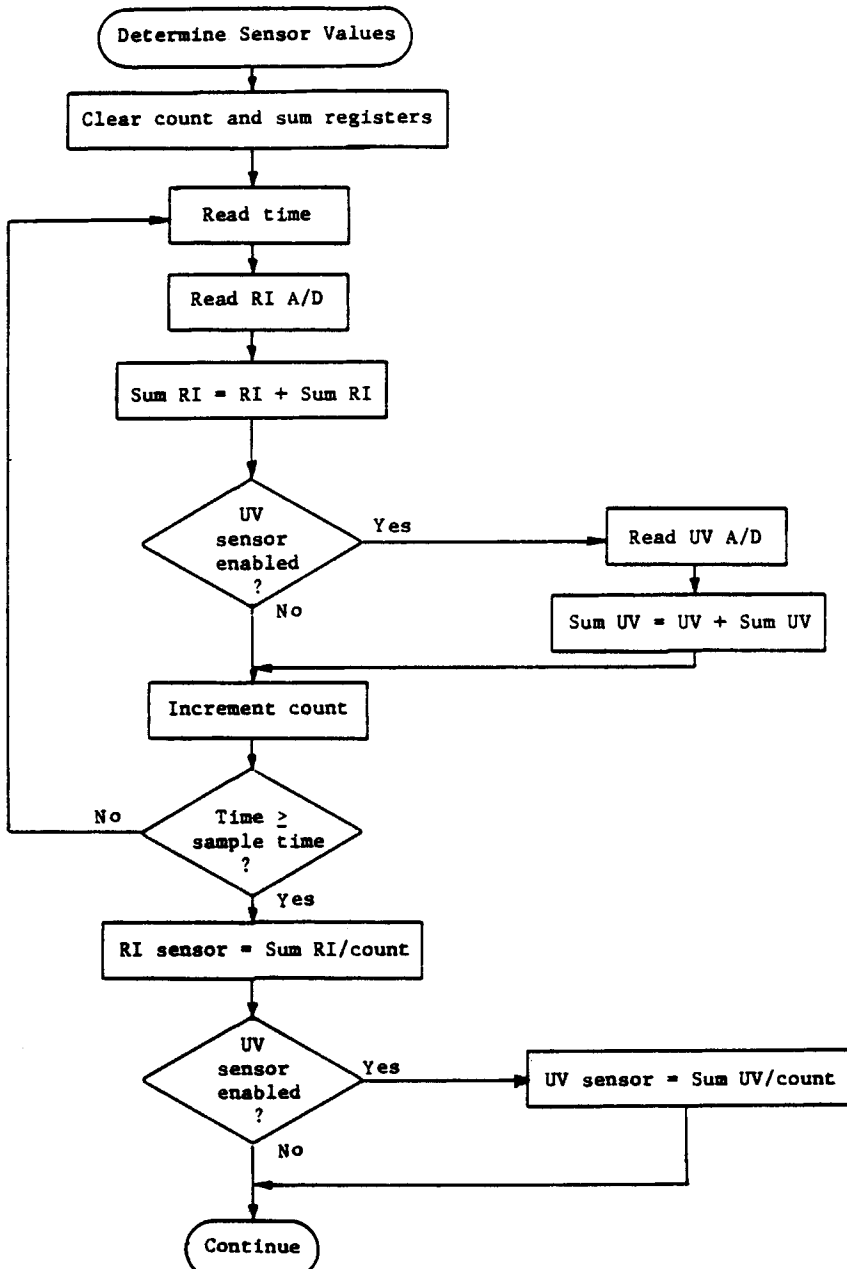


Fig. 3. Flow chart for GPC data collection.

Examination Routines

Once the raw data have been collected, it is often desirable to examine them in order to inspect their integrity or to establish curve bounds, peaks, and other parameters. In the Apple II Plus system, examination of the raw data can be done by either the "PRINT DATA" program, which simply prints out the raw data for different detectors between any two specified elution counts, or the "PLOT DATA" routine, which makes a high resolution plot of the chromatogram on the Apple II Plus display screen. Figures 7 and 8 provide the flow charts for the "PRINT DATA" and "PLOT DATA" programs, respectively.

A third program which allows the experimenter greater flexibility in examining and, if necessary, modifying raw data is "GPC EDIT." This program allows the examination of a raw data file in an interaction mode. If a small part of the data is in error because of some understood reason, the experimenter can alter the actual values of the data and write the results back into the data file. Other features of the GPC EDIT program include searching for a particular data value, searching for a maximum or minimum data value, and examining or altering the data values for any detector.

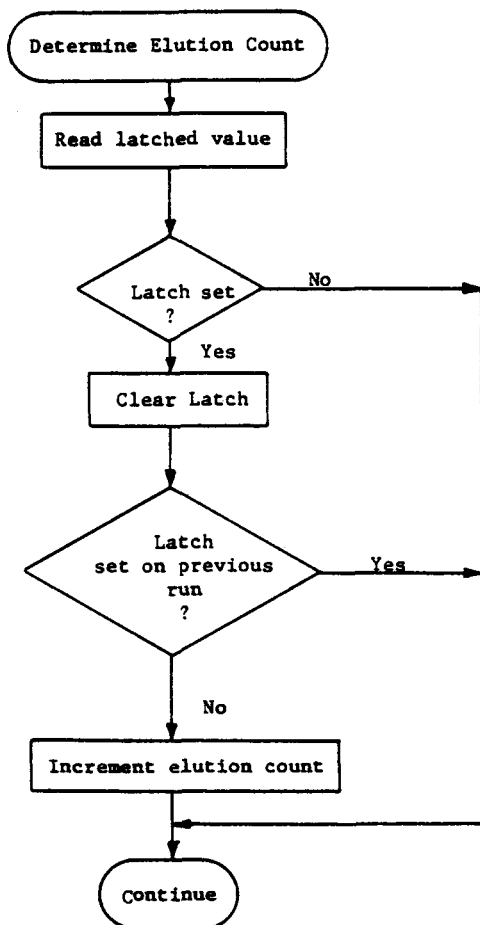


Fig. 4. Flow chart for "determine sensor values" block.

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RUN COLLECT DATA
      CALIBRATION FOR CONCENTRATION
      OF POLYMERS
      MAR 14, 1981 - 20:07:38

SAMPLE NUMBER? 30

FILENAME? A1.00 100K/30 3/81
DISK DRIVE? 2

POLYMERS? PS 100000
CONCENTRATION (MG/ML)? 1

RI ONLY? NO

SETTING OF RI DETECTOR? 8
SETTING OF UV DETECTOR? .5

TIME BETWEEN MEASUREMENTS (SEC)? 10
DURATION OF EXPERIMENT (MIN)? 210

ALL INPUT DATA CORRECT? YES

*** HIT 'RETURN' TO BEGIN ***

```

Fig. 5. Sample of microcomputer/user interaction for initiating data collection. Experimenter responses are underlined.

Data Reduction Routines

Data reduction consists of reading the raw data as stored in disk by the "COLLECT DATA" program and manipulating them in some predetermined fashion to produce the required results. In the case of GPC chromatograms the required results are:

- (i) area under the curve
- (ii) number- and weight-average molecular weights,
- (iii) z-average molecular weight,
- (iv) polydispersity ratio.

Factors which must be taken into account for the calculation of the above are:

- (i) fitting of the GPC-calibration curve,
- (ii) baseline fitting
- (iii) "skewing" of the curve due to axial dispersion, etc.

The data reduction or analysis can be broken down into two basic parts. The first part consists of those items which are required for the various types of data analysis. These are:

- (i) reading the raw data from the appropriate file,
- (ii) calculating from the calibration curve data the appropriate molecular weight corresponding to each data point in the raw data,
- (iii) fitting a baseline to the experimental curve in question.

The second part of data analysis consists of using the baseline-fitted data to calculate the desired values. Since the curve may be skewed due to imperfect resolution, it often becomes necessary to apply some form of correction. One such correction technique was proposed by Chang and Huang¹⁶ and is discussed in a later section.

The length of the program needed to do each of the above portions is about 1000 lines of BASIC code. The unwieldiness of such an approach and the logical division of the reduction task into two portions led to the following approach.

IRUN PRINT DATA

FILENAME? A1.00 100K/30 3/81
 DISK DRIVE? 2

START PRINT AT ELUTION CNT? 25
 END PRINT AT ELUTION CNT? 30

OUTPUT TO PRINTER? YES

CALIBRATION FOR CONCENTRATION
 OF POLYMERS

MAR 14, 1981 - 20:07:38

SAMPLE NUMBER: 30

FILENAME: A1.00 100K/30 3/81
 DISK: 2

POLYMERS: PS 100000
 POLYMER CONCENTRATION (MG/ML): 1

UV AND RI CURVES STORED

SETTING ON RI DETECTOR: 8
 SETTING ON UV DETECTOR: .5

TIME BETWEEN MEASUREMENTS: 10 SEC
 DURATION OF EXPERIMENT: 205.66 MIN

COUNT	TIME (MIN)	RI	UV
29	149	.688	1.136
	149.16	.739	1.212
	149.33	.782	1.292
	149.5	.822	1.37
	149.66	.868	1.456
	149.83	.919	1.545
	150	.966	1.636
	150.16	1.016	1.733
	150.33	1.064	1.834
	150.5	1.107	1.935
	150.66	1.146	2.023
	150.83	1.181	2.111
	151	1.219	2.195
	151.16	1.244	2.274
	151.33	1.277	2.342
	151.5	1.298	2.405
	151.66	1.324	2.461
	151.83	1.338	2.508
	152	1.352	2.551
	152.16	1.364	2.589
	152.33	1.377	2.62
	152.5	1.377	2.647
	152.66	1.38	2.668
	152.83	1.379	2.68
	153	1.379	2.687
	153.16	1.372	2.687
	153.33	1.362	2.682
	153.5	1.354	2.672
	153.66	1.351	2.659
	153.83	1.337	2.644
	154	1.322	2.625

Fig. 6. Sample of raw data output. Experimenter responses are underlined.

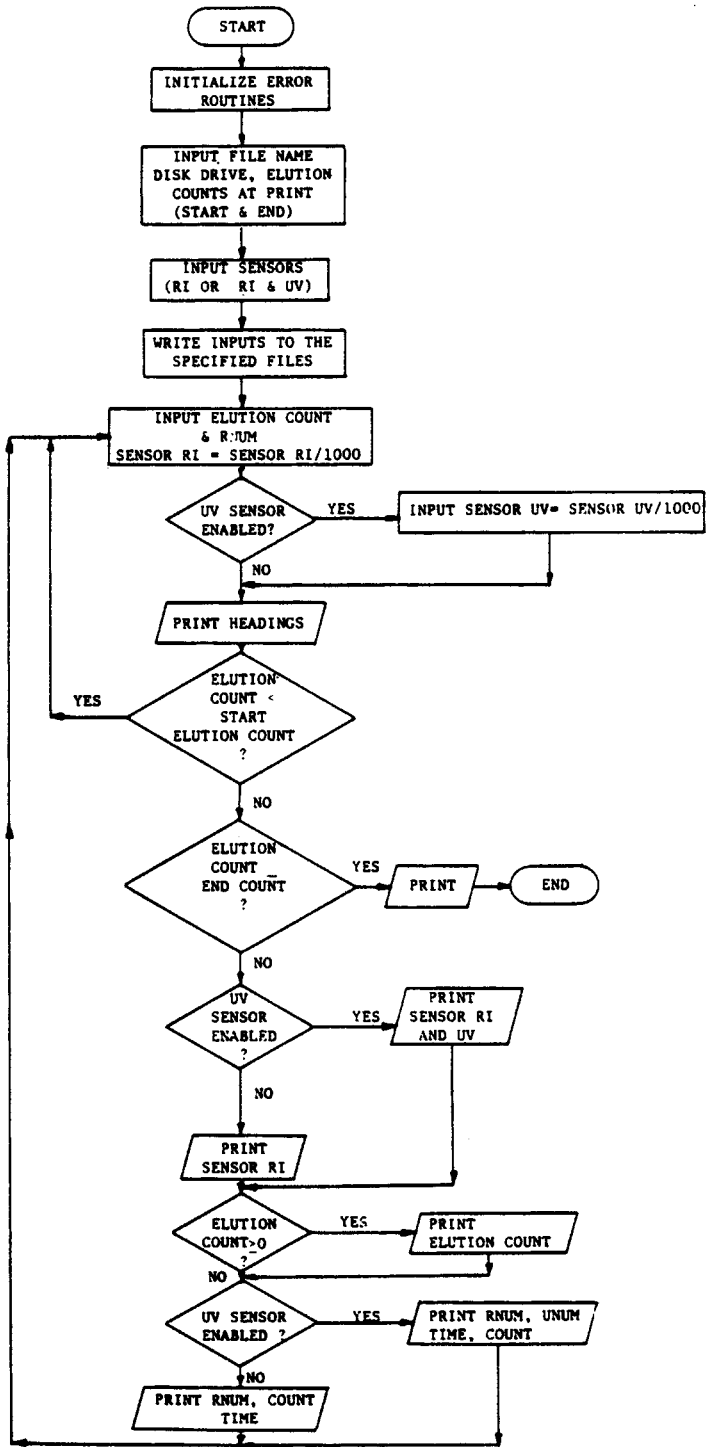


Fig. 7. Flow chart for "PRINT DATA" program.

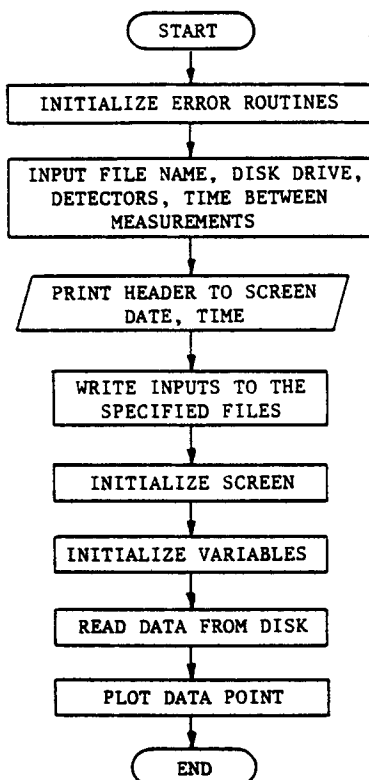


Fig. 8. Flow chart for "PLOT DATA" program.

The first task, which involves reading the raw data, calculating the molecular weight at each elution count, and doing the baseline fitting is executed by the "GPC REDUCE" program. This program is described in more detail below.

The second task, which involves the actual calculation of the result and any correction routine desired, may be done by a variety of programs depending on the actual reduction desired. Programs thus far implemented on the Apple II Plus system are a program for calculating areas and molecular weights with no smoothing or other correction techniques (GPC RAW) and a program for applying the Chang-Huang correction method to the data (GPC CORRECT).

Calibration Curve

The calibration curve can be fitted to the calibration data points in a number of ways:

- (i) straight line interpolation between any two experimentally derived points on the calibration curve,
- (ii) division of the calibration curve into a number of sections, each of which is a straight line,
- (iii) fitting a smooth curve (using some fitting routine such as a second-order least square fit) to the calibration points.

The "GPC REDUCE" program as presently implemented incorporates the second technique. This method can also be made to incorporate the first method by simply defining a straight line segment between any two adjacent calibration points.

Fitting a smooth curve to the data was not found to be necessary as, for the most part, the experimental chromatograms lie within the straight line sections of the calibration curve.

Baseline Fitting

One of the greatest problems in analyzing GPC data is that the baseline of the chromatogram drifts. The drift is especially true when the RI detector is being used. Some methods^{14,17} which have been suggested for correcting the baseline include:

(i) ignoring all drifts and assuming a horizontal baseline starting at the beginning of the experiment; this method is not generally acceptable for most detectors;

(ii) assuming zero drift from the beginning of the curve and so fitting a horizontal baseline from that point; this method works only when there is little baseline drift or when the peak of the curve is narrow;

(iii) establishing an originating and a terminating point for the curve and then joining these two points by a straight line; this method is most often used in fitting baselines to GPC curves;

(iv) attempting to establish higher order equations for the drift of the baseline by using various fitting techniques; the amount of computer power required for the relatively small increase in accuracy generally makes this method impractical.

The "CALC MOL WT" program and the "GPC REDUCE" program are capable of applying the baseline techniques described in (ii) and (iii). The flow charts for these two programs are given in Figures 9 and 10, respectively. The program provides a technique which is faster and more convenient than manual fitting, while the accuracy and precision would remain the same.

Molecular Weight and Area Calculations

The "CALC MOL WT" program is used to calculate the molecular weight averages of the polymer and also the area under the chromatogram. The beginning and ending points of the chromatogram are predetermined by the print and plot routines. The user also specifies the type of baseline fitting. The area under the curve is calculated by numerical integration using the trapezoidal method where the curve is divided into very small area strips so that the curvatures at the top can be approximated by a straight line.¹⁸ Since there are about 30 points between two elution counts, good accuracy is obtained.

The molecular weight averages are calculated from the weight fractions of the different molecular weight species by using the standard formulae.¹⁰ No smoothing is applied on the raw data. The molecular weight averages of any polymer can be computed using the polystyrene calibration data and a knowledge of the Mark-Houwink constants K and α for polystyrene and the polymer in question in the solvent used. The values of K and α for polystyrene in tetrahydrofuran are built into the main programs, while provision is made in the software such that the corresponding values for different polymers can be incorporated before analysis when it is necessary.

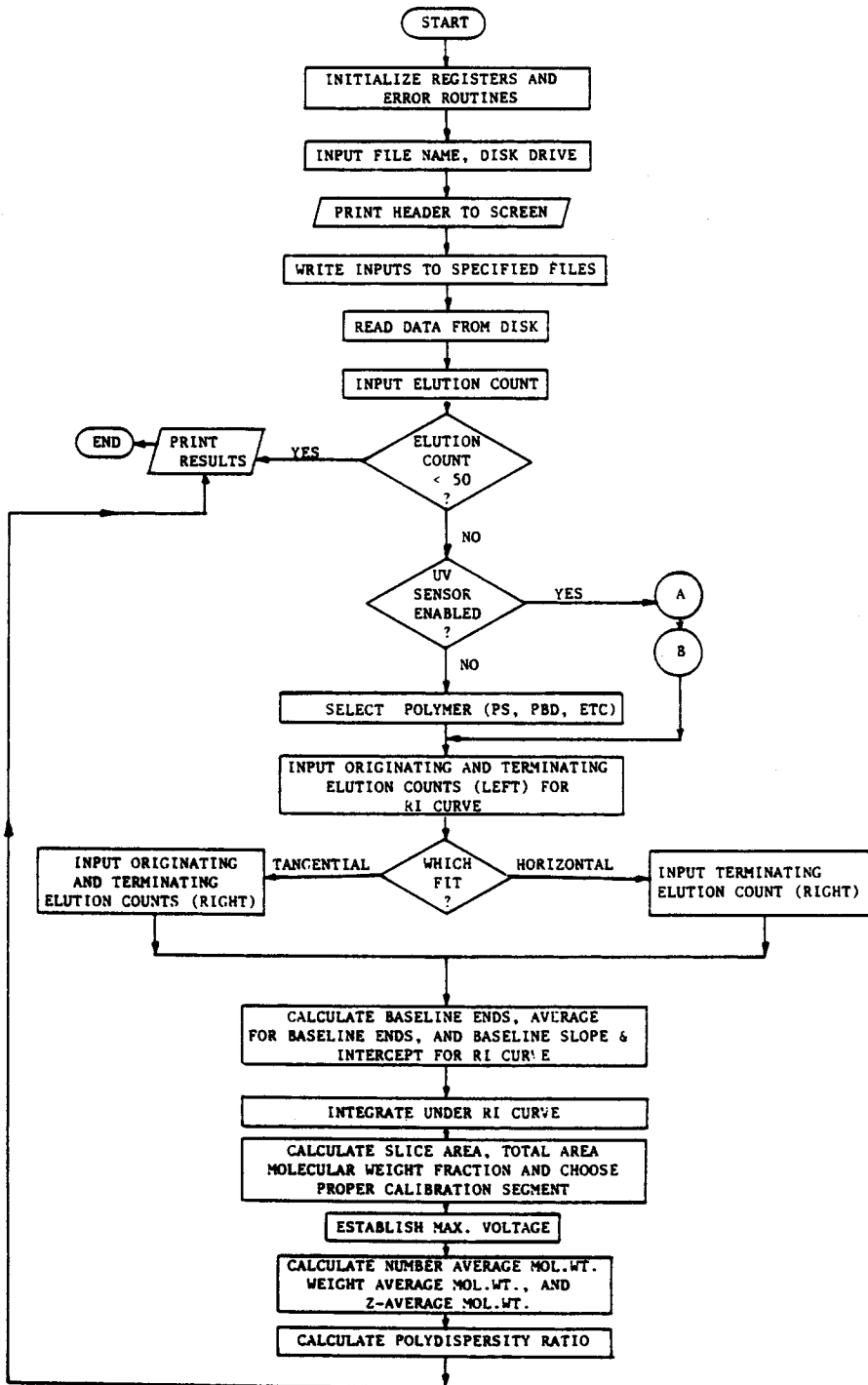


Fig. 9. Flow chart for "CALC MOL WT" program.

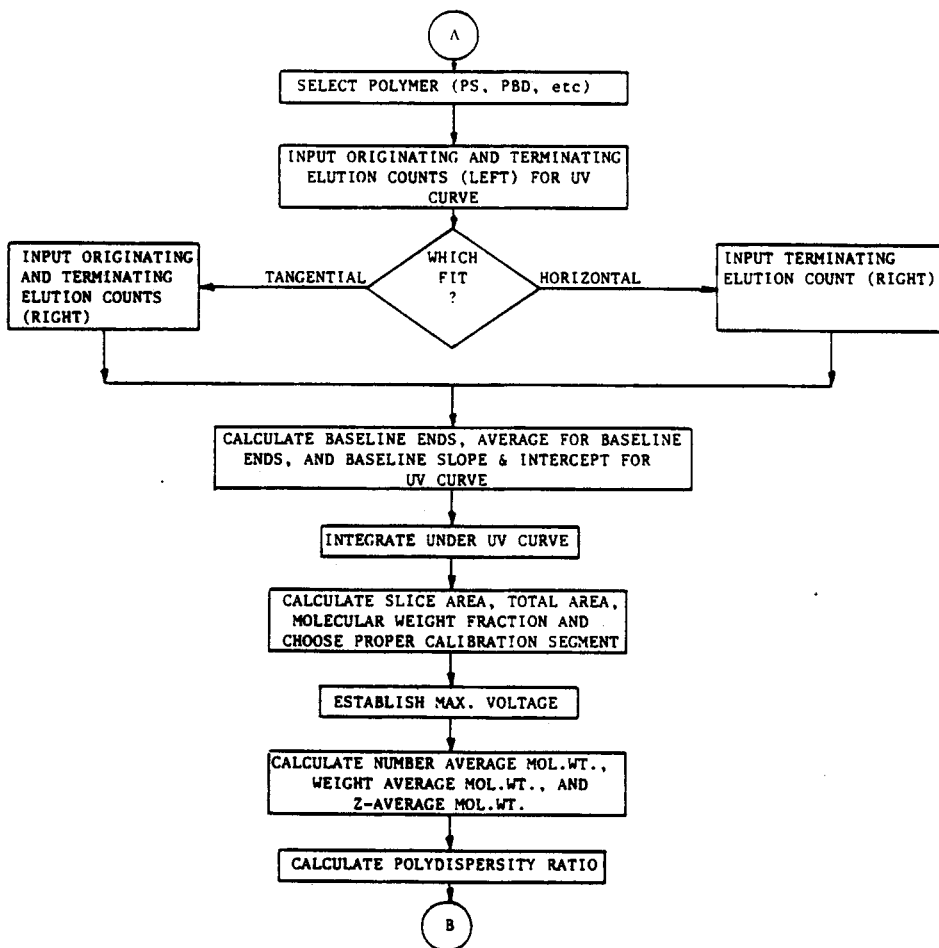


Fig. 9. (Continued from previous page.)

Chang-Huang Correction

The molecular weight averages and the polydispersity ratio calculated using a GPC chromatogram may be in error due to imperfect resolution resulting from axial diffusion and viscosity effects. These effects normally result in the calculated polydispersity ratio being higher than the actual value. Different correction techniques have been developed and reported in the literature.^{16,19-21} The Chang-Huang technique¹⁶ developed in our laboratory is an optimization method based on the steepest descent in the functional space. Solution is often obtained after two iterations. In this correction technique the data are first normalized and then smoothed using the seven-point cubic method.²² To calculate the corrected molecular weight averages, Chang and Huang have given two methods, namely, the first-order method and the second-order method. The "GPC CORRECT" program incorporates both those methods, in addition to data smoothing and normalizing routines. Figure 11 provides a flow chart for the "GPC CORRECT" program.

A sample output of data is shown in Figure 6. Approximately 30 data points are collected between two elution counts. By comparison, however, 5 or 6 data

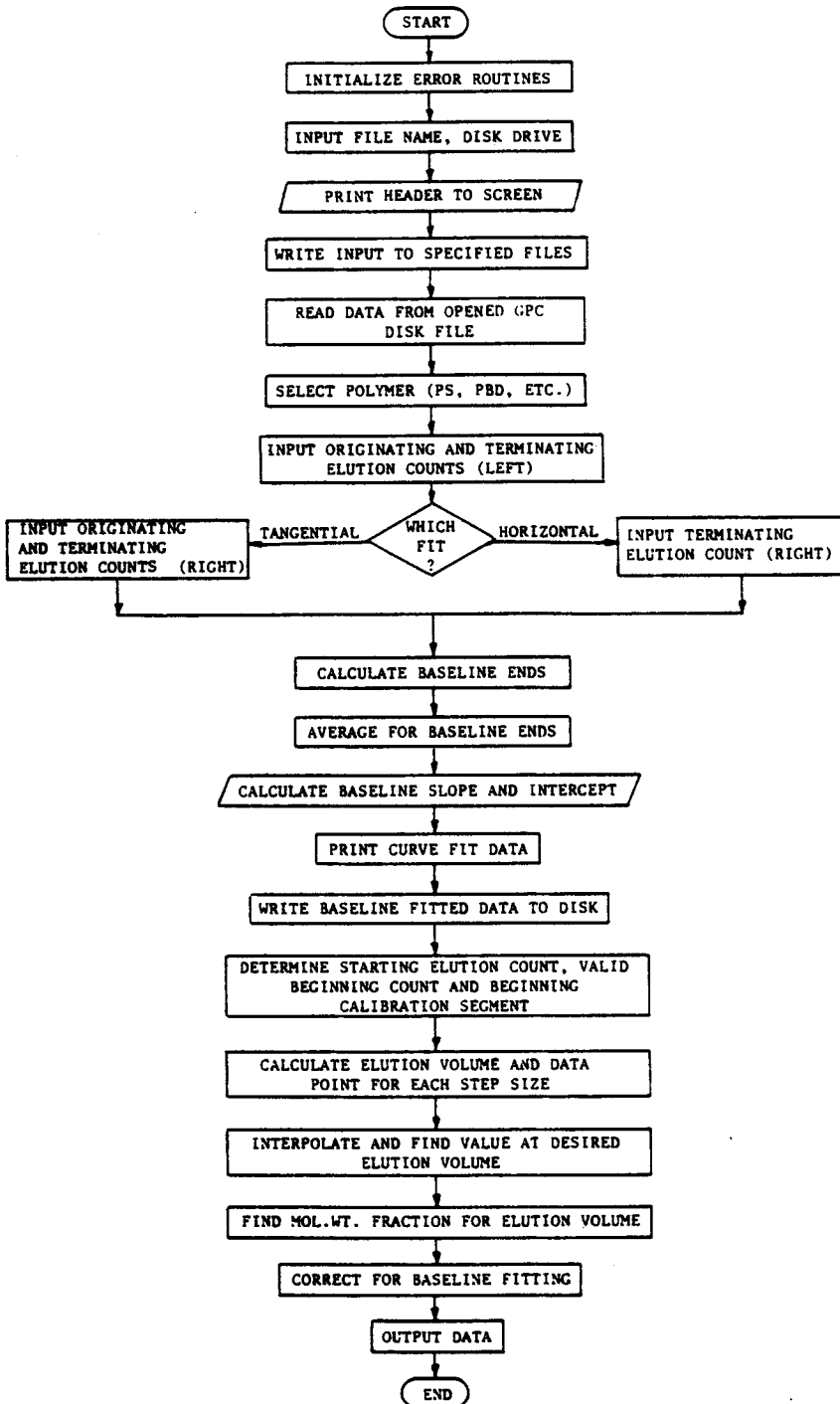


Fig. 10. Flow chart for "GPC REDUCE" program.

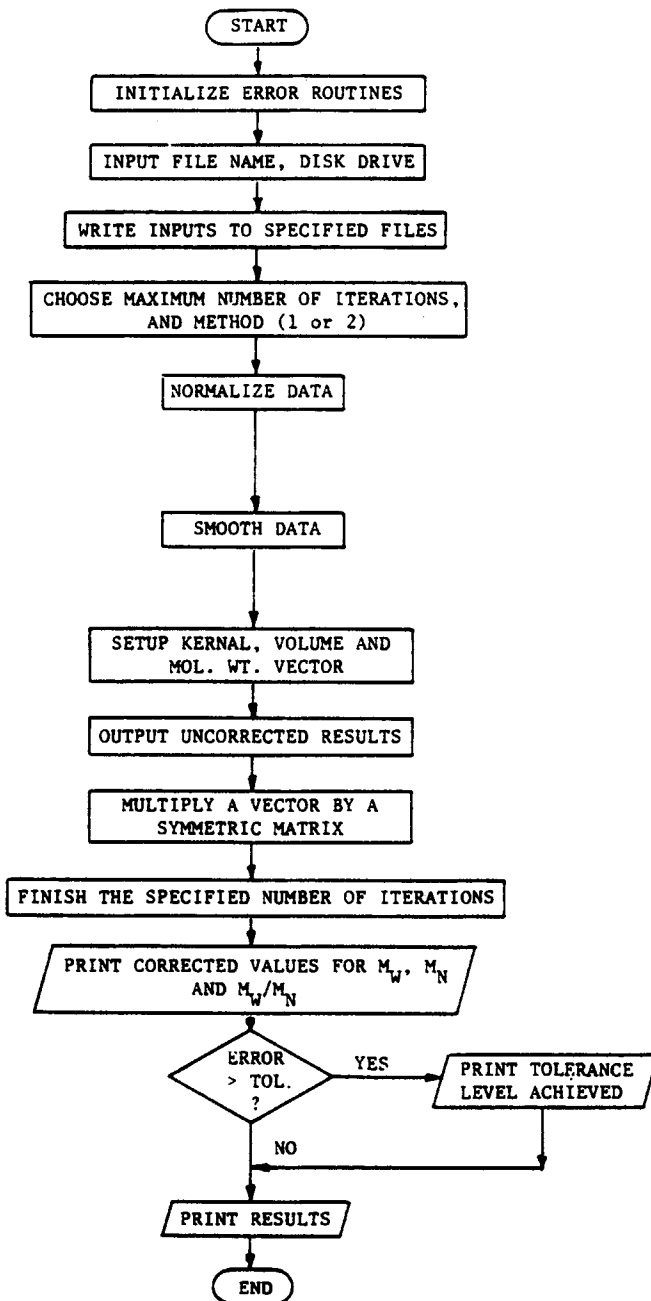


Fig. 11. Flow chart for "GPC CORRECT" program.

points per elution count provide the best results using between 5 or 10 iterations with the correction program. The time of analysis per sample is less than 1 h. If the correction program is not used, it takes only about 15 min for analyzing one sample and calculating the area, molecular weight averages, and the polydispersity ratio using all the data points. This compares with almost 2-3 hs using conventional manual means.

DISCUSSION

Some guidelines which were found to be of use in designing the system are:

(i) Hardware and software are almost always cheaper to buy than to develop separately. Thus, A/D converters, real time clocks, and high level language compilers and editors are almost always a good investment.

(ii) The user interface should be kept straightforward, with emphasis placed on the microcomputer clearly prompting the experimenter when some action on his part is required.

(iii) Storage of raw data is normally a good idea as it allows for easy checking or reprocessing at a future date, should results become suspect or more sophisticated data treatment programs became available.

(iv) Interface hardware should be kept to a minimum because it is the most difficult component to repair or replace should a failure occur at some future date.

(v) Where possible, programs should be written in a high-level language (such as PASCAL or BASIC) to allow rapid development and to reduce the possibilities of undetected errors.

(vi) The hardware and software should be designed so that more detectors can be easily added at a later date. One of the features of computer aided process work is that the amount of work tends to expand as the user becomes more at ease with it and more aware of its possibilities.

(vii) Adequate visual prompts should be provided so that the experimenter can verify at a glance the proper functioning of the equipment. In the GPC collection facility all data are displayed on the computer screen as they are collected. Also a chart recorder is kept connected to the detectors for curve verification.

CONCLUSIONS

Hardware and software for collecting and storing the GPC data from the UV and RI detectors, for printing and plotting them on the screen, and for the data reduction have been developed for the GPC-Apple II Plus System. The relatively large time constants present in GPC data allow the use of high-level languages. Computation of the areas under the chromatogram, the molecular weight averages, and the polydispersity ratio can all be done within 15 min per sample. A correction program for the molecular weights developed by Chang and Huang¹⁶ has been incorporated into the analysis programs. Other correction routines can be incorporated when needed, without any changes to the main molecular weight program. In comparison to manual calculations, these programs allow a reduction in the time required for analysis and an improvement in the accuracy and precision of the results.

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