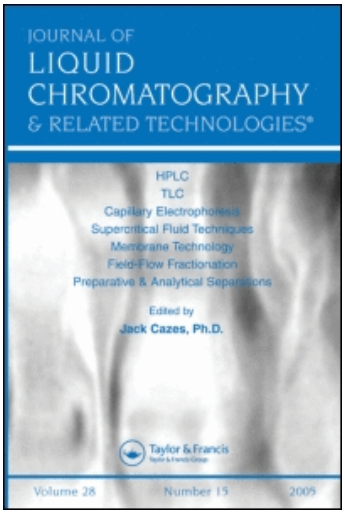

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Densimetric Detection in Sec. A Semi-Automated Method for Calculation of Molecular Weight Averages

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DENSIMETRIC DETECTION IN SEC. A SEMI-AUTOMATED METHOD FOR CALCULATION OF MOLECULAR WEIGHT AVERAGES.

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ABSTRACT

It is shown that detection by measurement of density (mass per unit volume) offers some advantages: the signals from such an instrument are inherently digital and integrated over each measuring interval, which makes calculation of molecular weight averages very easy. A BASIC program is described, by which data reduction can be performed with good accuracy by means of a low-cost minicomputer.

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INTRODUCTION

One of the most important advantages of steric exclusion chromatography (SEC) in the characterization of polymers with respect to their molecular weight distribution is the possibility to obtain molecular weight averages (M_n, M_w, M_z, M_η) as well as polydispersity (M_w/M_n) from a single chromatogram. The determination of molecular weight averages by a manual procedure is, however, rather laborious and presents many opportunities for operator errors. Hence, various automated data-handling systems have been developed,[1-10] which save time and improve the accuracy of the results. In general, there are two approaches towards automated data reduction in SEC:

1. Real-time data acquisition and off-line data processing
2. Combined real-time data acquisition and processing

Both of them involve usually the following steps, each of which may be subject to errors, as several authors have pointed out[11-15]:

1. Conversion of the analog signal from the detector (UV, RI etc.) into a digital form.
2. Transformation of elution times into elution volumes.
3. Definition of a baseline

4. Definition of start and end of a peak
5. Division of the peak into small slices (usually of equal elution volume intervals)
6. Assignment of a molecular weight to each slice (from a calibration curve)
7. Determination of the area of each slice (usually by approximation as a rectangle)
8. Calculation of molecular weight averages and polydispersity.

If peak spreading is not negligible, several additional steps may be necessary for the correction of molecular weight averages or even of the whole MWD.[1]

As we have shown in a previous paper,[16] the use of a density measuring device according to the mechanical oscillator method[17,18] as a detector in SEC[19-26] eliminates some of these steps and eliminates consequently some possible sources of error: The signals from such an instrument are inherently digital and integrated over each measuring interval. Data reduction could be performed by means of a programmable pocket calculator with good accuracy; but still many operator manipulations were required in this way.[16] Hence we have developed a much more convenient method, which involves storage of the raw data in the memory of a low-cost minicomputer prior to calculation of molecular weight averages. The main reason for the choice of an off-line method was

that it enables the operator to interact with the computer in the course of the calculations in order to avoid artefacts which might be produced by data reduction "on the fly".

DETECTION BY MEASUREMENT OF DENSITY

The measuring cell of a densimetric detector is an oscillating, u-shaped (glass or metal) tube, the period of which depends on the reduced mass of the oscillator, which itself results from the mass of the empty cell and the mass of the sample. As the sample volume is constant, the period of the cell represents the density of the sample.[17,18,20]

Period measurement is performed by counting the periods of an oven-controlled 5 Mc - quartz oscillator within a predetermined number of periods of the measuring cell.

A small change $\Delta\rho$ in density will cause a change ΔT in the period T_o:

$$\Delta\rho = 2A.T_o.\Delta T$$

wherein A is a constant for each individual cell.

The concentration c_i of a solute is given by

$$c_i = \frac{2A.T_o}{1-\rho_o.\bar{v}_i^*} \cdot \Delta T$$

wherein ρ_o is the density of the pure solvent and $\bar{v}_i^*$ is the (apparent) partial specific volume of the solute.

As the thermal volume expansion coefficient of most organic solvents is in the order of magnitude of $1.10^{-3}K^{-1}$, one will have to keep temperature constant within $\pm 1.10^{-4}K$, if a resolution in density of 1.10^{-7} g/cm^3 shall be achieved, which corresponds to a detection limit of approximately 1 ppm (in the cell) for a usual polymer-solvent system, such as polystyrene in tetrahydrofuran. The more feasible way is, however, the use of a reference cell for compensation of temperature variations.[24,25] By choosing a higher resolution for the reference cell combined with a sliding average a stable baseline can be achieved without an increase of baseline noise.[16]

EXPERIMENTAL

A detailed description of a densimetric detector has been given in a previous communication.[25] It consisted of two cells DMA 602 M of about 100 µl volume (A.PAAR KG, Graz, Austria), and a calculating unit developed in our laboratory. For all measurements, temperature was kept constant at 25±0.01°C using a thermostat Haake F3C. Both cells were arranged parallel in the thermostat circuit; a mixing chamber of about 10 l volume was placed

between thermostat and cells to keep temperature changes slow.

The calculating unit was connected via a VIC 1011B interface to a Commodore VC 64 computer equipped with a monitor, a VC-1541 floppy disk and a matrix printer Epson MX 80.

The chromatographic apparatus consisted of a pump LDC Constametric IIG, a Valco injection valve equipped with a 100 µl loop, a column Microgel M (Polymer Lab.) with an exclusion limit of about 5.10^6, its (mobile phase) volume was about 21 ml, a UV-photometric detector LDC Spectromonitor II and the densimetric detector. Chromatograms were also registered using a 3-channel strip-chart recorder (UV, density with and without temperature compensation).

The solvent (tetrahydrofuran) was distilled over benzophenon-potassium prior to use, polystyrene standards (from Pressure Chem.Co.,Pittsburgh,Pa. and Waters, Framingham,Mass.) were used as received.

All chromatograms were run at a flow rate of 1.00 ml/min, sample concentrations varied from 0.05 to 0.3% (w/v).

THEORETICAL CONSIDERATIONS

Several authors[11-15] have pointed out, that even in a perfect separation system under correct chromatographic

conditions various sources of error have to be taken into account, which might deteriorate the accuracy of molecular weight averages calculated from SEC:

1. Depending on the type of column used errors in the determination of elution volumes of only 0.1 % may cause errors in molecular weight of several per cent.[14,15] Even high quality pumps reproduce flow rates only within 0.3 %, which makes control of flow rates necessary (for example by the use of a low molecular weight internal standard).

2. Finite digitizer resolution as well as noise limit the precision of data, especially at low sample sizes. A sufficiently high sampling frequency (at least 20-30 points per peak) reduces these errors.[12,13,15] With densimetric detection, there are no problems with digitizer resolution; sampling frequency is, however, indirectly proportional to sensitivity, since higher resolution requires longer measuring times. Using 1000 periods of the measuring cell per interval one achieves a resolution in density of 2.8×10^{-7} g/cm^3 at measuring times of $\sim$4 sec (corresponding to $\sim$67 µl at a flow rate of 1.00 ml/min). This proved to be a good compromise: even with standards of very narrow MWD, 20-30 points per peak are obtained.

3. Noise also causes uncertainties in the definition of baseline height as well as of start and end of a

peak,[15] which leads to errors in the area of each slice and of the whole peak.

4. If a drift of the baseline adds to the effects mentioned above, especially the end of the peak may be poorly defined, which leads to serious errors especially in M_n.[15]

5. Additional errors may arise from variation of detector response with concentration or molecular weight. (The response of a differential refractometer for polystyrene in toluene varies up to a molecular weight of approximately 50000)[1].

6. A slight curvature of the calibration curve may also lead to erroneous molecular weights. In this case linear interpolation between standards should be superior to a calibration curve obtained by a least squares linear fit of the same data.[4] Starting from these considerations, our goal was the development of a program which should provide algorithms for minimizing these errors.

SEC - PROGRAM

The basic idea was that the operator should be able to examine the raw data before starting data processing, to eliminate artefacts, and to repeat any step or even the whole calculations. Definition of a baseline and inte-

gration should be performed within the limits entered by the operator, calculation of molecular weights should be possible using a linear calibration or interpolation between standards, alternatively. Flow rate changes should be compensated by the use of an internal standard.

A flow chart of the program is given in Fig.1. To illustrate how the program works, a typical report of a chromatogram and the calculations therefrom are shown in Figures 2 and 3. (The data entered by the operator are underlined.)

Before initializing the program, the expected number NE of values has to be entered (i.e. the number of measuring intervals of the detector within the time required for the whole chromatogram). When data acquisition has been completed (N=NE), the raw data are displayed on the screen for examination: If single values are in error for well understood reasons, they may be corrected. On entering the number of the first and the last value, the interesting part(s) of the chromatogram are plotted on the printer.

To integrate a peak, the operator has to define its start and end as well as a region before and after the peak, respectively, between the averages of which the program establishes a linear baseline. This procedure can be repeated, if there is more than one peak to be integrated.

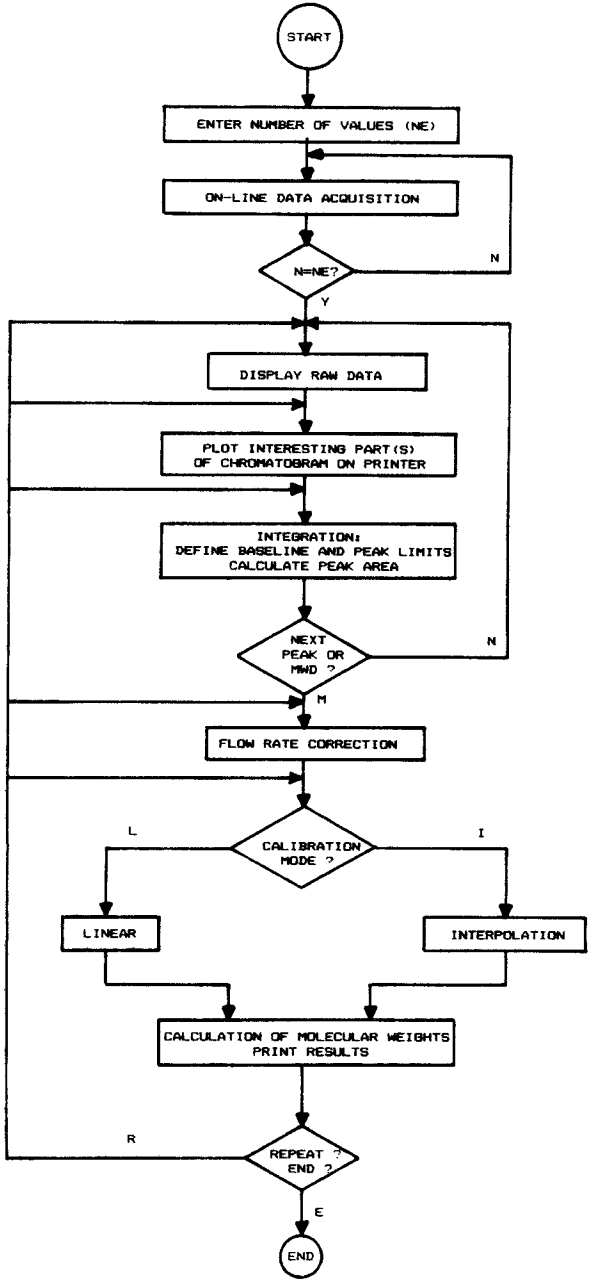

FIGURE 1

Flow chart of the SEC - program

DENSITY DETECTOR DMA 61: CHROM.NR. 7 DATE: 19.12.1983

SAMPLE: POLYSTYRENE 50000

COLUMN SET: MICROGEL M ELUENT: TETRAHYDROFURAN
CONCENTRATION: 0.1 % (W/V) INJECTED VOLUME: 100 MYL
FLOW RATE : 1 ML/MIN BASELINE: 19098910

N	T(MIN)	D(N)	DETECTOR RESPONSE
200	12.73	6	*
201	12.80	5	*
202	12.86	6	*
203	12.92	6	*
204	12.99	6	*
205	13.05	6	*
206	13.11	7	*
207	13.18	7	*
208	13.24	5	*
209	13.31	6	*
210	13.37	6	*
211	13.43	5	*
212	13.50	5	*
213	13.56	6	*
214	13.62	5	*
215	13.69	6	*
216	13.75	7	*
217	13.81	6	*
218	13.88	6	*
219	13.94	6	*
220	14.01	6	*
221	14.07	7	*
222	14.13	10	*
223	14.20	14	*
224	14.26	23	*
225	14.32	38	*
226	14.39	53	*
227	14.45	69	*
228	14.52	88	*
229	14.58	99	*
230	14.64	104	*
231	14.71	101	*
232	14.77	92	*
233	14.83	82	*
234	14.90	69	*
235	14.96	57	*
236	15.02	47	*
237	15.09	39	*
238	15.15	31	*
239	15.22	27	*
240	15.28	22	*
241	15.34	20	*
242	15.41	16	*
243	15.47	14	*
244	15.53	11	*
245	15.60	10	*
246	15.66	10	*
247	15.72	9	*
248	15.79	8	*
249	15.85	8	*
250	15.92	8	*
251	15.98	6	*
252	16.04	8	*
253	16.11	7	*
254	16.17	8	*
255	16.23	7	*
256	16.30	6	*
257	16.36	7	*
258	16.43	6	*
259	16.49	7	*
260	16.55	6	*
261	16.62	8	*
262	16.68	7	*
263	16.74	6	*
264	16.81	6	*
265	16.87	8	*
266	16.93	8	*
267	17.00	7	*
268	17.06	9	*
269	17.13	8	*
270	17.19	8	*

MAXIMUM AT N = 230 ELUTION TIME: 14.64 MIN
MINIMUM AT N = 201 ELUTION TIME: 12.8 MIN

FIGURE 2

Plot of a chromatogram. Operator responses are underlined.

INTEGRATION

BASELINE BEFORE PEAK : 200 - 221 START OF PEAK: 222
AVERAGE : 5.95 +- .65
BASELINE AFTER PEAK : 248 - 270 END OF PEAK : 247
AVERAGE : 7.26 +- .92

PEAK AREA = 980.02 NUMBER OF VALUES: 26

FLOW RATE CORRECTION

INTERNAL STANDARD : N(MAX) = 324 VE = 20.75 ML
FLOW RATE = 1.006 ML/MIN

*** CALIBRATION ***

DATE: 6.12.1983 ELUENT: THF FLOW RATE: 1 ML/MIN
STANDARDS: POLYSTYRENE CONC.:0.05 - 0.1 % VOLUME:100 MYL

600000	11.84
111000	13.88
50000	14.77
20500	15.66
9000	16.55
4000	17.31
2200	17.76
800	18.71
72	20.75

(LINEAR INTERPOLATION)

TABLE OF MOLECULAR WEIGHTS

N	VI	MI	WI(%)	%CUM
222	14.22	82025	.38	100.00
223	14.28	77450	.79	99.62
224	14.35	73131	1.70	98.83
225	14.41	69052	2.92	97.13
226	14.47	65201	4.76	94.21
227	14.54	61564	6.39	89.46
228	14.60	58131	8.32	83.07
229	14.67	54889	9.44	74.75
230	14.73	51828	9.95	65.30
231	14.79	48813	9.64	55.36
232	14.86	45780	8.72	45.71
233	14.92	42935	7.70	37.00
234	14.99	40267	6.37	29.30
235	15.05	37764	5.14	22.93
236	15.11	35418	4.12	17.79
237	15.18	33217	3.30	13.68
238	15.24	31152	2.48	10.38
239	15.31	29216	2.07	7.90
240	15.37	27401	1.55	5.83
241	15.43	25698	1.35	4.28
242	15.50	24101	.94	2.93
243	15.56	22603	.73	1.99
244	15.63	21199	.42	1.26
245	15.69	19928	.32	.84
246	15.75	18782	.31	.52
247	15.82	17702	.21	.21

MW = 48240 MN = 44719

MW/MN = 1.08

FIGURE 3

Output of calculations from the chromatogram shown in Figure 2. Operator responses are underlined.

Before starting the calculation of molecular weights, the actual maximum of an internal standard and its elution volume in calibration have to be entered, from which the program calculates the actual flow rate.

The calculation of molecular weights can be performed either using a linear calibration or a linear interpolation between standards. The molecular weights and elution volumes of the standards are read from a data file on the floppy disk. After completion of the calculations the results are printed, and the operator can decide whether to repeat any of the steps mentioned above or to finish the calculations.

RESULTS AND DISCUSSION

As has been pointed out in the previous sections, the accuracy of molecular weight averages and polydispersity calculated from SEC is determined by the following criteria:

1. choice of chromatographic conditions
2. quality of the separation system
3. reliability of the calibration curve
4. sensitivity and stability of the detector
5. reliability of data acquisition and data processing

Any deficiency in point 1-3 will result in more or less reproducible, systematic errors, inadequacy in point

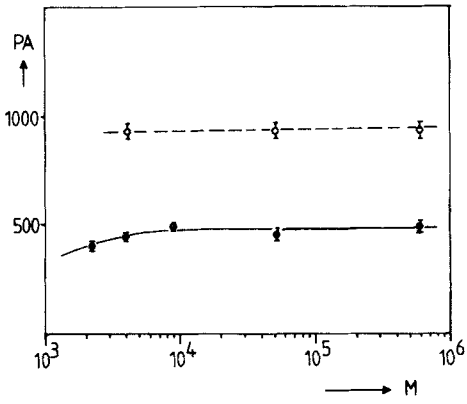

FIGURE 4

Peak areas as a function of molecular weight. Polystyrene standards 600000,50000,9000,4000,2200; Microgel M (60 cm), THF, 1.0 ml/min, injected volume 100 µl, sample concentration: 0.05 % (⊙), 0.1 % (○)

4 and 5 will cause irreproducible, random errors. Since this paper deals mainly with the performance of the detector and data handling, we have tested the accuracy as well as the reproducibility of the results obtained with our system by means of repeated analysis of polystyrene standards.

First of all we had to consider an often neglected source of error, which may occur in the low molecular weight range of the chromatogram, i.e. the variation of detector response with molecular weight. Although we have shown in a previous paper[21] that the response of the densimetric detector is not very sensitive to molecular weight, we have determined the peak areas obtained from

TABLE I

Molecular weight averages and polydispersity from repeated injections of polystyrene standard 60917 (Pressure Chem.Co.) on Microgel M in THF. Flow rate 1.0 ml/min, sample concentration 0.1 %, injected volume 100 µl.

Molecular weights reported by the distributor:

By light scattering M_W = 53700 ± 6 %

By intrinsic viscosity M_η = 47400 ± 6 %

By membrane osmometry M_n = 51150 ± 6 %

Kinetic molecular weight M_{nk} = 47000 ± 6 %

$M_w/M_n \leq 1{,}06$

M_w	M_n	M_w/M_n
48165	44985	1.07
49894	46820	1.07
48951	45807	1.07
49067	45813	1.07
49489	46307	1.07
49580	46096	1.08
49413	45780	1.08
48551	44911	1.08
49027	45753	1.07
50193	46908	1.07
48813	45289	1.08
48755	45207	1.08
48240	44719	1.08
49063	45652	1.07
49086 ± 1.2 %	45718 ± 1.5 %	1.074 ± 0.05

repeated injections of polystyrene standards (mol.weights from 600000 to 2200; sample volume: 100 µl, concentration: 0.05 and 0.1 %). As can be seen from Figure 4, only below a molecular weight of 4000 a significant decrease of peak areas is observed. The standard deviation of peak areas was typically less than 5 % even at sample sizes of 50 µg (100 µl, 0.05 %).

Baseline stability can be estimated from figures 2 and 3: In general, noise is less than ± 1 digit; after an equilibration period baseline drift within an average chromatogram does not exceed ±5 digits (1 digit corresponds to a density difference $\Delta\rho = 2.8 \times 10^{-7} g/cm^3$!).

Reproducibility and accuracy of molecular weight averages is demonstrated in table 1: even for a narrow MWD standard 25 ± 1 points per peak are obtained, M_w and M_n are determined with a standard deviation of 1.2 % and 1.5 % respectively.

Data reduction and printing of the results can be performed in this manner within less time than the excluded volume requires to pass the column (at 1 ml/min). Hence one may inject the next sample before processing the data from the last one, and start data acquisition thereafter.

CONCLUSIONS

It has been shown that the system described in this paper fulfills the requirements of high performance SEC,

as they have been formulated by Tchir, Rudin, and Fyfe[15]:

1. For an average polymer a sufficient number of points per peak is obtained
2. Noise level is less than 2 % even at sample sizes of 50 µg
3. Baseline level is defined to within 2 % at the same sample size
4. Peak width is defined to within 20 % (typically 5-10 %)

The reproducibility of molecular weight averages is typically better than ± 2 %, if an internal standard is used for flow rate correction.

Hence, the method described in this paper makes rapid and accurate determination of molecular weight averages possible by simple and inexpensive means.

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