A Universal Calibration in Gel Permeation Chromatography

Nobuo Donkai, Atushi Nakazawa, and Hiroshi Inagaki*

    The relationship between elution volume and separated molecular size in gel permeation chromatography (GPC) experiments was investigated for a number of polymers with lower molecular weights in tetrahydrofuran; polyethylene glycol, polytetrahydrofuran, polypropylene, polypropylene glycol, polystyrene, and n-paraffin. A unique feature of this investigation consists in the point that the experiments are conducted by taking it into account that the excluded volume effect in polymer coils tends to vanish in these low molecular weight polymers in solution. It was found that when elution volume was plotted in semilogarithmic scale against root mean square end-to-end distance of polymer chain, all data points obtained fell on a common line independent of the chemical structure of polymers tested. Thus it is proposed that the unperturbed dimension deduced from hydrodynamic experiments can be used as a universal parameter in GPC study, at least, for lower molecular weight polymers. This conclusion is further discussed by referring to the Mark-Houwink equation, and the Flory theory for the molecular weight dependence on intrinsic viscosity.

INTRODUCTION

    Since gel permeation chromatography (GPC) found practical use in the field of polymer science, it has become one of the most convenient tools for characterizing polymer substances.[1] Thus this technique has widely been applied to several types of polymers. However, some problems in utilizing GPC are still remaining unsolved. One of these may consist in the calibration of gel permeation columns employed, which has been made usually by plotting a parameter indicative of molecular size of test sample against elution volume in semi-logarithmic scale. To construct such a calibration curve for a given polymer homologous series, the need for a series of the species with different, known molecular weights and possibly narrow molecular weight distributions is apparent. It is quite clear, however, that this requirement cannot be fulfilled for any type of polymers, and it is often forced to refer to the calibration curve established for anionically prepared polystyrene, in place of that for another type of polymer without security if this curve be applicable. Thus much attention has been paid to date upon the problem of calibration that mainly consists in what type of parameter indicative of molecular size should be introduced in order to convert given calibration curves into a universal one that is independent of the chemical structure of polymers.

    At the very beginning, Moore and Hendrickson[2] suggested that for this parameter, the extended chain lenght might be universally related to elution

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* 吞海　信雄，中沢　　淳，稲垣　　博：Laboratory of Macromolecular Characterization, Institute for Chemical Research, Kyoto University, Uji, Kyoto.

volume. In other words: this chain length was supposed to be proportional to the molecular size of separated polymers in GPC and could be related to molecular weight by a "Q-factor" which is defined as the ratio of molecular weight to this chain length.

In recent studies, however, many authors[3~10] have agreed in taking the hydrodynamic volume of polymer in solution as the GPC parameter instead of the extended chain length. In fact, good correlation has been confirmed between elution volume and a product $[\eta] \times M$ for various types of polymers,[3~5] where $[\eta]$ and M mean intrinsic viscosity and molecular weight, respectively. Moreover, Cantow et al.[7] obtained a calibration curve common for polystyrene and polyisobutene samples, when the mean square end-to-end distance calculated from intrinsic viscosity was plotted against elution volume. On the other hand, Dawkins[9,10] showed that a close correlation between unperturbed root mean square end-to-end distance and elution volume for polymers held if they exhibit nearly equal exponents on molecular weight in the Mark-Houwink equation. This result suggests that the main factor governing the separation process in GPC is the hydrodynamic dimension of polymer coils so that the excluded volume effect in polymer coils should be taken into consideration. Few investigations[11] have, however, been reported to data, which were carried out at the Θ-point that allows one to discuss the separation process at the ideal state of polymer coils.

In the case of polymers with lower molecular weights, even if they are dissolved in any good solvent, it is possible to neglect the excluded volume effect.[12] Thus the present study deals with the GPC behavior of lower molecular weight polymers. We adopt the unperturbed dimension of linear macromolecules as the parameter indicative of molecular size and construct calibration curves to see if these curves can be superposed so as to obtain a universal curve.

THEORETICAL BACKGROUND

While the separation mechanism of GPC is not completely understood at present, a commonly accepted conception is that macromolecules passing through gel permeation columns are separated on the basis of their available pore volumes of porous gel particles containing voids in which solvent and polymer molecules can freely permeate.[13,14] Thus apparent dimensions of each single macromolecule may be related to an average volume of the pores, so that in the absence of specific interactions between polymer and gel, the volumes within the pores are considered, on the average, to be less available to larger macromolecules than to smaller ones. So far as one assumes such a hypothesis for the separation mechanism, our present problem to obtain a universal calibration curve may be reduced to what kind of parameter should be chosen as measure indicative of the chain dimension.

Generally speaking, there are two ways for the choice of the parameter. One of them will be to employ the statistical chain dimension and the other to employ the hydrodynamic chain dimension. However, only for intuitive and conventional reasons, we will discuss here on the choice of the latter quantity.

The hydrodynamic chain dimension of linear macromolecules is, above all,

reflected in the intrinsic viscosity, $[\eta]$, which is connected with molecular weight M by the well-known Mark-Houwink equation:

$$[\eta] = K M^a \qquad (1)$$

The exponent a is known to vary from 0.5 to ca. 0.8 depending on thermodynamic interactions of used solvents with macromolecules. The lower limiting value 0.5 is realized at the Θ-point, while the values greater than 0.5 are interpreted in terms of the excluded volume effect in macromolecular coils. For polymers with lower molecular weights (often smaller than 5×10^4), the exponent a becomes 0.5 independent of solvents used; that is to say, the following equation holds:[12]

$$[\eta] = K_0 M^{1/2},$$

where K_0 is rewritten K_θ (at Θ-point), which is proportional to the unperturbed mean square end-to-end distance $\langle R_0^2 \rangle$ characteristic of a given polymer chain. Relations under $[\eta]$, M, and $\langle R_0^2 \rangle$ are given by Flory and Fox[15] as

$$[\eta] = K_\theta M^{1/2} \alpha^3 \qquad (2)$$

and

$$K_\theta = \phi [\langle R_0^2 \rangle / M]^{3/2} \qquad (3)$$

with

$$\alpha^2 = \langle R^2 \rangle / \langle R_0^2 \rangle \qquad (4)$$

Here α is the linear hydrodynamic expansion factor of polymer chain due to the excluded volume effect, and ϕ is the Flory viscosity constant. According to the Flory theory[15] on the excluded volume effect, the expansion factor α is related to molecular weight by

$$\alpha^5 - \alpha^3 = C_F M^{1/2}, \qquad (5)$$

so that in a limiting case where solvent used is extremely good towards sample polymer, that is, for sufficiently large values of α,

$$\alpha \propto M^{1/10} \qquad (5')$$

It is clear from the above discussion that the unperturbed end-to-end distance $\langle R_0^2 \rangle$ may be taken as a parameter indicative of the molecular size for lower molecular weight polymers under the assumption that $\alpha = 1$ in eq. (2) for every good solvent, for the purpose of obtaining the universal calibration. Thus we may define a similar "Q-factor" for the value of $M/\langle R_0^2 \rangle^{1/2}$ as that proposed by Moore and Hendrickson, because the value of $M/\langle R_0^2 \rangle^{1/2}$ is independent of molecular weight as well as of solvent used for linear polymers composed of constant repeating units.

EXPERIMENTAL

GPC measurements were carried out on a commercial Shimadzu GPC Model 1-A unit at room temperature. A combination of three columns, each containing crosslinked polystyrene gel with 10^3, 10^2, and 10^1 Å nominal pore size, respectively, was made. Tetrahydrofuran was employed as solvent and the flow rate

was adjusted to 1.0 ml/min. Injected amount of polymer was always less than 5 mg. Polymers tested were n-paraffin, polyethylene glycol (PEG), polytetrahydrofuran (PTHF), polypropylene (PP), polypropylene glycol (PPG), and polystyrene (PST). Polypropylene[16] and polytetrahydrofuran[17] were fractionated samples, and polystyrene samples were the so-called monodisperse standard samples supplied by Pressure Chemical Co., Pittsburgh, U. S. A..

Elution volumes were determined at peak maximam of GPC curves, since all chromatograms obtained in this study gave quite sharp, symmetric curves of Gaussian type. Molecular weights were determined mainly by a vapor pressure

Table 1. Molecular Weight, Unperturbed End-to-End Distances and GPC Data.

Sample	Code	M	$\langle R_0^2 \rangle^{1/2}$	V_e (counts)
n-Paraffin				
	$C_{20}H_{42}$	283	15.6	21.4_9
	$C_{24}H_{50}$	339	17.9	21.0_0
	$C_{32}H_{66}$	451	19.7	20.4_0
	$C_{32}H_{74}$	507	20.9	20.2_1
Polyethylene Glycol				
	EG 300	297	13.2	21.2_4
	EG 400	399	15.3	20.7_5
	EG 600	557	18.1	20.0_9
	EG 1000	1,090	25.4	19.1_5
	U–4	3,230	43.7	17.6_2
	H–6	9,000	72.9	16.0_0
	H–10	11,100	80.9	15.6_6
	H–20	18,800	105.0	14.8_9
Polytetrahydrofuran				
	K–122	7,550	74.4	15.7_2
	K–123	14,200	102.0	15.0_0
	K–124	30,800	150.0	14.4_2
	K–102	72,500	231.0	14.3_3
Polypropylene				
	PPE	700	21.6	20.1_7
	IDB–1	1,170	27.9	19.5_6
	Fr. 8	2,000	36.5	18.5_0
	Fr. 7	5,000	57.7	17.1_9
	Fr. 6	7,600	71.1	16.5_0
	Fr. 5	10,800	84.8	15.8_4
Polypropylene Glycol				
	DNI–1	892	22.0	19.4_2
	DNI–2	1,550	29.0	18.3_3
Polystyrene				
		2,000	29.5	18.9_3
		4,800	45.6	18.1_5
		10,300	66.8	16.6_7
		19,800	92.7	15.8_5
		51,000	148.0	14.4_6

osmometer (Mechrolab model 301) with benzene at 37°C. Intrinsic viscosities of PTHF samples were determined in benzene at 30°C by Ubbelohde viscometer, just before GPC measurements, in order to eliminate the effect of thermal degradation of this polymer. Molecular weights of PTHF were calculated from the following equation :[17]

$$[\eta] = 1.31 \times 10^{-3} M_w^{0.60}$$

Table 1 summarizes the result of characterization of these sample polymers together with elution volume data.

RESULTS AND DISCUSSION

In Fig. 1 values of molecular weight M are plotted against elution volume V_e (counts) found for each polymer homologous series in semi-logarithmic scale. On these plottings, number-average and weight-average molecular weights are taken for PEG and PPG, and for the other polymer samples, respectively. However, both polyether samples will have sufficiently narrow distributions in their polymolecularity, as anticipated from the polymerization mechanism, so that the difference in molecular weight basis may be regarded to be negligible in further discussion. From Fig. 1 it is seen that distinct differences exist between the
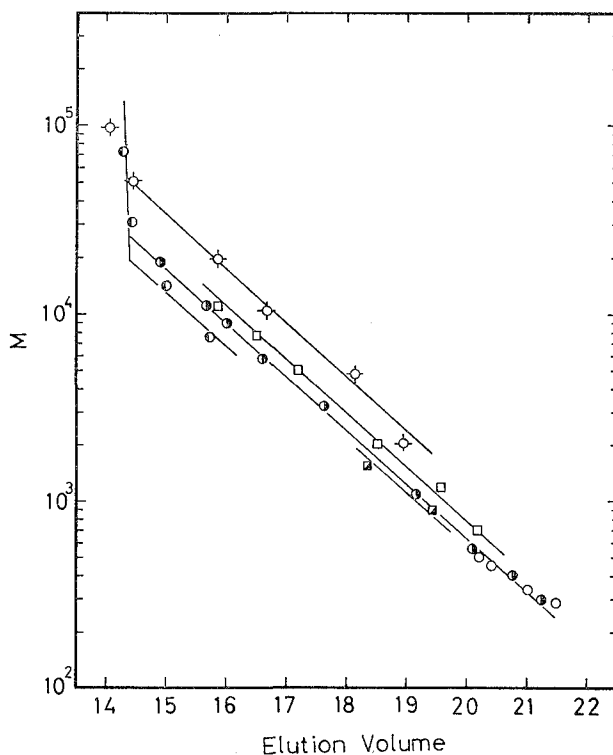

Fig. 1. Polt of molecular weight against elution volume for polymers in Table 1.
polystyrene –◯–, polyethylene glycol ◑, polytetrahydrofuran ◐,
polypropylene ▢, polypropylene glycol ◢, n-paraffin ◯.

calibration curves of each polymer homologous series, and this indicates that the introduction of molecular weight as parameter indicative of the size of macro-molecules does not permit us to construct the universal calibration curve.

Our next attempt was to introduce the root mean square end-to-end distance $\langle R_0^2 \rangle^{1/2}$ as the parameter. For the purpose, values of $\langle R_0^2 \rangle^{1/2}$ were evaluated with the aid of literature values of K_θ for each polymer homologous series.[18] The calculation was made on the basis of eq. (3) assuming 2.87×10^{-23} for Φ value. These values are listed in Table 2. A semi-logarithmic plot of $\langle R_0^2 \rangle^{1/2}$ against V_e for all polymer samples studied here is demonstrated in Fig. 2, from which one can confirm that all the data points fall approximately on a common single line. Exact inspection of the plot reveals, however, that slight but systematic deviations are seen among the data points for each polymer homologous series. These deviations may probably be not attributed to the failure in our working hypothesis but related to the choice of K_θ-value from those given in different literatures and also to the slight dependence of K_θ-value on solvent used. Despite such an incompleteness found in this plot as the universal calibration curve, one

Table 2. Unperturbed Dimensions of Linear Polymer Molecules.[18]

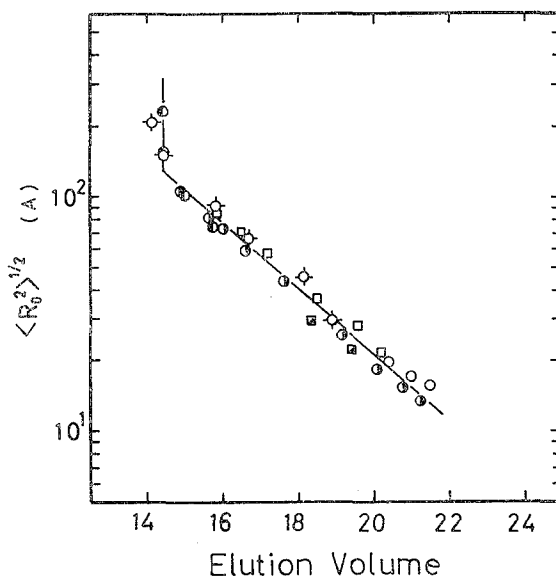| Polymer	$10^3[\langle R_0^2 \rangle / M]$ (Å)	Temp. (°C)
Polyethylene	1.86	100
Polyethylene glycol	1.27	35
Polytetrahydrofuran	1.58	30
Polypropylene	1.44	30
Polypropylene glycol	1.17	25
Polystyrene	0.93_5	30

Fig. 2. Plot of $\langle R_0^2 \rangle^{1/2}$ against elution volume for polymers in Table 1. Symbols as in Fig. 1.

may draw a conclusion that the plot between $\langle R_0{}^2\rangle^{1/2}$ and V_e could supply an approximate solution to the present problem.

In the next place we will discuss correlation between the two plots shown in Figs. 1 and 2, $i.\,e.$, those between $\log M$ and V_e, and between $\log \langle R_0{}^2\rangle^{1/2}$ and V_e. Within the range where the latter plot goes linear, the relationship between $\langle R_0{}^2\rangle^{1/2}$ and V_e can be approximated by

$$\log \langle R_0{}^2\rangle^{1/2} = AV_e + B, \tag{6}$$

where A and B are assumed as constants independent of polymer species so far as our result concerns. Putting eqs. (1), (2) and (3) into eq. (6) we have

$$(1+a)\log M - 3\log \alpha = 3AV_e + (3B + \log \Phi - \log K). \tag{7}$$

Provided the excluded volume effect will vanish in our polymer samples with lower molecular weights,[12] $i.\,e.$ $\alpha = 1$, this equation is rewritten as

$$\log M = [3A/(1+a)]V_e + C/(1+a) \tag{7'}$$

where C is again a constant. This dependence of slope of the plot between $\log M$ and V_e on the exponent a in the Mark-Houwink equation has been pointed out already by some investigators.[8,19,20] If it is sure that our lower molecular weight polymers in solution are really not influenced by the excluded volume effect, the a-value deduced from the experimental plot between $\log M$ and V_e should tend to 0.5. As is seen from Fig. 1, the plots between $\log M$ and V_e for each polymer homologous series are parallel to each other, and values of a calculated from the slopes were found to be in the proximity of 0.5 (see Table 3). Thus the aforementioned requirement was, in fact, fulfilled by the present study, and this indicates, in accordance with a suggestion made by Boni et al.,[8] that superposition of different plots between $\log M$ and V_e for different polymer homologous series could be attained if a certain, parallel shift of each plot is made by taking into account the difference in K_θ values characteristic of each polymer homologous series. At the same time, this result will give a basis to the observation that the plot between $\log \langle R_0{}^2\rangle^{1/2}$ and V_e became a universal calibration curve.

Table 3. Values of a Calculated from the Experimental Plots between $\log M$ and V_e Using eq. (7'),

Sample	a
Polystyrene	0.55
Polyethylene glycol	0.53
Polypropylene	0.56

On the other hand, Dawkins[9,10] pointed out that the plot between $\log \langle R_0{}^2\rangle^{1/2}$ and V_e gave a universal curve for a fairly a large number of polymer species even in the existence of the excluded volume effect. However, this seems somehow fortuitous as is clearly understood from eq. (7). In the experiment reported by Dawkins[9] for a limiting case where solvent used is extremely good, it should be assumed that α is nearly proportional to $M^{1/10}$, as anticipated from eq. (5'). Then we can obtain an expression of the same type of eq. (7') but with $(0.7+a)$

instead of $(1+a)$. This new equation will account for the result of Dawkins.

Use of $[\eta] \times M$ as a universal parameter representing average molecular size of perturbed polymer chains in good solvent was proposed by various authors.[3~5] When GPC plot between $\log [\eta] \times M$ and V_e, i.e.

$$\log [\eta] \times M = A'V_e + B'$$

A' and B' being constants, is connected with the Mark-Houwink equation (1), the resultant equation readily is written as

$$\log M = [A'/(1+a)]V_e + C'/(1+a) \qquad (8)$$

which tends to be equivalent to eq. (7'). On the basis of eq. (8), we estimated the a-values from the slope of the plots between $\log M$ and V_e constructed for the data reported by Benoit et al.[3] and compared them with those obtained from the dependence of $[\eta]$ on M. The results are summarized in Table 4. It is seen from this Table 4 that the a-values deduced from the two different sources are roughly in agreement and the slope of the $\log M$ versus V_e plot depends clearly on the a-value.

Table 4. The Comparison of a-Values Calculated from the Experimental Plots between $\log M$ and V_e Using the Relationship Existed in Two Calibration Curves with Observed Values.

	Sample	Solvent	a		
			obs.	$\log\langle R_0^2\rangle^{1/2}\text{-}V_e$	$\log[\eta]M\text{-}V_e$
Dawkins[9]	PST		0.80	0.80	0.75
	PMMA*	CHCl₃	0.80	0.80	0.75
	PDS**	(30°C)	0.77	0.77	0.77
Benoit et al.[3]	PST		0.70	0.91	0.66
	PMMA*	THF	0.70	0.79	0.53
	PVC***	(room temp.)	0.64	0.88	0.64

* Polymethyl methacrylate
** Polydimethyl siloxane
*** Polyvinyl chloride

In summary, it is found that the unperturbed root mean square end-to-end distance of linear polymers can be used as a universal parameter in GPC experiments for lower molecular weight polymers employed in this investigation. As far as this parameter has validity as a universal one, we may define a new "Q-factor" given by $M/\langle R_0^2\rangle^{1/2}$, and superposition of different polts between $\log M$ and V_e for different polymer homologous series can be attained by parallel shift of each plot taking into account this "Q-factor". It was made sure that the exponent a of the Mark-Houwink equation governs the slope of the plot between $\log M$ and V_e in systems in one of which the excluded volume effect is negligible, while in the other system this is not negligible.

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