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POLYMER MOLECULAR WEIGHT DISTRIBUTION ANALYSIS AT VERY HIGH SPEED USING ON-LINE DATA HANDLING

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SUMMARY

In the past several years, the use of gel permeation chromatography (GPC) has grown rapidly in the field of polymer analysis and characterization. Molecular weight and/or size distributions can be obtained from the information generated during a GPC run. However, the traditional GPC analyses have required long runs consuming large amounts of mobile phase (solvent) and time, and the data generated required considerable manipulation. The advent of porous glass size-exclusion column packings and very high-quality high-pressure pumps have led to tremendous reduction in the time and cost of GPC analyses. The use of an on-line data handling system allows a distribution analysis to be carried out in less than 15 min with unprecedented analytical precision.

INTRODUCTION

The determination of molecular weight distribution (MWD) is important in achieving an understanding of the differences in the physical behavior of polymers. The traditional method of analysis of polymers by gel permeation chromatography (GPC) has involved the use of porous polymer gels and columns with fairly large internal diameter. This has led to long analysis times (typically $1\frac{1}{2}$–4 h) and high mobile phase (eluent) consumption (typically 100 – 300 ml per analysis). More recently systems have been described which allow drastic reduction in analysis times and amounts of solvent consumed[1–3]. The system described here permits the analysis to be carried out in 15 min and requires only 11 ml of solvent.

Both the traditional and new high-speed molecular size distribution analyses generate raw data which must be laboriously manipulated and calculated to yield the results required by the analyst[4]. However, the proper evaluation of the data collected in a short time by traditional means is extremely difficult if not impossible. For this reason some methods have been developed using on-line computers to handle the chromatographic data[5] but these are generally complicated and require extensive knowledge of computer hardware and software.

In the rush to increase the speed of GPC analysis, many things have been overlooked. Since the time scale of analysis is a logarithmic function with molecular weight, very precise control of flow-rate is essential. Extremely small changes in absolute elu-

tion volume result in large changes in the weight-average molecular weight. In our system, the molecular weight range from 2,300,000 to 451,000 is covered in less than 60 sec. An error of 2 sec in elution time can represent an error of about 20,000 MW units. Manual systems are incapable of precisions in the range covered by a dedicated computer. The following study was made possible only because of the overall pumping reproducibility of the Model 1220 syringe pump.

The small columns used in high-speed MWD analysis require injection and detection systems of very small volume. The conventional GPC instruments are not adequate in this regard. Modern high-performance liquid chromatographs are particularly well suited to this type of problem.

A software program has been developed to collect and reduce the data obtained in a GPC analysis. The collection of the raw data is in the form of area slices as a function of time. A cumulative area percent analysis is generated from these raw data relative to the pertinent axis. In the case of GPC, this axis is molecular weight or size. Because of the reproducibility of the Model 1220 syringe pump, the calibration is in the form of molecular weight or size as a function of time. This software program is described in the following study.

EXPERIMENTAL

The instrument used in this study was a Perkin-Elmer Model 1220 liquid chromatograph equipped with a single 6000 p.s.i.g. constant flow syringe pump and a UV detector operating at 254 nm. The column system consisted of four 50 cm × 2.6 mm I.D. columns containing different Vit-X packings in the following order: Vit-X 328, Vit-X1068, Vit-X 15,300 and Vit-X 120,120. The characteristics of these porous glass packings have been described previously in detail by Telepchak[2].

The polystyrene standards were supplied by Pressure Chemical Co., Pittsburgh,

```
RUN TIMES/WIDTH    14.99    5.00    1

BASE LEVELS       7.73      7.33


RUN            NBS 706 APRIL 2 1974

INST    1  ,  SE  METHØD    50  ,  FILE     21    0:

% SMP       TIME      D(T)
   0         6.94   1.36,E  6,
  10         7.48   5.27,E  5,
  20         7.73   3.73,E  5,
  30         7.93   2.93,E  5,
  40         8.11   2.36,E  5,
  50         8.29   1.91,E  5,
  60         8.47   1.54,E  5,
  70         8.67   1.22,E  5,
  80         8.90   9.32,E  4,
  90         9.22   6.36,E  4,
 100.0      10.14   2.14,E  4,

AREA      5.26,E  6,
:
```

Fig. 1. Molecular weight distribution printout at the termination of an analysis. Sample: NBS-706 broad-molecular-weight polystyrene. Sample size, 5 μl of a chloroform solution; mobile phase, chloroform, 0.75 ml/min; detector, UV at 254 nm.

```
S
GCSE1 WØRDS:
   351
FILES:
GC
   7   8   9   18   22   23   24   25
SE
  20   21   26   27
METHØDS:

GC
   5
SE
 210 207 205 200 100   52   51   50
```

Fig. 2. Computer memory status report. The numbers shown represent chromatograms and methods stored in memory. The chromatograms are designated as files, and are identified as either GC type chromatograms or molecular weight (SE) types.

Pa., U.S.A.; chloroform used as the mobile phase (solvent) was of "Distilled in Glass" quality, obtained from Burdick and Jackson Labs., Muskegon, Mich., U.S.A.

The chromatograph was connected to the PEP-2 Chromatographic System via the standard interface. The system contained the software program for size exclusion analyses. The headings in the final distribution analysis printout (see Fig. 1) represent the following parameters. "Run Times/Width" represents the total analysis time in minutes followed by the initial "Lock Out" time in minutes during which no data are taken, and finally the width of each area slice in seconds. The "Base Levels" represent the baseline points encountered at the above times. "Run" is the sample naming line. The next line identifies the instrument, the analytical method employed, and the data file which is being analyzed. A register is kept of the methods and files residing in the memory of the data system. This register can be called up at any time by pressing the "S" key on the teletype (see Fig. 2). The column headings are "% SMP", "TIME" and D(T). These represent the percent of area corresponding to the adjacent time, and the molecular weight (or size) from the calibration. The molecular weight (or size) is expressed in exponential notation with three significant digits (*e.g.* 3.24 E5 means 3.24×10^5).

```
P50:
RUN

INST   1 ,  SE  METHØD   50 ,  FILE    18    0:

NØRM F.    100.0:

RUN TIMES/WIDTH     11.50,     5.00,     1,

BASE  TIMES    11.50,      .00,      .00,

BASE LEVELS      .00,      .00,

CUT INTERVAL  100,

    TIME     D(T)     ARF(T)
   6.64,  2.30,E  6,  1.00,E  ,
   7.57,  4.51,E  5,  1.00,E  ,
   9.73,  3.45,E  4,  1.00,E  ,
  11.67,  3.55,E  3,  1.00,E  ,
:
```

Fig. 3. This is a basic analytical method with a four-point calibration.

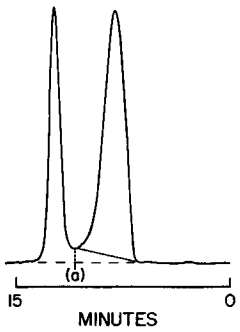


Fig. 4. This chromatogram shows some of the post analyses calculation limits allowable.

The analytical method for size exclusion is shown in Fig. 3. The line "Base Times" is used in conjunction with "Base Levels" to establish the limits of calculation of the experimental data. An example is shown in Fig. 4. The broken line represents the actual baseline of the analysis. Since the second narrow peak is an artifact (probably residual initiator), it does not represent the distribution. The "Base Time" (a) was chosen to exclude this peak. Another baseline criterion is represented by the dotted line. If the base level is not set by the operator, the baseline will be the solid line. The area of the distribution will be the area above this line and under the curve. If the base level is adjusted, the area will be that area above the broken line and under the curve.

```
P205:
RUN

INST   1  ,  SE  METHØD  205  ,  FILE    18    0:

NØRM F.    100.0:

RUN TIMES/WIDTH    327.67,      .00,    20,

BASE  TIMES    11.50,       .00,       .00,

BASE LEVELS      .00,       .00,

CUT INTERVAL  100,

    TIME      D(T)       ARF(T)
   6.63,   2.30,E  6,  2.30,E  6,
   6.74,   1.60,E  6,  1.60,E  6,
   6.95,   1.00,E  6,  1.00,E  6,
   7.30,   5.96,E  5,  5.96,E  5,
   7.49,   4.58,E  5,  4.58,E  5,
   7.64,   3.86,E  5,  3.86,E  5,
   7.76,   3.35,E  5,  3.35,E  5,
   7.87,   2.95,E  5,  2.95,E  5,
   7.97,   2.62,E  5,  2.62,E  5,
   8.07,   2.35,E  5,  2.35,E  5,
   8.17,   2.11,E  5,  2.11,E  5,
   8.26,   1.89,E  5,  1.89,E  5,
   8.35,   1.71,E  5,  1.71,E  5,
   8.44,   1.54,E  5,  1.54,E  5,
   8.54,   1.38,E  5,  1.38,E  5,
   8.64,   1.23,E  5,  1.23,E  5,
   8.74,   1.10,E  5,  1.10,E  5,
   8.85,   9.64,E  4,  9.64,E  4,
   8.98,   8.35,E  4,  8.35,E  4,
   9.31,   7.04,E  4,  7.04,E  4,
   9.59,   4.12,E  4,  4.12,E  4,
:
```

Fig. 5. PEP-2 printout of a method used to calculate a weight-average distribution and M_w.

The line "Cut Interval" in Fig. 3 determines the number of values printed out for the distribution. The additional column "ARF(T)" is the response factor column. The area slices accumulated during the analysis can be multiplied by either the appropriate detector response factor, the molecular weight at that slices, or the reciprocal of the molecular weight (see Fig. 5). The inclusion of the last two factors yields the weight-average molecular weight M_w, and the number-average molecular weight M_n. These numbers are obtained by dividing the "Area" numbers at the bottom of the distribution analyses by one another. These factors may also be used to derive weighted distributions. A normal analysis will consist of a set of data, and the printouts of three analyses using three different methods. The first is with "ARF(T)" equal to one. The second has the molecular weights of the slices in the "ARF(T)" column. The third has the reciprocal of the molecular weight in the "ARF(T)" column (see Fig. 6).

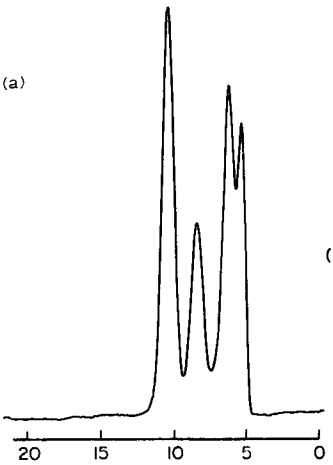
```
XP207:
RUN

INST   1  ,  SE  METHØD  207  ,  FILE    13    0:

NØRM F.    100.0:

RUN TIMES/WIDTH    327.67,       .00,    20,

BASE  TIMES    11.50,       .00,       .00,

BASE LEVELS       .00,       .00,

CUT INTERVAL     2,

     TIME      D(T)      ARF(T)
    6.63,   2.30,E   6,  4.35,E- 7,
    6.74,   1.60,E   6,  6.25,E- 7,
    6.95,   1.00,E   6,  1.00,E- 6,
    7.30,   5.96,E   5,  1.68,E- 6,
    7.49,   4.58,E   5,  2.18,E- 6,
    7.64,   3.86,E   5,  2.59,E- 6,
    7.76,   3.35,E   5,  2.99,E- 6,
    7.87,   2.95,E   5,  3.39,E- 6,
    7.97,   2.62,E   5,  3.82,E- 6,
    8.07,   2.35,E   5,  4.26,E- 6,
    8.17,   2.11,E   5,  4.74,E- 6,
    8.26,   1.89,E   5,  5.29,E- 6,
    8.35,   1.71,E   5,  5.85,E- 6,
    8.44,   1.54,E   5,  6.49,E- 6,
    8.54,   1.38,E   5,  7.25,E- 6,
    8.64,   1.23,E   5,  8.13,E- 6,
    8.74,   1.10,E   5,  9.09,E- 6,
    8.85,   9.64,E   4,  1.04,E- 5,
    8.98,   8.35,E   4,  1.20,E- 5,
    9.31,   7.04,E   4,  1.42,E- 5,
    9.59,   4.12,E   4,  2.43,E- 5,
:
```

Fig. 6. PEP-2 printout of a method used to calculate a number-average distribution and M_n.

RESULTS

In order to evaluate the reproducibility of the system, a mixture of polystyrene standards was analyzed in triplicate. The mixture consisted of standards of molecular weights 2,300,000, 451,000, 34,500, and 3,550. The data were accumulated and analyzed by the PEP as normal chromatographic data; a representative chromatogram and PEP printout for the first sample are shown (see Fig. 7). The absolute retention time reproducibility was better than ± 1 sec.

To evaluate long-term calibration stability, the system was run by two operators on different days for a period of two weeks. Standard retention times reproduced



(b) THRESHØLDS 128 8

RUN 2

INST 1 , GC METHØD 5 , FILE 9 0:

TIME	AREA	RRT	RF	C	NAME
6.66	2.2864	.666,	1.0000,	17.3656,	!
7.56	3.9043	.756,	1.0000,	29.6528,	!
9.75	2.3059	.975,	1.0000,	17.5136,	!
11.71	4.6696	1.171,	1.0000,	35.4656,	!

:

Fig. 7. (a) Chromatogram of narrow range standards under the same conditions as Fig. 1 and (b) the accompanying PEP printout.

to better than 0.04 min. The linearity and slope of the molecular weight calibration was determined from a semi-log plot of the molecular weights of the standards *vs.* their retention times; this plot is shown in Fig. 8. This calibration mode is an approximation and the development of an accurate calibration curve would require the use of more standards of known peak molecular weight.

It is important to note here that the calibration uses retention time instead of elution volume. This is possible because the pump used in the Model 1220 is a constant-flow pump; it delivers the selected flow-rate independent of column back pressure or carrier viscosity. Therefore, the retention times and volumes are directly related with a precision of $\pm 0.2\%$.

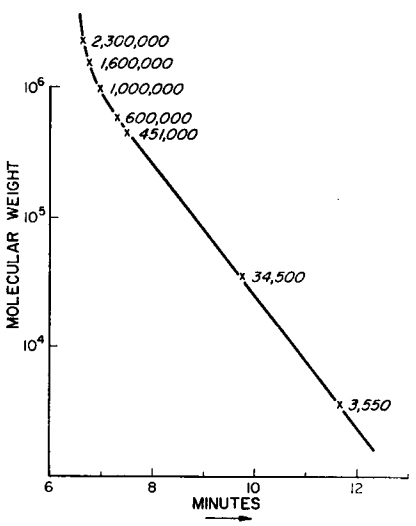
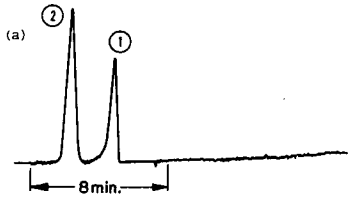


Fig. 8. Calibration plot.



(b) THRESHØLDS 256 64

RUN 5 PØLYSTYRENE STANDARDS QUANT

INST 1 , GC METHØD 5 , FILE 24 0:

TIME	AREA	RRT	RF	C	NAME
3.28	4.7262	.328,	1.0000,	31.1088, !	
5.78	10.1331	.578,	1.0000,	66.6976, !	

*

Fig. 9. (a) Chromatogram of a 1:1 w/w mixture of polystyrene standards of M_w 498,000 (1) and 2,100 (2) with (b) a PEP printout of the relative areas of the peaks.

In this study, the system was calibrated in molecular weight for linear polystyrenes. Since the column material (Vit-X) used is porous glass based, the calibration does not change with solvent or polymer type. The system can thus be calibrated in size or intrinsic viscosities to yield a universal calibration plot applicable to other polymer types.

To establish the calibration of the computational method, several points were taken from the curve. The times and molecular weights were typed into the method. The program does a log–linear interpolation between the identified points. At least three points should be taken in regions of curvature in the calibration plot. The calibration curve established by the PEP can be printed out with up to 100 points for comparison with the initial calibration data.

Methods of data reduction used in the past for molecular size distribution have ignored differences in molar and weight detector response factors at different molecular weights although the differences are significant. This is illustrated in Fig. 9, which shows the chromatogram of a 1:1 w/w mixture of two standards with the corresponding PEP printout given in Fig. 5. It is clear that the ratio of the two peak areas significantly differs from the ratio of the amounts present; it is 2.14:1 and not 1:1. Using the PEP-2 Chromatography Data System, response factors can be calcu-

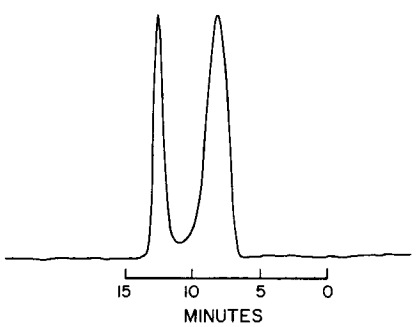


Fig. 10. Chromatogram of NBS-706 under the conditions of Fig. 1.

```
RUN TIMES/WIDTH    14.99    5.00    1

BASE LEVELS       7.32       7.33


RUN            NBS 706 APRIL 2 1974

INST    1  ,  SE  METHØD  200  ,  FILE     21    0:

% SMP       TIME      D(T)
   0         6.93   1.05,E  6,
  10         7.62   3.91,E  5,
  20         7.96   2.68,E  5,
  30         8.24   1.93,E  5,
  40         8.53   1.39,E  5,
  50         8.86   9.48,E  4,
  60         9.40   5.13,E  4,
  70        11.82   2.88,E  3,
  80        12.39   1.46,E  3,
  90        12.68   1.04,E  3,
 100.0      13.28   5.03,E  2,

AREA      8.72,E  6,
:
```

Fig. 11. Printout of distribution analysis of NBS-706 for both peaks.

lated and inserted in the same line as the retention time and molecular size (or weight); from this a corrected distribution curve can be obtained. Also, the ability to change response factors over a range of 10^{16} to 10^{-15} allows the distribution to be calculated based on molecular weight or M_w or M_n numbers, with a single calibration plot.

Finally we would like to show an actual analysis of a molecular weight distribution and the corresponding automated calculation. Fig. 10 shows the analysis of a broad molecular weight distribution sample (NBS-706). The corresponding printout is shown in Fig. 11. The total elapsed time between sample injection and data printout was 15 min. The values calculated for M_w and M_n were 252,000 and 128,000. The ratio M_w/M_n was 1.97. The given values were 258,000 and $<$136,000.

The chromatogram (Fig. 10) showed a large peak at the low-molecular-weight

```
RUN TIMES/WIDTH    11.49    5.00    1

BASE LEVELS       5.56       5.35


RUN     3

INST    1  ,  SE  METHØD   50  ,  FILE     27    0:

% SMP       TIME      D(T)
   0         6.83   1.65,E  6,
  10         7.50   5.07,E  5,
  20         7.84   3.29,E  5,
  30         8.10   2.39,E  5,
  40         8.34   1.81,E  5,
  50         8.55   1.41,E  5,
  60         8.76   1.10,E  5,
  70         8.97   8.48,E  4,
  80         9.23   6.25,E  4,
  90         9.60   4.05,E  4,
 100.0      10.76   1.03,E  4,

AREA      7.80,E  6,
:
```

Fig. 12. Printout of distribution analysis as in Fig. 11, but the second peak is excluded.

end. It is thought to represent residual initiator. Since the data file is maintained in the memory of the PEP, it can be reanalyzed with different methods; the program allows calculation of a complete distribution of molecular weights between any chosen limits. For example, Fig. 12 shows the result of post-analysis data manipulation of the original printout giving only the molecular weight distribution corresponding to the first peak, while Fig. 11 shows the printout of the entire distribution including the peak at low molecular weight.

CONCLUSIONS

The reproducibility and stability of the Model 1220 liquid chromatograph allows highly precise and repeatable molecular size (or weight) distribution analyses to be done in about 15 min. The Vit-X column system yields a highly stable calibration over the size range observed in this study. PEP-2 with the Molecular Size Exclusion Program allows extremely versatile handling of analytical data, and yields the required form of distribution printout. The calculation of M_n and M_w can be carried out from the original distribution printouts.

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