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GEL PERMEATION CHROMATOGRAPHY

XII. COMPUTER-ASSISTED GEL PERMEATION CHROMATOGRAPHY AND LOW-ANGLE LASER LIGHT-SCATTERING PHOTOMETRY

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SUMMARY

An automated gel permeation chromatograph-low-angle laser light-scattering system capable of measuring the molecular-weight distribution of soluble polymers without the necessity of calibration is described. The automation system eliminates most of the tediousness associated with molecular-weight distribution measurement. The molecular-weight distributions and molecular-weight averages of a broad range of polystyrene standards were determined using this technique. The results obtained are shown to agree closely with literature data.

INTRODUCTION

In an earlier article¹ we described the use of a recently developed low-angle laser light-scattering (LALLS) photometer² coupled to an experimental gel permeation chromatograph³ for measuring the molecular-weight distribution (MWD) of polymers without the necessity of calibration and band-broadening correction. It was demonstrated¹ that light-scattering photometers, when properly designed, could be used to monitor molecular weights of gel permeation chromatographic (GPC) effluents in a continuous fashion and in a real time mode. The raw data obtained consist of a dual trace [differential refractometer (DRI) and LALLS excess scattering] chromatogram from which the concentrations, the Rayleigh factors and the molecular weights of the GPC effluent fractions were calculated. Although the calculations involved in converting the raw data to molecular-weight averages and MWDs are simple, they are tedious. The manual digitization of the concentration and Rayleigh factor chromatograms is not only time consuming, it is also difficult to accomplish with high precision.

The continuous and real time characteristics of both the DRI and light-scattering intensity obtained from a directly coupled LALLS and GPC instrument makes this system ideally suitable for computer assisted data acquisition (direct digitization and storage of the LALLS and DRI analog signals) and reduction (reducing the data to molecular-weight distribution and averages). Consequently, some effort has been devoted to apply laboratory automation techniques to improve the performance of the gel permeation chromatograph-LALLS system.

Since the LALLS photometer does not need the high-precision flow-rate and temperature control required by the real time viscometer molecular-weight detector³, the LALLS was coupled directly to a commercially available chromatograph (a Modified Waters Assoc. Model 200) rather than to the high-precision experimental gel permeation chromatograph¹. As expected, the LALLS photometer performed equally well with the commercial gel permeation chromatograph as with the high-precision chromatograph. Narrow MWD polystyrenes (Pressure Chem., Pittsburgh, Pa., U.S.A.; Duke, Palo Alto, Calif., U.S.A.; and National Bureau of Standards, Washington, D.C., U.S.A.) covering a wide range of molecular weights were analyzed using the above system and the results obtained were compared with literature values.

INSTRUMENTATION

A commercially available chromatograph (Waters Assoc. Model 200 GPC) was modified to minimize the "hold up" volume of the mobile phase system and to relocate the DRI concentration detector so that outgasing could be minimized and coupling with the LALLS photometer facilitated. The modification consisted of the following:

(i) Removal of the original pumping system and replacing it with a Milton Roy "minipump" with a Laboratory Data Control Model 709 pulse dampener connected in parallel with the pump. Two sintered $5\ \mu\text{m}$ metal filters (one between the solvent reservoir and the pump and the other between the pump and the sample injection valve) were used to insure removal of particulates from the mobile phase. A diagram of the mobile phase modification is illustrated in Fig. 1.

(ii) The DRI detector was lowered to a point just above the siphon collector. This prevented the formation of gas bubbles in the DRI cell and also shortened the 0.015-in.-I.D. PTFE tubing connector (a 1 m \times 1/16 in. O.D. \times 0.015 in. I.D. PTFE tubing was used to connect the fractionating column, LALLS and DRI detectors).

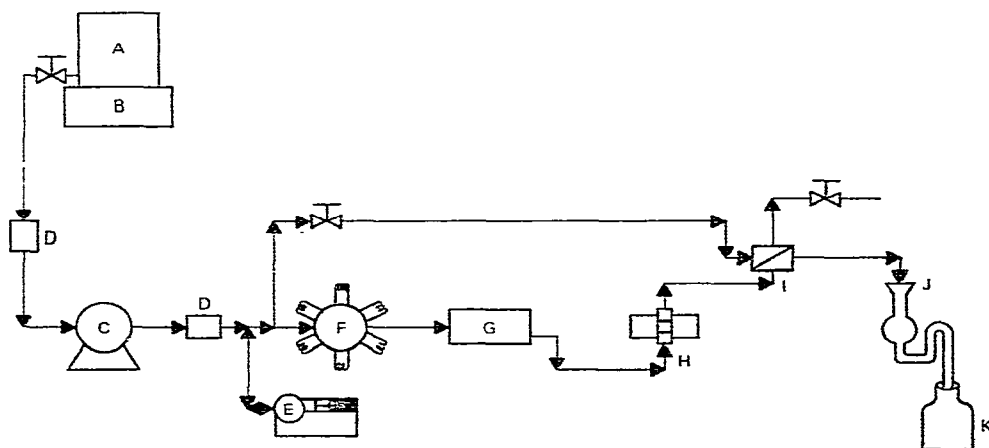


Fig. 1. Diagram of the modified GPC-200 mobile-phase lines. A = Solvent reservoir; B = magnetic stirrer; C = Milton Roy minipump; D = $5\text{-}\mu\text{m}$ sintered metal filter; E = LDC pulse dampener; F = Waters Assoc. automatic sample injection system; G = fractionating column; H = LALLS photometer; I = DRI; J = Siphon collector; K = waste bottle.

The above modification is not necessary for newer chromatographs with low holdup volume in the mobile phase lines and better located concentration detectors.

The LALLS photometer used in this work is essentially the same as the one described in the earlier article¹. However, some modifications have been made on the design of the sample cell and its associated filter. The modification made was mainly done to improve the plumbing into and out of the sample cell and to reduce the mixing volume of the sample filter. The black PTFE light-scattering cell was replaced with a stainless-steel sample cell with a black PTFE insert. The PTFE insert not only served as a low reflecting surface but also served as an effective sealing gasket between the two fused silica sample cell windows. A sketch of this setup is shown in Fig. 2.

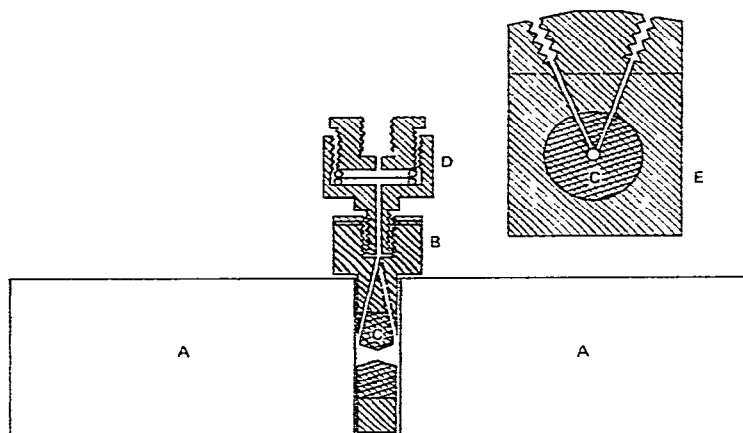


Fig. 2. Sketch of the improved cell design. A = Silica window; B = stainless-steel sample cell housing; C = black PTFE cell; D = modified millipore filter; E = stainless-steel cell housing.

The laboratory automation system hardware consisted of a signal amplifier for each of the analog signals (DRI and LALLS), a sensor-based computer (IBM System/7) for data acquisition, a host computer (IBM 360/195) for data reduction, a typewriter terminal (communication with the host computer) and a gas panel terminal (communication with the sensor-based computer). In our installation, the analog-to-digital converter is situated in the sensor-based computer, hence the analog signal had to be transmitted by a twisted pair of shielded cables over a distance of approximately 150 ft. To avoid an increase in the background electrical noise in the transmission lines, the low-level (0–100 mV) signals from both the DRI and LALLS detectors were amplified to 0–5 V at the chromatograph before being transmitted. A flow sheet of the data acquisition system is shown in Fig. 3.

The laboratory automation software consisted of a relatively simple data acquisition program which specifies to the sensor-based computer the following parameters:

- (i) Data sampling frequency—This parameter refers to the time interval between sampling of the analog signal. This parameter can be varied over a very wide range, *i.e.*, 10^{-4} –60 sec.
- (ii) The length of the experimental run—This parameter specifies the total number of hours or minutes required to complete the experiment.

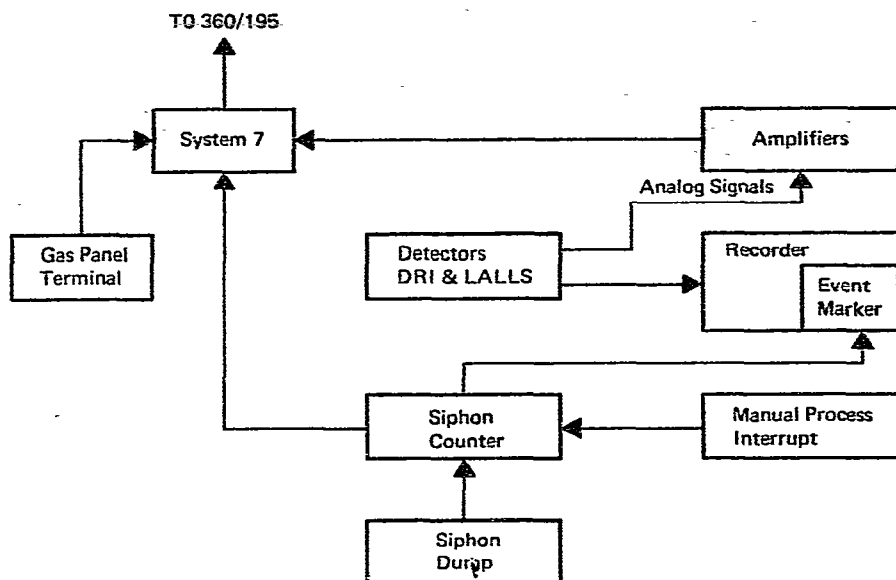


Fig. 3. Flow sheet diagram of the data acquisition system which shows the components of the laboratory automation hardware.

(iii) Data transmission and job submit option parameter—This parameter allows an option for complete automatic or manual data transmission from the sensor-based computer to the data processing (host) computer and data reduction at the termination of the analytical run. In the automatic mode, an unlimited number of samples (in our case we are limited to six samples, which is the maximum number our automatic sample injection system can hold per analytical run) could be processed without any intervention.

A flow chart of the data acquisition and reduction of the laboratory automation software is illustrated in Fig. 4. After the transmission of the data to the host computer, it is partitioned into six individual samples. Each sample chromatogram is reduced into MWD and molecular-weight averages via the following schematics:

(i) Both the DRI and LALLS signals are smoothed using a simplified least squares smoothing method⁴.

(ii) The peak and baselines of both the DRI and LALLS chromatograms are located from the digitized chromatogram data array.

(iii) The excess scattering intensities and concentrations are computed from the chromatograms using the following equations:

$$R_{\theta_i} = K_1 Y_i \quad (1)$$

and

$$C_i = K_2 x_i / \Sigma x_i \quad (2)$$

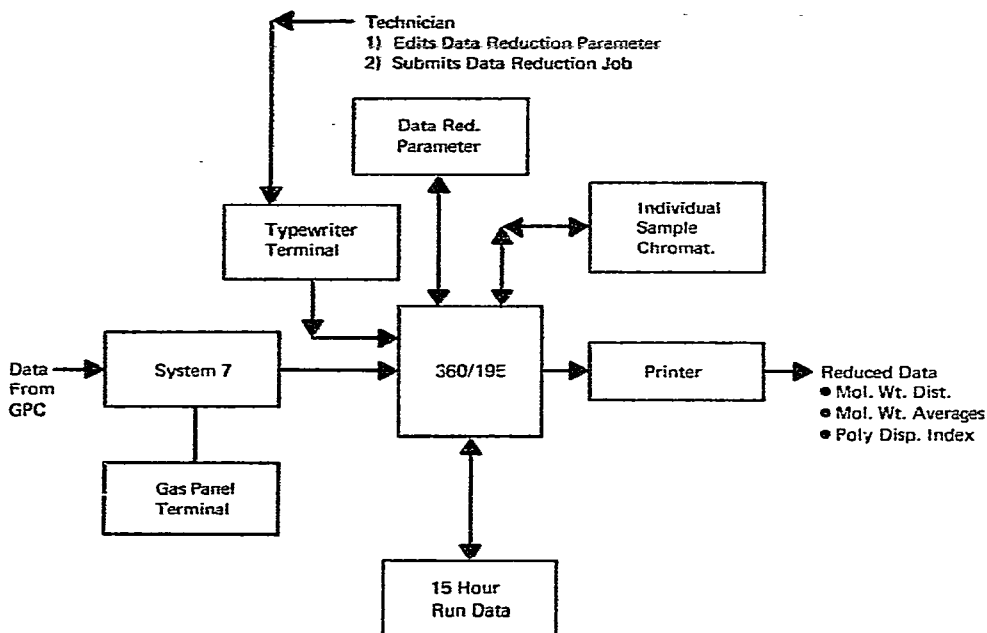


Fig. 4. Flow chart of the laboratory automation software which illustrates the different steps in reducing the raw data MWDs.

where Y_i and x_i are the digitized LALLS and DRI data and K_1 and K_2 are instrumental constants defined as follows

$$K_1 = F/P_0G \quad (3)$$

and

$$K_2 = WT/\Delta V \quad (4)$$

when F , P_0 and G are the attenuation factor, the transmitted light intensity, and the geometrical constant of the photometer, respectively¹, WT is the total sample weight injected, and ΔV is the volume increment of the effluent being considered.

(iv) The second virial coefficient, A_i , is obtained from the relationship

$$A_i = K_3 M_i^{-\alpha} \quad (5)$$

where K_3 and α are constants obtained empirically from a plot of A_i versus M_i . This is done by obtaining light-scattering data of various molecular weights and concentrations of the polymer in an off-line determination prior to the GPC-LALLS analysis.

(v) The molecular weight, M_i , is computed in an iterative mode using eqns. 5 and 6.

$$\frac{K_4 C_i}{R_{\theta_i}} = \frac{1}{M_i} + A_i M_i \quad (6)$$

where K_4 is the well-known polymer constant which depends on the viewing angle, the solvent refractive index and the refractive index increment.

(vi) The molecular-weight averages are calculated from the moments of the MWDs using Simpson's rule integration routines.

EXPERIMENTAL PROCEDURE

If high-accuracy MWD measurement is desired, the second virial coefficient, A_2 , must be determined as a function of M_w . A_2 can be determined in an off-line analysis (LALLS not coupled to the gel permeation chromatograph) by obtaining LALLS data at various concentrations of the polymer over a suitable range of molecular weights. If the polymer is not available over a range of molecular weights with relatively narrow MWD, A_2 can be obtained by repeated runs in the gel permeation chromatograph-LALLS system at various total injected weights of the polymer. Fig. 5 shows a plot of the second virial coefficients with molecular weights. The K_3 and α obtained from polystyrene in tetrahydrofuran were $7.41 \cdot 10^{-3}$ and 0.187, respectively.

The refractive index increment ($\Delta N/\Delta C$) for polystyrene in tetrahydrofuran was measured using a Brice Phoenix differential refractometer calibrated with KCl solution. The $\Delta N/\Delta C$ obtained was 0.212 ml/g. The refractive index used for tetrahydrofuran was 1.405.

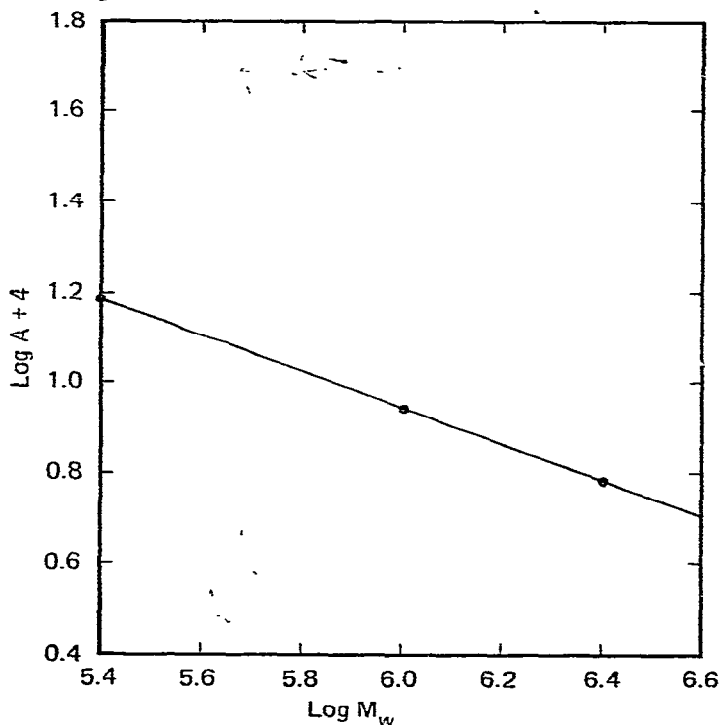


Fig. 5. Plot of the second virial coefficient (A) versus molecular weight (M_w) illustrating the strong dependence of A on M_w .

Sample solutions of various concentrations (the concentrations were adjusted according to molecular weights and the volume of the sample loops) were prepared. For example, for polystyrene of molecular weight $7.1 \cdot 10^6$ and a calibrated sample loop volume of 0.5202 ml, the concentration was $4.233 \cdot 10^{-4}$ g/ml or a total sample charge of 0.221 mg. For lower molecular weights, the concentrations and the sensitivity of the photometer were increased accordingly to give a good peak height of the LALLS chromatogram. The sensitivity of the DRI refractometer was likewise adjusted to provide a good signal-to-noise ratio.

After the correct range of LALLS and DRI detector sensitivities have been set, six samples can be analyzed in a completely automated mode. To start the analysis, the following sequence is followed:

(i) The calibrated sample loops of the automatic injector (Waters Assoc.) are loaded with six different sample solutions of precisely determined concentrations.

(ii) The data acquisition program is initialized through a gas panel terminal of the sensor-based computer.

(iii) The sample identifications and other parameters (K_1 , K_4 , K_3 and α) are entered into the data processing program (in the host computer) via a typewriter terminal.

(iv) Finally, the automatic injector is initialized and the samples are injected into the chromatograph at a programmed interval.

For six samples, at 1 ml/min flow-rate, the total time required to complete the analysis is about 15 h. In many cases, the length of time required to finish the analysis is not critical or of particular concern, for the analysis is usually conducted in the evening hours under conditions requiring no personnel attention or intervention. For example, if an analysis is started at 4:00 pm, the molecular-weight distribution and averages (in the form of digital printout and cumulative and distribution plots) of the six samples are usually ready the next morning.

DISCUSSION OF RESULTS

In common with other light-scattering techniques, one of the concerns in coupling the LALLS photometer to the gel permeation chromatograph is in making certain that particulate matter is filtered out from the effluent as completely as possible. Fortunately, LALLS has a very small scattering volume, *i.e.*, 0.02 μl . If particles were present in the sample cell in relatively low concentration, the probability that these particles reside in the scattering volume is not high and, furthermore, their residence times are normally short. Consequently, the particles appear as spikes in the LALLS chromatogram and not as a broad increase in the scattering of the solvent. Fig. 6 is an illustration of a case where the filter did not operate properly and a considerable amount of particulate matter (particle size in the order of less than 1 μm) from the column entered the LALLS sample cell. Despite the noisy signal one gets when particles are present in LALLS, the data obtained are usable, for the baseline of the spikes can still be reasonably interpreted as the scattering intensity without the particles. The same could not be said for light-scattering photometers with large scattering volumes where the scattering contribution of the particles could not be easily factored out. The presence of particles in a large scattering volume would not appear as individual spikes, but as the integral or the envelope of these spikes resulting in a

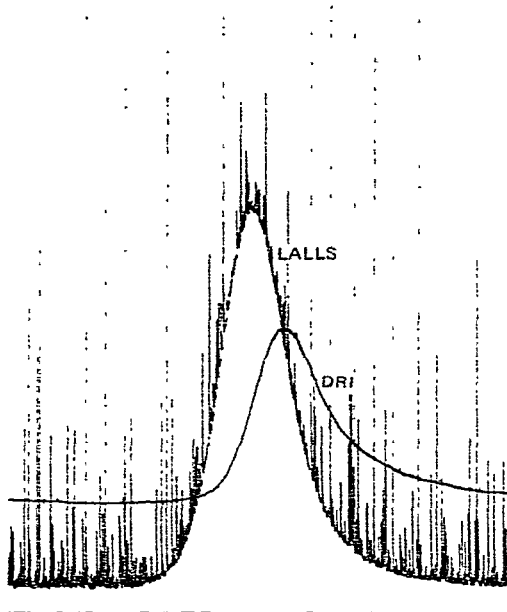


Fig. 6. Case where the filter did not operate properly and a considerable amount of particulate matter from the column entered the LALLS sample cell. Each spike represents a particle which passed through the scattering volume.

gross increase in scattering intensities in the same manner as an increase of polymer concentration or molecular weight in the solution.

The particles causing the spikes in Fig. 6 can be eliminated to a large extent by proper design of the filter cell and proper selection of the filtering medium. A dramatic result in the reduction of particles is illustrated in Fig. 7. It was found that by using a series of filtering media (two layers of a $0.2\text{ }\mu\text{m}$ FGL Millipore filter, topped with a $0.45\text{-}\mu\text{m}$ Flotronix membrane filter) the particles can be drastically reduced.

Table I shows the molecular weight averages of several narrow-MWD polystyrenes which are commonly used as calibration standard for GPC. Except for the Duke $7.1 \cdot 10^6$ sample, which is the highest-molecular-weight calibration standard we presently have, the weight average molecular weights (M_w) obtained by the normal gel permeation chromatograph (M_w calculated using a calibration curve but without

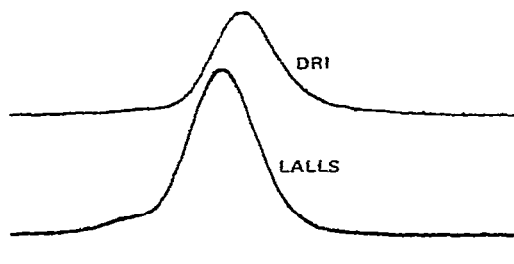


Fig. 7. LALLS chromatogram free of particles.

TABLE I

COMPARISON OF MOLECULAR-WEIGHT DISTRIBUTIONS OF POLYSTYRENE STANDARDS BY GPC AND GPC-LALLS METHODS

Sample	$M_w \times 10^{-5}$		$M_n \times 10^{-5}$		M_w/M_n	
	GPC	GPC-LALLS	GPC	GPC-LALLS	GPC	GPC-LALLS
Duke $7.1 \cdot 10^6$	69.0	73.0	54.3	70.6	1.28	1.03
PS- $1.8 \cdot 10^6$	17.4	16.0	7.94	15.4	2.19	1.04
PS- $670 \cdot 10^3$	6.10	6.05	5.01	5.64	1.22	1.07
PS- $411 \cdot 10^3$	4.46	4.30	3.80	4.17	1.17	1.03
PS- $179 \cdot 10^3$	1.81	1.76	1.62	1.67	1.12	1.06

band broadening correction) are all higher compared with the values obtained by the coupling of gel permeation chromatograph and LALLS photometer (direct calculation of molecular weight). On the other hand, the number average molecular weights (M_n) as obtained by GPC are in all cases lower than those obtained by GPC-LALLS. The net result is a higher M_w/M_n ratio for the GPC compared to that of the GPC-LALLS method. It is well known that for narrow-MWD polymers, the M_w/M_n calculated from GPC chromatograms and from a calibration curve without band broadening correction (reshaping of the chromatogram) give higher values than the actual. The question is: Does the M_w/M_n calculated directly from the concentration and LALLS chromatogram represents the actual M_w/M_n of the polymer. The answer to this question is, of course, negative. The M_w/M_n obtained by the GPC-LALLS method would be lower than the actual because the fractionating column does not have infinite resolution. That is, the GPC effluent is not composed of perfectly separated components but mixtures of different molecular weights, though their distribution may be narrow indeed. In fact, for a column with zero resolution (*i.e.*, columns packed with non-porous glass beads), a sample with a very broad distribution will have an M_w/M_n of unity via the GPC-LALLS method.

In this work, the fractionating column was composed of five columns with permeability limits extending from $6.5 \cdot 10^6$ to $5 \cdot 10^3$ Å (Waters Assoc. designation) with column efficiencies ranging from 800 to 1500 plates/ft. Hence, it was not surprising to have obtained M_w/M_n values close to the reported literature values of the polystyrene samples^{5,6}.

The odd result obtained for the Duke 7.1×10^6 polystyrene sample which gave a M_w by GPC lower than by the GPC-LALLS method could be explained by the uncertainty of the calibration curve for molecular weights higher than $7.2 \cdot 10^6$. The molecular weights of the fractions represented by retention volume earlier than the peak of the $7.1 \cdot 10^6$ sample were obtained by extrapolating the calibration curve beyond the retention volume of the highest molecular weight calibration standard. Since the calibration curve is logarithmic in molecular weight, a small error in the extrapolation can mean a large change in the value of the calculated M_w .

CONCLUDING REMARKS

It has been demonstrated that a light-scattering photometer coupled with a gel permeation chromatograph to measure light-scattering intensities of the chromato-

graph effluents in a continuous mode can be readily interfaced with a sensor based computer for on-line data acquisition and processing. In addition to the obvious advantages of laboratory automation, *i.e.*, elimination of the tedious and time-consuming manual data processing, high-precision data can be extracted from the chromatogram by signal-enhancement techniques. The presentation and formatting of the processed data is also much improved with the computer-assisted chromatograph.

The effluent from a gel permeation chromatograph has been shown to be unsuitable for light-scattering measurement (particularly if the light-scattering photometer has a relatively large scattering volume) without careful filtration. However, it has also been demonstrated that, with proper filter-cell design and a good choice of filter media, particles from the effluent can be effectively removed.

The MWD measurement of NBS polystyrene and other narrow-MWD polystyrene standards showed results that agree well with published results. The heterogeneity index (M_w/M_n) obtained by the GPC-LALLS technique appears to be slightly but consistently lower than those reported for the polystyrene standards. This has been attributed to the less than perfect resolution of the column.

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