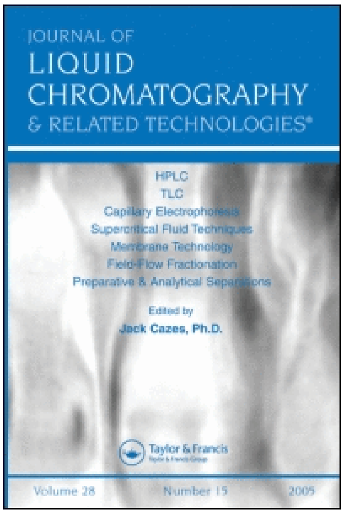

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UNIVERSAL CALIBRATION IN AQUEOUS SIZE EXCLUSION CHROMATOGRAPHY WITH ON-LINE DIFFERENTIAL VISCOMETRY USING COMMERCIAL TSK-PW COLUMNS

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ABSTRACT

Poly(ethylene oxide), poly(ethylene glycol), and poly(saccharide) exhibit universal calibration under aqueous SEC conditions with commercial TSK-PW columns using on-line, differential viscometry. The product of log(intrinsic viscosity * molecular weight) versus retention volume converges to a single curve for these polymers. Mobile-phase compositions of 0.1 N sodium nitrate and 20% aqueous acetonitrile at 35° C are used to examine this behavior. Mark-Houwink constants for these polymers are determined directly from on-line specific viscosity measurements.

INTRODUCTION

With the recent advent of differential viscometry detection for size exclusion chromatography (SEC), a variety of applications are described in the literature for polymer molecular weight characterization (1-4). Differential viscometry detection is based on the concept of universal calibration. Universal calibration requires as a prerequisite for molecular weight characterization, that in solution the polymer hydrodynamic volume (the product of intrinsic viscosity times molecular weight) determines polymer separation in SEC rather than molecular weight alone (5).

Haney and Armonas demonstrated universal calibration behavior using differential viscometry detection for a number of polymer/organic-solvent combinations (6). However, the behavior of water-soluble polymers under aqueous SEC conditions is less well defined (7). This is due to secondary effects such as hydrophobic interaction, ion exclusion, and intramolecular electrostatic repulsion. Mori recently reviewed the behavior of secondary effects of anionic sodium poly(styrene sulfonate) and pullulan, a poly(saccharide), on four types of commercial SEC columns (8).

Unlike organic-phase SEC methodology, a limited number of polymer standards and column supports are available for aqueous SEC work. Widely used commercial, monodisperse, water-soluble standards include poly(ethylene oxide), or PEO, poly(ethylene glycol), or PEG, both of which are slightly anionic (9), and nonionic poly(saccharide), or PSC, a polymaltotriose. Toyo Soda TSK-PW column supports have gained wide popularity for molecular weight characterization of synthetic, water-soluble polymers by aqueous SEC. These columns contain a hydrophilic polyether gel as the packing material (10,11). Recently, Waters/Millipore introduced their Ultrahydrogel line of columns for aqueous SEC which utilize a packing material based on hydroxylated poly(methylmethacrylate). TSK-PW columns, however, contain a higher level of residual carboxylic groups on the packing surface than Ultrahydrogel columns. This imparts a more hydrophilic character to the TSK-PW packings. Recent investigators have made the first attempts to examine universal calibration with Ultrahydrogel packings for aqueous SEC using single-capillary viscometers (12-14). Abnormal retention behavior of PEO was observed and it is not clear whether this is due to the less hydrophilic character of these packing materials. Earlier investigations of universal calibration for aqueous SEC, used off-line viscosity or literature data as the means to calculate intrinsic viscosity and hydrodynamic volume (8,10,11).

In this paper, we describe for the first time the conditions suitable for universal calibration with commercial TSK-PW columns using the on-line, Viscotek differential viscometry system. Expanding the use of differential viscometry detection to polymers analyzed by aqueous SEC requires this type of verification of universal calibration.

EXPERIMENTAL SECTION

The aqueous SEC work was carried out on a Waters 150C Gel Permeation Chromatograph (Waters/Millipore, Milford, Mass.), coupled to a Waters Model 410 differential refractometer and a Viscotek Model 100 differential viscometer (Viscotek Corporation, Porter, Tex.). A set of Toyo Soda TSK-PW columns of pore sizes 1000, 2000, 3000, 4000, and 5000 were used in series, each 7.5 mm ID x 30 cm (Toyo Soda Manufacturing Company). Data were acquired using Viscotek UCAL software (version 2.71) with an IBM-AT data system. The Narrow Distribution option was utilized for the calibration calculations. Two different mobile-phase compositions were employed for this study: 0.10 N $NaNO_3$ and 20% CH_3CN, vol/vol (Fisher Scientific Company, Pittsburgh, Pa.) in Milli-Q deionized water (Millipore Corporation, North Wales, Pa.). All SEC measurements were performed at 35^o C, with a measured flow rate of 0.92 ml/min. Injection volumes were 0.200 ml.

Six poly(ethylene glycol) standards, ranging from 975 to 17,500 molecular weight, six poly(ethylene glycol) standards, ranging from 43,500 to 800,000 molecular weight (American Polymer Standards Corp., Mentor, Ohio 44061), and eight poly(saccharide) standards, ranging from 5,800 to 853,000 molecular weight (Polymer Laboratories, Inc., Amherst, Mass. 01002), were used to construct the universal calibration curve. The molecular weights and polydispersities of the above standards are summarized in Table I. Poly(ethylene oxide) and poly(ethylene glycol) are both chemically identical with a repeat unit of $-(CH_2CH_2O)-$. Usually, the molecular weights greater than 20,000 are referred to as poly(ethylene oxide) and those less than 20,000 as poly(ethylene glycol).

TABLE I

POLYMER STANDARDS USED FOR UNIVERSAL CALIBRATION

(Suppliers indicated by code letter)

Type	Nominal Molecular Weight		M_w / M_n
Poly(ethylene oxide)	800,000	(A)	1.17
	580,000	(A)	1.04
	260,000	(A)	1.09
	145,000	(A)	1.07
	72,300	(A)	1.02
	43,500	(A)	1.05
Poly(ethylene glycol)	17,500	(A)	1.18
	10,730	(A)	1.04
	4,885	(A)	1.10
	2,500	(A)	1.08
	1,500	(A)	1.06
	975	(A)	1.09
Poly(saccharide)	853,000	(P)	1.14
	380,000	(P)	1.12
	186,000	(P)	1.13
	100,000	(P)	1.10
	48,000	(P)	1.09
	23,700	(P)	1.07
	12,200	(P)	1.06
	5,800	(P)	1.07

(A) American Polymer Standards Corp., Mentor, Ohio 44061
(P) Polymer Laboratories Inc., Amherst, Mass 01002

Concentrations of the standards used in the SEC analyses were approximately 0.40 mg/ml to 2.5 mg/ml, dependent on molecular weight. The poly(ethylene glycol) and poly(ethylene oxide) standards were prepared in the appropriate mobile phase by gentle agitation at room temperature. The poly(saccharide) standards required overnight swelling in the mobile phase, as suggested by the vendor. All samples were prefiltered through a 0.45 micron Millipore HV membrane prior to analysis.

RESULTS AND DISCUSSION

The Viscotek differential viscometer is based on a fluid analogue of a Wheatstone bridge, and has been described in detail elsewhere (1). When used as a viscosity detector with SEC, the differential viscometer measures specific viscosity as a function of molecular weight (1). The intrinsic viscosity, $[\eta]$, is defined as,

$$[\eta] = (\eta_p/c)_{c \rightarrow 0} \qquad [1]$$

where η_p is the specific viscosity and c is the concentration of the polymer solution as measured by the concentration detector, the differential refractometer. Since the differential viscometry detector measures specific viscosity in tandem with a differential refractometer, a universal calibration curve can be constructed: the product of log(intrinsic viscosity * molecular weight) versus elution volume. The universal calibration method originally described by Benoit (5) employs the hydrodynamic radius or volume, the product of $[\eta]$ times molecular weight, M, as the separation parameter. Thus, the calibration curves for any number of polymers will converge toward a single curve when plotted as $\log([\eta] * M)$ versus elution volume, V_r, rather than plotted the conventional way as log(M) versus V_r.

Concentration chromatograms from the differential refractometer and viscosity chromatograms from the differential viscometer are shown in Figures 1-A and 1-B, respectively, for PSC 48,000 and PEO 43,500 molecular weight. These data were acquired at 35^{o} C in a mobile phase of 0.10 N $NaNO_3$. These two standards, approximately equal in molecular weight, were chosen to illustrate two important features. First, although the PEO is 10% lower molecular weight than the PSC, it elutes earlier than the PSC standard. Second, its hydrodynamic volume is larger in solution than the PSC and is reflected in the viscosity chromatograms. PEO exhibits a significantly larger specific viscosity compared to PSC even though the sample concentrations are approximately equal. Data for these two standards are summarized in Table II.

From the measurement of specific viscosity with the differential viscometer, and the measurement of concentration and peak retention volume from the differential refractometer, a master universal calibration curve was constructed using the PEG, PEO, and PSC standards in a mobile phase of 0.10 N $NaNO_3$. This curve is seen in Figure 2. The plot of $\log([\eta]$

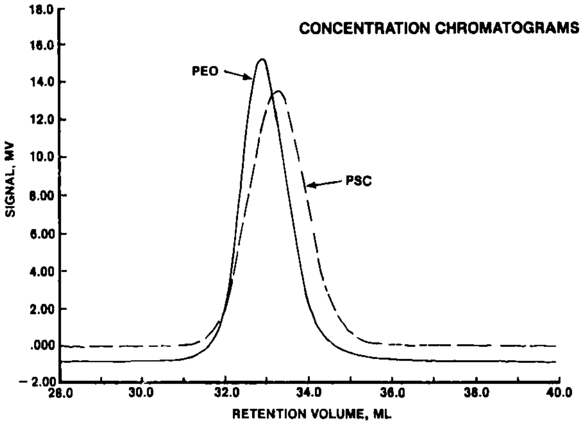

FIGURE 1-A. Differential Refractive Index (concentration) chromatograms for PEO 48,000 and PSC 43,500. Mobile phase is 0.10 N $NaNO_3$ at 35° C.

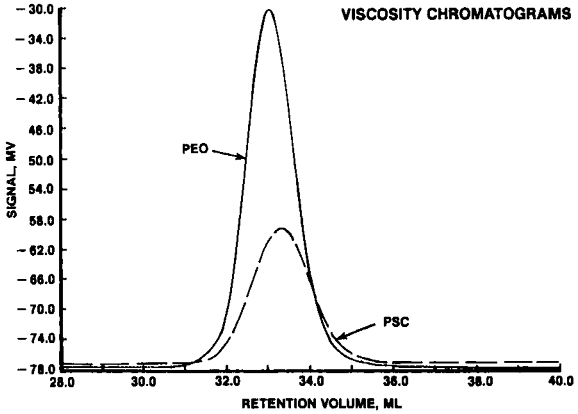

FIGURE 1-B. Differential viscosity chromatograms for PEO 48,000 and PSC 43,500. Same conditions as in Figure 1-A.

TABLE II

DATA FOR PSC 48,000 AND PEO 43,500 STANDARDS

	Concentration (mg/ml)	Retention Volume (ml)	[η] (dl/g)
PSC 48,000	0.530	33.29	0.270
PEO 43,500	0.540	32.93	0.578

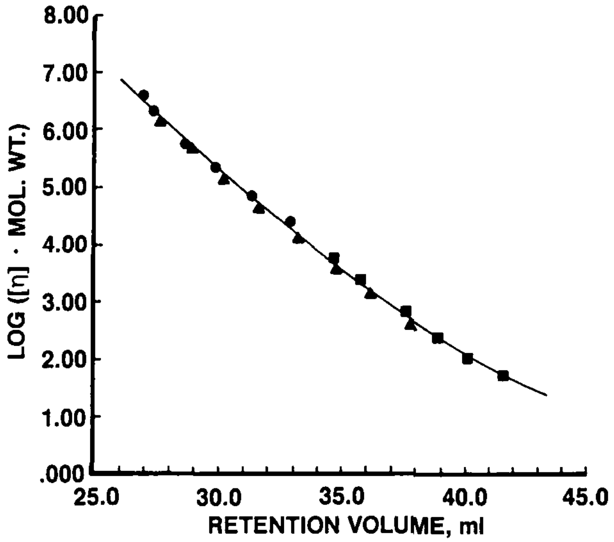

FIGURE 2. Universal calibration curve for PEG (■), PEO (●), and PSC (▲) in 0.10 N sodium nitrate at 35° C, using TSK-PW columns.

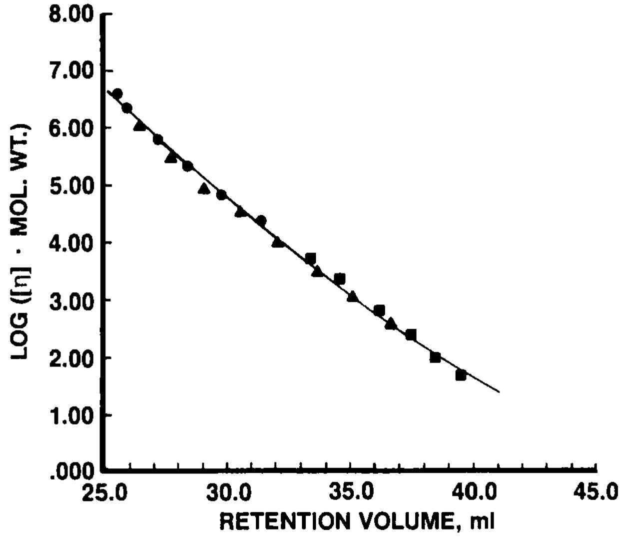

FIGURE 3. Universal calibration curve for PEG (■), PEO (●), and PSC (▲) in 20% aqueous acetonitrile at 35° C, using TSK-PW columns.

* M) versus retention volume shows that these polymers fall on a single calibration curve. The data show a small degree of scatter around the best fit curve (a third-order fit). The same data were acquired using the above standards with an aqueous mobile-phase of 20% CH_3CN. The universal calibration curve for this mobile phase is shown in Figure 3. The curve, however, is shifted slightly to lower retention volumes. This reflects the use of the nonelectrolyte mobile phase (20% CH_3CN) and/or a slight degree of swelling of the column support material by the acetonitrile. The TSK-PW column supports are a nonsilica, crosslinked polyether gel material which contains residual COOH groups (10,11). Pore sizes

range from 120 to 2000 angstroms, with plate counts of approximately 15,000 per meter. The TSK-PW supports exhibit more hydrophobic character than untreated and treated silica glass (8).

The importance of these results is that universal calibration is obeyed under these conditions. The absence, or at least the near absence, of nonexclusion effects occurs during the chromatography of these type of hydrophilic, water-soluble polymers. Even high molecular weight poly(ethylene oxide) and poly(saccharide) exhibited this response, in lieu of any adsorption effects. Most failures of universal calibration are normally due to the failure of the size exclusion mechanism (6,8). To our knowledge, this verification of universal calibration behavior has not been previously demonstrated with TSK-PW column supports using PEG, PEO, and PSC, and on-line differential viscometry detection.

Mark-Houwink constants for PEG, PEO, and PSC were obtained from the on-line, measured intrinsic viscosities of these standards, and by relating them with molecular weights. These values are summarized in Table III. Mark-Houwink plots of log[η] versus log(M) for 0.1 N $NaNO_3$ and 20% aqueous acetonitrile are shown in Figures 4 and 5, respectively. Although PEG and PEO are chemically equivalent, a distinct difference is observed in the slope of the curves for these polymers at approximately 10,000-20,000 molecular weight. This behavior has been observed for many polymers where the

TABLE III

MARK-HOUWINK CONSTANTS FOR PEG, PEO, AND PSC

	0.1 N NaNO$_3$ (35° C)		20% Aqueous CH$_3$CN (35° C)	
	a	K	a	K
PEG	0.58	1.01E-3	0.60	8.73E-3
PEO	0.70	3.47E-4	0.73	2.45E-4
PSC	0.66	2.14E-4	0.59	3.72E-4

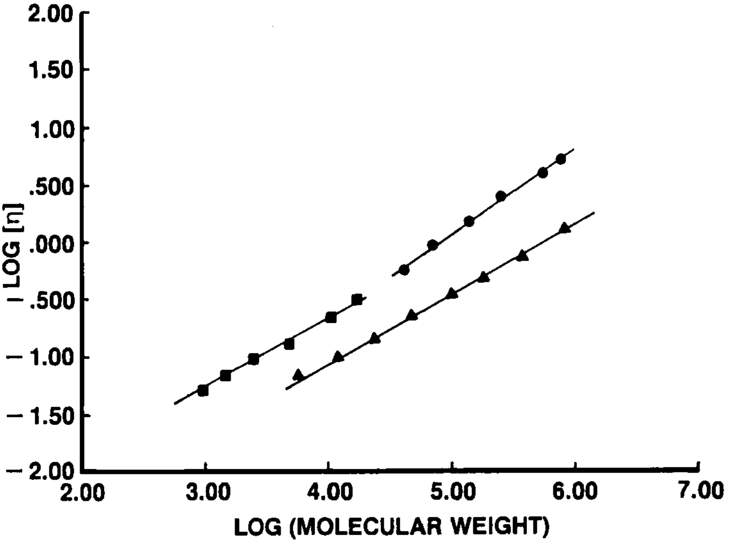

FIGURE 4. Mark-Houwink plots for PEG (■), PEO (●), and PSC (▲). Same conditions used as in Figure 2.

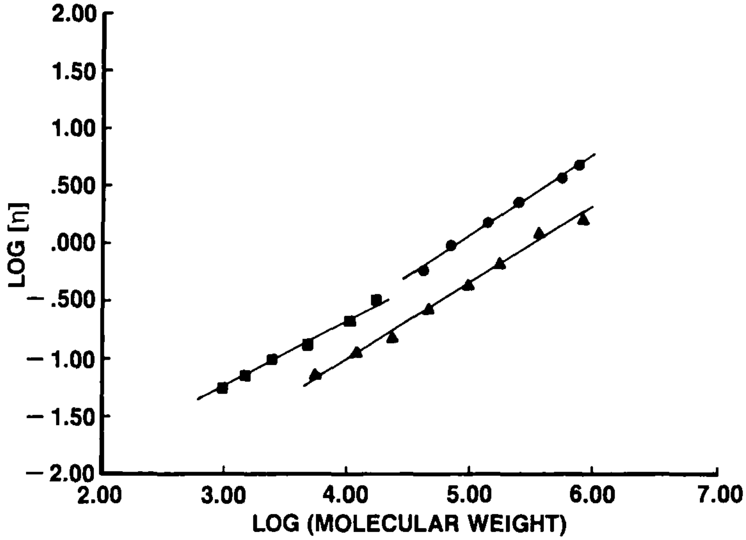

FIGURE 5. Mark-Houwink plots for PEG (■), PEO (●), and PSC (▲). Same conditions used as in Figure 3.

"a" value is near 0.5 or even lower for molecular weight less than 20,000. With PEO and PEG, this is observed only in some solvents such as dimethylformamide while absent in others such as benzene (15). However, this behavior was not exhibited with the PSC standards.

A broader study of universal calibration behavior for aqueous size exclusion chromatography remains for further investigation using these polymer standards and column supports with other ionic strength conditions and organic-modified, mobile-phase eluants. As more applications of this technique proliferate, characterization of absolute molecular weight distribution with on-line differential viscometry requires the necessary validation of this behavior.

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