

Universal Calibration Assessment in Aqueous Gel Permeation Chromatography

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SYNOPSIS

It was the objective of this paper to assess the applicability of the universal calibration method to aqueous GPC/SEC with nonionic and anionic polymers using the Viscotek differential viscosity detector. Three water-soluble polymers—polyacrylic acid, dextran, and polyethylene oxide—were chromatographed using four Ultrahydrogel™ columns with 0.3M NaCl and 0.1M KH₂PO₄ as the mobile phase adjusted to pH 7. Three distinct calibration curves were obtained. Upon addition of 10% methanol, a reasonably good universal calibration curve was obtained. However, quantitative analysis of the data exhibited about 5% deviation in average M_w and M_n for sodium polyacrylate as calculated from the single curve as opposed to about 40% when calculated from the composite curve. The applicability of three theoretical models for the universal calibration method was assessed, and a recommendation was made for future work. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

Although the concept of universal calibration in GPC is generally accepted for randomly coiled macromolecules in nonaqueous systems, some questions remain on its applicability in aqueous systems.^{1,2} Dubin and Principi³ described the failure of universal calibration for aqueous size exclusion chromatography (SEC) of rodlike vs. random coil macromolecules, although Dawkins and Hemming⁴ illustrate the validity of universal calibration for rigid rod and random coil polymers using poly(γ -benzyl-L-glutamate) and polystyrene in *N,N*-dimethyl acetamide. For nonionic and ionic randomly coiled macromolecules in aqueous systems, the rod/coil question is not an issue, but secondary variables, which include ion-exclusion and hydrophobic interactions between the polymer solute and the stationary phase, can enter the chromatographic process.⁵

Nagy et al.⁵ reported on the sensitivity of the universal calibration relation of poly(2-vinyl pyridine) to the concentration of sodium nitrate in the 0.01–

0.5N concentration range on Synchrom Catsec™ columns. Dawkins et al.⁶ reported that universal calibration was obtained for poly(ethylene oxide) (PEO), poly(ethylene glycol), and pullulan polysaccharide at high molecular weights, but at low molecular weights, the pullulan diverged and eluted later when using macroporous cross-linked polyacrylamide with an aqueous mobile phase. Lesec and Volet,⁷ using Ultrahydrogel™ columns from Waters Associates and Shodex OH-Pak™ from Showa Denko, noted a solute concentration effect on the universal calibration curve of pullulans and PEOs and suggested the need for extrapolation to zero concentration. Kato et al. reported a congruent universal calibration curve for PEO and sodium polyacrylate using TSK PW columns with 0.3M aqueous NaCl.⁸ In their chromatograms, the intrinsic viscosities were determined separately on the whole polymer.

It was therefore the objective of this work to assess the experimental and theoretical bases for the applicability and accuracy of the universal calibration method in an aqueous environment using GPC in combination with a differential viscometer. The polymers examined were dextran, PEOs, sodium polyacrylates (SPAs), and sodium polystyrene sul-

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fonate (SPSS). Chromatograms were obtained with different mobile phases, polymer concentrations, and column configurations. The experimental procedures, results, theoretical considerations, and discussions follow.

EXPERIMENTAL

Samples

The standard samples employed were broad molecular weight distribution (MWD) dextrans (peak MWs 60,100, 110,000, 185,000, 360,000, 400,000) and SPAs (peak MWs 115,000, 193,800, 392,600, 589,700, 1100,000) having an M_w/M_n of about 1.5, as well as narrow MWD PEOs (peak MWs 105,000, 260,000, 400,000, 580,000, 865,000) and SPSSs (peak MWs 31,000, 88,000, 145,000, 345,000) having an M_w/M_n of about 1.1 and were obtained from American Polymer Standards Corp. The molecular weight values quoted by the manufacturer were assumed to be correct.

Equipment

All the experiments on this project were performed on a Waters 150C GPC/ALC system (Waters/Millipore Corp., Milford, MA) coupled in parallel to a Viscotek differential viscometer, Model 100 (Vis-

cotek Corp., Porter, TX). Both detectors were maintained at 30°C. The data were collected through Viscotek Unical™ software Version 3.02 using the broad standards universal calibration procedure, since the narrow standards universal calibration procedure gave spurious results with the broad MWD standards of SPA and dextran.

Procedure

The chromatographic conditions are displayed in Table I. The sodium nitrate solution was prepared in deionized water. The phosphate buffer mobile phases were prepared with 0.1 or 0.2 M KH_2PO_4 and brought to pH 7 with NaOH.

RESULTS AND DISCUSSION

The starting point of this work was gel permeation chromatography using two Ultrahydrogel™ columns, one 125 Å and one linear column with aqueous 0.1 M NaNO_3 as mobile phase. The universal calibration curves for several dextrans and SPAs in this medium are displayed in Figure 1. The divergence of the two relationships at low molecular weight was clearly noticeable and believed to be primarily due to poor fractionation. This led us to explore increasing the number of columns.

Table I Experimental Conditions for GPC

Liquid chromatograph	Waters 150C ALC/GPC
Columns	7.8 mm × 30 cm Ultrahydrogel Set 1: 125 Å and 1 linear column Set 2: 1000, 500, 250, and 120 Å Set 3: 2000, 1000, 500, and 250 Å
Temperature	30°C
Flow rate	1 mL/min
Detection	Differential refractive index and Viscotek differential viscometer
Sample concentration	0.5–2.0 mg/mL
Injection volume	0.1 mL
Mobile phases	(1) 0.1M NaNO_3 in water (2) 0.3M NaCl, 0.1M KH_2PO_4 , pH 7 (3) 10% methanol in water, 0.3M NaCl, 0.1M KH_2PO_4 , pH 7 (Ref. 9) (4) 10% acetonitrile in water, 0.2M KH_2PO_4 , pH 7

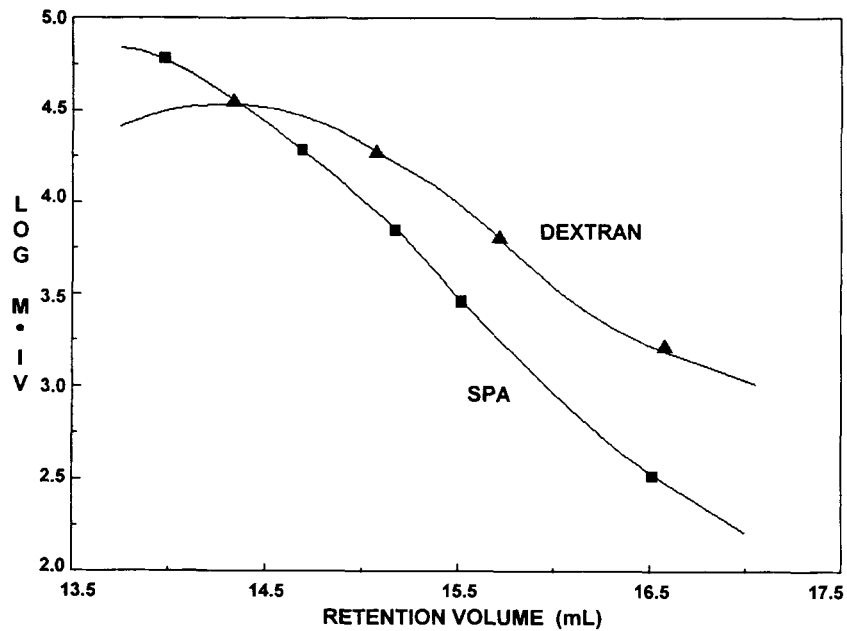


Figure 1 GPC universal calibration plot using two columns: (triangles) dextran; (squares) SPA.

Figure 2 displays the universal calibration curves for dextran, PEO, and SPA when four Ultrahdragel columns (120, 250, 500, and 1000 Å) were utilized at a higher ionic strength mobile phase, 0.3 M NaCl, with 0.1 M KH₂PO₄ at pH 7 in an attempt to improve

the fractionation and eliminate secondary effects. In this case, the curves for the three polymers did not diverge but were distinct and did not overlap.

Figure 3 displays similar data for the three polymers, but the sample concentration was cut by $\frac{1}{2}$

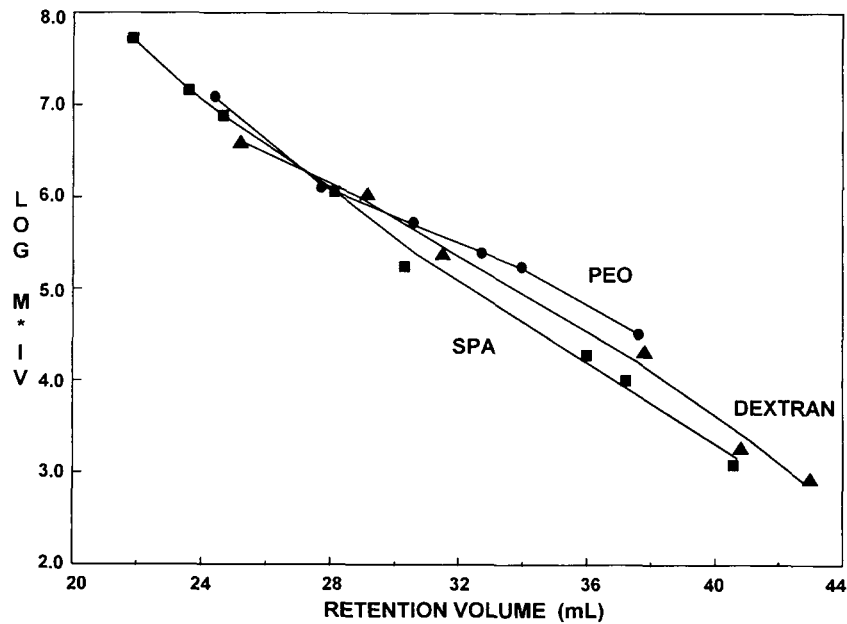


Figure 2 GPC universal calibration plot using four columns: (circles) PEO; (triangles) dextran; (squares) SPA.

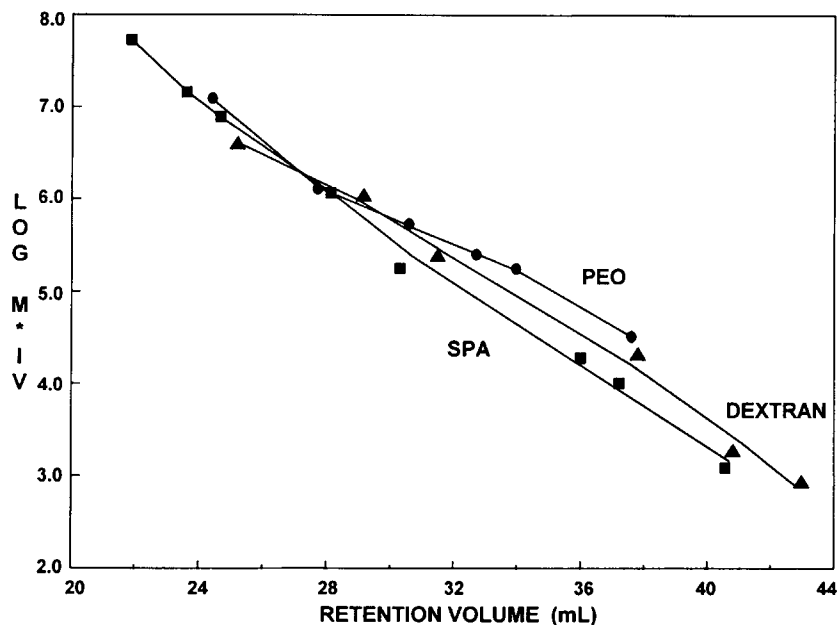


Figure 3 GPC universal calibration plot using four Ultrahydrogel columns at lower sample concentration: (circles) PEO; (triangles) dextran; (squares) SPA.

(0.5–1.0 mg/mL), and the 120 Å column was replaced by a 2000 Å one to observe if the fractionation could be further improved, in case the columns were overloaded, to yield a better universal calibration fit. No improvement in the universal calibration relationship was observed.

In the next experiment, the polarity of the solvent was decreased by the addition of 10% methanol in order to decrease or eliminate any hydrophobic interactions. The results are illustrated in Figure 4 and exhibit a much improved universal relationship. However, the relationship for each polymer appeared

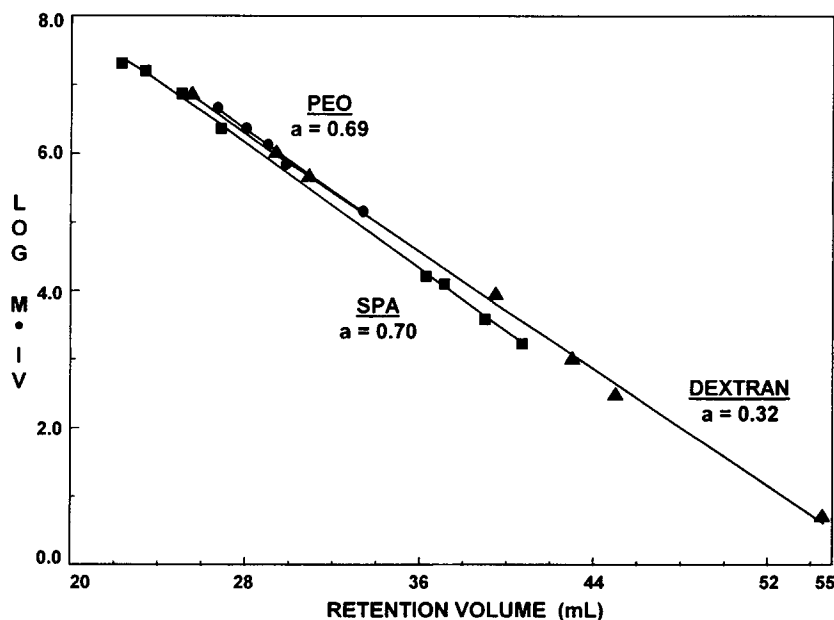


Figure 4 GPC universal calibration plot using four columns with 10% methanol in the mobile phase: (circles) PEO; (triangles) dextran; (squares) SPA.

to be distinct, so that a numerical analysis of the data was made as shown in Table II. When the number- and weight-average molecular weights were calculated for SPA using the single SPA curve, the mean deviation was about 5%, but when the composite curve was used, the mean deviation was about 40%, which was somewhat high. This composite deviation (40%) was of the same order as that observed by Haney and Armonas for broad MWD organic-soluble polymers.¹⁰

The polarity of the solvent was further decreased by the addition of 10% acetonitrile to water, which is a recommended mobile phase for SPSS.¹¹ In this case, the mobile phase contained 0.2M KH₂PO₄ and the polymers examined were dextran, PEO, SPA, and SPSS. It was observed that the data could be fit into two relationships: one for the polyanions and one for the nonionic polymers dextran and PEO, as illustrated in Figure 5. The data, however, exhibited excessive scatter so that this mobile phase was not further pursued. An attempt was next made to investigate the theoretical basis of the universal calibration to determine if the deviations were of a theoretical nature.

THEORETICAL CONSIDERATIONS

Three theoretical models for determining the hydrodynamic volume of polymers were considered. According to the Einstein-Simha model for viscosity, the product of intrinsic viscosity times molecular weight is related to the hydrodynamic volume by the shape factor ν as follows^{12,13}:

$$[\eta] \times \text{Mol. Wt.} = \nu \times N \times V$$

where $[\eta]$ is the intrinsic viscosity; N , Avogadro's number; and V , the hydrodynamic volume. The above equation would indicate that the hydrodynamic volume of a polymer depended on the shape factor as well, a point further amplified by Casassa.¹⁴ However, since the mobile phase contained a high salt concentration, the polymers may be considered to be random coils, so that this model did not explain the deviations observed here.

The Flory-Fox model, as modified by Ptitsyn and Eizner, suggested that the product of intrinsic viscosity times molecular weight was related to the hydrodynamic volume by the value of the Mark-Houwink exponent as follows^{15,16}:

$$[\eta] \times M = \phi(1 - 2.63E + 2.86E^2)(6^{1/2}R_g)^3$$

where ϕ is the universal constant = 2.86×10^{23} , $E = (2a - 1)/3$, and a is the Mark-Houwink exponent:

$$[\eta] = KM^a$$

The above model would suggest that the universal calibration depended on the value of a . The results of this work, as shown in Figure 4 for SPA, dextran, and PEO, did not exhibit a dependency on the value of a . For example, SPA and PEO had values of a equal to 0.70 and 0.69, respectively, but their universal calibration curves did not overlap. For dextran, a was equal to 0.32, which was indicative of extensive branching, but its curve overlapped that of PEO. Therefore, this model likewise did not explain the deviations observed in the universal calibration curves.

Table II Evaluation of Universal Calibration Plot for Polyacrylic Acid: 10% MEOH, 0.3M NaCl, 0.1M KH₂PO₄, pH 7

Sample of PAA	Mol. Wt.	% Deviation	
		Single Curve	Composite Curve
1	$755 \times 10^3 M_w$	+11	+41
	M_n	-3	+49
2	$495 \times 10^3 M_w$	-6	+10
	M_n	-4	+19
3	$245 \times 10^3 M_w$	-4	+20
	M_n	-1	+30
4	$131 \times 10^3 M_w$	+8	+60
	M_n	+3	+67
Mean deviation		4.8%	37%

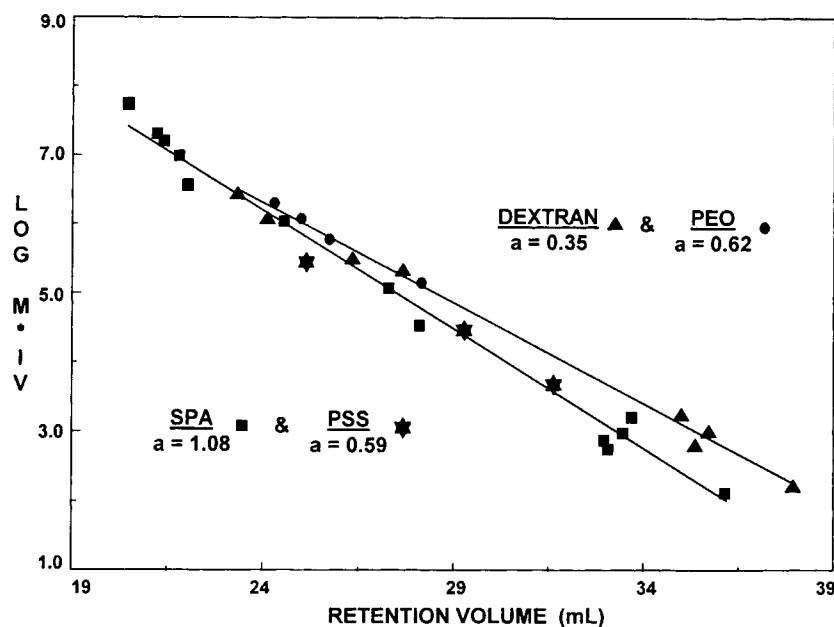


Figure 5 GPC universal calibration plot using four columns with 10% acetonitrile in the mobile phase: (circles) PEO; (triangles) dextrans; (squares) SPA; (hexagons) SPSS. Nos. denote Mark-Houwink exponent (see text).

The Hester and Mitchell model took a different approach. In this model, the hydrodynamic diameter was related to the natural log of the distribution coefficient K_d as follows¹⁷:

$$\ln \frac{(V_R - V_0)}{(V_T - V_0)} = -K(M[\eta])^{1/3}/D$$

where V_R is the retention volume; V_0 , the void or interstitial volume; V_T , the total void and pore volume; K , the proportionality constant; and D , the mean pore diameter. A plot of the left side, which was called $\ln K_d$, vs. $(M[\eta])^{1/3}$ should be a straight line intersecting the ordinate at $\ln K_d = 0$. According to this model, universal calibration depended on the hydrodynamic diameter of the macromolecule. A Hester and Mitchell plot of the data of Figure 4 is illustrated in Figure 6. It was readily observed that a better fit for the universal calibration relation was not obtained. Hence, none of these theoretical models resolved or explained the differences observed in the universal calibration curves for anionic and nonionic polymers. The simple Einstein relationship was equally satisfactory.

Finally, a universal calibration plot of the $\log[\eta]_p M_p$ for the peak values of the whole polymer was made manually to see if the mode of data treat-

ment made a difference in the relationships. The Viscotek Unical Version 3.02 broad standards procedure was based on a modified Hamielec method and used fractionation data from the on-line differential viscometer.¹⁸ The results, as illustrated in Figure 7, indicated that the deviations were not due to the mode of data treatment. In this case, a universal curve was obtained for PEO and dextrans, but clearly a different curve for SPA.

The results presented above indicate that under the best conditions found and employing the Ultrahydrogel columns it appears that two universal calibration relationships are obtained: one for anionic polyelectrolytes and the other for nonionic polymers. A plausible explanation for this behavior is ionic exclusion of the SPA, which makes it elute early even though the ionic strength was high. This appears plausible as the Ultrahydrogel packing consisted of a cross-linked hydroxylated polymethacrylate gel with residual carboxyl groups, which could repel the polyanion.¹⁹

Hence, the issue here was not considered to be of a theoretical nature. Therefore, the deviations observed were tentatively attributed to unresolved differences in the ionic interactions of the polymers and the substrate. Investigations with different columns and mobile phases may help resolve these issues.

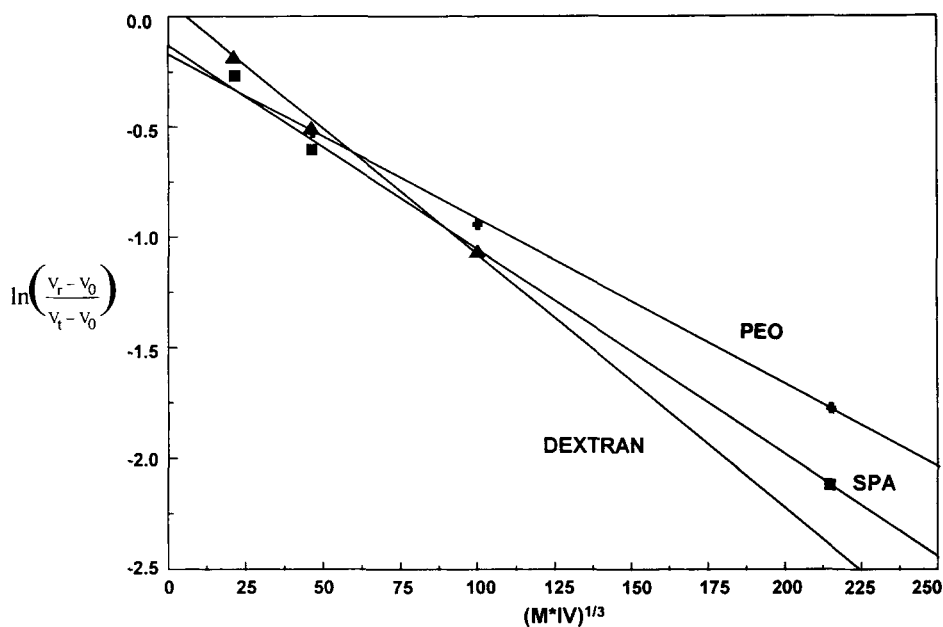


Figure 6 Aqueous universal calibration by the Southern method using data of Figure 4: (pluses) PEO; (squares) SPA; (triangles) dextran.

SUMMARY

In summary, it may be stated that the universal calibration relation was not always valid in aqueous GPC. The validity had to be verified for each system under consideration. Addition of methanol to the mobile phase improved the universal calibration re-

lationships for SPA, dextran, and PEO using Ultrahydrogel columns so that the mean deviation was about 40%. The deviations observed for randomly coiled macromolecules in water were not attributed to a theoretical deficiency, but to ionic and/or hydrophobic interactions of the solutes with substrate.

We thank Unilever for permission to publish this paper.

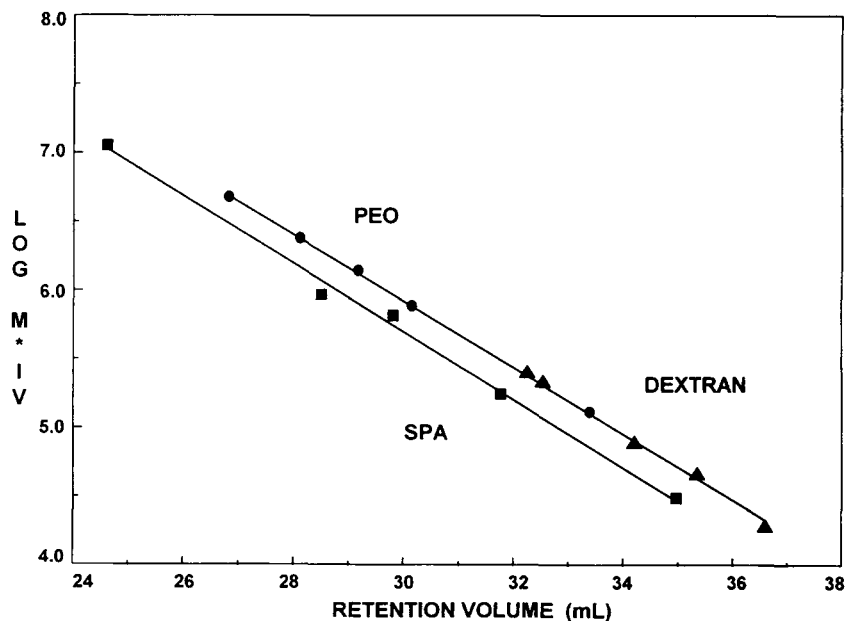


Figure 7 Universal calibration plot using data for whole polymers: (circles) PEO; (triangles) dextran; (squares) SPA.

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