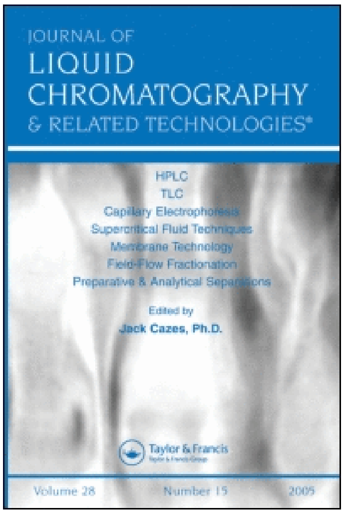

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STUDIES ON MOLECULAR WEIGHT DETERMINATION USING REFRACTIVE INDEX AND MULTI-ANGLE LASER LIGHT SCATTERING DETECTORS IN SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

The response of differential refractive index and light scattering detectors, used in size exclusion chromatography, depends on the concentration and mass of the polymer respectively. In order to evaluate the detection capabilities of these detectors, polystyrene sample SRM 706 and three commercial polybutadiene rubber samples were analyzed. Molecular weight data obtained by using these detectors have been compared in the present work.

INTRODUCTION

Size exclusion chromatography (SEC) is a rapid and convenient means to determine molecular weight (MW) and molecular weight distribution (MWD) of macromolecules[1,2]. A differential refractive index (DRI) detector, placed after SEC columns, is usually employed to detect the molecular species eluting according to their hydrodynamic size. A correlation between DRI detector response, which is proportional to the concentration of the eluting species, and MW of the unknown sample is required for deriving MW data from SEC. Universal calibration approach, suggested by Benoit *et al*[3], has been found to be applicable to a wide range of polymers[4-6] by using Mark-Houwink relation : $[\eta] = K\ M^{\alpha}$, where $[\eta]$ is the intrinsic viscosity, M is the viscosity average MW and K and α are the Mark-Houwink constants. To enable computation of MW of an unknown polymer, generally narrow MWD polystyrene (PS) standards are used on account of their easy commercial availability. But the knowledge of Mark-Houwink constants for PS and the unknown polymer for the same solvent system and operating conditions are required *a priori*. Since commercial availability of well characterized narrow dispersity polymers other than PS and values of the related Mark-Houwink constants in literature are limited, calibration of SEC columns is often complicated. A mass sensitive detector, connected to SEC system along with the conventional DRI detector, has been found to be helpful in addressing such problems[7].

In the present study, a broad MWD PS standard SRM 706 and three commercial polybutadiene rubber (PBR) samples have been evaluated for MW and MWD by SEC and SEC coupled with a multi-angle laser light scattering (SEC/MALLS) detector. A comparative study of the MW data obtained by using two different approaches has been presented in this work. MALLS detector has also been used as a stand-alone unit for determining weight-average MW of SRM 706 and the values obtained compared with those available in the literature.

EXPERIMENTAL

Materials

Narrow MWD PS calibration standards, ranging from 2K to 1800K, and SRM 706 were obtained from Pressure Chemical Co., U.S.A. and National Institute of Standards and Technology, U.S.A., respectively. PBR samples, received from three different commercial sources, consisting of > 96 % *cis*-1,4 content, were produced by using cobalt (#1 and #2) and nickel (#3) catalyst systems. HPLC grade toluene (S.D.Fine-chem, India) was used as mobile phase and solvent for polymers after filtering through 0.1 mμ filter (Alltech Associates, U.S.A.).

Sample Preparation

PS standards (0.1-0.2 % w/v, depending on MW), were dissolved in toluene and filtered through 0.45 mμ membrane filter (Millipore Corporation, U.S.A.) before injection. PBR solutions (0.3 % w/v) were filtered first through a Whatman No. 41 filter paper and later through 0.45 mμ membrane filter. The undissolved polymeric gel was discarded and a part of this solution was vacuum evaporated for calculating polymer concentration. The solution was appropriately diluted before injection.

SEC

Waters 590 solvent delivery system, U6K injector, R401 DRI detector and 745 B data module, equipped with GPC calibration module, were used for SEC measurements. A set of four Ultrastyragel columns (Waters Division of Millipore, U.S.A.) having nominal porosity 500, 10^3, 10^4 and 10^5 was used at a flow rate of 0.9 ml/min. The detector sensitivity and data module attenuation used were (⁻) 32 and 16 respectively. A 250 μl Hamilton syringe was used for injection. The values of Mark-Houwink constants, K and α for PBR in toluene at ambient temperature, taken as 5.19×10^{-4} dL/gm and 0.679 respectively for the present work[8], were also calculated by the procedure suggested by Houquiang *et al*[9] employing MW data at different retention volumes from SEC/MALLS measurements. Thus intrinsic viscosity [η] of the 12 narrow dispersity PS standards was calculated by using Mark-Houwink equation ($K=0.997\times10^{-4}$ dL/gm, $\alpha=0.73$ for PS in toluene)[10] and then universal calibration curve equation $\ln [\eta].M\ (= K.M^{\alpha+1})$ and RV was computed, where M is the peak MW and RV the corresponding retention volume.

SEC/MALLS

For light scattering measurements in flow mode, Waters liquid chromatograph ALC/GPC 150C and DAWN-F MALLS photometric detector (Wyatt Technology Corpn., U.S.A.) were connected together, as described schematically by Jackson *et al*[11]. A pulse dampener (Model: free Flow, Alltech Associates, U.S.A.) and 25 mm high pressure filter with 0.2 mμ membrane filter (Millipore Corporation, U.S.A.) were connected after pump for reducing its pulsations and on-line filtration of mobile phase. The column set and other chromatographic conditions were same as used for SEC measurements, except the DRI detector sensitivity and scale factor used were (⁻)64 and 10 respectively. The data manipulation and calculations were done using ASTRA software (supplied by Wyatt Technology Corpn., U.S.A.) with the help of an IBM AT computer and 80387-SX math co-processor, connected to a printer.

The calibration constant of MALLS was calculated by ASTRA while DRI detector constant,(dn/dV), was determined by sequentially injecting 200 µl of four solutions of 233K narrow MWD PS standard having different concentrations at a flow rate of 0.1 ml/min. As the PS solution passed through RI detector cell, the maximum voltage for each concentration was noted. DRI difference (dn) was calculated as concentration (gms/ml) times DRI increment for PS (taken as 0.11)[12]. The DRI detector calibration constant was given by the various values of slope (dn) versus voltage least-squares fit, obtained with the help of a computer programme. The DRI increment (dn/dc) for PBR in toluene at 632.8 nm and MW data were calculated using ASTRA[13].

MALLS

Weight-average MW of PS and PBR samples was determined by DAWN-F87 software (Wyatt Technology Corpn.) after disconnecting it from 150 C and replacing the flow cell with sample vials. The calibration was done by measuring the scattering intensity, I(θ), of toluene at 90°, given by the equation:

$$I(\theta) = \frac{V_{90} - V_{90,dark}}{V_L - V_{L,dark}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where V_{90} and V_L are the measured voltages for toluene at 90° and the laser monitor respectively while the subscript 'dark' indicates the corresponding voltage when the incident radiation source is turned off. The calibration constant A_{inst} is calculated as :

$$A_{inst} = \frac{R(\theta)}{2 \cdot I(\theta)} \qquad \ldots\ldots\ldots\ldots\ldots (2)$$

where $R(\theta)$ is the Rayleigh ratio[7] for toluene (taken as 1.406×10^{-5} for the present work)[13] at 632.8 nm, the wavelength of incident radiation. The 15 photo detectors of MALLS, at different fixed angles ranging from 26.57° to 128.66°, were normalized relative to 90° by measuring the scattering intensity of a 2 % solution of 25K narrow MWD PS standard in toluene, known to act as an isotropic scatterer for the incident radiation wavelength. The normalization coefficient of the ith detector, N_i, was calculated by DAWN-F87 according to the equation given below:

$$R(\theta)_i = N_i \cdot I(\theta)_i \qquad \text{...............} \quad (3)$$

where $R(\theta)_i$ and $I(\theta)_i$ are the normalized excess Rayleigh ratio and the scattering intensity respectively for the ith detector at angle θ; the normalization coefficient for 90° detector taken as unity.

The weight-average MW for a given sample was determined by measuring the scattering intensities of four solutions at different concentrations using DAWN-F87. Data manipulation and calculations were done using SKOR-F87 and AURORA (Wyatt Technology Corpn.) software using Zimm plot technique[14].

RESULTS AND DISCUSSION

The MW-retention time (RT) relationship from SEC measurement using 12 narrow MWD PS standards ranging from 2K to 1800K was found to be : log MW = 10.755 - 0.164 RT. The standard error of estimate and correlation coefficient were calculated as 0.056 and 0.998 respectively. The value of DRI constant for use in ASTRA was found to be 4.195×10^{-4} RIU per volt while the calibration constant A_{inst} for batch mode measurements was calculated as 2.14×10^{-5}. The MW averages for SRM 706 determined from SEC, MALLS and SEC/ MALLS alongwith literature data[15-21] are given in Table 1. The weight average MW obtained in the present study using different setups, and those available from literature are in excellent agreement, except that of Mori[15] who applied a concentration correction method to the results obtained by using 0.4% polymer solution. A comparative study of the cumulative MWD of SRM 706 by SEC and SEC/MALLS (Fig. 1) indicates that the low MW tail, consisting of about 12 % polymer, has a poor detector response from MALLS while rest of the data are slightly over estimated probably due to higher sensitivity of the light scattering detector. The smoothened data chromatograms obtained from DRI and at 9th detector of MALLS, which corresponds to 68.2° fixed angle and 66.34° scattering angle, are shown in Fig. 2. The MALLS response, being MW dependent, is different from concentration dependent DRI detector signal. Consequently, the low MW peak appearing at 30 ml elution volume in DRI chromatogram is not detected by MALLS.

The average value of DRI increment for the three samples, calculated by injecting known amounts of PBR using ASTRA[13], was found to be 0.040 which is close to the value obtained by direct measurement[22]. The SEC/MALLS chromatogram of a typical sample (light scattering signal at 90° fixed angle) is shown in Fig. 3. The SEC response is smooth while MALLS chromatogram shows similar multiple peaks in all the three samples, probably due to the presence of solute aggregates which remain unnoticed by DRI. The MW averages of these samples obtained by SEC and SEC/MALLS are given in Table 2. It is observed from the data that MW averages for PBR, obtained by mass detection technique, are higher as compared to SEC. Number-average and z-average MW values calculated from measured values of weight-

TABLE 1

Molecular Wight Data of Polystyrene Standard SRM 706

Method	$\bar{M}_w x10^{-5}$	$\bar{M}_n x10^{-5}$	$\bar{M}_z x10^{-5}$	$\bar{M}_w/\bar{M}_n$	Ref.
SEC	2.57	1.25	3.91	2.05	*
	2.85	1.37	—	2.08	15
	2.56	1.21	—	2.12	10
LALLS	2.60	—	—	—	16
	2.59	—	—	—	17
	2.58	—	—	2.10	NIST
MALLS	2.60	—	—	—	*
SEC/VIS	2.61	1.03	—	2.53	18
SEC/ LALLS	2.76	1.59	3.97	1.73	19
	2.63	1.49	4.17	1.77	20
SEC/ MALLS	2.63	1.72	4.04	1.53	21
	2.61	1.30	4.04	2.01	*

SEC = Size exclusion chromatography, LALLS = Low angle laser light scattering, MALLS = Multi-angle laser light scattering, SEC/LALLS = SEC coupled with LALLS detec tor, SEC/MALLS = SEC coupled with MALLS detector, SEC/VIS = SEC coupled with a viscometric detector, NIST = National Institute of Standards and Technology, U.S.A., $\bar{M}_w$ = Weight-average molecular weight, $\bar{M}_n$ = Number-average molecular weight, $\bar{M}_z$ = z-averge molecular weight, * = Present work

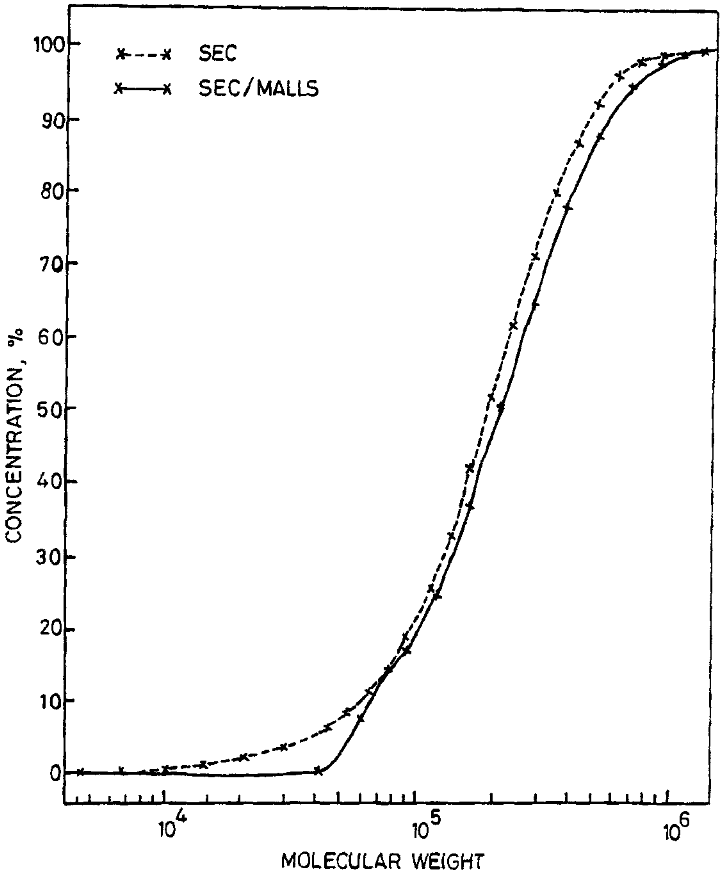

FIGURE 1 Cumulative molecular weight distribution of SRM 706

average MW, which is believed to be over estimated due to aggregation, are also higher. Since the MW averages for PS and PBR in the present study were determined using the same configuration, connecting tubes and operational parameters and the agreement for PS data obtained by SEC and SEC/MALLS being excellent, reasons for variation in PBR data were studied further. Thus the Mark-Houwink constants K and α for PBR used in the present study[8], were determined by the method suggested

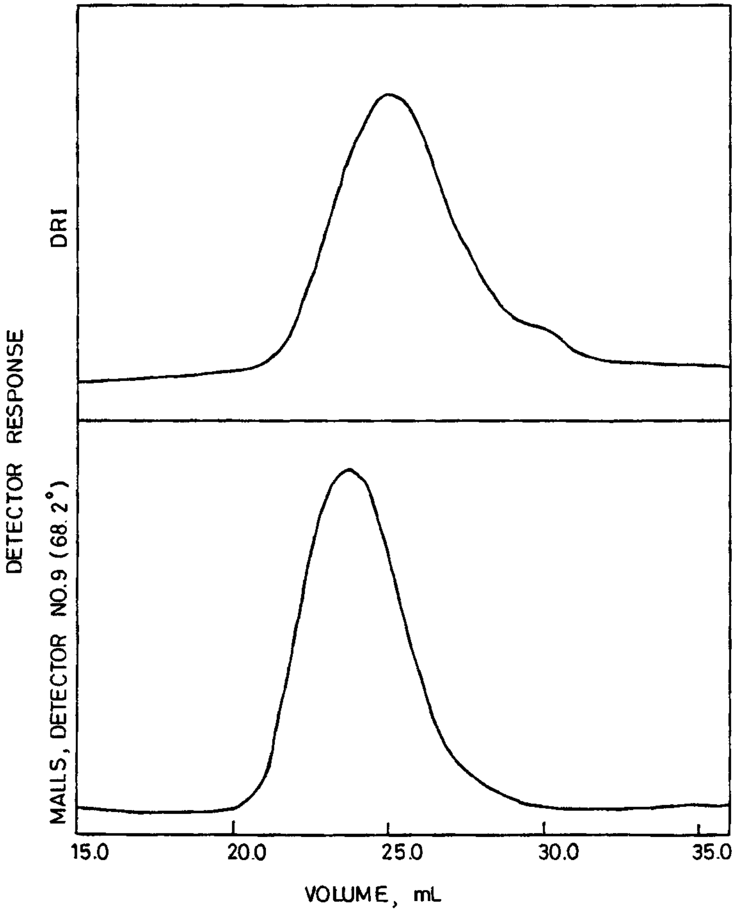

FIGURE 2 Smoothened data chromatograms from DRI and MALLS detectors

by Houquiang *et al*[9] using the weight-average MW data obtained by MALLS. The computed universal calibration curve was : $\ln [\eta].M = 34.828 - 0.728\ RV$, from which the values of intrinsic viscosity $[\eta]_i$ for the ith slice corresponding to the retention volume RV_i for a particular sample were calculated. The average values of K and α, calculated from

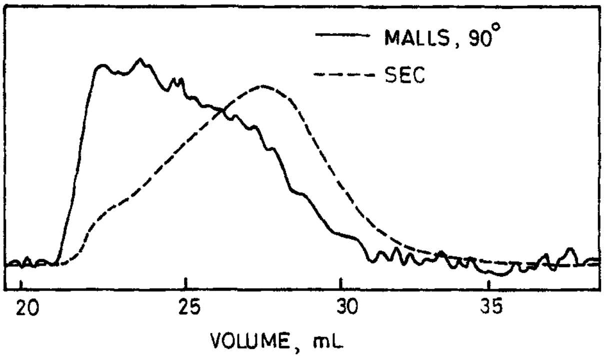

FIGURE 3 SEC/MALLS chromatogram for a typical PBR sample

TABLE 2

Molecular Weight Data on Polybutadiene Rubber Samples

Sample	#1		#2		#3	
Method	SEC	SEC/ MALLS	SEC	SEC/ MALLS	SEC	SEC/ MALLS
MW	$x10^{-5}$	$x10^{-5}$	$x10^{-5}$	$x10^{-5}$	$x10^{-5}$	$x10^{-5}$
$\bar{M}_w$	2.56	2.66	2.40	2.59	2.53	2.68
$\bar{M}_n$	0.88	1.07	0.76	1.10	0.80	1.00
$\bar{M}_z$	5.43	6.75	5.09	6.65	5.61	6.67

$\bar{M}_w$ = Weight-average molecular weight, $\bar{M}_n$ = Number-average molecular weight, $\bar{M}_z$ = z-average molecular weight the MW data of SEC/MALLS for the three samples, were found to be 4.96×10^{-4} dL/gm and 0.69 respectively which are close to the values used in the present work. The values of constants adopted for PS[10] in the present study were in the middle range of literature values covering a range of 2K to 900K. The values of these constants used for the present work were, therefore, found to be comparable and utility of SEC/MALLS in determining these values for unknown systems established. Ambler[8] has reported that nickel based catalysts produce predominantly linear PBR, cobalt based catalyst was considered to show similar behavior. The higher values of MW averages are, therefore, considered to be due to the presence of aggregates present in the solution.

CONCLUSIONS

The SEC/MALLS data for PS and PBR are found to be comparable with the results obtained by SEC and the setup provides a rapid means for determining the Mark-Houwink constants for the polymeric systems that follow the principle of universal calibration. It has also been found useful in calculating differential refractive index incremant for unknown polymeric systems, required for light scattering studies, from the mass of solute present in the peak.

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