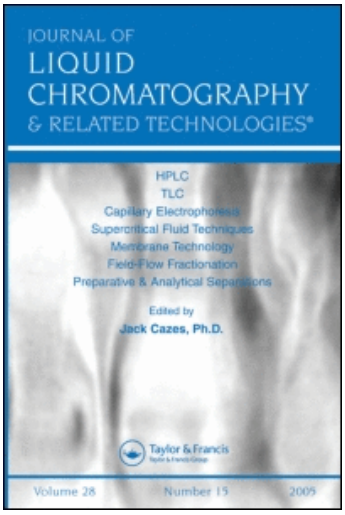

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Specific Refractive Index Increment Measurements on Macromolecules Using a Waters R401 Differential Refractometer

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To cite this Article Berkowitz, Steven A.(1983) 'Specific Refractive Index Increment Measurements on Macromolecules Using a Waters R401 Differential Refractometer', Journal of Liquid Chromatography & Related Technologies, 6: 8, 1359 — 1373
To link to this Article: DOI: 10.1080/01483918308064857
URL: http://dx.doi.org/10.1080/01483918308064857

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(8), 1359-1373 (1983)

SPECIFIC REFRACTIVE INDEX INCREMENT MEASUREMENTS ON MACROMOLECULES USING A WATERS R401 DIFFERENTIAL REFRACTOMETER

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ABSTRACT

An accurate and simple method for determining the specific refractive index increment, dn/dc, of synthetic polymers during size-exclusion chromatography has been developed using a modified Waters R401 differential refractive index (DRI) detector. The only modification required on the R401 involved the replacement of the standard white light source with a monochromatic source. The use of this instrument offers a number of advantages over more classical instruments and procedures normally employed in evaluating this parameter. The only requirement which must be met in order to successfully measure this parameter is the conservation of mass (in terms of the amount of polymer injected on to the columm and which passes through the DRI detector). However, in some cases, even this requirement may be relaxed. The application of this procedure will be demonstrated for several synthetic polymers in mixed and single solvent systems.

INTRODUCTION

Intensity light scattering measurements represent a classical technique for obtaining molecular weight and shape information on macromolecules (1). Due to several experimental difficulties associated with this technique, it has in general not been suitable for routine analytical work. Hence its use has been limited. However, with the development by

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Kaye and co-workers (2,3,4) of a low-angle laser light scattering instrument with several novel features which eliminate or minimized most of these difficulties intensity light scattering measurement has become more popular, especially in its application to the area of size-exclusion chromotography (SEC) (5). With this renewed interest in light scattering (LS) has come a need for determining the refractive index increment (dn/dc), a key parameter used in light scattering theory and one which must be determined when new classes of polymers or old polymers in new solvents systems are investigated. Classically this parameter has been measured on a differential refractometer such as the Brice-Phoenix, and more recently, the Chromatix KMX-16, which are dedicated instruments for determining small differences in refractive index in a static mode. In conducting LS measurements on a new class of wholly aromatic polymers, which are only soluble at room temperature in a very strong mixed solvent and whose solutions were stable for only a short time ($\approx$ 1 day), a formidable problem in measuring the specific refractive index increment at constant chemical potential, $(\partial n/\partial c)_{\mu}$, was encountered. This parameter, however, was successfully evaluated by employing a modified Waters R401 differential refractive index (DRI) detector. Although this instrument finds wide use as a universal concentration detector in SEC and liquid chromatography, we have found no published use of this instrument for determining accurate dn/dc values of macromolecules. In using this instrument, it has become apparent that it offered a number of advantages over other differential refractometers. Hence the subject of this paper is concerned with the evaluation of this instrument in measuring dn/dc.

MATERIALS

A narrow distribution sample of polytetrahydrofuran, PTHF, having a molecular weight of 31,700 was obtained from Altex Scientific, Inc. Narrow distribution samples of polymethylmethacrylate, PMMA, and polystyrene, PS, having molecular weights of 92,000 and 200,000 respectively were obtained from Pressure Chemical Company. Broad distribution (MWD≈2) samples of poly (ethylene terephthalate), PET, having weight average molecular weight of 42,000 and 65,000 were obtained from Celanese Corporation. Tetrahydrofuran (THF), methyl alcohol (MeOH) and toluene were obtained from Burdick and Jackson Laboratories, Inc., and used as received. Hexafluoroisopropanol (HFIP) was obtained from DuPont Company and distilled once before use.

METHODS

Light scattering measurements were conducted with a Chromatix KMX-6 light scattering photometer. Static measurements of dn/dc and the refractive index increment at constant chemical composition, $(\partial n/\partial c)_c$, were made at room temperature at wavelengths of 546nm and 633nm using a Brice-Phoenix and/or a Chromatix KMX-16 differential refractometer.

SEC was conducted using a Waters M-45 pump to deliver solvent, a Waters U6K injector to apply samples on to the SEC-columns, and a Waters R401 DRI detector to monitor elutant. The light source on the R401 was replaced with a Hg-Vapor or quartz iodine lamp which was coupled to the detector using fiber optics. The emission line at 546nm and radiation centered at 633nm were isolated with bandpass

interference filters. Columns used in this work were either Waters 60Å μ-Porasil or 100Å μ-Styragel columns (which separate low molecular weight compounds). On some occasions, a Waters E-linear μ-Bondgel column was used with a 60Å μ-Porasil column. SEC traces were recorded on a Waters 730 data module.

The use of low molecular weight SEC-columns, either by themselves or in conjunction with other SEC-columns, causes the bulk solvent bathing the polymer sample and the low molecular weight additives and contaminates present in the sample to be well separated from the polymer peak (see Figure 1). This occurs as a result of the partitioning effect between the small molecules and the much larger polymeric material during the passage of the injected material through the porous packing material. Hence the area of the polymer peak in Figure 1 is directly proportional to the polymer mass injected on to the columns. Areas computed from DRI detector traces recorded during SEC experiments are related to the following parameters:

$$\text{Area} = (\beta)(\text{DRF})(dn/dc) \qquad \text{Eq. 1}$$

where β is a constant equal to the following collection of experimental known parameters:

$$\beta = \left[\begin{array}{c}\text{chart}\\ \text{speed}\end{array}\right]\left[\begin{array}{c}\text{DRI}\\ \text{detector}\\ \text{gain}\end{array}\right]\left[\begin{array}{c}\text{volume}\\ \text{injected}\end{array}\right]\left[\begin{array}{c}\text{concentration}\\ \text{of sample}\end{array}\right]\left[\begin{array}{c}\text{solvent}\\ \text{flow rate}\end{array}\right]^{-1}$$

Eq. 2

If the detector response factor, DRF, is known, one can calculate the dn/dc value for the polymer in the mobile solvent during

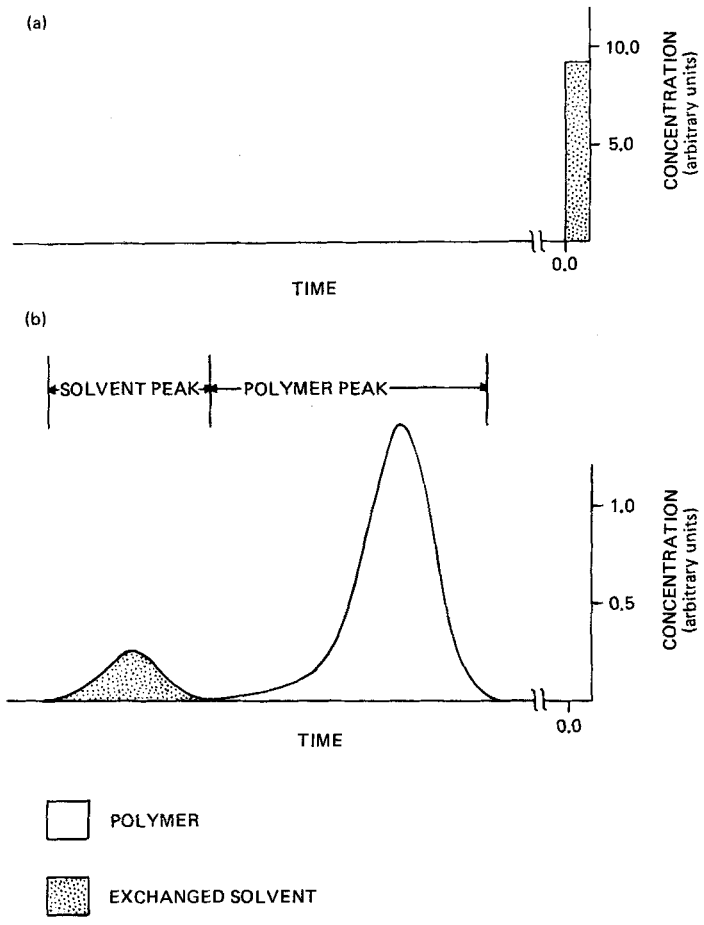

FIGURE 1. Hypothetical SEC-chromatograms of a polymer sample upon (a) injection and (b) elution.

SEC using Equation 1. Unfortunately, for a white light source, which is used in the standard Waters R401 DRI detector, DRF as well as dn/dc are both functions of wavelength, λ, (the former being a convolution problem of the intensity-wavelength spectrum of the light source and the response-wavelength spectrum of the detector). However, by using monochromatic light both DRF and

dn/dc become single value parameters permitting the use of Equation 1 to evaluate dn/dc by calibrating the DRI detector with a polymer having a known (k) dn/dc. Calibration is done by injecting an accurate volume and concentration of the polymer standard on a SEC-column bank. From several such injections, the average area is determined and combined with the known dn/dc and experimental terms contained within β. Similarly, a second equation can also be set up for the polymer sample having the unknown (unk) dn/dc. Since DRF is a constant for any given detector-light source combination and optical alignment, the combining of both equations eliminates the DRF term and on rearrangement yields the following equation for calculating the unknown dn/dc:

$$(dn/dc)_{unk} = \frac{(Area)_{unk}(\beta)_k}{(Area)_k(\beta)_{unk}} (dn/dc)_k \qquad \text{Eq. 3}$$

Since all the terms on the right side of Equation 3 are either measurable from data gathered or known, the unknown dn/dc can be evaluated. In performing the calibration, we have assumed conservation of mass (in terms of the amount of polymer injected on to the columns and which passes through the DRI detector), see Equation 2. It should be pointed out that the calibration procedure can be conducted in one solvent and dn/dc measurements conducted in another solvent (particular attention should be made in this situation to difference in flow rate between calibrating and experimental solvents).

RESULTS

The ability to accurately determine dn/dc values for macromolecules with the R401 detector during SEC was evaluated by com-

paring experimental values determined with this instrument to literature values. The polymers which we have chosen for this comparison work were PMMA and PTHF. The dn/dc value for both these polymers in THF at 546nm and 633nm were obtained from data listed by Huglin (6) and Chromatix (7). Actual experimental values determined with the R401 detector, using PS as our calibrating standard (the dn/dc values used for PS at 546nm and 633nm in THF were 0.194 (6) and 0.1845 (7) ml/g respectively), were found to be in good agreement with the literature values as shown in Table 1. A comparison of dn/dc values obtained for PET in HPIF using a Brice-Phoenix, KMX-16, and a R401 differential refractometers was also made. Date for this work is shown in Table 2. In this case, PMMA was used as the calibrating standard. The dn/dc value for PET in HFIP obtained with the R401 detector is in good agreement with the values determined using the other two instruments.

In situations where macromolecules are dissolved in a mixed solvent, preferential solution effects can seriously complicate dn/dc measurements (6,8,9). In this case, two important and different types of refractive index increments can be defined and measured. The first is measured at constant chemical composition, $(\partial n/\partial c)_c$. This simply involves measuring the refractive index difference between the mixed solvent and a solution made by the direct addition of the polymer sample to the mixed solvent. The second is measured at constant chemical potential, $(\partial n/\partial c)_\mu$. This requires the exhaustive dialysis of a polymer solution against the mixed solvent until thermodynamic equilibrium is attained. The refractive index difference between the dialyzed polymer solution and dialyzate is then determined. The difference

TABLE 1

Comparison of dn/dc Values Determined for PMMA and PTHF in THF with Literature Values.

Wavelength (nm)	dn/dc (ml/g)			
	PMMA		PTHF	
	Measured	Literature	Measured	Literature
633	0.084±0.002 *	0.083 **	———	———
546	0.087±0.005(4) §	0.0871±0.0001(2) †	0.063±0.004(4) †	0.063±0.001(2)

* Errors listed in this table are standard deviations.
** Data obtained from reference (7).
† The average value computed from data given in reference (6).
§ The number given within the parentheses represents the number of different samples used to calculate the mean.

TABLE 2

Comparison of dn/dc Determined on Several DRI Refractometers at 633nm for PMMA and PET in HFIP.

DRI Detectors	dn/dc (ml/g)	
	PMMA	PET
Brice-Phoenix	0.191	0.255
KMX-16	0.190	0.257
R401	———	0.259
Averages	0.191±0.001 *	0.257±0.004

* Errors listed in this table are standard deviation of the average value determined for each detector.

between these two refractive index increments for a binary solvent is given by the following relation:

$$(\partial n/\partial c)_\mu - (\partial n/\partial c)_c = \alpha\,(\partial n_o/\partial \phi) \qquad \text{Eq. 4}$$

Where α is the preferential absorption coefficient of solvent component 1 over the other solvent component, (this term is responsible for the difference in solvent composition near the macromolecule with respect to the bulk solvent) and $(\partial n_o/\partial \phi)$ is the change in the solvent refractive index with change in volume fraction of solvent component 1. Only in the case where either α is zero or all the solvent components have the same refractive index are these two refractive index increments the same. Hence, if the kinetics of exchange of the preferentially bound material on a polymer in a mixed solvent system is very rapid, in comparison to the length of time required for the polymer material to pass through the SEC column, the polymer sample which elutes from the column bank will be present in a solvent environment analogous to that which would be obtained by an exhaustive dialysis experiment. Hence the $\partial n/\partial c$ measured in this situation at any instant of time by the R401 detector would be equivalent to $(\partial n/\partial c)_\mu$ (assuming the pressure differentials between the column inlet and outlet are small or have little effect on $\partial n/\partial c$). This was experimentally verified by measuring both types of refractive index increments for a PS sample (NBS 705) of known molecular weight in a mixed solvent of 80% toluene and 20% methyl alcohol. The KMX-16 was used to determine $(\partial n/\partial c)_c$, and intensity light scattering measurements made during SEC experiments (see Figure 2) were used to determine $(\partial n/\partial c)_\mu$ by using the following equation:

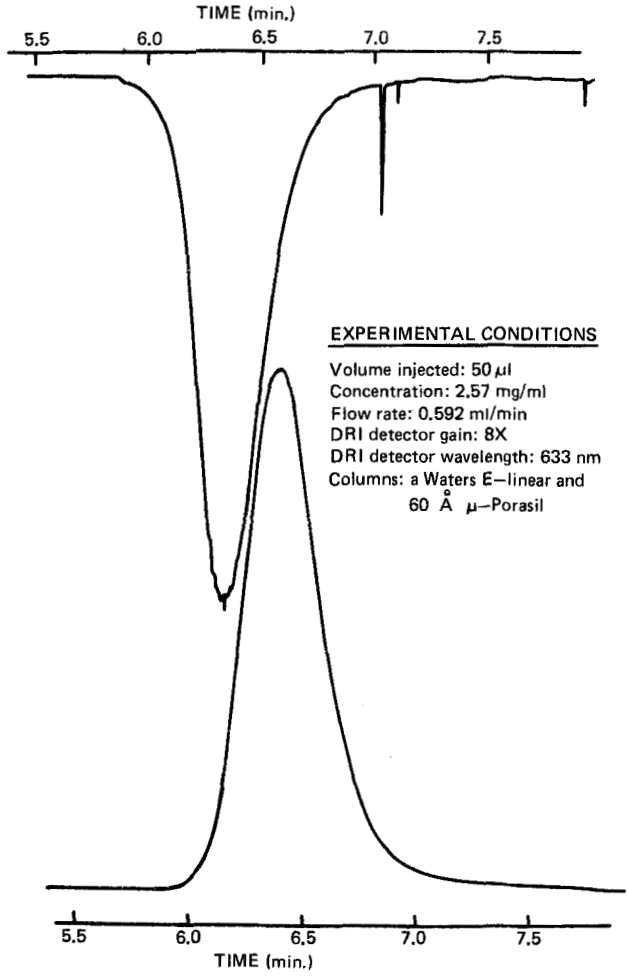

FIGURE 2. SEC-chromatograms of the NBS 705 polystyrene sample obtained from the modified R401 DRI detector (bottom) and the KMX-6 LS photometer (top) in toluene/methyl alcohol (80:20).

$$(\partial n/\partial c)_\mu = [\bar{R}_\theta/\bar{M}_w k'c]^{1/2} \qquad \text{Eq. 5}$$

where $\bar{R}_\theta$ is the excess Rayleigh ratio, $\bar{M}_w$ is the weight average molecular weight, K' is an optical constant, and c is concentration. The second and higher virial terms in equation 5

TABLE 3

$(\partial n/\partial c)_c$ and $(\partial n/\partial c)_\mu$ Values Determined for PS in Toluene: Methanol (80:20) at 633nm.

INSTRUMENT	KMX-16	LS	R401
Types of $(\partial n/\partial c)$	$(\partial n/\partial c)_c$	$(\partial n/\partial c)_\mu^*$	$(\partial n/\partial c)_\mu$
Experimental Value (ml/g)	0.150 ± 0.006 (3) † §	0.209 ± 0.009 (7)	0.207 ± 0.007 (3)

* The $\overline{M}_w$ determined at the National Bureau of Standards by LS was 179,300, and by sedimentation equilibrium was 189,800. Hence the average of 184,600 was used in Equation 4.

† Errors listed in this table are standard deviations.

§ The number given within the parentheses represents the number of different samples used to calculate the mean.

were neglected due to the dilute concentrations used and their low values. The refractive index increment determined with the R401 detector, using PS in THF as the calibrating standard, is shown in Table 3 to be in good agreement with the experimentally measured value for $(\partial n/\partial c)_\mu$.

DISCUSSION

In a review given by Huglin (6) on specific refractive index increment measurements, the Waters model R4 (which is now referred to as the model R401) DRI detector was mentioned as one of many commercially available differential refractometers capable of being used to evaluate dn/dc. However, the lack of monochromatic light and the mode in which the sample is introduced into the instrument were listed by this author as major drawbacks in using it in measuring dn/dc. In this paper, we have demonstrated the overlooked capability of this instrument to accurately deter-

mine dn/dc values of macromolecules during SEC. In so doing, we have realized that in comparison to other differential refractometers, this instrument offers the following advantages:

1. A very small amount of sample (microgram quantities) is required to conduct measurements; (this is especially important in dealing with biopolymers).

2. Baseline (or solvent) measurements are continuously monitored both before and after polymer material is eluted from the detector.

3. The amount of time required to perform measurements is very short, the procedure itself is very simple and readily automated using any automatic injector.

4. In performing LS measurements during SEC, both $\bar{M}_w$ and dn/dc values can be determined from the same experimental run.

5. The R401 detector is inexpensive in comparison to other differential refractometers. In addition it is not dedicated to static measurements, but can be used as a detector in liquid chromatography techniques.

6. This procedure allows $(\partial n/\partial c)_\mu$ and preferential solution effects to be evaluated in mixed solvent systems without the need to conduct lengthy dialysis experiments. This is particularly important in cases where chemically resistant membranes are not available and where polymer-solvent systems are stable for only a

short period of time. Although the concept of studying preferential solvation during SEC with a DRI detector has already been demonstrated by Berek et al (10) and Campos et al (11), the procedure outlined in this paper, which makes use of the polymer peak, differs in approach to that used by Berek et al, and Campos et al, which uses the solvent peak (see Figure 1). By using the polymer peak, we have eliminated potential problems discussed by Berek et al, and problems which might arise from the presence of contaminants in the sample solution (such as air, water, etc.) which would elute in the solvent peak region and lead to erroneous results.

Although the procedure in this paper offers the above advantages in determining dn/dc values, several important assumptions were necessary. This includes the following:

1. Conservation of mass is valid for the polymer-solvent-column resin system used.

2. Pressure effects on dn/dc are negligible.

3. Thermodynamic equilibrium between polymer and solvent components is reached before polymer is eluted from the column.

In the case where assumption "1" is in question or is known not to be valid the use of a second detector, such as a UV-Vis or IR spectrophotometer (if the macromolecule contains a chromophore and if the solvent is transparent at the appropriate spectral

regions), which is capable of giving concentration values from known extinction coefficients should permit the correct concentration to be determined. In fact, point by point dn/dc values across the chromatogram can be calculated. In the case where assumptions "2" and "3" are suspected, measurements conducted over a wide range of flow rates should allow the correct dn/dc value to be determined by extrapolation to conditions corresponding to zero flow rate. The extrapolated value would be equivalent to conditions of infinite time and zero pressure differential between the column inlet and outlet.

In conclusion, we have clearly demonstrated the ability of the Waters R401 detector to correctly determine dn/dc values for macromolecules. Although the accuracy of this procedure is 1-2% less than that capable from static measurements using standard differential refractometers, which is due mainly to the greater accuracy in the calibrating salt solution standards used for static type measurements in comparison to the calibrating polymer solutions used in this procedure, the advantages gained more than outweight this small loss in accuracy.

ACKNOWLEDGEMENTS

The author would like to thank Mrs. Rosemarie Peterson and Susan Williams and Ms. Geraldine Hogue for their technical asistance during the course of this work, and Dr. Paul Harget for his helpful comments on reviewing this manuscript.

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