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Modification of a Gel Permeation Chromatograph for Automatic Sample Injection and On-Line Computer Data Recording*†

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Summary

Gel permeation chromatograms are usually subjected to mathematical analyses of varying degrees of complexity to obtain molecular weight distribution and analytical data. Hand reduction of the recorder output data is difficult and time consuming even on an occasional basis. This paper describes in detail the modifications necessary to adapt the Waters Gel Permeation Chromatograph Model 200 for on-line data acquisition by a computer equipped with an analog to digital converter (IBM 1800). Since the operation is done in the background mode, time sharing with several other users or instruments is possible. Modifications to the auto injection unit are also described. This system has proven to remove all of the usual hand data reduction steps and to be extremely flexible for nonroutine analyses.

Gel phase or permeation chromatography (GPC) has become a routine analytical procedure for the qualitative and quantitative analysis of materials soluble in organic solvents. The technique has been applied to low, medium, and high molecular weight materials. The method is commonly used for the determination of molecular

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† II in a series on the automation of analytical instruments.

weight distributions on a wide variety of soluble medium and high molecular weight polymers. Since the time scale of an individual chromatogram is long with respect to most other analytical methods, maximum use of a given instrument can be obtained only if samples are interleafed. That is, injected so that several samples are being chromatographed in a given column at one time; spaced so that elution peaks do not overlap. For most GPC systems this is best done by some form of automatic injection system. This permits loading of a large number of samples at a convenient time for injection as column space becomes available.

For molecular weight determinations extensive calculations must be performed on data taken from the GPC peak. Although two computer programs have been described for the necessary calculations, data reduction from the curves is done by hand (*1*, *2*). This is a slow and exacting process subject to many errors. Some form of direct digital recording of the chromatographic data independent of the recorder chart would greatly improve the convenience and ultimate accuracy of the data processing and calculated molecular weight distributions.

The subject matter of this paper is the detailed discussion of the modifications necessary to a Waters Model 200 GPC for automatic operation with on-line, time share recording of data by an IBM 1800 computer. In addition, the computer programs for the calculation of M_w, M_n, M_z, M_v, M_w/M_n, graphical representation of recorded data, integral distribution, per cent distribution and per cent by volume eluted are outlined.

The problems of recording chromatographic type data where the independent variable is time on a time shared computer have been discussed before (*3*). For our particular monitor system no experiment is allowed to place undue demands on the computer (*4*). Data collection is accomplished using software timers and each instrument is serviced at the user's specified time interval, DT. There is no guarantee, however, that the requested time interval between data points will be realized. To overcome this, the real time at which the data point is collected is recorded along with the differential refractometer deflection. The differential refractometer is the detector in the apparatus. Since GPC is an extremely "slow" experiment, the nominal DT is 30.0 sec. However, interaction with the system and other users can result in a 5% variation. The actual interval, however, can be measured to an accuracy of 1 msec. A record at equal abscissa increments may be readily generated using a simple numerical linear interpolation.

During the course of a GPC run the measurement of the flow rate must be made. Here a demand/response mode of operation is utilized. The emptying of the siphon counter causes the computer to immediately respond and record the time at which the event occurred. This is a perfectly acceptable mode of operation since normally these "interrupts" occur at ~5.0 min intervals. The same method is used to record the infrequent auto-injection markers.

INSTRUMENTATION

Indexing Pen Modification

Under normal operating conditions a 15 mV signal is injected via a relay closure into the data recording each time the elution siphon counter empties. This causes the output of the differential refractometer to be displaced by 15 mV for approximately 15 sec. Should a computer reading be made of the differential refractometer output during a count marking cycle, an error of ~15 mV would be introduced. Thus, prior to any attempt at direct digital data recording of GPC curves, it is necessary to remove the siphon counter markers from the differential refractive index recording.

This requirement was met by the addition of a Perkin-Elmer event marker for a Speedomax W recorder, No. 124491. The pen coil was actuated by tapping a signal from Pins 13 and 14 of the relay plug-in, see Fig. 1. The relay is numbered KHP17A11. Since the output of Pins 13 and 14 is 110 V ac, a simple voltage dropping and rectifier arrangement is necessary to obtain 24 V dc to operate the pen coil. The 15 mV signal from the siphon counter is suppressed by the removal of the 1.5 V battery shown in Fig. 2.

In addition to the siphon counter marks, it is also necessary to record the injection point of the automatic sample injector. The necessary modifications to the auto inject system are shown in Fig. 3. With the above modifications, the siphon counter and refractometer signals are completely isolated from one another. These two signals are now in the correct form for direct connection to the 1800 computer.

Modifications to the Auto-Injection Unit

The Waters auto injector is an adequate solution to the problem of automatic sample injection. However, the attachment was found to have one flaw as received. The cycle of the loop, once started, goes

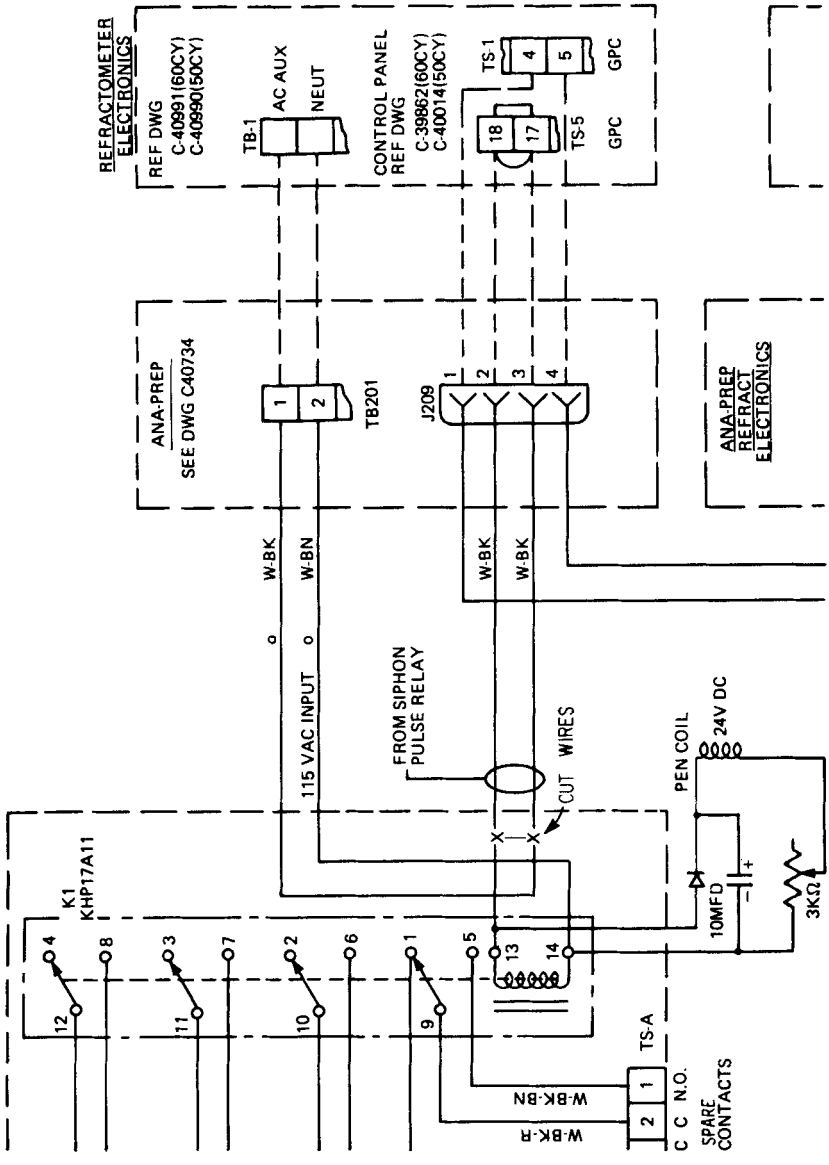

FIG. 1. Attachment of the event marker pen coil.

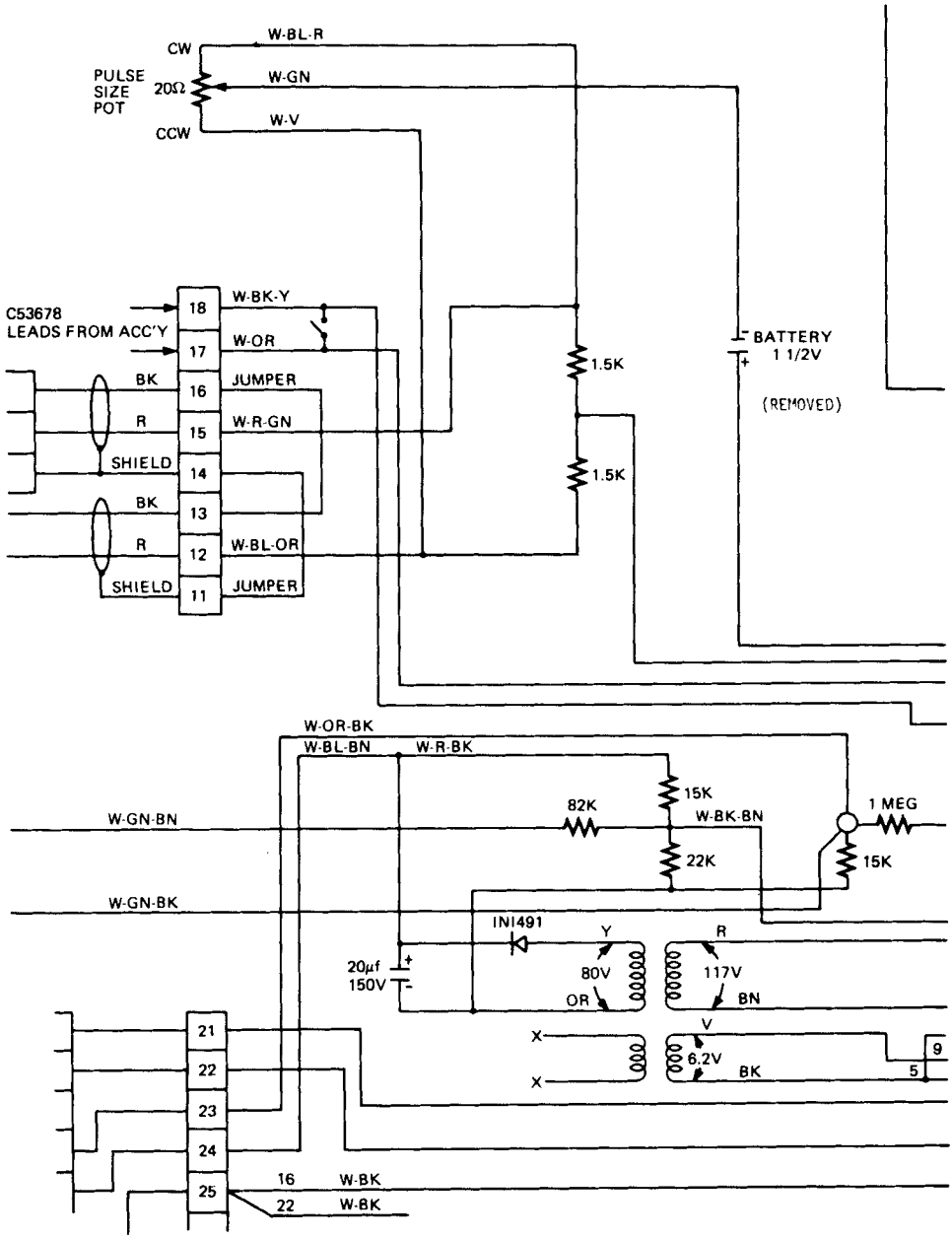

FIG. 2. Battery location.

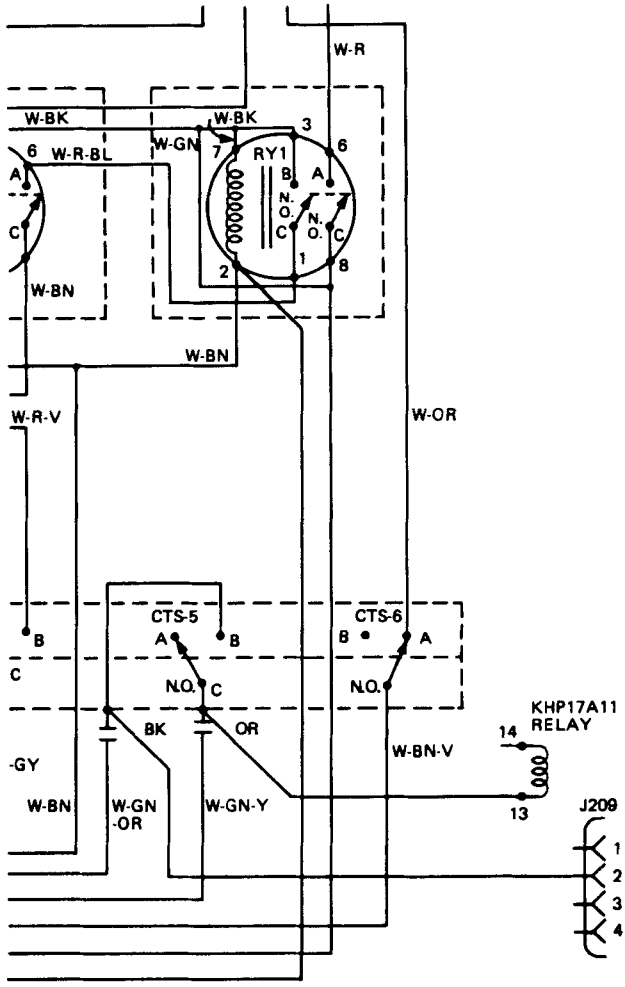

FIG. 3. Addition of the auto injector pulse to the volume count signal to the IBM 1800 and the event marker.

on until the main power switch of the auto injector is turned off manually. This operation results in empty (solvent filled) loops being injected after the last "loaded" sample. Were the refractometer less sensitive, this would be no problem. However, the solvent placed in the first loop when the first sample was injected is not identical to the machine solvent after the sixth loop is injected. This results in a series

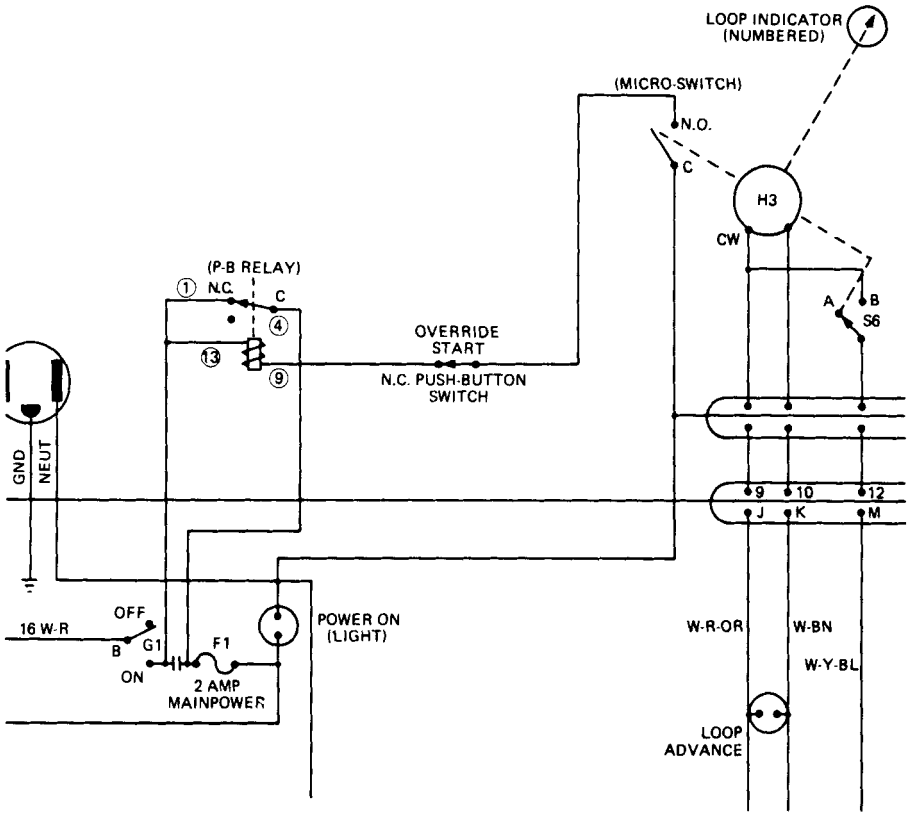

FIG. 4. Modification of the auto injector for automatic stop after six injections.

of baseline variations corresponding to each blank injection. Since with proper interleafing and column choice the average peak requires only 1.5 to 2 hr for actual elution, only 9 hr are required to go through the six sample cycle. This leaves, if the machine is run overnight, 7 hr for blank injection. The resultant unstable baseline ruins the next day's work.

To solve this problem, a microswitch was placed on the motor which rotates the loop. Corresponding to each numbered loop position is a mechanically attached cam. The microswitch is located between the first and sixth loop (cam) position. After the sixth injection, a relay operated by the switch causes the main power to the injector unit to shut off. The power to the auto injector remains off until the power

override switch is reset. The circuit modifications are shown in Fig. 4. This requires a small change in the operating sequence as follows:

Loading

1. Turn on the main power switch and press the override switch. Wait for the loop assembly to advance to the 1-2 position.
2. Flush the loop with solvent and inject sample.
3. Press the loop advance switch (hold for approximately 5 sec) to advance to the next loop and repeat Step 2 until all loops have been filled. Each loop *must* be filled with either *sample* or *solvent*.
4. After loading the 6-1 position, press the loop advance switch and advance the loop to the cutoff switch.
5. Press the override switch to restart. The loop will advance to the 1-2 position which is the initial injection position.

Injection

1. Set timer to 3 counts and then to the desired injection interval.
2. Turn on pulse counter. The injection sequence will then begin after 3 counts.
3. After the last loop has been injected the microswitch will turn off the auto injector.

Data Collection—Hardware

Direct connection of a GPC to a digital computer is a relatively simple task. The interface bears many similarities to that recently described for gas–liquid chromatography (*3*). Only two direct connections between the IBM 1800 and the GPC are required. These are: 1) a shielded twisted pair conductor connected across the recorder input from the refractometer. This connection does not alter the operation of the GPC refractometer or the recorder. This pair carries the analog signal ($\pm$100 mV) to the 1800 analog-digital converter. 2) A twisted pair conductor connected across Pins 3 and 7 of the siphon counter relay KHP17A11, Fig. 1. The other end of this pair is connected to a contact interrupt at the 1800. A count or pulse from the siphon which closes relay KHP17A11 produces a resistance drop ($>$10000 to $<4\Omega$) which is recognized as a contact interrupt at the computer. This latter connection causes the time of the siphon pulse to be automatically recorded at the computer.

The analog output of the refractometer is reduced at the computer by a factor of 3 (chosen so that the recorded signal is always on scale) using a simple voltage divider, and the reduced signal is connected to a

low-level filtered analog input on the relay multiplexer of the 1800. No other treatment of the signal is required. The relay multiplexer input provides filtering with a time constant of 0.3 sec.

The remainder of the hardware consists of a panel at the GPC having a momentary closure switch which is connected to a second contact interrupt at the 1800. This switch is used to initiate action by the computer. In addition, toggle switches on the same panel at the GPC are connected to seven bits of a digital input group at the 1800. Of these switches, six are used to specify run times (i.e., how long the 1800 records the GPC output) of 0.5 to 9 hr, and one to determine if the switches are to be read or if data are to be collected. Two bits of digital output from the 1800 are used, one to switch on a light on the GPC panel indicating that data collection is in progress and the other to close a relay completing the siphon counter/contact interrupt circuit. This latter precaution is necessary to prevent the siphon counter from generating interrupts at the 1800 when analog data collection is not actually being carried out. The interface is completed by a 1053 typewriter near the GPC which prints out run status information.

No attempt has been made thus far to provide automatic operation of the various functions of the GPC by the computer. The programmable Waters auto injection system is efficient. All that is required of the 1800 is to record the "real time" at which an automatic injection has occurred. This is performed by the counter monitoring circuit. Automatic gain control on the refractometer output is unnecessary, for the dynamic range of the 1800 analog to digital converter is more than adequate to handle the entire linear range of the refractometer.

Data Collection—Software

Three programs are used for data collection from the GPC. Two, "GELIN" and "COUNT," are interrupt core leads. These are loaded from disk and executed each time an interrupt is generated at the instrument (e.g., by depression of the momentary closure switch discussed above).

```
GEL-PERMEATION CHROMATOGRAPH
NO. DATA POINTS = 720
RUN TIME = 6.0     INTERVAL = 30.00 SECS.
```

FIG. 5. Printout of 1053 Typewriter run status information.

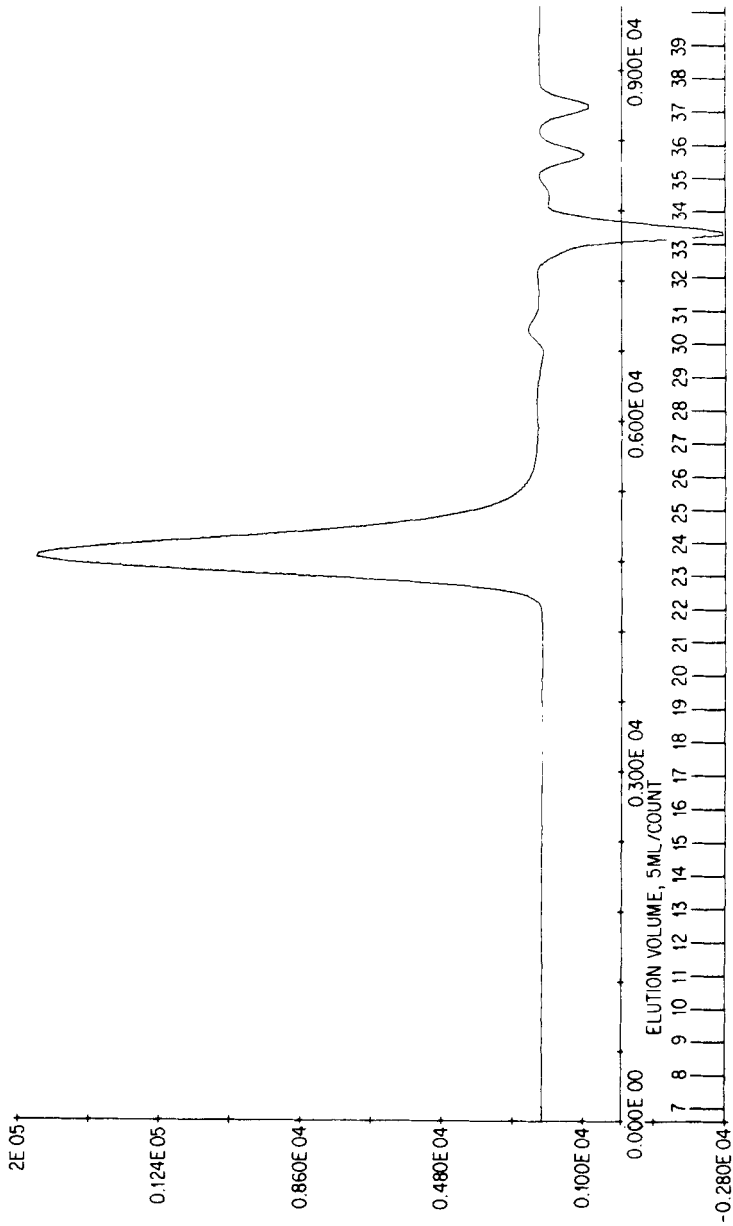

FIG. 6A. Calcomp plot of 4000 styrene sample.

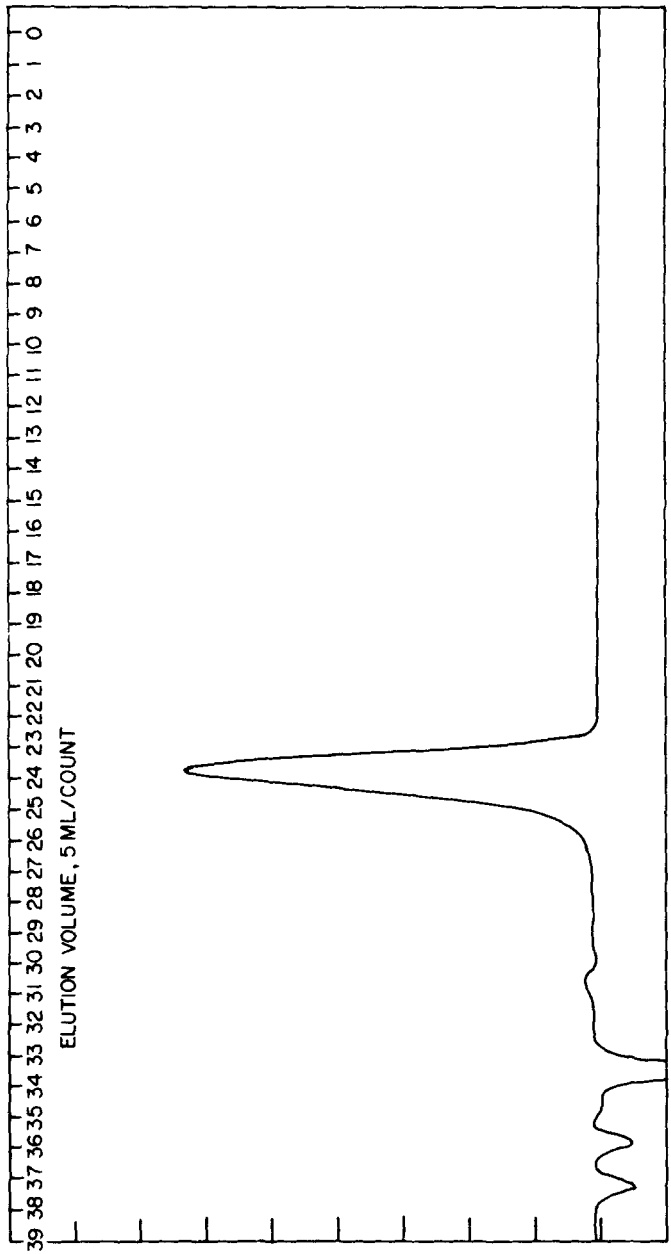

FIG. 6B. Gel chromatograph recorder output of 4000 styrene sample.

The function of "GELIN" is twofold: 1) to read the data switches, set up the run parameters [run time, time interval between points (DT), and number of data points (NPTS)], and to type these out for the operator to check at the 1053 typewriter; 2) to initiate data collection, switch on the "run" light, and activate a relay that starts the monitoring of count markers. The programming for the time-shared acquisition of data is a part of the TSX computer monitor system described previously (*4*). All that is required in the GPC program is a simple Fortran subroutine call with the appropriate arguments (DT, NPTS). See Fig. 5 for 1053 message.

The program "COUNT" is loaded and executed each time an interrupt is generated by the siphon emptying. The program's function is to read and record the computer "time-of-day" clock and to store these time values on disk. The first value in this data file contains the time at which data collection started. With this initial value, at the conclusion of a run all of the subsequent count markers can be related to the recorded differential refractometer readings. It should be emphasized that these interrupts are generated *only* while data collection is in progress.

At the conclusion of the run time the third core load, "GELEX," is queued and upon execution the time interval and refractometer output files are read. A record is provided at equal abscissa increments by interpolation of the digital record. The "count" file is then read and reconstructed in terms of real time in seconds from the run start. The interpolated record and the count markers, together with injection markers, are then plotted on the Calcomp plotter. Thus, the two sets of data (count interrupts and refractometer output) are reconstructed to give the conventional gel chromatogram at the plotter. This record is identical to the output of the GPC recorder with the exception that, irrespective of sample size, the chromatogram may be scaled to utilize the full width of the plotter. Such a plot is contrasted to the recorder plot in Fig. 6.

Punched cards suitable for off-line analysis of the above data are obtained by executing a simple nonprocess program "GPNCH."

Data Reduction—Software

Once a digitized record of a chromatogram or set of chromatograms has been collected, the type of reduction which may be applied is extensive. It is convenient to analyze our data off-line in the nonprocess

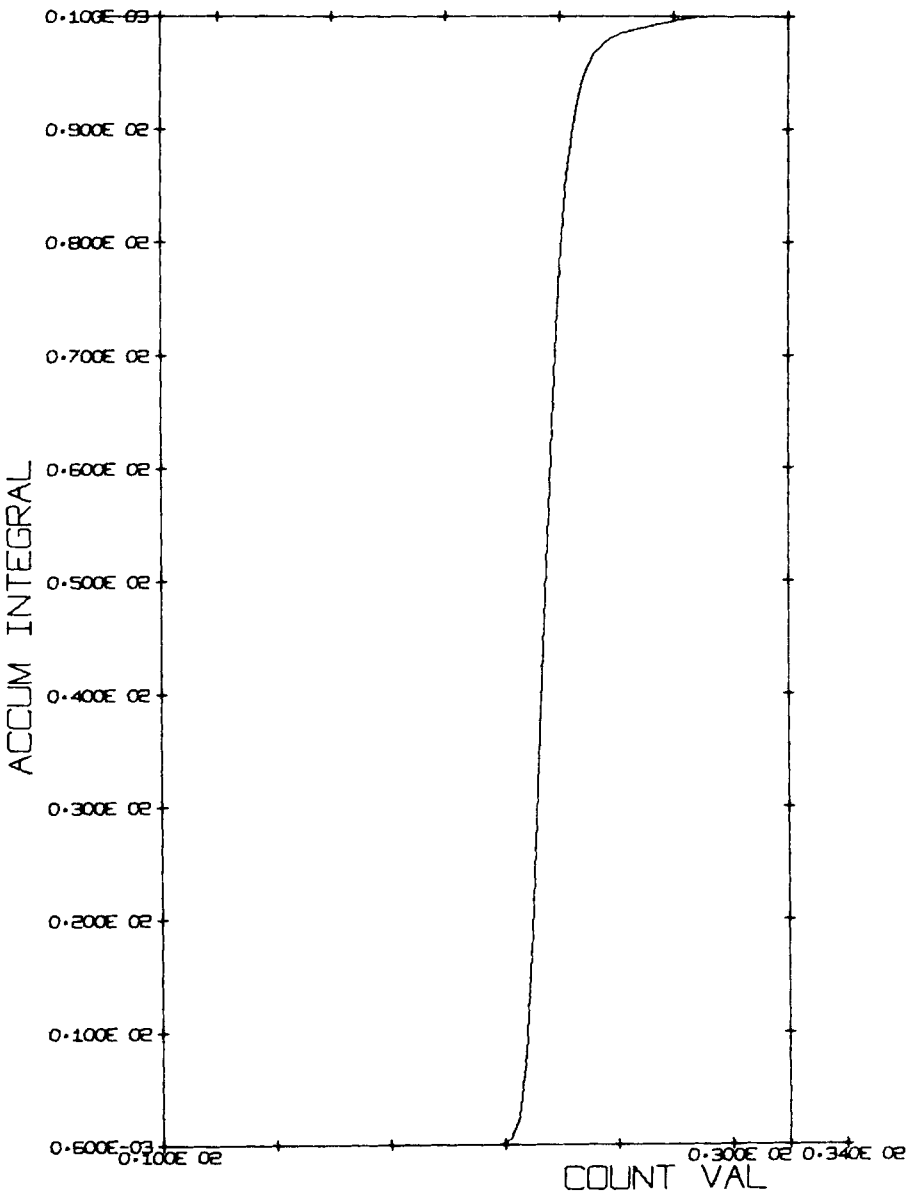

FIG. 7. Integral distribution curve from Program GPC1.

RUN IDENTIFICATION

BD-690181 POLYSTYRENE 4000 8/21/69

INTEGRATION LIMITS START = 12 FINISH = 23 FROM RUN INITIATION

START = 18 FINISH = 29 FROM INJECTION POINT

BASE-LINE DETERMINED AT 12 AND 34 VOLUME COUNT

FRACTIONAL COUNT VALUE = 0.25

VOL. COUNT (5 ML.)	TIME (SECS)	TRUE COUNT (5 ML.)	AREA (MV. SECS.)	PERCENT AREA	ACCUM. PERCENT AREA
12.0000	3250.8006	18.0000	0.1173	0.0006	0.0006
12.2500	3321.9000	18.2500	0.2745	0.0016	0.0023
12.5000	3392.9999	18.5000	0.1437	0.0008	0.0031
12.7500	3464.0994	18.7500	0.0243	0.0001	0.0032
13.0000	3535.2002	19.0000	0.3502	0.0020	0.0053
13.2500	3606.3001	19.2500	0.8594	0.0050	0.0104
13.5000	3677.3996	19.5000	0.3088	0.0018	0.0122
13.7500	3748.4995	19.7500	0.1325	0.0007	0.0129
14.0000	3819.6003	20.0000	0.0000	0.0000	0.0129
14.2500	3891.6003	20.2500	0.0000	0.0000	0.0129
14.5000	3963.6003	20.5000	0.0000	0.0000	0.0129
14.7500	4035.6003	20.7500	0.0000	0.0000	0.0129
15.0000	4107.6013	21.0000	0.0000	0.0000	0.0129

15.2500	4177.8001	21.2500	0.0000	0.0000	0.0129
15.5000	4247.9999	21.5000	0.5179	0.0030	0.0160
15.7500	4318.1988	21.7500	2.6519	0.0155	0.0316
16.0000	4388.4005	22.0000	14.1645	0.0832	0.1149
16.2500	4459.5009	22.2500	80.9777	0.4760	0.5909
16.5000	4530.6003	22.5000	332.0036	1.9518	2.5427
16.7500	4601.6997	22.7500	942.5332	5.5410	8.0838
17.0000	4672.8011	23.0000	1827.6154	10.7444	18.8282
17.2500	4743.0009	23.2500	2617.4763	15.3879	34.2161
17.5000	4813.1997	23.5000	2883.4424	16.9515	51.1677
17.7500	4883.3986	23.7500	2579.3120	15.1635	66.3312
18.0000	4953.6013	24.0000	1989.3811	11.6954	78.0266
18.2500	5024.7007	24.2500	1349.6815	7.9346	85.9613
18.5000	5095.8001	24.5000	850.8946	5.0023	90.9636
18.7500	5166.8995	24.7500	517.2921	3.0411	94.0047
19.0000	5238.0009	25.0000	310.2628	1.8240	95.8288
19.2500	5309.1003	25.2500	189.5566	1.1143	96.9431
19.5000	5380.1997	25.5000	119.4729	0.7023	97.6455
19.7500	5451.2992	25.7500	78.9908	0.4643	98.1099
20.0000	5522.4005	26.0000	53.9512	0.3171	98.4270
20.2500	5593.5009	26.2500	40.3798	0.2373	98.6644
20.5000	5664.6003	26.5000	31.3992	0.1845	98.8490
20.7500	5735.6997	26.7500	26.2657	0.1544	99.0034
21.0000	5806.8011	27.0000	22.6152	0.1329	99.1364
21.2500	5877.9005	27.2500	19.9101	0.1170	99.2534
21.5000	5949.0009	27.5000	22.2061	0.1305	99.3839
21.7500	6020.1003	27.7500	24.7020	0.1452	99.5291
22.0000	6091.2007	28.0000	24.6913	0.1451	99.6743
22.2500	6161.3995	28.2500	22.7965	0.1340	99.8083
22.5000	6231.5993	28.5000	18.8729	0.1109	99.9192
22.7500	6301.7982	28.7500	13.7392	0.0807	100.0000

GEL-PERMEATION DATA REDUCTION

STD-700042 PS STD 4000 HI-4 1/19/70

COUNT	AREA	LOG. MOL. WT.	MOL. WT.
24.0000	0.1690	4.3791	23940.8
24.2500	0.0000	4.2813	19112.1
24.5000	0.0000	4.1906	15510.9
24.7500	0.3836	4.1070	12796.4
25.0000	17.1669	4.0302	10720.8
25.2500	94.0825	3.9592	9104.9
25.5000	337.5266	3.8928	7814.2
25.7500	909.2407	3.8299	6760.6
26.0000	1850.3046	3.7690	5875.8
26.2500	2871.1673	3.7086	5112.9
26.5000	3536.1746	3.6473	4439.9
26.7500	3709.3164	3.5839	3836.7
27.0000	3345.0807	3.5173	3291.0
27.2500	2765.7160	3.4468	2797.7
27.5000	2029.7957	3.7204	5252.9

NO. AVERAGE MOL. WT. = 4412.899

WT. AVERAGE MOL. WT. = 4742.558

Z-AVERAGE MOL. WT. = 5096.742

DISPERSIVITY = 1.074

VISCOSITY AV. MOL. WT. = 4567.6401

FIG. 9. Table of molecular weight from GPC2.

job stream of the 1800 using two programs that give the user some flexibility of operation. The data may be supplied to these programs either from magnetic disk or from data cards (GPNCH).

The program "GPC1" performs simple tasks such as background reduction, linear base line drift correction, digital smoothing (five point quadratic), and integration between fractional count values. The record, which was collected by the computer as integers ranging between ± 32767 (*5*), is converted to millivolts of true output from the instrument. As an option the user may request a Calcomp plot of the integral distribution curve, see Fig. 7. Also, an interpolated printout of the refractometer deflection at 0.25 count values is produced. The same program produces a set of punched cards in a format suitable for use with the Chevron Research Programs (*1*, *2*). A printout is shown in Fig. 8.

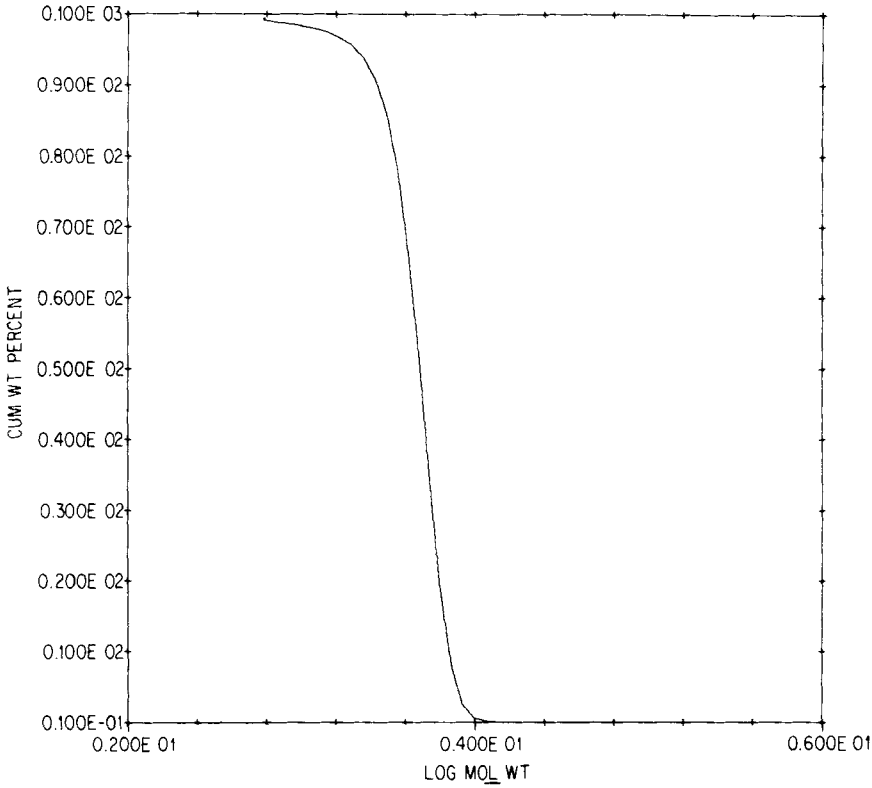

FIG. 10. Molecular weight integral.

The second program, "GPC2," uses the table of fractional count vs. areas generated by GPC1 to give a molecular weight distribution. The molecular weight (M_i) corresponding to a particular elution volume is determined by interpolation into a table of calibration data. The distributions are obtained using the conventional equation

$$\bar{M} = \frac{\Sigma n_i M_i^j}{\Sigma n_i M_i^{j-1}}$$

where n_i is the number of molecules of weight M_i.

M = number-average mol wt if $j = 1$
= weight-average mol wt if $j = 2$
= Z average mol wt if $j = 3$

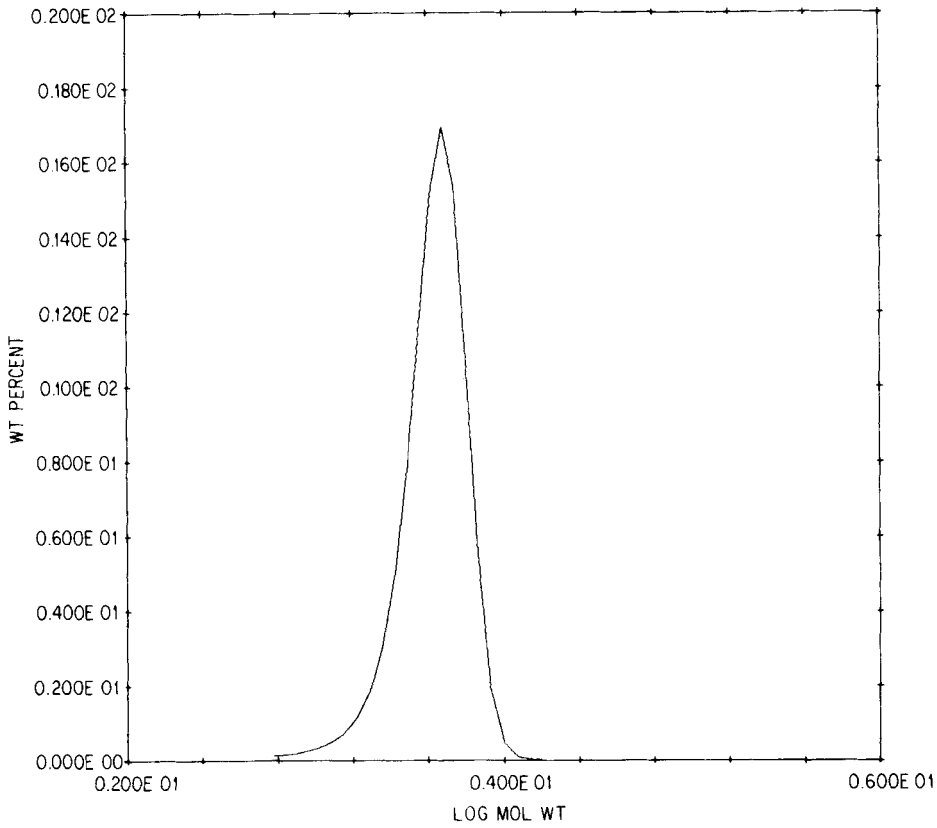

FIG. 11. Molecular weight differential.

Viscosity-average molecular weight may also be calculated from GPC2. The relationship

$$\bar{M}_v = \left\{ \frac{\Sigma n_i M_i^{(1+a)}}{\Sigma n_i M_i} \right\}^{1/a}$$

where a = polymer–solvent interaction parameter. This a must be determined by an alternate method.

The GPC2 output is in the form of a table, Fig. 9, and Calcomp plots of accumulated area vs. log molecular weight and area vs. log molecular weight (a differential plot with 0.25 count as the step). Figures 10 and 11 show these plots. It is planned that several calibration curves shall

be included in GPC2 and the user permitted to specify the desired curve.

CONCLUSION

With the data collection system described in this study it is possible to obtain useful molecular weight distribution data and liquid chromatographic analyses in a semi-automatic manner. The programs "GELIN," "COUNT," and "GPNCH," remove most of the work from data reduction. Although programs "GPC1" and "GPC2" are very simple, they provide useful data given adequate calibration. The punched cards furnish data in an ideal form for more sophisticated treatment.

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4. H. M. Gladney, *J. Comp. Phys.*, **2**, 255 (1968).
5. The word size of the IBM 1800 Computer is fifteen bits plus a sign bit, as a consequence the absolute value of the largest integer number which can be represented in a single word is $2^{15} - 1$.

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