

tential utility of *ortho*-carborane ligand for the synthesis of metal carbonyl carbene complexes. Further work concerned with the application of *ortho*-carborane ligand to the synthesis of other new types of metal carbonyl carbene complexes is currently underway in this laboratory.

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**Supplementary Material Available.** Tables of bond distances and bond angles, anisotropic thermal parameters, positional parameters for hydrogen atom; listings of observed and calculated structure factors. Supplementary materials are available from one of the authors (S. O. Kang) upon request.

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## On-Line Determination of $dn/dc$ for Size Exclusion Chromatography Coupled with a Light Scattering Detector

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The internal standard method, which is widely used in chromatographic techniques, is applied to the size exclusion chromatography for the on-line determination of  $dn/dc$  value of the sample. This method is found to provide the  $dn/dc$  value with suitable accuracy in determining the absolute molecular weight and the molecular weight distribution of the polymers when a light scattering detector is used.

### Introduction

Size exclusion chromatography (SEC) is perhaps the most frequently employed technique for the molecular weight de-

termination of macromolecules.<sup>1</sup> It utilizes the exclusion phenomenon of macromolecules from small pores when they pass through a series of columns packed with porous materials. Therefore the technique separates the macromolecules

in terms of their size in the solution of the carrier solvent not by their absolute molecular weight. Nonetheless, the technique has a number of advantages over many other characterization methods: it provides not only the average value but the distribution also, it is fast and relatively easy to use, and the required amount of sample is very small and so on.

With the development of light scattering detectors (LSD) it has become possible to determine the absolute molecular weight.<sup>2,3</sup> In order to obtain the absolute molecular weight, however, one needs an additional parameter, namely the specific refractive index increment ( $dn/dc$ ) of the polymer solution.<sup>4,5</sup> If the  $dn/dc$  value for the polymer/solvent system is not already compiled in the literature, it has to be measured independently and usually obtained by use of an off-line differential refractometer. However, this procedure requires an independent costly instrument and extra time, typically much longer than the SEC analysis itself. Also it needs a fair amount of sample usually far more than that for SEC/LSD analysis itself. Occasionally these shortcomings can be serious obstacles in using SEC/LSD.

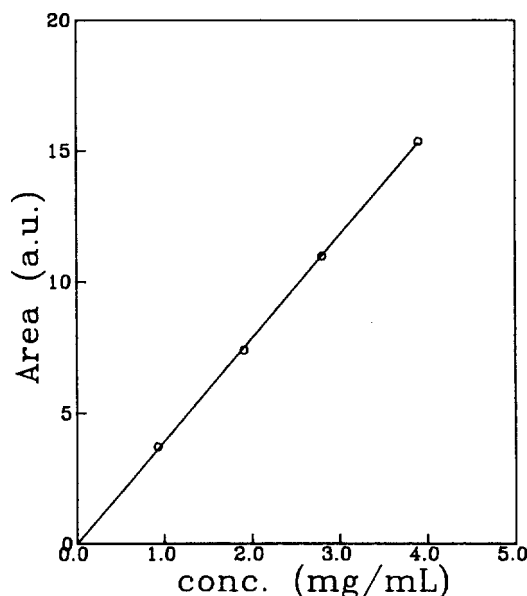
In this report, we would like to demonstrate the use of internal standard to measure the  $dn/dc$  value by use of a typical refractive index detector, which is one of the standard detectors for an HPLC system. Thus one can measure the absolute molecular weight and its distribution directly by on-line SEC/LSD analysis without a separate  $dn/dc$  measurement.

## Experimental

A small molecular weight polystyrene with narrow molecular weight distribution ( $M_w$ : 1,700) from Polymer Laboratories was used as the internal standard. A commercial polystyrene resin (GP150) and a poly(styrene-stat-acrylonitrile) (SAN350) were gifts from Miwon Petrochemical Co. A poly(dimethylsiloxane) (PDMS) resin was acquired from Lucky DC Silicone Co. Poly(styrene-stat-methyl methacrylate) (PS-co-PMMA) was synthesized in this laboratory by a typical solution radical polymerization method using toluene and AIBN as the solvent and the initiator, respectively. Tetrahydrofuran, chloroform and toluene were fractionally distilled before use with proper drying agent by reported procedures.<sup>6</sup>

The size exclusion chromatography system consists of an isocratic pump (LDC, Constametric 3200), an injector (Rheodyne 7125 equipped with a 100  $\mu$ L loop), 4 columns (Showa Denko KF series), a low angle laser light scattering (LALLS) detector (LDC, KMX 6), a refractive index (RI) detector (LDC, RefractoMonitor IV) and a UV/Visible absorption detector (LDC, Spectromonitor 3000). Solvents were degassed by a membrane degasser (LDC) and filtered by an in-line filter (Alltech) before it reaches the columns. Columns were put in an oven (FIATron, CH-460) and the oven temperature was kept at 25  $^{\circ}$ C.

The samples for the SEC analysis were prepared gravimetrically and converted to g/mL unit from the density of the materials. Flow rate of the solvent was kept at 1.0 mL/min for all the measurements. Off-line  $dn/dc$  values were measured by a differential refractometer at 633 nm wavelength (LDC, KMX 16) at 25  $^{\circ}$ C. All the data analyses were performed by the PCLALLS<sup>TM</sup> software provided by LDC with



**Figure 1.** Dependence of RI detector response peak area on the concentration of a commercial polystyrene (GP150). Injection volume of the polystyrene/THF solution was 100  $\mu$ L.

some necessary modification elaborated in the following section.

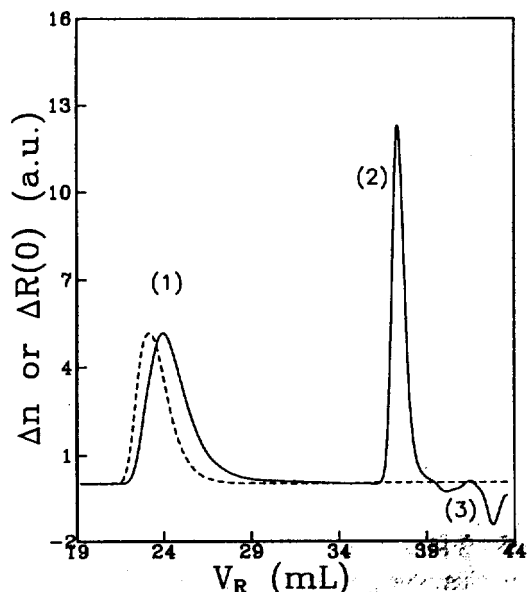
## Results and Discussion

In Figure 1 is shown the dependence of RI detector response, *i.e.*, integrated area of the detector signal, on the concentration of GP150 solution injected. The injection volume was kept constant as 100  $\mu$ L. The area of the detector response peak is proportional to the amount of the injected sample and the  $dn/dc$  value, *i.e.*,

$$A = K_{RI} \cdot (dn/dc) \cdot m \quad (1)$$

where  $A$  is the integrated area of the detector response,  $K_{RI}$  is the instrumental constant of the RI detector, and  $m$  is the mass of the injected sample, which is calculated from the concentration and the injection volume of the internal standard solution. There is no specific reason to use the small molecular weight polystyrene as the internal standard other than (1) it is eluted just before the solvent peak so that the overlap with the polymer peak of interest can be minimized; (2) it has a large  $dn/dc$  value to provide good sensitivity in RI detection; (3) it does not absorb light in the visible wavelength region. The transparency at the visible wavelength region is important to obtain a linear relationship between the elution peak area and  $dn/dc$  for this type of RI detector which uses a tungsten lamp light source. Any molecule which meets the above criteria can be used as a reasonable internal standard material.

A good linear relationship is evident in Figure 1. This linearity is not only a necessary condition for the reliable  $dn/dc$  measurement, but it is also required for the SEC/LALLS analysis itself since the method relies on the signal intensity of a concentration detector, such as RI or UV/Vis detector, to determine the concentration of the polymer eluted in each fraction. Then the molecular weight of the



**Figure 2.** RI (solid line) and LALLS (dashed line) chromatograms of GP 150 (1) and a standard polystyrene ( $M_w$ : 1,700) (2) mixture. The internal standard is eluted just before the solvent peak (3). The injection amount of GP150 and the internal standard were 16.8  $\mu\text{g}$  and 12.3  $\mu\text{g}$ , respectively. The injection volume was 100  $\mu\text{L}$ . From  $(dn/dc)_{std}$  of 0.192,  $A_{sam}/A_{std}$  of 1.32, and  $m_{std}/m_{sam}$  of 0.732,  $(dn/dc)_{sam}$  was calculated to be 0.186 according to Eq. (6).

polymer in the  $i$ th fraction  $M_i$  and the overall weight average molecular weight  $\langle M_w \rangle$  are calculated from the scattering intensity by the following formulae.

$$R_i = K_L \cdot (dn/dc)^2 \cdot c_i \cdot M_i \quad (2)$$

$$\langle M_w \rangle = \frac{\sum M_i \cdot c_i}{\sum c_i} \quad (3)$$

where  $K_L$  is the instrumental constant for LALLS detector, and  $R_i$  and  $c_i$  are the Rayleigh factor (normalized scattering intensity) and the polymer concentration in the  $i$ th fraction, respectively.

A typical SEC chromatogram of GP150 taken with an internal standard is displayed in Figure 2. The solid line represents the RI detector response while the dashed line is the output of the LALLS detector. In the RI chromatogram, two peaks of GP150 and the internal standard are completely separated down to the baseline and it is relatively easy to carry out the area integration for the polymer and the internal standard separately. The internal standard peak does not appear in the LALLS chromatogram since the molecular weight of the internal standard is low enough not to scatter a detectable amount of light. The  $dn/dc$  value of the polymer sample can be calculated from the injected amount of the polymer and the internal standard by Eq. (1) as follows.

$$A_{sam} = K_{RI} \cdot (dn/dc)_{sam} \cdot m_{sam} \quad (4)$$

$$A_{std} = K_{RI} \cdot (dn/dc)_{std} \cdot m_{std} \quad (5)$$

where the subscripts "sam" and "std" stand for the polymer sample and the internal standard, respectively. Then it follows that

**Table 1.** Comparison of  $dn/dc$  Values Obtained by On-line Internal Standard Method and Off-line Differential Refractometry

Sample	Solvent	$dn/dc$		Deviation (%)
		On-line	Off-line	
GP150	THF	0.186	0.192	-3.1
	Chloroform	0.157 $\pm$ 0.003*	0.159	-1.3
	Toluene	0.111	0.115	-3.5
PS-co-MMA	THF	0.140	0.142	-1.4
	Chloroform	0.109 $\pm$ 0.003*	0.109	0.0
SAN350	THF	0.163	0.169	-3.5
	Chloroform	0.135 $\pm$ 0.004*	0.132	2.2
PDMS	Toluene	-0.0930	-0.0958	2.9

\*The average and one standard deviation of 5 repeated measurements.

$$(dn/dc)_{sam} = (dn/dc)_{std} \cdot (A_{sam}/A_{std}) \cdot (m_{std}/m_{sam}) \quad (6)$$

Therefore, the instrumental constant  $K_{RI}$  is not required to obtain  $(dn/dc)_{sam}$  by this method while one has to have prior information of  $(dn/dc)_{std}$ ,  $m_{std}$ , and  $m_{sam}$ . The value of  $(dn/dc)_{std}$  needs to be measured by a differential refractometer separately, however, the value can be used repeatedly for the given solvent system once its value is compiled. The  $dn/dc$  values of the standard polystyrene in three popular SEC solvent systems, namely, THF,  $\text{CHCl}_3$ , and toluene, were determined by off-line differential refractometry as 0.192, 0.150, and 0.111, respectively. These values are in good agreement with the literature values.<sup>7</sup> The values of  $m_{std}$  and  $m_{sam}$  can be taken as the product of concentration and the injection volume. Or it can be calculated from another concentration detector response, for example a UV/Vis detector, if the calibration constant for the concentration detector is known. Either method was found to work well.

Another useful feature of the internal standard method can be inferred from Eq. (6). Since  $(dn/dc)_{sam}$  depends on the ratio of  $m_{std}/m_{sam}$ , the possible uncertainty in the injection volume is canceled out and the exact value of the injection volume is not necessary. Therefore in a fixed volume injector such as the Rheodyne type injector with the fixed volume loop, one only needs to know the concentration of the standard and the sample to be injected.

This feature can not be exploited fully for SEC/LALLS analysis, since the exact amount of the injected sample is also a necessary parameter to determine the  $c_i$  which is in turn necessary to calculate the molecular weight *via* Eqs. (2) and (3). However, it is certainly desirable to enhance the accuracy in determining the  $dn/dc$  value. Since the scattered light intensity is proportional to the square of  $dn/dc$  value, the error in the determination of  $dn/dc$  is doubled in the final molecular weight.

In Table 1 are listed the results of the on-line  $dn/dc$  determination of a few different polymers in comparison with the values measured by KMX 16. The deviation is at most ca. 3%. Although the precision appears not as good as the off-line measurement with an independent differential refractometer, it is clear that the internal standard method is quite acceptable for routine SEC/LALLS analysis and can be used

**Table 2.** Comparison of Weight Average Molecular Weights Determined by on-line Internal Standard SEC/LALLS and Batch LALLS

Sample	Solvent	$\bar{M}_w$ ( $\times 10^5$ )	
		SEC/LALLS	Batch LALLS
GP150	THF	250	253
	Chloroform	249	245
	Toluene	241	240
PS-co-MMA	THF	397	421
	Chloroform	401	404
SAN350	THF	103	100
	Chloroform	109	106
PDMS	Toluene	295	287

for a variety of polymer-solvent pairs with good reproducibility. A few % difference among the same polymer/solvent system can be easily found in the open literatures.<sup>4,7</sup>

At this point, the use of white light source in the RI detector has to be justified. As is well known,  $dn/dc$  value is a function of the wavelength, thus it has to be measured at the same wavelength as that of the light scattering detector. However, most of the RI detectors for HPLC system use a white light source, some modification of the instrument is necessary if one wishes to measure  $dn/dc$  with a monochromatic light source at a specific wavelength. We also first tried to use a wide band interference filter for this study, but soon found that it was not worth much. As can be found easily from the compiled  $dn/dc$  values in the literature, the ratio of  $dn/dc$  values at the different wavelengths is almost constant for most of different polymer/solvent systems.<sup>7</sup> Since we are dealing with  $(dn/dc)_{\text{sam}}/(dn/dc)_{\text{std}}$ , the wavelength dependence is largely canceled out. As a result, the use of white light source provides us more consistent results than the case of using an optical filter where the signal to noise ratio is aggravated due to the loss of light intensity. In any sense, however, we are not claiming that the use of a white light source is superior, but simply reporting that the internal standard method can be practical enough to provide acceptable results. Needless to say, it should be better to use an RI detector operating at the same wavelength as that of light scattering detector, if available.

Some characterization results of polymers including GP150 shown in Figure 2 are summarized in Table 2. In the table, the weight average molecular weights obtained by internal standard SEC/LALLS method are compared with the value determined by static light scattering (batch mode of LALLS with off-line  $dn/dc$  measurement). The agreement is surely satisfactory and can be taken as a successful proof of the applicability of the internal standard method.

This type of on-line  $dn/dc$  measurements have been attempted previously for SEC.<sup>8-12</sup> In the studies, however, the calibration method was used so that the instrumental constant,  $K_{RI}$  was measured separately and the  $dn/dc$  value was calculated from the peak area and the instrumental constant. As the authors pointed out in their report<sup>11,12</sup> and we also confirmed the problem separately, the instrumental constant is not reproducible with sufficient precision for a long

time span, thus it needs to be measured within a short period to ensure the accuracy of the measurement. However, it is a painstaking job and in this respect, we believe that internal standard method is superior to the calibration method.

In case of the analyses of relatively small molecular weight polymers, high molecular weight polymers can be used as internal standards instead. If the pre-mixing of the polymer sample with an internal standard is not desirable, a known amount of the standard material can be injected separately with some time difference. In this way, more variety of standard materials can be used at the cost of some characteristic advantages of internal standard. In fact, we learned recently that this method was implemented in a commercial SEC analysis software.<sup>13</sup> We found that this method was acceptable only if the injection volume was well defined and the injection time difference was not large.

In summary, we have shown that the internal standard method can be used with suitable accuracy and greater efficiency to measure  $dn/dc$  value of polymer samples without relying on independent off-line  $dn/dc$  measurement. This method is simple, fast, economical and does not require extra specimen for the off-line  $dn/dc$  measurement. The absolute value of average molecular weight and the distribution can be obtained through a single SEC run with quite acceptable precision.

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