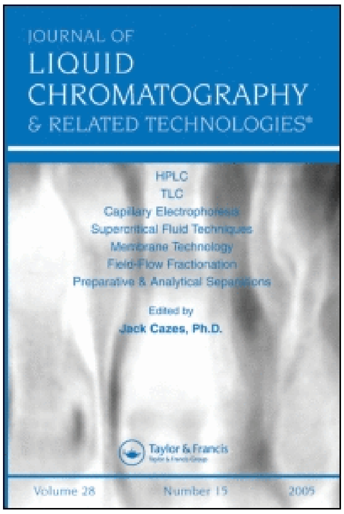
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Elution Behaviors and Rationalized Calibration for Low-Molecular-Weight Polymers in Size Exclusion Chromatography

Sadao Mori[a]; Akira Yamakawa[a]
[a] Department of Industrial Chemistry, Faculty of Engineering Mie University, Japan

To cite this Article Mori, Sadao and Yamakawa, Akira(1980) 'Elution Behaviors and Rationalized Calibration for Low-Molecular-Weight Polymers in Size Exclusion Chromatography', Journal of Liquid Chromatography & Related Technologies, 3: 3, 329 — 342

To link to this Article: DOI: 10.1080/01483918008069338

URL: http://dx.doi.org/10.1080/01483918008069338

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 3(3), 329-342 (1980)

ELUTION BEHAVIORS AND RATIONALIZED CALIBRATION FOR LOW-MOLECULAR-WEIGHT POLYMERS IN SIZE EXCLUSION CHROMATOGRAPHY

Sadao Mori and Akira Yamakawa
Department of Industrial Chemistry, Faculty of Engineering
Mie University, Tsu, Mie 514, Japan

Downloaded At: 19:18 24 January 2011

ABSTRACT

Molecular weight relationships among oligostyrene, n-hydrocarbon, epoxy resin, p-cresol novolak resin, and oligoethylene glycol having the same retention volume were discussed using SEC gels of different pore sizes in chloroform and tetrahydrofuran. Gel capacity and the maximum number of components resolvable increased with the use of chloroform except the case of epoxy resin. Different elution behaviors of oligomers in different eluents make it difficult to use molar volumes or effective chain lengths as calibration parameters. The influence of the pore size and shape of the gel on the elution order among oligomers was negligible except some cases. Molecular weight conversion equations for several oligomers based on molecular weight of oligostyrene or n-hydrocarbon were derived. These equations make it possible to use oligostyrene or n-hydrocarbon as a reference standard when molecular weights of oligomers are measured.

INTRODUCTION

One of the goals of size exclusion chromatography (SEC or GPC) is to estimate molecular weight of materials, especially low molecular weight compounds such as oligomers and a column system in SEC must be calibrated using appropriate samples of known molecular weight, e.g., monodisperse polystyrenes. However, in

Copyright © 1980 by Marcel Dekker, Inc.

SEC, the molecules are separated based on their molecular volume occupied in solution, as opposed to either a linear dimension of a molecule or molecular weight (1). Molecular weight conversion to correct values of the molecule in question is required when a polystyrene-based calibration curve is applied to estimate molecular weight of materials other than polystyrene. For high polymers, so called the universal calibration method is used to construct a calibration curve for the polymer concerned. But this method is not adequate for oligomers, because the intrinsic viscosity of such low molecular weight substances is hardly measured precisely.

Two specific measures of the size of low-molecular weight materials are proposed: the effective chain length describing by the number of carbon atoms in the n-hydrocarbon of the same effective length (2,3) and the molar volume (1,4-6). Lambert obtained an equation to calculate the molar volume of n-hydrocarbons (5) and estimated the molar volume values of several polar and non-polar compounds using a set of these values for n-hydrocarbons as a standard (6). Because of solute-solvent and solute-gel interactions, the effective chain length and the molar volume of materials must correlate experimentally with a standard such as n-hydrocarbons in order to use these values as calibration parameters. These parameters, however, do not give any information about molecular weight of the material.

The present work is concerned with the examination of the molecular weight relationship between polystyrene or n-hydrocarbon and epoxy resin, p-cresol novolak resin (PCR novolak), or polyethylene glycol (PEG) both of which elute at the same retention volume. Solute-solvent association, the effect of the width of the network openings in the gel beads, and other factors affecting the molecular weight relationships among these oligomers having the same elution volume are discussed.

Downloaded At: 19:18 24 January 2011

MATERIALS

Molecular weights of polystyrene standards, purchased from Pressure Chemical Co., were 600, 2200, 3600, and 10000. PEGs (diethylene-,triethylene-, and tetraethylene glycols and PEG 200, PEG 1000, PEG 1540, PEG 2000, and PEG 3400), n-hydrocarbons, and epoxy resins (EPIKOTE 1001 and 1004 which are the products from bisphenol A and epichlorohydrin) were obtained from several chemical supply houses. PCR novolak resins were synthesized from p-cresol and formaldehyde in our laboratory.

Columns were Shodex GPC A802 and A801 (50 cm x 8 mm i.d.) and JSP 101 (50 cm x 7.2 mm i.d.). These columns have the exclusion limits of 10000, 2000, and 5000, respectively, by polystyrene molecular weight. The column substrate was a rigid, cross-linked polystyrene gel. Shodex columns were provided by courtesy of Showa Denko Co., Ltd. through Hikari Kogyo Co., Ltd., Chuo-ku, Tokyo 104, Japan and JSP columns by Jasco Syntex Co., Ltd., Hachioji, Tokyo 192, Japan.

Downloaded At: 19:18 24 January 2011

METHOD

A JASCO (Nihon Bunko Kogyo Co., Ltd., Hachioji, Tokyo 192, Japan) TRIROTAR high performance liquid chromatograph was used with a differential refractometer Shodex SE-11 (Showa Denko Co., Ltd., Minato-ku, Tokyo 105, Japan). The following column-solvent combinations were used: two Shodex A802 - tetrahydrofuran (THF), a JSP 101 - THF, two Shodex A801 - THF, two Shodex A802 - chloroform, and a JSP 101 - chloroform. Flow rate were 1.0 ml/min for Shodex series and 0.5 ml/min for JSP series. A sample injection volume was 0.1 ml. A sample concentration was selected between 0.2 and 2.0 %(w/v) to obtain suitable responses from the detector attenuated at x8. All experiments were carried out at ambient temperature.

RESULTS AND DISCUSSION

The high efficiency of the columns used is demonstrated by the separation of several oligomers as shown in Figure 1. Calibration curves shown in Figures 2 and 3 obtained by plotting peak elution volumes and molecular weights represent the elution behavior of the oligomers in both solvents, THF and chloroform. The n-hydrocarbons are non-polar compounds and are assumed to elute without the solute-solvent association or the adsorption on the gel. Choosing n-hydrocarbons as reference standards, the elution behaviors of other oligomers are summarized as follows:

1. Oligostyrenes, epoxy resins, PEGs which eluted at the same retention volumes as the n-hydrocarbons in THF eluted earlier than the corresponding n-hydrocarbons if chloroform was used as solvent.
2. In chloroform, PCR novolak resins eluted later than the n-hydrocarbons which eluted at the same retention volumes as PCR novolak resins in THF.
3. Epoxy resins and PEGs which eluted at the same retention volumes as oligostyrenes in THF eluted earlier than the corresponding oligostyrenes in chloroform.

The volume between the total permeation and the exclusion limit, which corresponds to the inner volume V_i of the gel, of the column with a chloroform eluent was about 1.2 times that of the column with a THF eluent. Exception was the case of epoxy resin where the value for THF was larger than that for chloroform. The numbers of theoretical plates of oligomer species and the resolution of adjacent peaks for the column with a chloroform eluent were also much larger than in the case of a THF eluent. (Reverse results were obtained in the case of epoxy resin.) It can be concluded that the increase of gel capacity (V_i/V_o) with the use of a chloroform eluent results in the increase of the maximun number of components resolvable and that this effect depends on the nature of the sample to be separated.

Downloaded At: 19:18 24 January 2011

Downloaded At: 19:18 24 January 2011

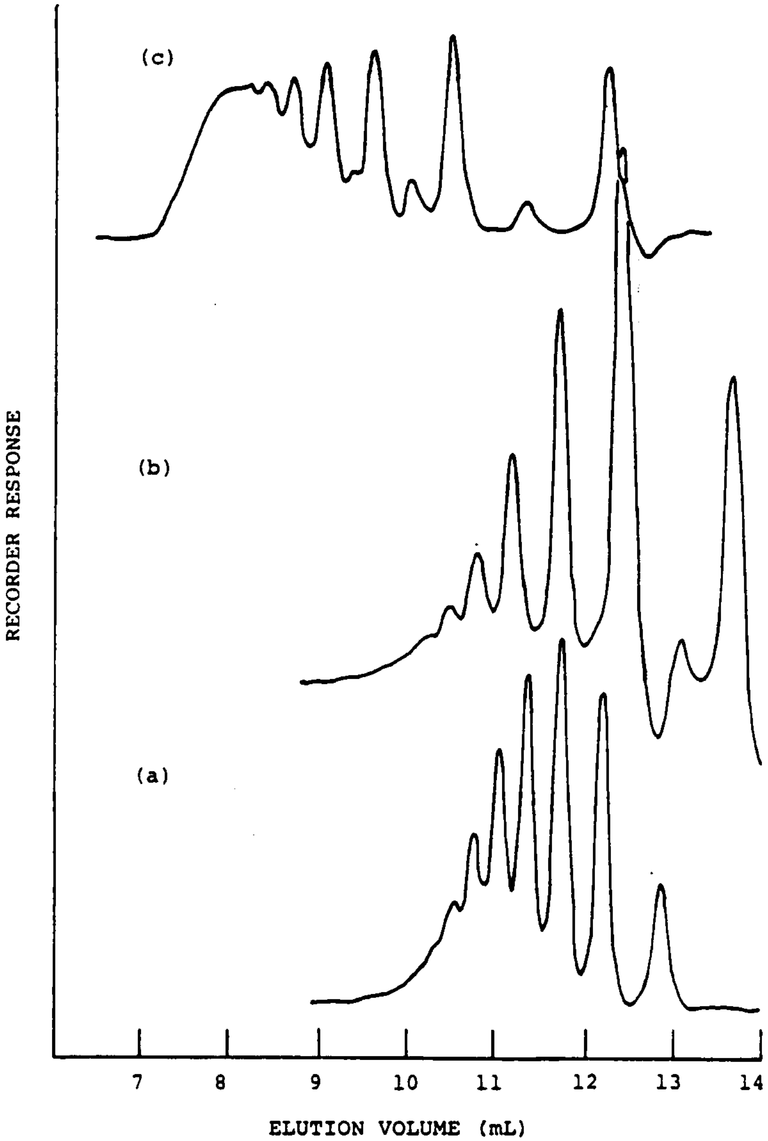


FIGURE 1. Size exclusion chromatograms of oligomers. A JSP101 column. THF eluent at 0.5 ml/min. (a) Polystyrene 600; (b) p-cresol novolak resin; (c) epoxy resin (EPIKOTE 1001).
]

Downloaded At: 19:18 24 January 2011

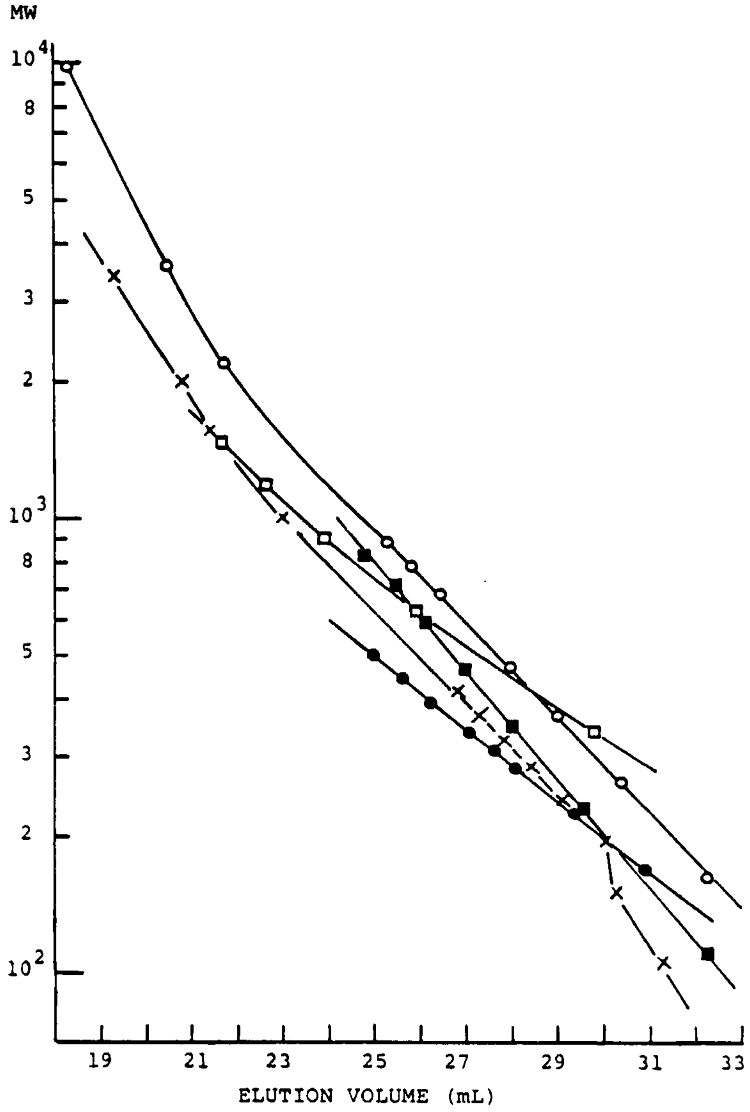


FIGURE 2. Molecular weight vs. elution volume relationships (1). Two Shodex A802 columns. THF eluent at 1.0 ml/min. (○) Pst; (●) n-hydrocarbon; (□) epoxy resin; (■) PCR novolak resin; (✕) PEG.

Downloaded At: 19:18 24 January 2011

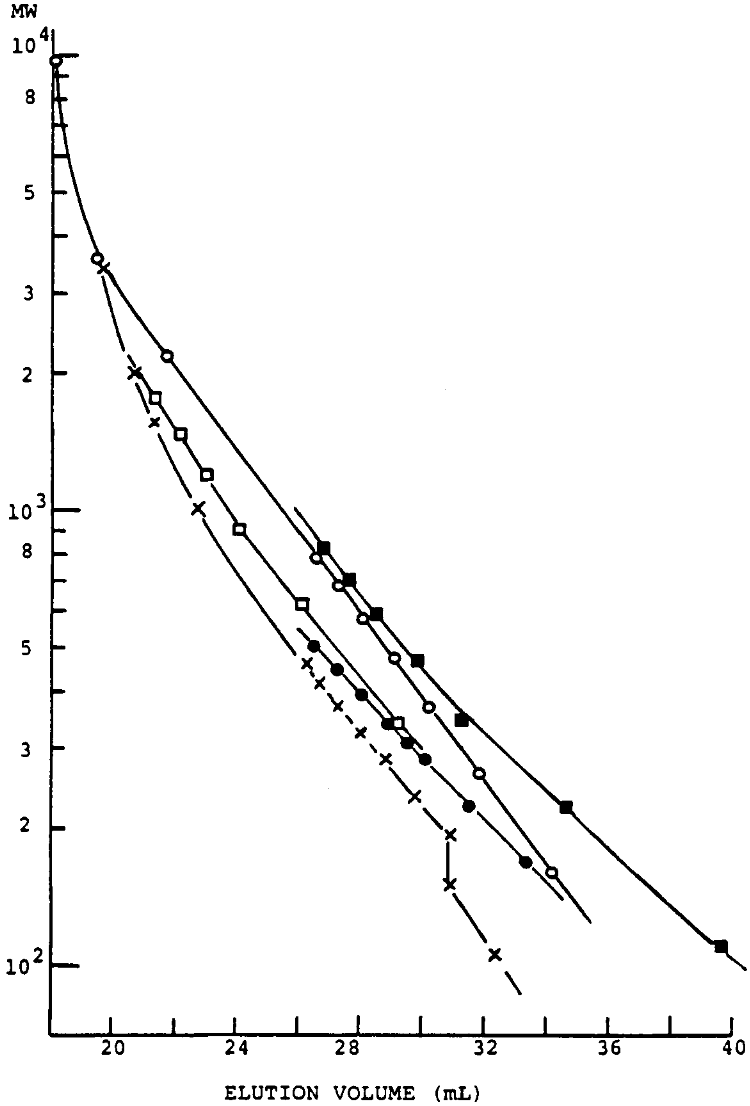


FIGURE 3. Molecular weight vs. elution volume relationships (2). Two Shodex A802 columns. Chloroform eluent at 1.0 ml/min. Symbols the same as in Figure 2.

When THF is used as the elution solvent, the polar molecules are larger than the estimated values as a result of hydrogen bonding with THF (4,7). A plot of elution volumes versus the logarithm of the molar volume for glycols falls above a similar line obtained for the n-hydrocarbons and this noncoincidence has been assumed to be due to adsorption of the glycols onto the gel surface (1). As a possible elucidation for the observation 1, smaller molecular volume of n-hydrocarbons in chloroform than that in THF might be considered rather than the adsorption of n-hydrocarbons onto the gel surface in chloroform. Oligostyrenes and PCR novolak resins, which were estimated to have the same molecular volumes on the basis of their simple molecular structures, had the similar retention volumes in chloroform. However, in THF PCR novolak molecules behaved as they had larger molar volumes by 160. Two THF molecules might associate with two phenolic hydroxyl groups at both ends. No evidence was observed that all the hydroxyl groups associated with THF molecules. The observation 3 leads us to postulate that the molecules of epoxy resins and PEGs in chloroform are actually larger than the molecules of oligostyrenes eluted at the same retention volumes in THF as a result of the solute-solvent association.

The data for PEG obtained with a Shodex A801 - THF system indicate that the molar volume values of PEGs from diethylene glycol to nonaethylene glycol with respect to those of the reference n-hydrocarbons are larger by about 80 to 105 than the estimated values from density and molecular weight. If the molar volume of THF is 74.3 (ref.4), then the above result implies that one to one and a half THF molecules are attached to glycols by hydrogen bonding. The elution behaviors of diethylene- and triethylene glycols might be assumed the intramolecular ring conformations. By the separate experiment of SEC with water as an eluent, the linear relationship on elution volume vs. log molecular weight was obtained throughout this range.

The pore size and shape of the gel might modify the elution order of solutes in SEC. Dimer (n=2) of PCR novolak resin in a

Downloaded At: 19:18 24 January 2011

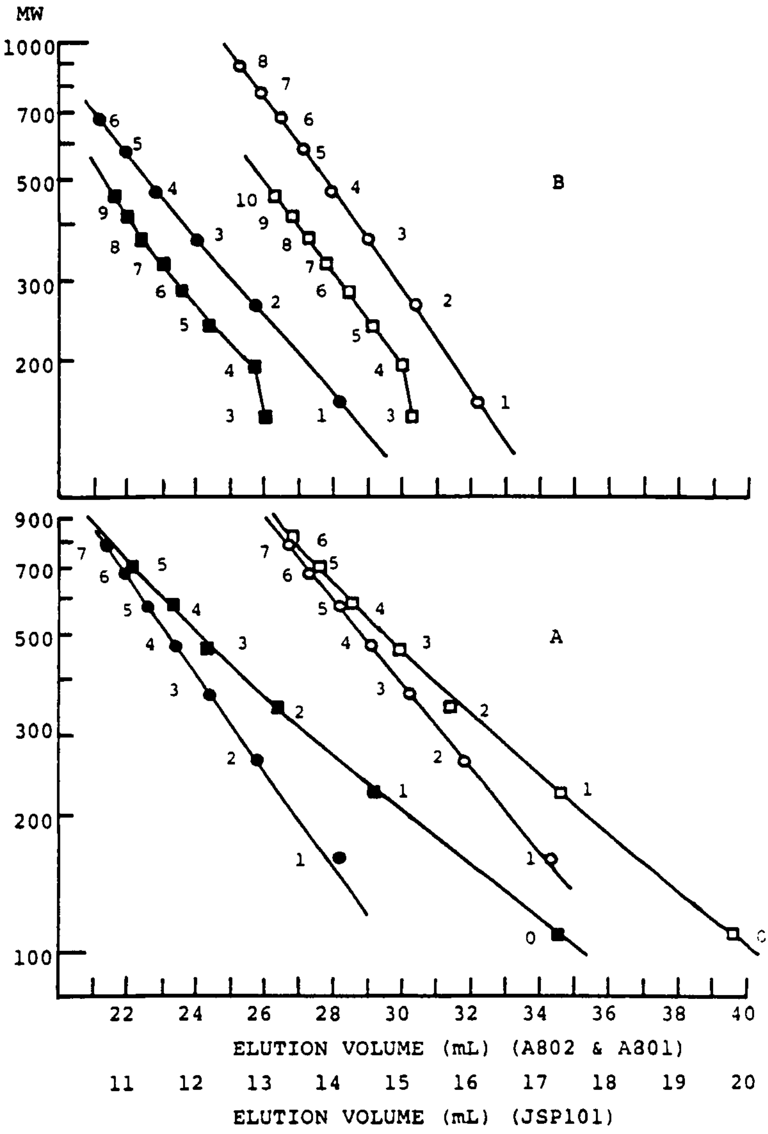


FIGURE 4. The effects of the pore size and shape of the gel. (A) (○) Pst and (□) PCR novolak in a Shodex A801 - chloroform system; (●) Pst and (■) PCR novolak in a JSP101 - chloroform system. (B) (○) Pst and (□) PEG in a Shodex A802 - THF system; (●) Pst and (■) PEG in a Shodex A801 - THF system. Numbers on curves refer to the number of monomer unit of the oligomers.

Downloaded At: 19:18 24 January 2011

TABLE 1

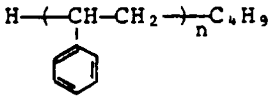
Comparison of Molecular Weights Between Oligostyrenes and Other Oligomers Having the Same Elution Volume

Eluent	Chloroform		THF		
Column	A802	JSP101	A802	JSP101	A801
Pst M.W.			Oligomer M.W.		
			n-Hydrocarbon		
400	295	295	260	265	265
600	400	400	350	355	365
			Epoxy Resin		
500	360	350	470	460	470
700	490	480	600	590	600
			PCR Novolak		
300	380*	420*	210	210	210
400	470*	500*	295	295	300
600	650	650	470	470	480
			PEG		
400	225	220	273	265	280
600	320	315	400*	390*	440*
800	420	410	535*	510*	600*

* Inconsistency between columns was observed.

Downloaded At: 19:18 24 January 2011

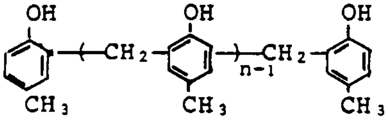
Shodex A802 - chloroform system eluted before dimer (n=2) of oligostyrene and that in a JSP101 - chloroform system after the latter dimer. Tetramer (n=4) of oligostyrene in a Shodex A802 or JSP101 - THF system eluted at the position between retention volumes of n=6 and n=7 of PEGs and that in a Shodex A801 - THF system n=7 and n-8. Similarly, n=5 of oligostyrene in A802 eluted between n=8 and n=9 of PEGs and that in A801 n=9 and n=10. These results are shown in Figure 4. Numbers n refer to the number of monomer unit of the oligomers as listed below;



Pst

$$H\!-\!\left(\!-OCH_2\!-\!CH_2\!-\!\right)_n\!-OH$$

PEG



PCR novolak

Downloaded At: 19:18 24 January 2011

Molecular weight of each oligomer having a elution volume the same as that of oligostyrene in the individual column were obtained in a linear range of the plot of elution volume versus molecular weight and are summarized in Table 1 which suggests the slight influence of gel dimensions. No inconsistency between columns in the same solvent was observed to measure molecular weight of an oligomer using oligostyrene as a reference standard except a few cases. The discrepancy of molecular weight of PCR novolak obtained from both systems, Shodex A802 - chloroform and JSP101 - chloroform, was observed in a lower molecular weight region and that of PEG from A802 - THF and A801 - THF in a higher molecular weight region. It may be concluded that the influence of the pore size and shape of the gel on the estimation of molecular weight relationships between oligostyrenes and other oligomers was negligible except a few cases, though the mechanism of this exceptional influence is not clear. This conclusion will make the next treatment possible.

A linear relationship has been obtained by plotting log molecular weight of oligostyrene and that of epoxy resin, PCR novolak, or PEG having the same elution volume in the same solvent. From this relationship was derived a conversion equation to obtain a molecular weight of an oligomer in question by knowing a molecular weight of oligostyrene which eluted at the

TABLE 2
Molecular Weight Conversion Equations for Several Oligomers Based on Oligostyrene Molecular Weight and MW Range Applicable

Eluent Oligomer	Chloroform	THF
n-Hydrocarbon	$M = 3.07 \times M_s^{0.76}$	$M = 2.45 \times M_s^{0.779}$
	$170 \le M \le 510$	$170 \le M \le 510$
Epoxy Resin	$M = 0.70 \times M_s^{1.001}$	$M = 4.50 \times M_s^{0.748}$
	$340 \le M \le 1760$	$340 \le M \le 1760$
PCR Novolak	$M = 5.47 \times M_s^{0.747}$	$M = 0.362 \times M_s^{1.122}$
	$345 \le M \le 830$	$105 \le M \le 830$
(for JSP101	$M = 12.2 \times M_s^{0.62}$	
	$345 \le M \le 830$)	
PEG	$M = 0.506 \times M_s^{1.004}$	$M = 0.967 \times M_s^{0.945}$
	$195 \le M \le 3400$	$195 \le M \le 3400$
	(for A801	$M = 0.779 \times M_s^{0.989}$
(for A802	$195 \le M \le 2000$)	$195 \le M \le 1000$)

M : Molecular Weight of Oligomers, M_s: MW of Oligostyrene.

same elution volume of the former. Results are listed in Table 2. An oligostyrene calibration curve can be converted to a calibration curve of oligomer in question using this conversion equation. Because of the lack of availability of pure components of oligomers of higher molecular weight, calibration was limited to the molecular weight enough to separate individual components except PEG which were up to MW = 3400. The extrapolation of the equation to higher molecular weight might be permitted. For example, an epoxy resin, EPIKOTE 1004, had $\overline{M}_n = 1350$ by this

Downloaded At: 19:18 24 January 2011

method (a Shodex A802 - chloroform system) and $\bar{M}_n$ = 1470 by VPO (chloroform at 45 ^{o}C). Weight average molecular weight, $\bar{M}_w$, of this resin was 2610 and species from molecular weight 340 (n = 0) to 8300 were included.

The conversion equations based on n-hydrocarbons are listed in Table 3. The upper limit of these equations is the molecular weights of the oligomers that would elute at the same retention volume as n-hexatriacontane (C_{36}) which was the highest n-hydrocarbon available in our laboratory.

These conversion equations make it possible to use oligostyrene or n-hydrocarbon as a reference standard when molecular weights of these oligomers are measured.

Downloaded At: 19:18 24 January 2011

TABLE 3
Molecular Weight Conversion Equations for Several Oligomers Based on n-Hydrocarbon Molecular Weight and MW Range applicable

Eluent Oligomer	Chloroform	THF
Oligostyrene	$M = 0.229 \times M_h^{1.316}$	$M = 0.316 \times M_h^{1.29}$
	$160 \leq M \leq 890$	$160 \leq M \leq 890$
Epoxy Resin	$M = 0.155 \times M_h^{1.320}$	$M = 2.66 \times M_h^{0.908}$
	$340 \leq M \leq 625$	$340 \leq M \leq 625$
PCR Novolak	$M = 1.802 \times M_h^{0.982}$	$M = 0.0595 \times M_h^{1.533}$
	$465 \leq M \leq 710$	$225 \leq M \leq 710$
PEG	$M = 0.300 \times M_h^{1.163}$	$M = 0.207 \times M_h^{1.293}$
	$195 \leq M \leq 415$	$195 \leq M \leq 415$

M : Molecular weight of oligomers, M_h : MW of n-hydrocarbons.

REFERENCES

1. Cazes, J. and Gaskill, D. R., Factors Affecting the Gel-Permeation Chromatographic Fractionation of Low-Molecular-Weight Compounds, Separ. Sci., 2, 421 (1967).

2. Hendrickson, J. G. and Moore, J. C., Gel Permeation Chromatography. III. Molecular Shape versus Elution, J. Polym. Sci., Part A-1, 4, 167 (1966).

3. Hendrickson, J. G., Molecular Size Analysis Using Gel Permeation Chromatography, Anal. Chem., 40, 49 (1968).

4. Cazes, J. and Gaskill, D. R., Gel-Permeation Chromatographic Observation of Solvent-Solute Interaction of Low-Molecular Weight Compounds, Separ. Sci., 4, 15 (1969).

5. Lambert, A., Rationalisation of the Sizes of Small Molecules in Gel Permeation Chromatography, J. Appl. Chem., 20, 305 (1970).

6. Lambert, A., Rationalised Sizes of Small Molecules in Gel Permeation Chromatography, Anal. Chim. Acta, 53, 63 (1971).

7. Edwards, G. D. and Ng, Q. Y., Elution Behavior of Model Compounds in Gel Permeation Chromatography, J. Polym. Sci., Part C, 21, 105 (1968).

Downloaded At: 19:18 24 January 2011