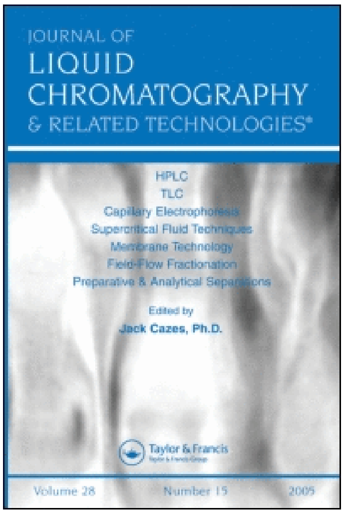

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The "SIZE" of Macromolecules and Some Observations on Their Mass

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THE "SIZE" OF MACROMOLECULES AND SOME OBSERVATIONS ON THEIR MASS

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ABSTRACT

The determination of the "radius of gyration" by light scattering (LS) techniques is reexamined in view of some misunderstandings of the concept presented in a recent paper. Light scattering measurements made over a broad range of scattering angles may be used to extract this mean square radius even though the initial slope of the scattering variation may be experimentally inaccessible. The underlying theory and measurement precision (which can result in erroneous conclusions) are examined and discussed. The combination of HPSEC/LS (with universal calibration) permits deduction of molecular size for certain linear polymers that would be inaccessible to light scattering alone. The measurements of number and weight average molecular weights by viscometry and LS are discussed in view of recent developments.

INTRODUCTION

A recent article by Yau and Rementer (1) claims that "... there appears to be a narrow window in which reliable r_g values can be obtained by SEC-MALLS [multiangle laser light scattering] technology. The lower limit is approximately 10 nm, which corresponds roughly to a MW value of 100,000 PS, and the upper value is about 30 nm corresponding to 500,000 PS. For $r_g > 30$ nm, one needs to know polymer conformation for accurate r_g determination, because particle scattering function of large particles is known to be highly dependent of particle morphology ... ." This statement is at variance with light scattering theory (2,3,4) whereby the "radius of gyration" may be derived from differential light scattering measurements. The purpose of this article is to examine critically this pronouncement by Yau and Rementer and point out how they arrived at their erroneous conclusions. In addition, it is important to explain how such problems may be avoided in the future. Other comments from the Yau-Rementer paper are analyzed and some general conclusions made concerning universal calibration and viscometric determinations. Goldwasser's (5) recent derivation concerning the deduction of number average molecular weights without the use of a concentration detector from measurements of relative viscosity is compared with Jackson's (6) similar formulation for the determination of weight average molecular weights from light scattering measurements (without a concentration detector).

The measurements reported by Yau and Rementer were made on 24 August 1989, as part of an in-house (Wyatt Technology Corporation) preliminary study of calculational procedures with the then-current EASI® software. They were never intended for publication. Indeed, had the authors indicated their intention to use these data as the basis for announcing the limitations of differential light scattering techniques, it may not have been necessary for this paper to have been written.

THEORY

The measurement of the variation of scattered light intensity from an ensemble of particles (molecules) as a function of scattering angle is sometimes referred to as DLS or differential light scattering (7) which comes from the physical concept of a differential scattering cross section measurement. These measurements are also sometimes referred to by the terms TILS (total intensity light scattering) or MALLS (multiangle laser light scattering). The former term is vague and conveys a concept of *integration* over all angles; the latter is misleading in that it implies that a laser light source is required, although such measurements were made many years before lasers were invented. The important concept conveyed by the term differential light scattering (DLS) is the angular *variation* of scattered light intensity. Recordings of these variations are referred to simply as DLS *patterns*. In recent years, however, DLS has been used to refer to dynamic light scattering (more precisely referred to as photon correlation spectroscopy). Accordingly, the simpler abbreviation LS will be used throughout this paper.

For the case of particles whose refractive index is almost the same as their surrounding media, the theory which describes their interaction with light may be greatly simplified by the introduction of the Rayleigh-Gans-Debye (RGD) approximation (4,8). Particles of mean radius R and refractive index n are said to satisfy this approximation theory *if and only if*

$$|n/n_0 - 1| << 1 \tag{1}$$

and

$$2kR\,|n/n_0 - 1| << 1\ , \tag{2}$$

where the refractive index of the media in which they are illuminated is n_0, $k = 2\pi n_0/\lambda_0$, and λ_0 is the wavelength of the incident light in vacuum. The RGD approximation puts no upper limit on R as long as both Eqs. (1) and (2) are satisfied. Macromolecules in solution are particularly well described by this approximation.

An impressive element of this approximation theory is the ability to extract a measure of the particle (polymer) mass-weighted mean square distance from the particle center of gravity by examining the angular variation of scattered intensity in the limit of very small scattering angle. For a given particle (molecule) of mass M, Guinier (9) showed that the angular variation of light scattered from it could be written in the form

$$P\ (\theta) = 1 - (2\ k \sin\ \theta/2)^2 \langle r_g^2 \rangle / 3 \tag{3}$$

independent of the configuration of the particle (molecule) so long as

$$(2\ k \sin\ \ \theta/2)^2 \langle r_g^2 \rangle \Big/ 3 << 1\ , \tag{4}$$

where

$$\langle r_g^2 \rangle = \frac{1}{M} \int_g r^2\, dm\ . \tag{5}$$

The symbol g refers to integration with respect to the particle center of gravity.

The quantity on the left-hand side of Eq. (5) [derived, for example, by Debye (2); see also Mijnlieff and Coumou (10)] is called the mean square radius of the particle with respect to its center of gravity. The integration is over each mass element *dm* measured with respect to and at a distance *r* from the molecule's center of gravity. The misnomer "radius of gyration" is commonly used, but such a kinematic term is conceptually irrelevant to the meaning of Eq. (5). In addition, the mean square radius is *not* the same as the hydrodynamic radius determined from viscometric or diffusion measurements. For certain *linear* molecules, there is sometimes a relation between the hydrodynamic radius and the root mean square radius, r_g (= $<r_g^2>^{1/2}$), such as presented by Yau and Rementer in their Eq. (16).

The Yau-Rementer Eq. (16) represents a combination of the Fox-Flory equation with the Ptitsyn-Eizner equation and relates the root mean square radius derived from LS with the hydrodynamic radius derived from viscosity. Certainly for the case of linear coiling molecules, this relation is valid. From viscosity, the hydrodynamic volume of a molecule is directly proportional to the product of its intrinsic viscosity and its molecular weight and from the Yau-Rementer stated relation, one should be able to calculate the light scattering root mean square radius. Thus for r_g sizes inaccessible to light scattering measurements, one should be able to use this procedure to calculate an r_g value. We shall return to this possibility later.

For macromolecules, the excess Rayleigh ratio $R(\theta)$ is measured over a range of scattering angles θ and concentrations c. From these data, the weight average molecular weight, M_w, the second virial coefficient, A_2, and the z-averaged (11) mean square radius are derived from the relation developed by Zimm (3)

$$\frac{R(\theta)}{K^*c} = M_wP(\theta)\left[1 - 2A_2M_wP(\theta)c\right] + \cdots + O\,(c^2) \tag{6}$$

The polymer concentration is c, in g/mL,

$$R(\theta) = f\left[I(\theta) - I_s\,(\theta)\right] / I_0, \tag{7}$$

and

$$K^* = 4\pi^2 (dn/dc)^2 n_0^2 / \left(N_A \ \lambda_0^4\right). \tag{8}$$

The vertically polarized, incident light intensity is I_0, $I_s(\theta)$ is the angular variation of the light scattered from the pure solvent, $I(\theta)$ the corresponding quantity for the solution of dissolved molecules, f is an absolute calibration constant, and N_A is Avogadro's number.

Equation (6) may be put into a form more linear in $\sin^2(\theta/2)$ by inverting it to yield

$$K^*c / R(\theta) = \frac{1}{M_w P(\theta)} + 2A_2c + O\left\{ [P(\theta) - 1]\, c^3 \right\}. \tag{9}$$

Following Debye (2), $P(\theta)$ may be represented as a simple power series in $\sin^2(\theta/2)$, *viz.*

$$P(\theta) = 1 - \alpha_1 \sin^2(\theta/2) + \alpha_2 \sin^4(\theta/2) - \alpha_3 \sin^6(\theta/2) + \cdots \tag{10}$$

where

$$\alpha_1 = (2k)^2 \left\langle r_g^2 \right\rangle / 3\,, \tag{11}$$

$$\alpha_2 = \frac{(2k)^4}{120\,M} \int_g r^4 \, dm\,, \tag{12}$$

etc. (See Ref. 10.)

Measurements at various angles and concentrations of the left-hand side of Eqs. (6) or (9) permit a least squares fit to the parameters on the right-hand side. Thus, in theory, the *angular* variation of the measurements of $R(\theta)$ permits the derivation of the various coefficients α_1, α_2, ... α_n implicit in $P(\theta)$ on the right-hand side of these equations, as well as M_w. The determination of A_2 requires measurement at more than one concentration. For high performance size-exclusion chromatography, HPSEC, the concentrations at the light scattering detector are generally very low and the second virial coefficient term often may be set equal to zero. This is certainly not required, however, since A_2 is rather easily determined (even as a function of M_w, if necessary) by making two measurements at two different concentrations. Once the coefficients α_1, α_2, ... have been obtained, the coefficient α_1 is used to extract the root mean square radius, or "size" of the molecule. The deduction of $<r_g^2>$ from α_1 has absolutely nothing to do with the conformation of the molecule as Guinier (9) and Debye (2) showed. Thus, as long as a value of α_1 may be derived, a value of $<r_g^2>$ may be calculated independent of the molecular shape. This important point appears to have been misconstrued by Yau and Rementer.

Since the Guinier relation, Eq. (3), is true for $(2k \sin\theta/2)^2 <r_g^2> / 3 << 1$, $<r_g^2>$ is found conventionally (following the method of Zimm) by measuring the slope of the angular variation at very small angles. For larger molecules, however, the smallest accessible angles may be too large to yield an accurate value of $<r_g^2>$, and the initial slope concept becomes unsatisfactory. Yet the right hand side of Eq. (6) or (9) is valid for all angles, so the accurate extraction of α_1 may permit the deduction of $<r_g^2>$ *even though the initial slope cannot be measured.* Ptitsyn (13) also pointed out this fact when he stated: "... If we expand *P(θ)* in a series with respect to *θ*, then, for particles of any shape the coefficient of the first term of the series following unity is equal to 1/3 of the mean square of the radius of gyration of the particle. ..." Yau and Rementer use their Fig. 24 erroneously in this regard. By assuming (i) that the conformation of the molecule is known and (ii) that light scattering data can only be fit to a *linear* form for a molecule of $r_g = 35$ nm, they conclude mistakenly that said light scattering measurements yield an r_g result 20% in error. Were the second term used as well, there would be no significant error. The purpose in expanding *P(θ)* in the series of Eq. (10) is to be able to extract the coefficients of the linear term and derive the proper $<r_g^2>$ *irrespective of the shape of the molecules.* Accurate measurements of very large $<r_g^2>$ values have been reported (12). Indeed, these confirmations of the theory are discussed at length in Kerker's text (8): "... the Rayleigh-Gans treatment of the light scattering data gave excellent results for radii up to at least 400 nm ..." (*cf*, p. 446).

But light scattering deductions of $<r_g^2>$ are not without their problems. As the molecular size falls far below about λ/40, the experimental uncertainties begin to overwhelm the precision of the values derived, and the average value of α_1 goes to zero (isotropic scatterers). Yet, statistically, a large set of measurements of α_1 will yield highly fluctuating individual values, some of which will be negative. There is nothing unphysical about this result. *It is exactly what is expected as the precision* *of the measurements diminishes.* Yau and Rementer have correctly stated a known fact that there is a lower limit to the molecular size derived only from light scattering measurements. However, precision can always be improved by

a) making more measurements,
b) measuring a sample at a higher concentration if concentration effects are negligible, and
c) using a shorter wavelength light source, a viable approach.

EXPERIMENTS

The data reported by Yau and Rementer were collected using ASTRA™ 1.14 software. A toluene solution containing one-tenth percent each of three narrow polystyrene standards (nominal molecular weights 47 K, 200 K, and 600 K) was prepared and injected using a 100 µl loop (0.1 mg mass of each standard). Three Ultrastyragel columns (Waters Chromatography Division, Milford, Massachusetts, two linear, one 1000 Å) were placed between the injector and DAWN-F unit which was followed by a Waters 410 RI detector. The DAWN unit used an SF 10 cell resulting in a detectable angular range of 12.4° to 152°. Data were reduced dropping the lowest, 12.4°, detector. The lowest detector angle was therefore about 20°. Yau and Rementer's Fig. 20 reveals that something was wrong with the separation. We shall return to this matter presently.

Because of the severity of the claims by Yau and Rementer, it was decided to remeasure the samples. In the event that there might be any lack of sensitivity due to particularly low concentrations, the injected solution contained 0.1%, 0.2%, and 0.3% of the 600 K, 200 K, and 47 K samples (rather than the earlier 0.1% value for each). The solvent was selected as THF (for greater LS response). The flow rate was 1.0 ml/min. and the injection loop was 100 µl. A K5 cell replaced the SF10 cell, resulting in the accessible angular range from 20° to 160°. A single PSS (Mainz, Germany) linear SDV 60 cm column was used. The new ASTRA 2.0 software was used for collection and further processing was done with the program EASI®. Measurements were made at a wavelength of 632.8 nm.

RESULTS AND DISCUSSION

Figure 1 presents a plot of the new data collected at all angles. The RI signal is shown in the background. Figure 2 contrasts the RI and 90° light scattering signals for the three peaks analyzed. Note that the RI signal is

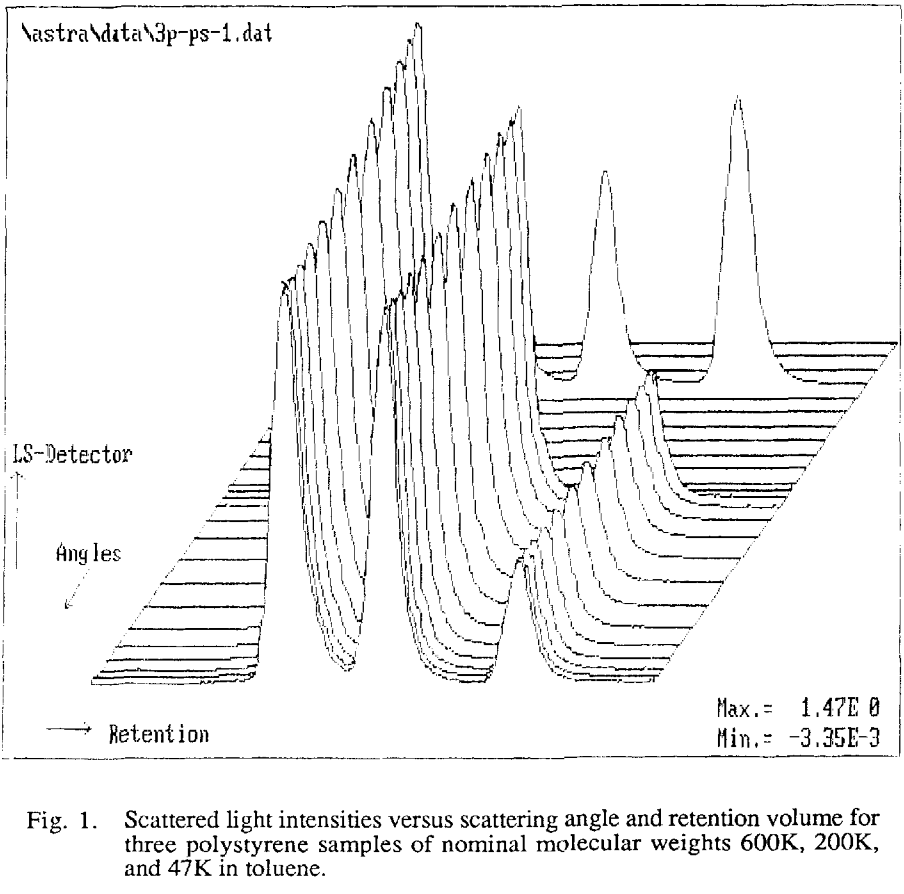

Fig. 1. Scattered light intensities versus scattering angle and retention volume for three polystyrene samples of nominal molecular weights 600K, 200K, and 47K in toluene.

proportional to the concentration, while the light scattering signal is proportional to the product of concentration and molecular weight. From the peak maxima to the minimum values selected (vertical bars), the concentration varies by a factor of several hundred. Thus, the precision of the mass values calculated could be affected by fluctuations of the calculated concentrations. Precision of the size determinations is very dependent on the signal-to-noise ratios measured which, in turn, depend critically on the concentration. Figure 3 shows the result of these calculations: the molecular weight is plotted (log scale) against elution volume. Note the two sets of curves: at the left are the data from these recent measurements

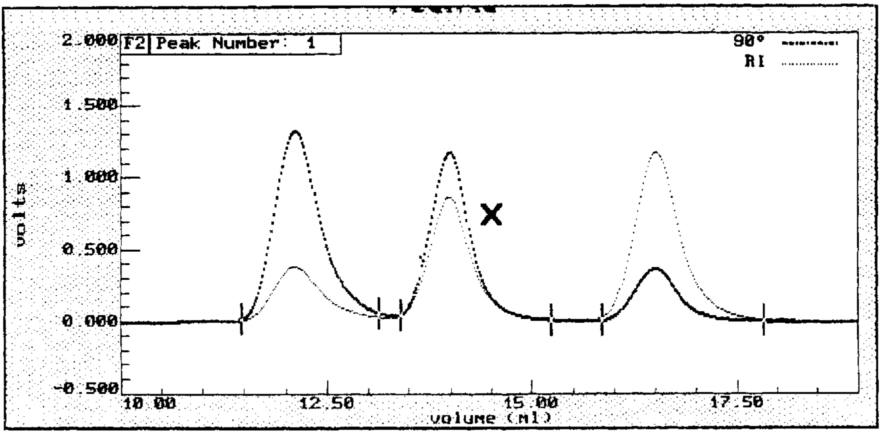

Fig. 2. Refractive index detector and 90° light scattering response with three peaks marked for samples of Fig. 1.

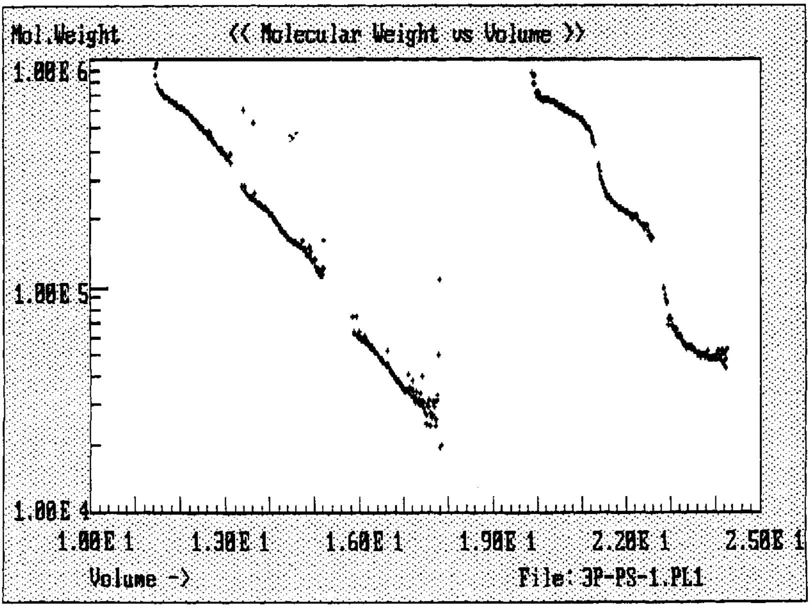

Fig. 3. Molecular weight as a function of elution volume: Data from Fig. 1 experiment at left, Yau-Rementer separations at right.

while a replot of the Yau-Rementer data (their Fig. 20) is shown at the right. Here is clear evidence of the *non-linear* and anomalous behavior of the columns used to collect the latter data. Unfortunately, these were the only columns available to perform the measurements at that time.

Despite the faulty columns and questionable separations achieved, Yau and Rementer continue their analysis by presenting a plot of log r_g versus log M in their Fig. 23 together with the comment: "... the slope of this so-called polymer conformation plot should equal ... the value of the exponent α in the r_g versus MW relation of Eq.20. The data in Fig. 23 provide α value of 0.43, which is obviously in error ... ." As noted earlier, as the resolution limits of LS are approached, the derived values of $<r_g^2>$ will fluctuate about zero for the 47K sample. There will be positive values and negative values whose average, over the range of slices measured, should correspond to the z-average square radius. The standard deviation of the slope determination increases as the slope approaches a mean value of zero. Once the standard deviation exceeds the value of r_g, no valid estimate of r_g can be made. A logarithmic plot of the root mean square radii versus the corresponding mass requires taking the square root of some negative values, and these result in some imaginary (though statistically significant) *r.m.s.* radius values. Since the logarithmic plot cannot include such points, they are automatically dropped by the software. Thus, the Yau-Rementer Fig. 23 shows three sets of points. Those corresponding to the 47 K fraction are shown to the left of the figure as a set of vertically stacked points. Many points corresponding to negative values of $<r_g^2>$ are missing and, therefore, the derived slope is erroneously calculated.

Figure 4 presents the Fig. 23 data re-plotted with the 47 K contribution dropped since the complete set of points cannot be used in the derivation of the conformation slope. Note that the 0.62 ± 0.02 slope (based on a concentration weighted least squares fit) agrees very well within the range of expected values. (One cannot properly analyze data on a logarithmic scale whose fluctuations include an appreciable fraction of imaginary values.) Also, plotting r_g distribution functions of molecules separated by HPSEC requires good separations.

Figure 5 is a similar plot of the data collected for this present paper. The slope 0.58 ± 0.02 at a 95% confidence level is within the expected range of values. Again, the data corresponding to polymer molecules whose root mean square radii are less than about 10 nm (with large statistical fluctuations) cannot be used with impunity for the derivation of conformational characterizations.

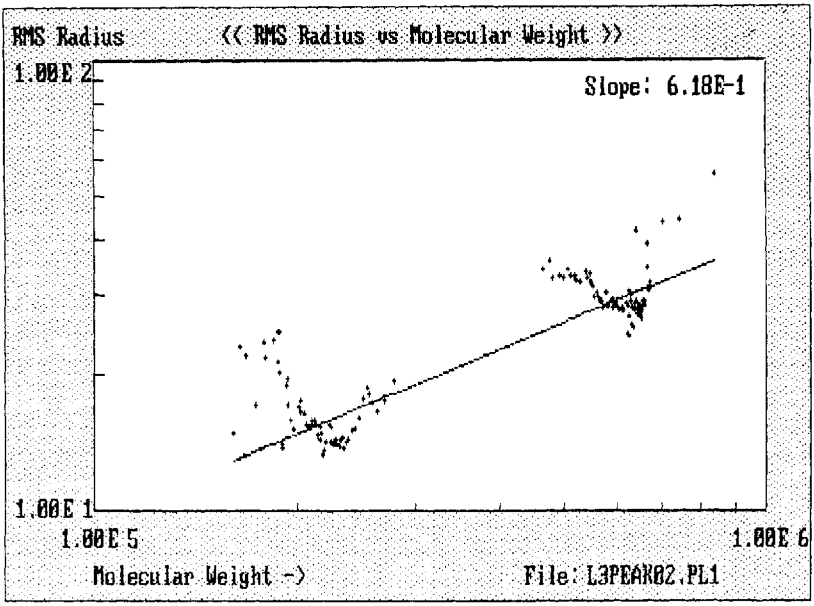

Fig. 4. Replot of Yau-Rementer Fig. 23 data with 47K data removed.

Although the use of Eqs. (6) or (9) to derive molecular weights from the light scattering data results in good precision even as the concentration becomes vanishingly small, the same cannot be said of $<r_g^2>$ deductions as indicated by Yau and Rementer (1). At the center of the peak, where the concentration is a maximum, the precision of the derived $<r_g^2>$ values is greatest. That this precision drops off rapidly as one reaches the baseline has been noted most recently by C. Johann (14) who showed that by considering peak values only, he could plot log r_g against log M_w results and obtain excellent conformational data over a very wide range of molecular weights and sizes. Figure 6 presents his results for both THF and toluene measurements of a variety of polystyrene standards. Note that the values of r_g he reports exceed the Yau-Rementer "limits" by more than a factor of 10.

Yau and Rementer are correct in ascribing increasingly less accurate $<r_g^2>$ values from light scattering measurements as molecular sizes approach the

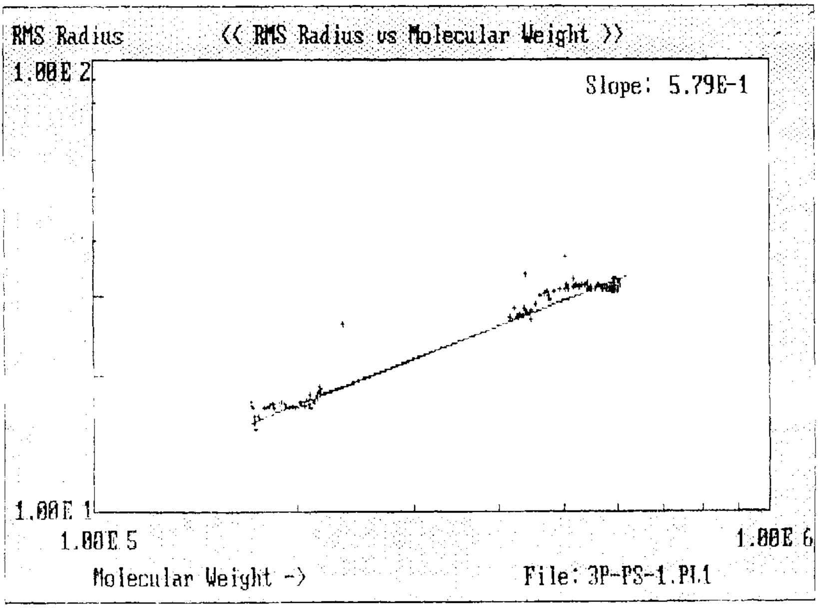

Fig. 5. Log r_g versus log M_w for values generated from the data of Fig. 1.

resolution limits of the experiments (affected by wavelength, measurement time, sample concentration, and column resolution). However, their belief that $<r_g^2>$ cannot be obtained from light scattering for $r_g > 30$ nm unless the molecular confirmation is known is simply not true. While there certainly is an upper limit to r_g derivable from light scattering, it is due to the stringent requirements of the RGD approximation [Eqs. (1) and (2)] rather than any dependence on particle morpholy. A reading of the papers previously cited by Debye (2), Zimm (3), Mijnlieff and Coumou (10), as well as Guinier (9) confirms this fact.

Yau and Rementer have demonstrated implicitly that plots such as shown in Figs. 4 and 5 (produced by current versions of EASI) may not be optimally calculated. The least squares analysis of log r_g vs. log M should certainly take into account the statistical fluctuations associated with each point. At present, the points are weighted by the concentration associated with each (M, r_g) pair. More sophisticated weighting procedures are presently under development that will use more extensive statistical weighting techniques when establishing a suitable fit.

One of the problems with all software packages is that, at times, anomalous results are produced that require awareness by the user of the physical basis for the anomaly. A case in point is the result presented by Yau and Rementer in their Table IV where they show a light scattering derived value of r_g for the 47 K sample that is *larger* than the value for the 228 K polymer! Yet the exemplar for the lower molecular weight shown in their Fig. 19 has a slightly negative slope which would result in an ascribed radius of zero. Yau and Rementer should have been somewhat more skeptical of the result, and reviewed the raw data which contained a few anomalously large values that, when averaged into the smaller values, distorted the final weighted values. These anomalous values may have occurred because, for some few slices, very large particulates / aggregates passed through the sample cell. These points should have been deleted and the data reprocessed. Analysis of the 47 K data shown in Fig. 1 (greater concentration than used in the Yau-Rementer experiments) yields a z-average r.m.s. radius of 6 ± 3 nm, in good agreement with the calculated result based on their Eq. (16).

UNIVERSAL CALIBRATION FILLS THE GAP

Implicit in the Yau-Rementer paper is an interesting conclusion. If one can measure (for a certain set of columns and chromatographic conditions) the exact elution volumes for a set of narrow distribution standards whose Mark-Houwink coefficients and molecular weights are known (the latter from light scattering), then a universal calibration (15) curve may be constructed *without need to measure the corresponding intrinsic viscosities.* (The latter may be calculated from the Mark-Houwink coefficients and the molecular weight.) The resulting universal calibration curve may be used therefore in conjunction with a LS measurement (plus a concentration detector) to read off the intrinsic viscosity for an unknown linear polymer and then calculate the root mean square radius (r_g) from the Fox-Flory relation and the Ptitsyn-Eizner formula (see Yau and Rementer, Fig. 13). The Mark-Houwink coefficients can be derived for an unknown linear polymer by measuring several sets of ($[\eta]$, M). For polystyrene in THF, for example, the Mark-Houwink coefficients K and a are 1.418×10^{-4} and 0.72, respectively, from Yau and Rementer's Fig. 13. Thus the universal calibration approach combined with LS will permit the derivation (for linear polymers) of r_g values where LS determinations of these quantities are unreliable because they are too close to the lower limits of detection. These measurements are easily performed without a viscometer: the LS results plus a universal calibration curve are all one needs. Thus light scattering and viscometry approach the Universal Calibration (UC) concept reciprocally. Whereas, the viscosity instruments measure intrinsic viscosity (IV) and derive molecular weights, the combination of light scattering with UC results in a derivation of IV from the light scattering determination of mass. As Yau and Rementer have pointed out, the light scattering results always "... give good estimates of the MW values ...". Vilenchik (16) has developed some techniques by which such "paper" viscometers may be used to extract Mark-Houwink coefficients of samples. He has shown how the Mark-Houwink coefficients may be derived directly from the analysis of light scattering data from a broad MW standard and from those how to derive the appropriate hydrodynamic radii.

Although the combined LS/universal calibration technique seems suitable to provide more precise r_g values (where light scattering alone begins to fail), there are some lower limits to its applicability. As Yau and Rementer have pointed out for the 47 K fraction of polystyrene, viscometry/universal calibration combined with the Fox-Flory and Ptitsyn-Eizner equations yield a reasonable value of r_g. However, as molecular weights fall much below 10 K or 20 K, it becomes more difficult to derive intrinsic viscosity values since the actual concentration must be determined, usually by means of a refractive index detector. Since the latter responds to changes in concentration inversely as *dn/dc*, and since *dn/dc* changes significantly below this range of molecular weight, one cannot derive molecular weights accurately from measurements of relative viscosity and concentration. Indeed, for the universal calibration curve presented in their Fig. 3, the lowest polystyrene standard molecular weight plotted is about 10 K. Universal calibration may fail as molecular weights fall much below about 10 K unless very well calibrated standards and detectors are available in this range. Analyses of highly branched polymers from universal calibration may be subject to even greater uncertainties.

NUMBER AND WEIGHT AVERAGE MOLECULAR WEIGHT FROM HPSEC/VISCOMETRY AND HPSEC/LS

Goldwasser (5) has recently shown that the number average molecular weight of a sample may be determined without a concentration detector. His final result as expressed in the Yau-Rementer formulation is:

$$M_n = \frac{sample\ amount}{v} \Big/ \Sigma\ (ln\ \ \eta_{rel} / hv)_i\,. \tag{13}$$

Equation (13) contains a factor $v \equiv v_i$, the volume eluted in each slice i (assumed equal at constant elution rate). This term was dropped (or set equal to 1) in the Yau-Rementer paper. The logarithm of the relative viscosity, η_{rel}, $ln\ \eta_{rel} = c_i\,[\eta]_i$ (at vanishingly low concentrations) where $[\eta]_i$ is the intrinsic viscosity at slice i. Since $(hv)_i = [\eta]_i M_i$ and $ln\ \eta_{rel} \approx c_i\,[\eta]_i$ at the usual SEC concentrations, the value read from the universal calibration curve is

$$M_n = \frac{\Sigma\, c_i}{\Sigma\,(c_i / M_i)}\,, \tag{14}$$

as has been shown. Thus, by measuring the relative viscosity $\eta_{rel\,i}$ at each slice i by means of an on-line viscometer and retrieving the $(hv)_i$ calibration curve, the number average molecular weight may be derived in principle, assuming that

1) a universal calibration curve may be established over the entire range of molecular weights and intrinsic viscosities for the unknown sample,
2) the total injected mass is known and recovered, and
3) the unknown polymer conforms to the universal calibration curve.

Assumption 1) requires a previous calibration procedure and therefore is an indirect measurement since the unknown polymer cannot be used to generate the universal calibration curve. The universal calibration curve must span the entire range of eluting volumes of the unknown.

Assumption 2) requires that the total elution peak be considered in the summation of Eq. (13). However, the only basis for including any values in the summation is that there is a detectable signal on the viscometer.

Assumption 3) is important since the unknown polymers generally should obey random coil behavior (*e.g.*, linear polystrene in tetrahydrofuran) though there are many exceptions.

The Goldwasser measurement requires only a viscometer so that η_{rel} may be measured during a HPSEC separation. The most important advantage of the

Goldwasser approach is the ability to determine M_n for copolymers and polymer blends by HPSEC/viscometry. In addition, this method avoids the tedious measurements by membrane and/or vapor pressure osmometry over a wide MW range. Such measurements of M_n cannot be performed using a "paper viscometer" with a LS measurement.

A parallel and equally important result was obtained by Jackson (6) who showed that the *weight* average molecular weight may be derived from light scattering, also without the need for a concentration detector. His result permits the accurate calculation of weight average molecular weights, especially for molecular weight distributions that have very high molecular weight fractions undetectable by typical RI detectors at the required low concentrations.

At each slice i, we begin with Eq. (9):

$$K^*c_i / R_i(\theta) \approx \frac{1}{M_iP(\theta)} + 2A_2c_i\,, \tag{15}$$

assuming that A_2 may be treated as a constant over the elution range of the sample. If A_2 is a function of molecular weight, it must be determined by a dual injection procedure. In general, for HPSEC, the value of A_2c will be very small in comparison to $1/M_i$. By analytical techniques discussed earlier, the value of $R_{i0} \equiv R_i(0)$ may be derived at each slice. Thus at $\theta = 0$, Eq. (15) yields

$$K^*\, c_i / R_{i0} = \frac{1}{M_i} + 2A_2\, c_i\,. \tag{16}$$

Rearranging yields

$$M_ic_i = R_{i0} \,\big/ (K^* - 2A_2R_{i0})\,. \tag{17}$$

But the definition of the weight average molecular weight is just

$$M_w = \Sigma\, M_i\, c_i / \Sigma\, c_i\,. \tag{18}$$

Combining (17) and (18) gives the final result

$$M_w = \frac{1}{\Sigma\, c_i} \sum \frac{R_{i0}}{K^* - 2\, A_2\, R_{i0}} \,. \tag{19}$$

However, $\Sigma\, c_i\, v_i = v\, \Sigma\, c_i, = W_T$ where $v_i \equiv v$ is the volume eluted during the collection of a slice and W_T is the total injected mass. (We assume, as before, that the collection is made at equal intervals, *i.e.* $v_i = v$.) Therefore,

$$M_w = \frac{v}{W_T} \sum \frac{R_{i0}}{K^* - 2\, A_2\, R_{i0}} \,. \tag{20}$$

In the limit of very small A_2R_{i0}, Eq. (20) simplifies to

$$M_w = \frac{v}{W_T\, K^*} \sum R_{i0} \,. \tag{21}$$

This result is independent of c_i and may be derived, therefore, without a concentration detector.

Equation (21) has only two requirements: that the total injected mass is recovered, and that K^* is constant over all slices. This requires that *dn/dc* be constant which is not generally true for heterogeneous copolymers, nor low M_w (<10 K) polymer species. On the other hand, for random copolymers, a weight averaged, single value of *dn/dc* will yield a reliable value of M_w over a broad range of copolymer species. The direct, absolute scattering result is valid and, of course, of great physical importance. Since high molecular weight components, even at very low concentrations, are easily detected by light scattering, Eq. (20) has a far broader range of applicability than any viscometric analogue. The viscometric result, Eq. (13), may have its major application to heterogeneous copolymers greater than 20 K daltons, whereas the light scattering result, Eq. (20), would be most valuable when applied to large linear and/or branched polymers. In addition, the light scattering result is absolute and requires no universal calibration. It is thus valid for species that do not fall on the universal calibration curve. (Both moments, M_n and M_w, however, are important for characterizing a polymer.)

The root mean square radius for most molecular solutions yielding measurable dissymmetry may be determined from LS independently of any *a priori* knowledge of *dn/dc* or *c* as long as the latter is small, which is generally true for

HPSEC measurements. The means by which such values are extracted will depend, of course, upon experimental conditions and the technique employed. If the variation of K^*c/R_θ is linear over a reasonable angular range, then the slope of the extrapolated curve will yield $<r_g^2>$. On the other hand, for more complex behavior, an analytical extraction of the coefficient α_1 of Eq. (10) will suffice.

It is important to note, however, that the weight average molecular weight, second virial coefficient, and *z*-average square mean radius may always be obtained without HPSEC separation using the classical method of Zimm (3). The significance of Eq. (20), however, is that for HPSEC separations that have large, unresolved fractions beyond the exclusion limits of the columns used, the weight average molecular weights may always be calculated for the complete injected sample even though only a fraction of it has a measurable distribution. A measurable distribution of molecular weights from light scattering combined with HPSEC requires both an RI signal (for concentration) and LS signals (for absolute molecular weights). The same is true for determining distributions from viscometric measurement though the extraction of such distributions requires the applicability of UC.

CONCLUDING REMARKS

There are other scientists who have felt that there may be some limit to the range of mean square radii extracted from light scattering measurements. Most importantly, Kratochvil in his text (17) *Classical Light Scattering from Polymer Solutions* expresses considerable misgivings about the "... curved angular dependencies... " of $K^*c/R(\theta)$ plots and how such curvature introduces "... a great deal of arbitrariness into the determination of the initial slope of a curve the shape of which is not known in advance. ..." Indeed, he stresses that "... the actual cause of the curvature in a particular case is only rarely known. ..." Eventually, he is left to conclude that there seems "... to be an interval of molar masses of polymers, approximately between 5×10^5 and 2×10^6 g/mol, in which the determination of both M_w and $<r_g^2>^{1/2}$ is most accurate. On either side of this interval, the relative experimental error increases. ..." Now, although this is quite different from Yau and Rementer's stricture, for a completely different reason, it does point to another apparent restriction at variance with the underlying Rayleigh-Gans-Debye approximation. The data of Johann shown in Fig. 6, for example, exceed these limits by more than an order of magnitude. Since Kratochvil did not seem to

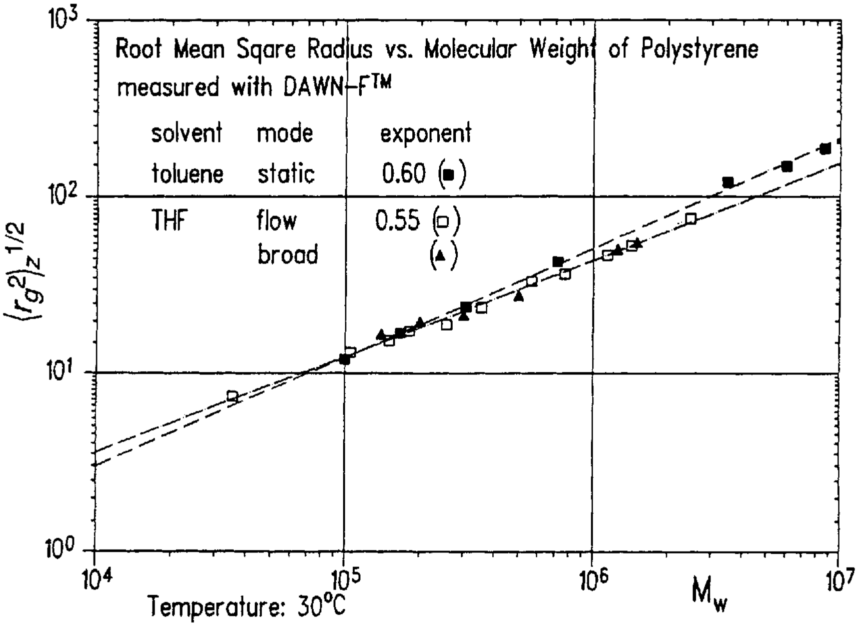

Fig. 6. Log r_g versus log M_w for various polystyrene standards from peak data measurements of Johann (14). HPSEC measurements made in both toluene and THF.

consider Debye's more general formulation, Eq. (10), and the possibility of extracting the coefficient of the term proportional to $\sin^2(\theta/2)$ by methods (10) that do not require direct measurement of the initial slope, we conclude that his statements are overly pessimistic.

But, just what is $<r_g^2>$? While $<r_g^2>$ is directly related to the average end-to-end distance of a Gaussian coil, even taking into account the hindered rotations (18) of the links, its relationship to the intramolecular distances becomes more complex when "volume effects" (interaction between links which become close to each other as well as between links and the molecules of the solvent) are included (13). For this reason, measurements aimed at deducing link sizes and intramolecular distances are often performed in two types of solvents: good solvents where volume effects are very important, and poor solvents (theta solvents) where Gaussian statistics are obeyed and molecular dimensions are smaller (13,19).

Although, for linear molecules, r_g seems to be proportional to the hydrodynamic radius, as has been discussed earlier in this and Yau and Rementer's paper, in *general such a relation would not be expected.* For example, for the case of branched polymers, the hydrodynamic radius derived from viscometry measurements, is not affected by branching to the same extent as the radius of gyration derived from light scattering (20). Although $<r_g^2>$ is a quantity whose dimensions correspond to an area, its physical meaning should be thought of in terms of a spatially weighted molecular mass distribution which depends critically on the solvent itself and the stiffness of the molecule. Thus, a molecule whose mass is confined to a homogeneous spherical shell of radius R, for example, and a molecule whose mass is distributed uniformly throughout a homogeneous sphere of radius R both have the *same* hydrodynamic radius and projected area. Yet, their $<r_g^2>$ values are considerably different; the former being equal to R^2, whereas the latter is 3/5 R^2. From a macromolecular point of view, it would seem obvious that the important parameter is $<r_g^2>$, not R^2. Preoccupation with the latter (via viscometry) deprives one of measuring and appreciating how macromolecules are put together, how they interact with the solvent, how they aggregate, and how they form the complex structures and new materials currently being developed. The mean square radius and the associated higher moments of the mass distribution, if they can be measured (for larger molecules), yield, therefore, details of mass distribution within the molecule and, thus, the *shape* of the molecules (2).

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