The Use of a Densitometer as a Detector for Gel Permeation Chromatography

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Synopsis

A commercially available densitometer, DMA 60 (Anton Paar, Austria), in combination with a Model DMA 602-W flow cell was used as a detector in a Waters Model 200 GPC equipped with differential refractive index and ultraviolet absorbance detectors. The density measuring cell was thermostatted to ±0.004°C and the sample injection concentration was 1.5 mg/mL. Polystyrene samples of molecular weights ranging from 9000 to 860,000 were used. The optimum period was found to be 10,000 oscillations, a setting which gave good resolution and a sufficient number of data points to define the chromatogram. The molecular weight averages calculated using the density outputs compared well with those obtained through the conventional UV detector. A sliding average technique was applied to the densitometer data for reducing the baseline noise. It was found that concentrations as low as 1.0 mg/mL (≈ 0.114%) could be used to obtain densitometer chromatograms that yield molecular weight averages comparable to those obtained from the ultraviolet detector. This densitometer thus appears suitable to be used as an additional GPC detector for routine analyses.

INTRODUCTION

Gel permeation chromatography (GPC) is a widely used technique for the determination of molecular weight averages and molecular weight distributions (MWD) of polymeric materials. In a gel permeation chromatograph a differential refractive index (RI) detector is normally used either alone or in conjunction with some other detectors such as an ultraviolet absorbance (UV) detector[1] or an infrared spectrometer,[2] connected in series along the eluant path. Photometric detectors can be used only for light-absorbing materials while the RI detectors can be used for most polymeric materials. A density-based detector will find considerable importance where RI or UV detectors fail, for example, when the difference of refractive indices is very small [as in the case of poly(dimethyl siloxane) in tetrahydrofuran[3,4]] or when the polymer chain contains no functional group absorbing strongly in the UV (polybutadiene at 254 nm).

The adaptation of a flow densitometer as a detector for GPC has been demonstrated in the literature by Francois et al.,[3] Trathnigg and co-workers,[5–10] and Elsdon et al.[11] with encouraging results. In the present work, however, lower polymer concentrations and improved data treatment procedures were adopted to achieve greater flexibility and wider use of densitometric detection.

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THEORETICAL BACKGROUND

The densitometer used in this study is based on the mechanical oscillator method. The principle of this type of densitometer is based on the concepts of Kratky et al.,[12] and is also discussed in detail by Trathnigg.[7,10] It is recounted here for the sake of completeness.

The density is determined by measuring the period of oscillation of a vibrating U-tube filled with the liquid sample. The period of oscillation, T, is given by

$$T = 2\pi \left(\frac{M + \rho V}{C}\right)^{1/2} \tag{1}$$

where M is the mass of the U-tube, ρ is the density of the sample, V is the vibrating volume, and C is the elasticity constant of the U-tube. Therefore,

$$T^2 = A\rho + B \tag{2}$$

where $A = 4\pi^2 V/C$ and $B = 4\pi^2 M/C$, which are instrument constants. The difference in densities between the samples is given by

$$\Delta\rho = \rho_2 - \rho_1 = (1/A)(T_2^2 - T_1^2) \tag{3}$$

However, in the case of GPC detection, we are not concerned with absolute densities, but only with a variable which is proportional to the polymer concentration. The difference in densities between a polymer solution and pure solvent can be written as

$$\Delta\rho = \rho_2 - \rho_1 = C_2(1 - \bar{v}_2^*\rho_1) \tag{4}$$

where ρ_2 and ρ_1 are, respectively, the densities of polymer solution and the pure solvent, C_2 is the polymer concentration, and $\bar{v}_2^*$ is the apparent specific volume of the polymer solute.

Equating (3) and (4), we obtain

$$C_2 = \frac{(T_2^2 - T_1^2)}{A(1 - \bar{v}_2^*\rho_1)} = k(T_2^2 - T_1^2)$$

where K is a constant. This assumes that $\bar{v}_2^*$ is independent of the polymer molecular weight and concentration, which is not strictly true.[13-15] However, Trathnigg[6] has shown that the density of a polymer solution does reflect the concentration of the polymer with adequate accuracy within a wide range of molecular weight and is therefore suitable as a detection variable in GPC.

When ΔT and $\Delta\rho$ are small, it can be shown that

$$\Delta\rho \propto \Delta T$$

Thus, a plot of ΔT as a function of elution volume will provide a distribution curve for a polymer analyzed through GPC.

EQUIPMENT USED

Description of the Densitometer and Its Operation

The densitometer system consisted of a model DMA 602-W measuring flow cell with a heat exchanger and a DMA 60 processing unit (counter), manufactured by Anton Paar K.G., Austria.

The DMA 60 processing unit consists of an eight-decimal figure digital period meter and an electronically controlled quartz time base. The quartz time pulses every 10^{-5} s. The "period select" switch enables the counting of time periods for completion of 1000, 2000, 5000, 10,000, 20,000 or 50,000 periods of the vibrating U-tube. The period meter measures the time for the number of preselected oscillator periods by counting the number of the clock pulses for the number of oscillations selected by the "period select" switch. In this way, it is possible to perform period measurements of high resolution within very short times. The position of the "period select" switch determines the compromise between resolution and measurement time.[16]

The DMA 602-W measuring cell consists of an oscillator or sample tube, made of borosilicate glass and fused onto a dual wall glass cylinder. The thermostat liquid flows between the double walls of the glass cylinder. The sample U-tube oscillates in a gas of high thermal conductivity contained in the inner most space of the double-walled cylinder. Between the double walls of the glass cylinder where the thermostat liquid flows is a glass heat exchanger through which the GPC effluent first flows before reaching the oscillating U-tube. The volume of the heat exchanger is approximately 2 mL, and that of the measuring cell approximately 1 mL. The actual volume of the measuring cell which influences the time for the preselected number of oscillations to occur is 0.7 mL. The inside diameter of the glass tubing is approximately 2 mm.[17] The lags in the efflux time due to the extra tubing, the heat exchanger, and time for the reading to be taken are incorporated in the calibration curve and do not affect the results.

The temperature of the measuring cell is controlled by a Hetofrig cooling bath type CB7. The Hetofrig bath has a volume of about 12 L and is well stirred for maximum temperature stability. The thermostat bath is equipped with a compressor for cooling, which works continuously at its maximum effect when switched on. The heating system works in opposition to the cooling system and achieves a claimed temperature stability of <0.002°C/°C and 0.004°C/°C for the best and worst cases, respectively. The temperature of the bath cannot be accurately controlled with the compressor off below 35°C due to the energy dissipated in stirring and circulating. The bath reaches temperature stability within 20 min of switching on.

The gel permeation chromatograph used is a Waters Model 200 equipped with a UV detector using a monochromatic light of wavelength 254 nm and an RI detector using white light. The detectors were positioned along the eluant path and their outputs were recorded on a dual-channel Texas Instruments recorder.

The densitometer was connected in series with the RI and UV detectors. A microvalve was positioned between the UV detector and the densitometer. This allows the eluant to flow through the RI and UV detectors only or through all three detectors. This enables the densitometer to be easily isolated from the GPC system when not in use or when being used for another purpose, for example, as a static cell for density measurements of solutions.

EXPERIMENTAL

The characteristics of the commercial polystyrene samples used are given in Table I. These polymers are all narrow molecular weight distribution samples and supplied by Pressure Chemical Company. The solvent used was "Baker

TABLE I
Characteristics of Polystyrene Samples Used For GPC Calibration

Sample	Molecular weight	$\overline{M}_w/\overline{M}_n$ (supplied by manufacturer[a])
PS 4000	4000	<1.06
PS 9000	9000	<1.06
PS 17,500	17,500	<1.06
PS 37,000	37,000	<1.06
PS 100,000	100,000	<1.06
PS 233,000	233,000	<1.06
PS 390,000	390,000	<1.10
PS 860,000	860,000	<1.15

[a] Pressure Chemical Co.

analyzed" reagent grade tetrahydrofuran supplied by J. T. Baker Chemical Co., which was also used as the eluant in the GPC.

The gel permeation chromatograph was used with four columns each 4 ft long containing Styragel packing of the following size designation: 2000–5000 Å, 15,000–50,000 Å, 150,000–170,000 Å, and 5,000,000 Å. The flow rate was 1 mL/min and the injected sample size was 2.0 mL.

The UV and RI outputs were obtained as analog plots on the strip chart recorder. The digital readings from the densitometer were recorded manually. The value of ΔT, being the difference between the value of T for the solution and that for the pure solvent, was plotted as a function of elution volume. This gives the densitometer chromatogram for the polymer solution.

Separate calibration curves were obtained for the UV and densitometer detectors, using polystyrene standards. These calibration curves were then employed for the determination of molecular weights of the samples.

RESULTS AND DISCUSSION

The chromatograms plotted from the densitometer outputs are presented in Figures 1–8. The molecular weight averages calculated are presented in Tables II–V.

In Figures 1–4 are described densitometer chromatograms for the polystyrene sample PS 100,000 run at different periods. The "period select" setting of 5000 or 5 K gives a density reading after counting 5000 periods, while a period of 50,000 or 50 K will provide density measurements after counting 50,000 periods. Thus, although the latter output is counted over more periods, and hence corresponds to a larger difference from the pure solvent baseline, the number of points obtained will be proportionately smaller. In other words, at a setting of 5 K, a reading is obtained approximately every 18 s while it takes about 3 min for every reading at a setting of 50 K. The actual value of the output, however, is greater with a higher value of the setting. Thus, a compromise must be struck between the resolution and the number of points obtained.

Comparing Figures 1–4 it is seen that there are approximately two points between elution counts in Figure 4 (period = 50 K), four points between elution counts in Figure 3 (period = 20 K), and eight or nine points between counts in Figure 2 (period = 10 K). It is difficult to define the curve in the case of 50 K,

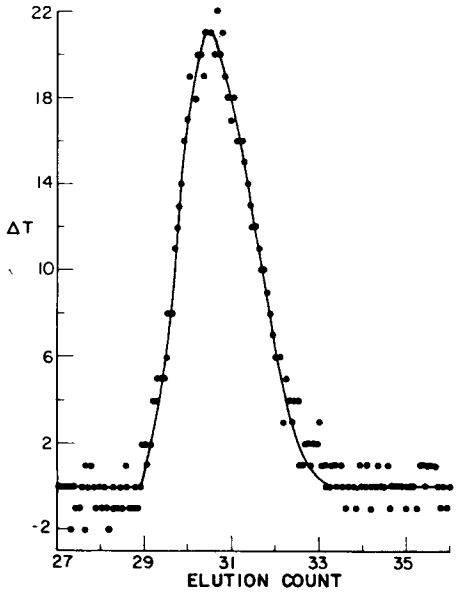

Fig. 1. Densitometer chromatogram for PS 100,000. Concentration = 1.5 mg/mL. Densitometer period = 5 K.

and prior knowledge of the peak position and shape from Figures 1 and 2 was used. Although four points per elution count is usually sufficient for analyzing a chromatogram for molecular weight averages, it is still not sufficient to define the curve accurately. A similar technique of smoothing between the points by

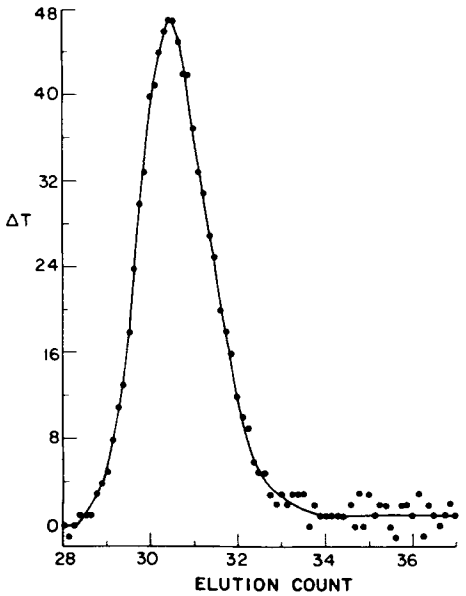

Fig. 2. Densitometer chromatogram for PS 100,000. Concentration = 1.5 mg/mL. Densitometer period = 10 K.

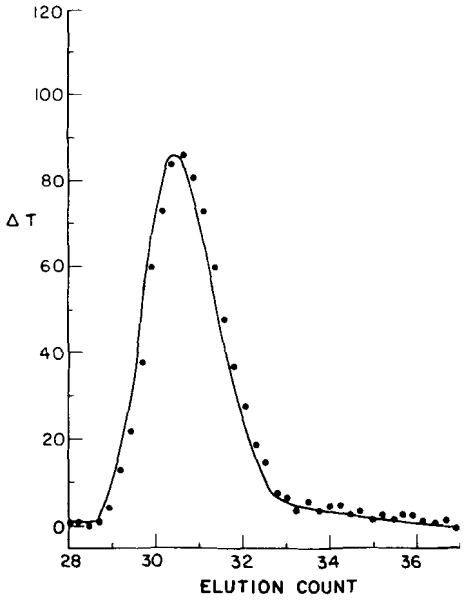

Fig. 3. Densitometer chromatogram for PS 100,000. Concentration = 1.5 mg/mL. Densitometer period = 20 K.

drawing the curve through the midpoint of the time interval as shown in Figure 4 was also used in Figure 3. On the other hand, Figure 1 (period = 5 K) has many points, but also a larger drift or "noise" in the baseline. This is due to poorer resolution, because a fluctuation of one digit in the final decimal place constitutes a large relative error. The scatter of the points seen near the peak also

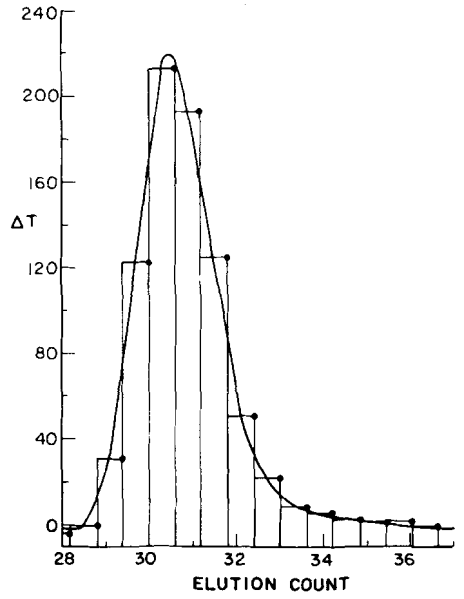

Fig. 4. Densitometer chromatogram for PS 100,000. Concentration = 1.5 mg/mL. Densitometer period = 50 K. Curve drawn through midpoints of the time intervals.

TABLE II
Molecular Weight Averages of PS 100,000[a]

Densitometer period		$M_w \times 10^{-3}$	$M_n \times 10^{-3}$	M_w/M_n
5000	Uncorrected	89.94	71.43	1.26
or 5 K	Corrected	88.77	78.60	1.13
10,000	Uncorrected	99.38	74.28	1.34
or 10 K	Corrected	97.49	82.66	1.18
20,000	Uncorrected	92.43	68.02	1.36
or 20 K	Corrected	90.76	78.10	1.16
50,000	Uncorrected	95.83	70.08	1.37
or 50 K	Corrected	91.59	77.00	1.19

[a] Concentration = 1.5 mg/mL.

makes it difficult to define the curve properly. Hence, for reasons both of resolution and of having sufficient number of data points to define the chromatogram accurately, Figure 2 (period = 10 K) can be selected as the best compromise. This is further confirmed from the calculated molecular weight averages of the polymers determined from each of these chromatograms (Table II) and compared with the results obtained from the UV output (Table III). Thus, period 10 K was selected for further experiments. For gel permeation chromatographs with shorter columns, shorter periods would likely be optimum.

TABLE III
Molecular Weights Averages of Polystyrene Samples[a]

Sample	Detector		$\overline{M}_w \times 10^{-3}$	$\overline{M}_n \times 10^{-3}$	$\overline{M}_w/\overline{M}_n$
PS 9000	UV	Uncorrected	8.96	5.91	1.52
		Corrected	8.50	6.52	1.30
	Densitometer	Uncorrected	9.07	5.56	1.63
		Corrected	8.27	6.26	1.32
PS 17,500	UV	Uncorrected	18.58	12.68	1.47
		Corrected	17.55	13.66	1.29
	Densitometer	Uncorrected	19.10	13.05	1.46
		Corrected	18.49	14.93	1.24
PS 37,000	UV	Uncorrected	39.60	28.40	1.40
		Corrected	36.60	30.40	1.20
	Densitometer	Uncorrected	39.01	27.09	1.44
		Corrected	36.56	29.63	1.24
PS 100,000	UV	Uncorrected	94.30	70.98	1.33
		Corrected	92.54	79.17	1.17
	Densitometer	Uncorrected	99.38	74.28	1.34
		Corrected	97.49	82.66	1.18
PS 233,000	UV	Uncorrected	257.10	198.30	1.30
		Corrected	249.00	217.30	1.15
	Densitometer	Uncorrected	259.50	124.30	2.09
		Corrected	244.90	194.70	1.26
PS 390,000	UV	Uncorrected	430.00	295.00	1.46
		Corrected	415.00	324.00	1.28
	Densitometer	Uncorrected	417.00	285.00	1.46
		Corrected	396.00	307.00	1.29
PS 860,000	UV	Uncorrected	960.00	639.00	1.50
		Corrected	952.00	721.00	1.32
	Densitometer	Uncorrected	938.00	646.00	1.45
		Corrected	893.00	697.00	1.28

[a] Concentration = 1.5 mg/mL; densitometer period = 10 K.

The calibration for GPC was done with polystyrene standards of known molecular weight averages and at a period of 10 K.

A number of polystyrene samples, with molecular weights ranging from 9000 to 860,000 was selected, and their UV and densitometer chromatograms were analyzed. The concentration used in all these cases was 1.5 mg/mL. The Chang–Huang correction technique[18] for axial dispersion, developed in our laboratory, was applied to the data and the corrected molecular weight averages determined. The uncorrected and corrected molecular weight averages, obtained from UV and densitometer chromatograms of each polymer, are presented in Table III. The results obtained for the densitometer agree very well with those obtained using the UV output, and indicate that very little mixing is occurring so that the distribution is not being appreciably broadened nor is the resolution being diminished.

Sliding-Average Technique

One of the most important aspects of analyzing a chromatogram is the fitting of the baseline. This problem is more crucial when one constructs a chromatogram from data collected at different time intervals, especially when there is a sizeable scatter or noise in the data. Thus, this problem is a real one in the case of densitometer outputs. Although manual curve fitting was applied to the data discussed so far, a better mathematical technique was felt necessary for defining the baseline. The averaging of a set of points and then plotting the average in the mean position can be an effective technique for smoothing a curve and reducing the noise on the baseline. The next average is taken one data point later.

Three levels of averaging were done. First, three consecutive points were taken, averaged, and the results plotted (Fig. 5). Similarly five points of the same data were taken at a time, averaged, and the results plotted (Fig. 6). Finally, seven points were taken, averaged, and a plot was obtained (Fig. 7). It is clearly seen from these figures that, as more points were averaged, the noise in the baseline became less. It was also observed that the averaging of five points was adequate to define a proper baseline. If the data were handled by a computer, greater numbers of points can be averaged.

The molecular weight averages calculated with and without the sliding average technique are presented in Table IV. This technique was found to be of much

TABLE IV
Molecular Weight Averages of PS 100,000[a]

No. of points averaged		$\bar{M}_w \times 10^{-3}$	$\bar{M}_w \times 10^{-3}$	$\bar{M}_w/\bar{M}_n$
1	Uncorrected	99.38	74.28	1.34
	Corrected	97.49	82.66	1.18
3	Uncorrected	99.18	71.22	1.39
	Corrected	95.57	76.93	1.24
5	Uncorrected	99.03	73.26	1.35
	Corrected	93.15	80.44	1.16
7	Uncorrected	102.96	74.58	1.38
	Corrected	100.27	82.87	1.20

[a] Sliding average method using different numbers of points. Concentration = 1.5 mg/mL; densitometer period = 10 K.

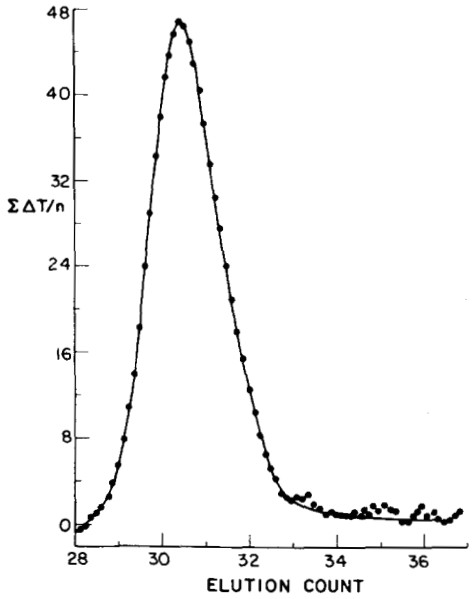

Fig. 5. Densitometer chromatogram for PS 100,000 using sliding average technique. The number points averaged at a time (n) = 3. Concentration = 1.5 mg/mL. Densitometer period = 10 K.

use in the case of lower resolution and higher scatter of data as, for example, with a period of 5 K or less (Fig. 1). These results show that, even with a larger number of points being averaged, very little broadening of the distribution is being introduced.

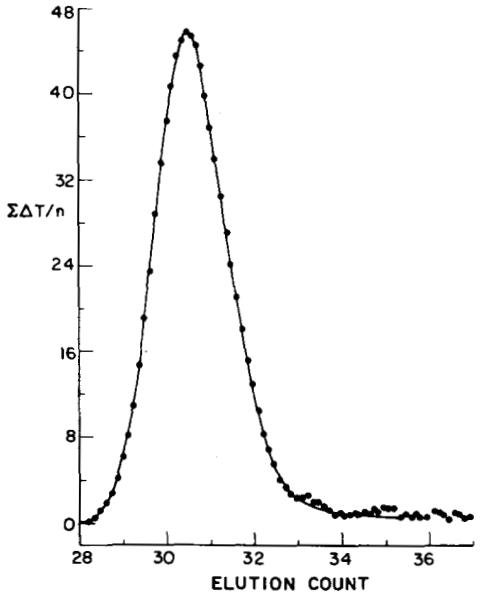

Fig. 6. Densitometer chromatogram for PS 100,000 using sliding average technique. The number of points averaged at a time (n) = 5. Concentration = 1.5 mg/mL. Densitometer period = 10 K.

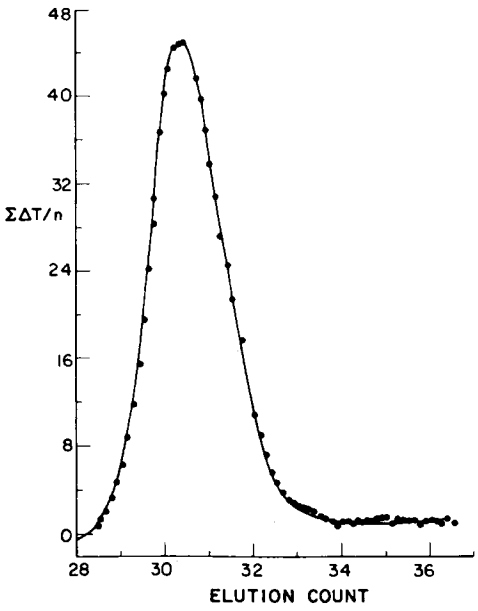

Fig. 7. Densitometer chromatogram for PS 100,000 using sliding average technique. The number of points averaged at a time (n) = 7. Concentration = 1.5 mg/mL. Densitometer period = 10 K.

The Effect of Concentration

The response of the densitometer is greater at higher polymer concentrations. The value of ΔT is higher, and hence the relative error of the background noise at higher concentrations is smaller. Some of the earlier workers, therefore, used reasonably high concentrations,[10,11] normally not used in general GPC applications. The densitometer used in the present work has a reported accuracy[17] of $\pm 1.5 \times 10^{-6}$ g/cm^3. Hence, although the concentration of 1.5 mg/mL used in this work is within the normal levels such that the densitometer could be used as a detector in the same way as UV or RI detectors for routine analyses, investigations were carried out to find out the lower limits of concentration that can be used without losing accuracy.

Two lower concentrations of 1.0 mg/mL and 0.5 mg/mL were investigated for the same polymer PS 100,000. The densitometer chromatograms obtained for these concentrations are presented in Figure 8. As is to be expected, the peak heights are proportionately lower than that for a concentration of 1.5 mg/mL. The molecular weight averages calculated from these chromatograms are presented in Table V. It is seen that the results for the 1.0 mg/mL solution are still comparable to the results of the UV chromatogram (Table III). The results for the 0.5 mg/mL solution give a broadening effect. Also, its $\overline{M}_n$ values are much lower. This is possibly due to the fact that near the baseline the ΔT values are so low that the contributions of the species on either side of the distribution are severely reduced. The whole chromatogram, in effect, has been skewed toward the low molecular weight end.

Thus, the lowest concentration that can conveniently be used with the present densitometer is about 1.0 mg/mL ($\approx$ 0.114%).

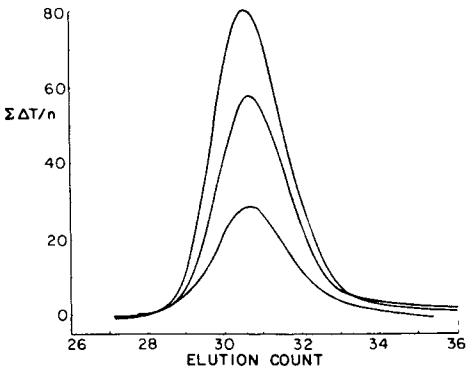

Fig. 8. Densitometer chromatograms for PS 100,000 at various concentrations: (i) 0.5 mg/mL; (ii) 1.0 mg/mL; (iii) 1.5 mg/mL. Densitometer period = 20 K. Sliding average technique was applied with $n = 5$.

CONCLUSIONS

The automatic digital densitometer DMA 60 (Anton Paar K.G., Austria) with a Model DMA 602-W flow cell was connected to a Waters Model 200 GPC unit equipped with RI and UV detectors, in series with the dual detector system. Polystyrene samples of different molecular weights ranging from 9000 to 860,000 were analyzed using the densitometer. The molecular weight averages calculated using the densitometer chromatograms agreed very well with those calculated using the conventional UV output. Using a period of 10 K was found to be the ideal because at this value a compromise was achieved between the number of data points and resolution. Shorter periods would likely be optimum for chromatographs with columns of shorter length. A sliding average technique was applied to the data to reduce the noise in the baseline. Concentrations as low as 1.0 mg/mL ($\approx$ 0.114%) were used, and the results of molecular weight averages calculated from the densitometer chromatogram were found to agree well with those obtained from a conventional UV detector. This suggests that the densitometer used here can be used as a routine GPC detector.

The authors wish to thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for the Special Equipment Grant for the purchase of the densitometer. The authors also thank Mr. Newton Milne for his help in the installation of the equipment.

TABLE V
Molecular Weight Averages of PS 100,000[a]

Concn (mg/mL)		$M_w \times 10^{-3}$	$M_n \times 10^{-3}$	M_w/M_n
0.5	Uncorrected	91.38	49.97	1.83
	Corrected	86.70	53.88	1.61
1.0	Uncorrected	94.98	68.79	1.38
	Corrected	91.69	68.01	1.35
1.5	Uncorrected	97.61	69.15	1.41
	Corrected	92.51	75.70	1.22

[a] Sliding average method using 5 points and varying concentrations. Densitometer period = 20 K.

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