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Short Communication

Estimation of inter-detector lag in multi-detection gel permeation chromatography

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ABSTRACT

An analytical method for estimating the lag between concentration and molecular-mass-sensitive detectors has been developed. This method is simple in its approach and does not require any knowledge about the molecular mass distribution of the polymer sample used in the analysis. The elution behavior results obtained using the lag value from this method correlate well with those obtained from the traditional gel permeation chromatography peak calibration method.

INTRODUCTION

In recent years, the use of multiple detectors for gel permeation chromatography (GPC) has become more common. Probably the two most common detectors coupled with the traditional differential refractive index (DRI) detector are the differential viscometric (DV) detector and the light scattering (LS) detector. An advantage of coupling either or both of these detectors to the DRI (or some other concentration sensitive) detector is the ability to identify the molecular mass of a polymer in each elution volume increment. However, accurate determination of these absolute molecular masses requires a precise estimation of the inter-detector lag such that the concentration and LS or DV signals corresponding to any elution slice are correlated correctly. Several methods for estimating this lag have been reported in the literature [1–5] that are based on a variety of experimental techniques or analyses. We present here a different method for estimating the detector lag between a concentration detector and a second detector in which the signal is dependent on the molecular mass of a polymer sample. This straightforward method is analytical in its approach and does not require any knowledge about the molecular mass distribution (MWD) of the polymer sample used in the analysis.

THEORY

In GPC, the elution volume of a polymer molecule depends on its hydrodynamic size, not its mass. In most cases, the sample injected in GPC contains molecules of the same class, e.g., linear, star, comb, so that the sizes of molecular species increase as the molecular masses increase. Thus, in general, increasing elution volumes correlate with decreasing molecular masses

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(exceptions being non-homogeneous samples, such as those containing mixtures of both linear and branched polymer molecules). In a multi-detection GPC run, one signal (e.g., DRI) depends only upon the concentration of the solute molecules, while the molecular-mass-dependent signal (LS or DV) is a function of both the concentrations as well as the molecular masses of these eluting molecules. Therefore with increasing elution volumes, the molecular-mass-dependent signal will generally peak before the concentration signal because of the constantly decreasing contribution of the molecular masses of the eluting solute molecules. Consequently, the peaks corresponding to these two signals would be offset even if the two detectors were at the same position in the flow system. In reality, different detectors are at different positions in the flow system which leads to a lag between their signals.

Light scattering detector

If we consider the case of the LS detector, we have the signal measured from any elution slice as being proportional to the excess Rayleigh ratio, R_θ

$$R_\theta = Kc[MP(\theta) - 2A_2cM^2P^2(\theta)] \quad (1)$$

where c is the concentration of the polymer of molecular mass M in that elution slice, and A, is the corresponding second virial coefficient. P(B) = $1-2\mu^2$(rf) /3! + ... is commonly termed as the structure factor (where $\mu = (4\pi/\lambda)\sin(\theta/2)$, A being the wavelength of the incident light, and r_g the radius of gyration) and K is an optical constant.

In a GPC experiment, realistic upper estimates for the concentration are approximately 10^{-4}–10^{-5} g/ml at the peak of the concentration chromatogram, and generally A_2 = 10^{-3}–10^{-4} mol ml/g^2. As a result, the A_2 term in eqn. 1 can usually be neglected for GPC-LS calculations. Therefore, for our purposes, eqn. 1 can be written as:

$$R_\theta = KcMP(\theta) \quad (1a)$$

(although this simplification does not have an effect on the final outcome of our analysis).

For further simplifying the analysis, we would like to set P(B) ≈ 1. If $P(\theta) \neq 1$, then the analysis becomes slightly more complex since $P(\theta)$ is a function of r_g, and r_g, in turn, varies with the elution volume. For a low-angle laser light scattering (LALLS) detector, since θ is small, $P(\theta) \approx 1$ in any case. For a multiple-angle laser light scattering (MALLS) detector, P(8) ≠ 1 for higher-angle detectors unless $r_g \ll A$ *(i.e.,* the scattering molecules are small). However, it is preferable, if possible, to use LS signals from a higher-angle detector (such as 90°, for example) since they are less susceptible to noise due to extraneous scatterers. Therefore, when this analysis is applied to MALLS detectors, the preferred strategy is to use higher-angle LS signals from scatterers small enough such that $P(\theta) \approx 1$.

With the assumption of $P(\theta) \approx 1$, the dependence of the LS signal on the elution volume, v, can be written as:

$$\frac{dR_\theta}{dv} = \frac{\partial R_\theta}{\partial c}\cdot\frac{dc}{dv} + \frac{\partial R_\theta}{\partial M}\cdot\frac{dM}{dv} \quad (2)$$

From eqn. la, we obtain

$$\frac{\partial R_\theta}{\partial c} = KM \quad (3a)$$

and

$$\frac{\partial R_\theta}{\partial M} = Kc \quad (3b)$$

The elution dependence of polymer molecules from GPC columns can generally be written as an equation of the general form:

$$\log M = B - Dv \quad (4)$$

Hence,

$$\frac{dM}{dv} = -2.303D\cdot 10^{B-Dv} = -2.303DM \quad (5)$$

The concentration peak occurs at $dc/dv = 0$, while the LS peak occurs at $dR_\theta/dv = 0$.

From eqn. 2, we see that for $dR_\theta/dv = 0$,

$$\frac{\partial R_\theta}{\partial c}\cdot\frac{dc}{dv} = -\frac{\partial R_\theta}{\partial M}\cdot\frac{dM}{dv}$$

$$\Rightarrow KM\frac{dc}{dv} = -Kc\frac{dM}{dv} \quad (6)$$

Using eqn. 5, we rewrite the above condition as:

$$\left[\frac{dc}{dv}\right]_{dR_\theta/dv=0} = 2.303Dc \quad (7)$$

Since all quantities on the right-hand side of eqn. 7 are finite (even though small), we can conclude that under practical GPC conditions, $dc/dv \neq 0$ when $dR_\theta/dv = 0$ (unless the sample is perfectly monodisperse). Thus the peaks of the concentration and the LS chromatograms generally cannot be matched to get the detector lag since they both do not represent the same elution slice.

Given all this, in order to find the inter-detector lag, we need to find the elution slice on the concentration chromatogram that should correspond to a known slice on the LS chromatogram. We choose, as our reference slice, the peak of the LS signal. Referring to eqn. 7, we see that the elution slice corresponding to $dR_\theta/dv = 0$ is the slice on the concentration curve (at elution volume v^*) where

$$\frac{1}{c}\cdot\frac{dc}{dv} = 2.303D \quad (7a)$$

The quantity on the left-hand side is easily evaluated by fitting the peak portion of the concentration chromatogram with a smooth curve, and then calculating the fractional slope as a function of elution volume. The offset between the peaks of the concentration and the LS signals is given by $v_{\text{peak concentration}} - v^*$. The inter-detector lag then is the difference between the $v_{\text{peak LS}}$ and v^* for the data as *it is received by the collection instrument* (since $v_{\text{peak LS}}$ and v^* would coincide if there was no lag).

Once the concentration and LS chromatograms have been obtained for the sample being utilized in the lag estimation procedure, a value for D (the slope of the log molecular mass vs. elution volume curve for this polymer-column combination) is required. It is possible to determine the true value of D independently from a traditional GPC calibration procedure, such as the peak calibration method [6]; this true value of D should directly yield, in one step, the correct detector lag using eqn. 7a. However, the availability of the true value of D is not a requirement; some reasonable estimate of D can be used as an initial value in an iterative procedure. The first calculation yields a first estimate of the detector lag. At this point, the GPC-LS calculations should be carried out for the sample with this value of detector lag. These calculations will yield an estimated molecular mass vs. elution volume plot for this sample. The slope of this plot should be entered as the next estimate for D in a new iteration of the above procedure to yield another detector lag value and hence another apparent molecular mass vs. elution volume plot. These iterations should be carried out until the detector lag converges to a constant value. With this value, the slope of the molecular mass vs. elution volume plot is the true D for this polymer-column combination. The final convergence value does not depend on the initial estimate of D. It should be noted here that this iterative procedure is more cumbersome given the necessity of careful evaluations of D from the sample data.

Differential viscometry detector

In a viscometric detector, the signal being measured is the pressure drop of the fluid as it flows through a capillary tube. This pressure drop is proportional to the viscosity, η, of the fluid. For polymer solutions, the intrinsic viscosity, $[\eta]$, is evaluated by the equation:

$$\frac{\ln\left(\frac{\eta_s}{\eta_o}\right)}{c} = [\eta] + k''[\eta]^2 c \quad (8)$$

where the subscript "o" refers to the solvent, and "s" refers to solution containing the polymer at concentration c, k" is referred to as the Kraemer constant. For low concentrations (typical in GPC experiments), $k''[\eta]^2 c \ll [\eta]$. Therefore, the pressure drop measurement, p, of any elution slice allows the evaluation of the intrinsic viscosity of that slice through the relationship:

$$[\eta] = \frac{1}{c}\ln\frac{p}{p_o} \quad (9)$$

This intrinsic viscosity is dependent upon the polymer molecular mass as $[\eta] = K'M^\alpha$, where K' and α are the Mark-Houwink constants for a polymer. Substituting for $[\eta]$ from the Mark–Houwink equation and rearranging:

$$\ln\frac{p}{p_o} = cK'M^\alpha \quad (10)$$

Differentiating, we get

$$\frac{\mathrm{d}(\ln \frac{p}{p_o})}{\mathrm{d}v} = K'c\frac{\mathrm{d}(M^\alpha)}{\mathrm{d}v} + K'M^\alpha\frac{\mathrm{d}c}{\mathrm{d}v} \quad \text{(II)}$$

Substituting from eqn. 4 and setting $\mathrm{d}(\ln p/p_o)/\mathrm{d}v = 0$,

$$K'M^\alpha\frac{\mathrm{d}c}{\mathrm{d}v} = -K'c\frac{\mathrm{d}(10^{\alpha(B-Dv)})}{\mathrm{d}v}$$

$$= 2.303D\alpha K'c(10^{\alpha(B-Dv)}) \quad (12)$$

Thus the elution slice on the concentration curve corresponding to $\mathrm{d}(\ln p/p_o)/\mathrm{d}v = 0$ is given by:

$$\frac{1}{c}\cdot\frac{\mathrm{d}c}{\mathrm{d}v} = 2.303D\alpha \quad (13)$$

The detector lag can then be evaluated in a method similar to that mentioned in the *Light scattering detector* section. However, in this analysis, the constant α needs to be pre-determined in order to carry out the calculations. One could either use a polymer sample for which a is available in the literature, or use narrow standards of the polymer to determine α from intrinsic viscosity experiments.

EXPERIMENTAL

Experiments were carried out on the DAWN-F (Wyatt Technologies, Santa Barbara, CA, USA) MALLS detector (A = 6328 A) coupled to a 150-C (Waters, Milford, MA, USA) GPC system. The chromatography was carried out using a combination of Waters Ultrahydrogel 500 and 2000 columns, with a guard column, at 25°C. The mobile phase was water (containing 0.02% NaN_3 as a bacteriostat) with a flow-rate of 0.92 ml/min. The analyses were performed using LOTUS l-2-3 (Lotus, Cambridge, MA, USA) and the ASTRA program provided with the DAWN-F. Curve fitting was carried out with MICROSOFT EXCEL (Microsoft, Redmond, WA, USA). Polymers utilized in the studies were narrow-MWD polyethylene oxide (PEO) standards, molecular masses ranging approximately from 50 000 to 900000 (Toyo Soda, Japan), and a broad-MWD PEO sample, nominal molecular mass 200000 (Polysciences, Warrington, PA, USA). The detector lag estimation analyses were carried out on the DRI signals 'from narrow-MWD PEO samples of molecular mass less than 100000. The $v_{peak\ LS}$ for each sample was evaluated from the 90° LS detector signal. The resulting mean value of the detector lag was then used in subsequent molecular mass calculations with the GPC-LS data.

RESULTS AND DISCUSSION

The analyses on the low-molecular-mass polymer samples yielded an estimated detector lag of 0.124 ± 0.009 ml. The elution behavior of a number of different PEO samples was then obtained using the mean value of the detector lag in the molecular mass calculations. The elution behavior, thus obtained, for the broad- and narrow-MWD PEO samples overlapped, as they should. Fig. 1 shows the data for the elution dependence of the broad-MWD sample.

As pointed out elsewhere [2], the slope of the elution curve is sensitive to the inter-detector lag. Too high a value of detector lag leads to an artificially flat elution curve, while too low a value leads to too steep a curve. (This is especially noticeable for narrow-MWD samples, since changing the value of the detector lag leads to significant changes in the polymer concentration assigned to any elution slice. Calculations

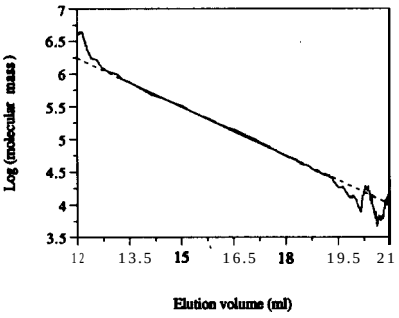

Fig. 1. Data showing elution behavior of broad-MWD PEO sample calculated from GPC-LS data with the detector lag value obtained by this method (rough line) and reference elution curve from traditional peak calibration method using narrow-MWD PEO samples (smooth line). The reference elution curve is extrapolated for clarity (dashed line).

for broad-MWD samples are generally less susceptible to this problem since their concentration chromatograms are spread out.) Therefore, we can use the slope of the elution curve as the parameter to ascertain the accuracy of our method. For this purpose, the absolute reference elution curve was obtained from a traditional GPC peak calibration method using only the DRI detector with narrow-MWD standards (of the same polymer as the samples used for the analysis). Shown in Fig. 1 is the elution dependence (linear over the evaluation range) corresponding to such a GPC peak calibration method using the narrow-MWD PEO standards. Examination of Fig. 1 shows that the elution curve obtained by using the estimated detector lag correlates well with the peak calibration data. Since the detector lag obtained by our analysis yields (from the GPC-LS data) an elution curve for a polymer-column combination that agrees with the reference curve, the value of the lag must be correct.

The methods described by Balke et *al.* [4] and Kuo et al. [5] determine the inter-detector lag (for GPC-LS and GPC-DV systems, respectively) by comparison with reference data obtained from calibration with narrow-MWD standards. In a sense, the philosophical underpinnings of our one-step method (i.e. utilizing the slope from a reference curve directly for the calculation of the inter-detector lag) are similar to these methods, the major difference being that they obtain the inter-detector lag through an empirical approach, while we use an analytical one. On the other hand, our iterative method, though not as simple, obviates the necessity of the reference data for obtaining the inter-detector lag.

Some points should be noted here: (1) It is easier to carry out the lag estimation with reasonably narrow-MWD samples since they provide high dc/dv values. However, broad-distribution samples are also utilizable, as long as the peak slices can be easily identified. If the iterative procedure is being followed, the intermediate estimates of D (from the sample data) must be evaluated carefully since the data range is small. (2) The calculation for the offset of the two peaks are carried out on the concentration curve. Generally concentration detectors have a less noisy signal than molecular-mass-sensitive detectors since the former are not affected by very small concentrations of large contaminants (mainly dust). Still, the calculations of the fractional slope must be accurate, since the accuracy of the method directly depends on this quantity. (3) If we use the D value from a previous iteration in the next iteration of the analysis, the effects of column dispersion do not bias our values. This is consistent with the fact that one cannot expect the detector lag to be a function of the column efficiency. (4) Since the method described here utilizes GPC columns in-line, both the concentration- and molecular-mass-dependent signals have lower noise by virtue of the columns eliminating most extraneous scatterers in the flow field (particularly important for aqueous systems) as well as suppressing the pressure fluctuations due to the pump. (5) This analysis assumes the elution curve to be linear over the elution volume range under consideration. Generally, this should hold, especially for narrow-MWD samples, but if this linearity condition does not hold, the analysis would need to be modified if it is to be utilized.

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