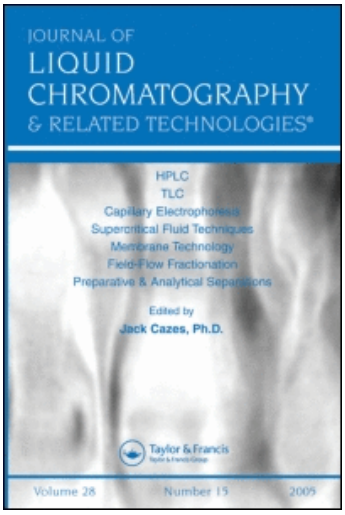

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Elution Behavior of Low Molecular Weight Compounds in Gel Permeation Chromatography

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ELUTION BEHAVIOR OF LOW MOLECULAR WEIGHT COMPOUNDS IN GEL PERMEATION CHROMATOGRAPHY

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ABSTRACT

Elution behavior of organic compounds in gel permeation chromatography was investigated using chloroform as eluent. In aliphatic hydrocarbons, the elution counts decreased linearly with increasing the molecular volumes. In aromatic hydrocarbons, the relation between molecular volume and elution count slightly shifted toward lower counts. The elution counts in esters, ketones, amides, alcohols and carboxylic acids always fell in lower elution counts than expected by aliphatic hydrocarbons. This fact suggests that all these compounds are solvated by eluent molecules. Amines and chlorides exhibit an adsorption effect on cross-linked polystyrene gel. These compounds are eluted behind the corresponding hydrocarbons for given molecular volumes, which were obtained by dividing molecular weight by density.

INTRODUCTION

Gel permeation chromatography (GPC) is very useful, not only for polymer, but also for simple organic compounds such as hexane and benzene. Generally, organic compounds are expected to elute

according to molecular volume like high polymers. However, we can not conclude that elution counts of organic compounds depend only upon molecular volume. According to published papers,[1)-6)] so called universal calibration[7)] is not always observed for organic compounds. The order of elution may depend upon the kinds of solvents, i.e., eluent, polarity of compounds and experimental conditions, although the compounds are approximately eluted according to molecular volume.

As described above, it is very important to investigate the elution behavior of organic compounds as a function of molecular volume in GPC. In this study, experiments were carried out using chloroform as eluent. The logarithmic molecular volumes were plotted against elution counts. The elution behavior of esters, alcohols, etc. is discussed on the basis of elution behavior of aliphatic hydrocarbons.

EXPERIMENTAL

Instrument and Procedure

A Toyo Soda Model-807 high speed liquid chromatograph was used equiped with the two columns ($G2000H_8$) which were packed with cross-linked polystyrene gel. The columns have a nominal exclusion limit of 250Å. The instrument was operated using chloroform as eluent with a flow rate of 1.0ml/min. at ambient temperature. 100 µl of a 0.5 to 1.0% chloroform solution of samples was injected into the columns.

Samples

Aliphatic and aromatic hydrocarbons were supplied by various chemical producers. Esters and alcohols were supplied by Applied Science Laboratories, U.S.A., and ketones and carboxylic acids by Polyscience Corp., U.S.A. and P-L Biochemicals, Inc., U.S.A., respectively. Amides and amines were supplied by various Japanese and American chemical producers. The densities of these compounds were taken from the literature or estimated from those of homologous or similar compounds. The densities of solid compounds in

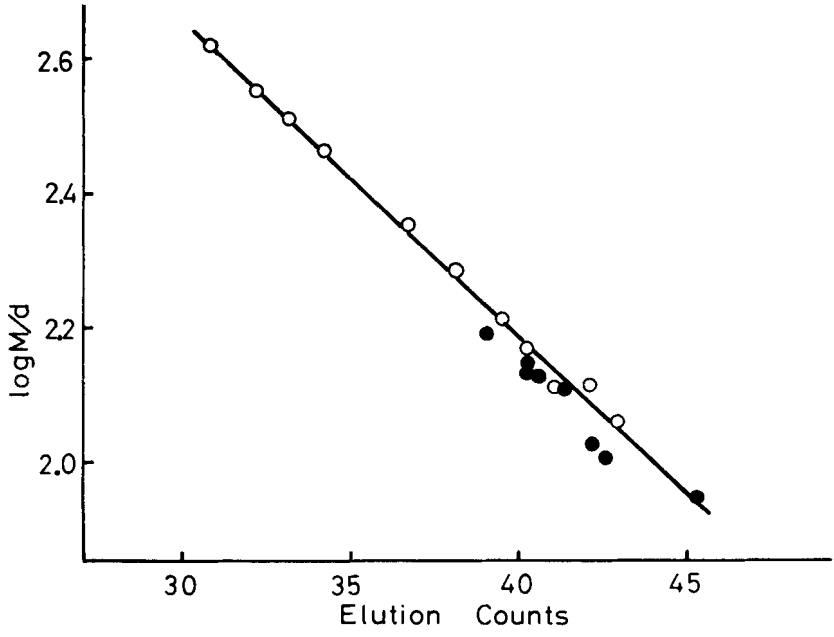

FIGURE 1. Logarithmic molecular volumes vs. elution counts of hydrocarbons.
○ : aliphatic, ● : aromatic.

solution were conventionally obtained by measuring the density of solution containing a given amount of the solute as follows:

$$\rho_2 = \frac{W_2}{\frac{V_1 + W_2}{\rho_3} - V_1} \quad \text{.....(1)}$$

where ρ is the density, W is the weight, V is the volume, and subscripts 1, 2, 3 mean solvent, solute and solution, respectively.

RESULTS AND DISCUSSION

The relationship between molecular volume vs. elution count is expected to be linear, since the columns used in this study are

TABLE 1

Molecular Volumes and Elution Counts of Hydrocarbons

Compound	Molecular Volume	Elution Count
n-Tetracosane	423.9	30.8
n-Eicosane	359.4	32.1
n-Octadecane	328.4	33.1
n-Hexadecane	294.0	34.2
n-Dodecane	228.6	36.7
n-Decane	195.9	38.1
n-Octane	164.0	39.4
n-Heptane	147.5	40.2
n-Pentane	115.2	43.0
p-Cymene	157.4	39.0
Cumene	139.4	40.3
Tetralin	136.8	40.3
Diphenyl	134.1	40.6
Ethylbenzene	123.1	41.3
Styrene	114.8	42.5
Toluene	106.8	42.1
Benzene	89.4	45.3

commercially supplied. According to the usual application of GPC, the relationship will be hereafter called calibration curve. The molecular volumes of samples were obtained by dividing M by d (M: molecular weight, d: density). Strictly speaking, the molecular volume of organic compounds in solution may slightly deviate from M/d. However, we need not be concerned with such small differences in this study.

The calibration curve for aliphatic hydrocarbons is shown in FIGURE 1. and TABLE 1. As expected, elution counts increase linearly with decreasing molecular volumes. The elution counts of aromatic hydrocarbons shift slightly toward lower counts for a

TABLE 2

Molecular Volumes and Elution Counts of Esters

Compound	Molecular Volume	Elution Count
Methyl Behenate	410.9	30.2
Methyl Arachidate	378.0	30.8
Ethyl Stearate	359.0	31.3
Ethyl Oleate	356.9	30.9
Methyl Stearate	345.1	31.4
Methyl Myristate	279.3	33.2
Methy Laurate	246.3	33.7
Methyl Caprate	213.4	34.5
Methyl Caprylate	180.3	35.6
Phenyl Benzoate	160.5	37.8
Methyl Caproate	147.2	36.9
Phenyl Acetate	126.3	39.6
Methyl Benzoate	125.0	39.4
Diethyl Carbamate	121.2	37.6
Methyl Acetate	79.3	39.7

TABLE 3

Molecular Volumes and Elution Counts of Ketones

Compound	Molecular Volume	Elution Count
2-Nonanone	173.3	35.4
2-Octanone	156.3	36.1
2-Heptanone	140.8	36.6
2-Hexanone	123.5	37.4
2-Pentanone	106.5	38.0
2-Butanone	89.6	39.0
Acetophenone	117.4	39.1
Acetylacetone	102.5	38.5

TABLE 4

Molecular Volumes and Elution Counts of Amides

Compound	Molecular Volume	Elution Count
Erucamide	338.0	31.5
Stearamide	283.0	32.6
Oleamide	281.5	32.7
N,N-Dimethylformamide	77.4	37.9

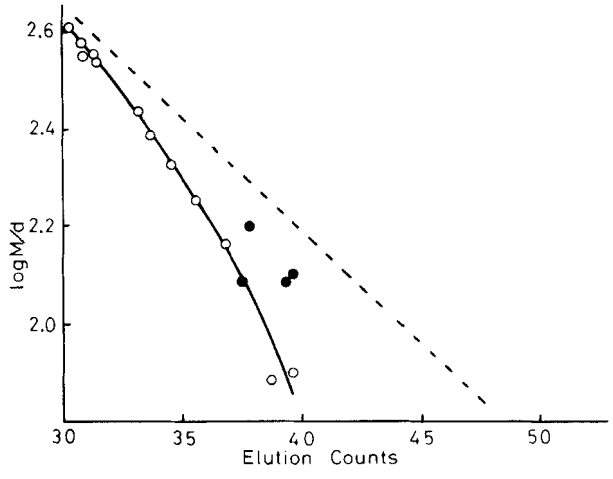

FIGURE 2. Logarithmic molecular volumes vs. elution counts of esters.
○ : alipahtic, ● : aromatic,
------- : hydrocarbons.

given molecular volume. This fact suggests that aromatic hydrocarbons exhibit interactions between solute and solvent molecules. Moreover, as will be discussed later, the effect of adsorption of solutes on the cross-linked polystyrene gel has to be also taken into account.[3),8)] At any rate, the calibration curve obtained for aliphatic hydrocarbons indicates the most reliable relationship between elution count and the molecular volume which is expressed by

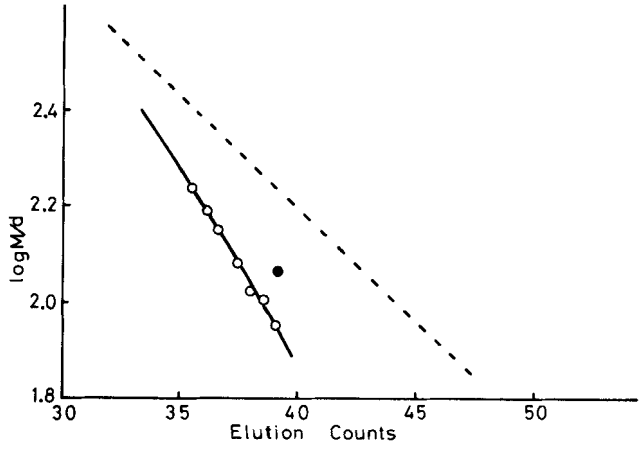

FIGURE 3. Logarithmic molecular volumes vs. elution counts of ketones.
○ : aliphatic, ● : aromatic,
-------- : hydrocarbons.

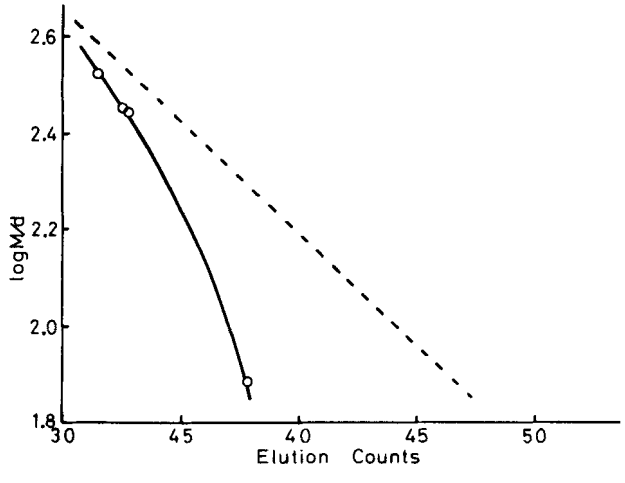

FIGURE 4. Logarithmic molecular volumes vs. elution counts of amides.
○ : aliphatic,
-------- : hydrocarbons.

M/d. The elution behavior of other compounds will be always discussed on the basis of the curve for aliphatic hydrocarbons.

The elution behavior of esters, ketones and amides is shown in TABLES 2-4 and FIGURES 2-4. Like hydrocarbons, all these compounds are principally separated according to molecular volumes; the elution counts decrease with increasing molecular volumes. However, the compounds are eluted earlier than hydrocarbons, although experimental points in the lower molecular weight region are somewhat scattered. Similar tendency is observed for alcohols and carboxylic acids, as shown in FIGURES 5, 6 and TABLES 5, 6. Apparently, carboxylic acids are not so different from other polar compounds. However, the shapes of the peaks are skewed toward higher elution counts, as shown in FIGURE 7.

Two reasons are considered for these deviations: one is due to repulsion between solutes and cross-linked polystyrene gel,[1] the other is due to the association of solute molecules with solvent or other solute molecules. The latter is more probable. The effec-

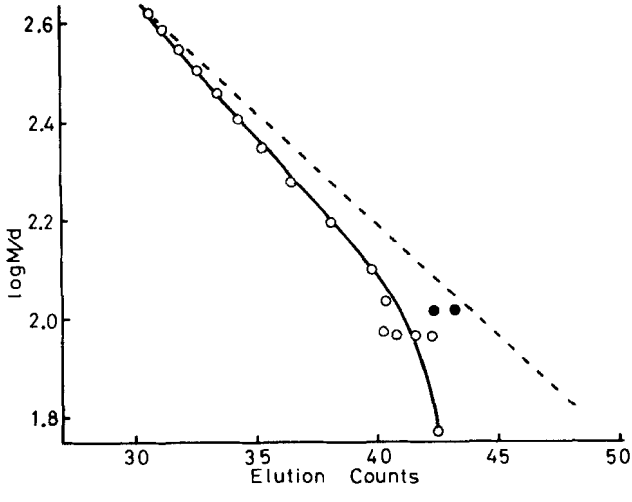

FIGURE 5. Logarithmic molecular volumes vs. elution counts of alcohols.
○ : alipahtic and alicyclic, ● : aromatic,
------- : hydrocarbons.

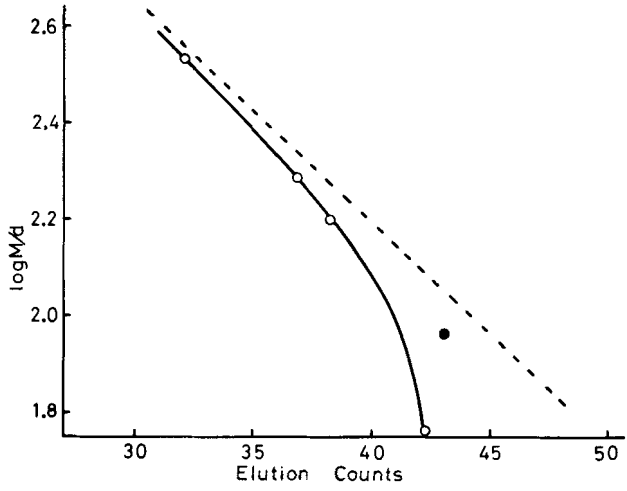

FIGURE 6. Logarithmic molecular volumes vs. elution counts of carboxylic acids.
○ : aliphatic, ● : aromatic,
------- : hydrocarbons.

TABLE 5

Molecular Volumes and Elution Counts of Alcohols

Compound	Molecular Volume	Elution Count
1-Tetracosanol	420.5	30.7
1-Docosanol	387.9	31.3
1-Eicosanol	355.2	31.9
1-Octadecanol	322.4	32.7
1-Hexadecanol	289.6	33.5
1-Tetradecanol	257.1	34.4
1-Dodecanol	224.3	35.4
1-Decanol	191.3	36.6
1-Octanol	158.4	38.2
1-Hexanol	125.3	39.8
n-Amyl Alcohol	108.7	40.4
t-Butyl Alcohol	94.9	40.2
s-Butyl Alcohol	92.3	40.8
n-Butyl Alcohol	92.0	41.6
Cyclopentanol	91.4	42.3
m-Cresol	105.0	43.2
Benzyl Alcohol	103.8	42.3

TABLE 6

Molecular Volumes and Elution Counts of Carboxylic Acids.

Compound	Molecular Volume	Elution Count
Stearic Acid	340.0	32.0
n-Capric Acid	192.9	36.8
n-Caprylic Acid	158.5	38.2
Acetic Acid	57.2	42.2
Benzoic Acid	92.0	43.0

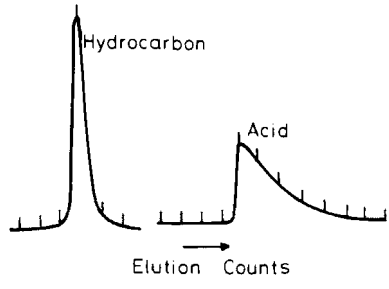

FIGURE 7. Elution peaks of hydrocarbon and carboxylic acid.

tive volumes of solutes should be enlarged if association takes place. Let us express the deviation of effective molecular volumes from M/d by ΔV_m,

$$\Delta V_m = V_o - V_c \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where V_c is identical to M/d, V_o is the effective molecular volume. ΔV_m is schematically shown in FIGURE 8. If a polar group in a compound gives rise to association with eluent molecules, ΔV_m should be positive. FIGURE 9 clearly proves this idea. Generally, with decreasing molecular volume ΔV_m increases. Ketones, amides and esters have a similar tendency. ΔV_m for these compounds approaches

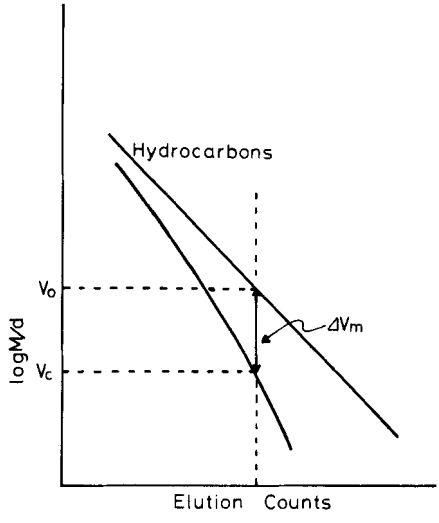

FIGURE 8. Deviation of molecular volumes of polar compounds from those of corresponding hydrocarbons.

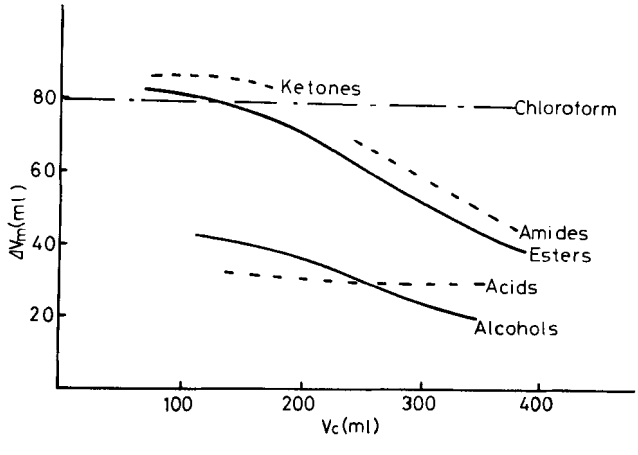

FIGURE 9. ΔV_m as a function of molecular volume in various solutes.

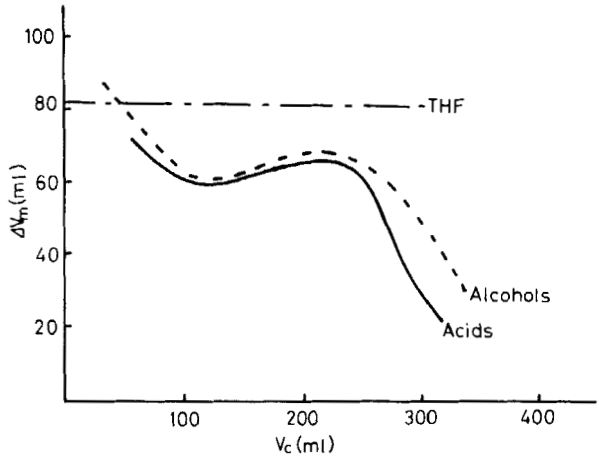

FIGURE 10. ΔV_m as a function of molecular volume in THF. These relationships were derived from data by Chang.

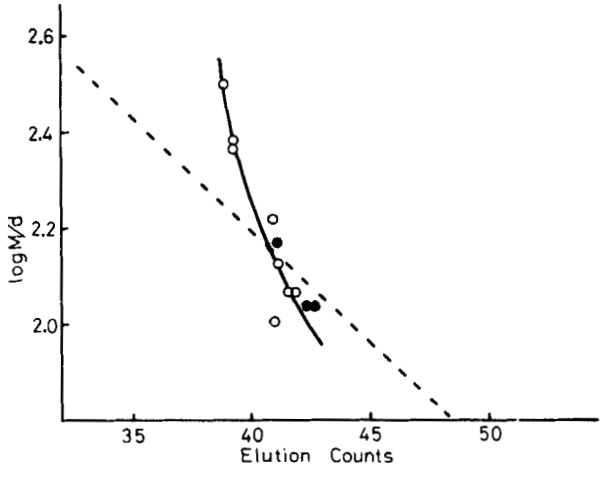

FIGURE 11. Logarithmic molecular volumes vs. elution counts of amines.
○ : aliphatic and alicyclic, ● : aromatic,
------- : hydrocarbons.

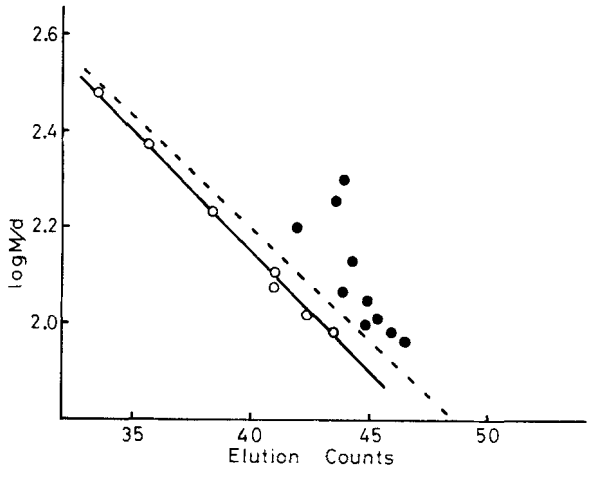

FIGURE 12. Logarithmic molecular volumes vs. elution counts of chlorides.
○ : aliphatic, mono- and di-chlorides,
● : other chlorides, ------- : hydrocarbons.

TABLE 7

Molecular Volumes and Elution Counts of Amines

Compound	Molecular Volume	Elution Count
Stearylamine	312.7	39.0
Tri-n-butylamine	238.2	39.4
Laurylamine	231.3	39.3
n-Octylamine	166.3	41.0
n-Hexylamine	133.1	41.2
n-Amylamine	115.6	41.7
Cyclohexylamine	115.0	41.8
s-Butylamine	100.4	41.0
N,N-Dimethylbenzyl-amine	147.8	41.2
Benzylamine	109.5	42.5
N-Methylaniline	108.9	42.7

TABLE 8

Molecular Volumes and Elution Counts of Chlorides.

Compound	Molecular Volume	Elution Count
Cetyl Chloride	301.5	33.5
Lauryl Chloride	235.9	35.6
n-Octyl Chloride	171.1	38.3
1,5-Dichloropentane	128.1	41.0
n-Amyl Chloride	120.9	40.9
n-Butyl Chloride	104.4	42.4
1,2-Dichloropropane	97.5	43.4
1,2,4,5-Tetrachloro-benzene	199.8	43.9
Hexachlorobenzene	181.5	43.5
Hexachlorocyclo-pentadiene	160.3	41.8
α-Chloronaphthalene	136.2	44.2
o-Chlorotoluene	117.1	43.8
Hexachloroethane	113.2	44.9
1,1,2,2-Tetrachloro-ethylene	104.5	45.3
1,1,1-Trichloroethane	99.6	44.7
Carbon Tetrachloride	97.1	45.8
1,1,2-Trichloroethane	92.5	46.4

the molecular volume of chloroform at M/d = 200. Carboxylic acids and alcohols exhibit a different pattern and their ΔV_m's are lower than those for the above compounds. This tendency is also similar to behavior in tetrahydrofuran (THF); the results were derived from the data of Chang (FIGURE 10).[2] However, the extent of solvation seems to be different from that in chloroform. At the present stage, it is very difficult to predict the magnitude of ΔV_m by an appropriate method. This problem will be fully discussed in the next paper with respect to infrared and NMR spectra.

Amines have specific behavior as shown in FIGURE 11 and TABLE 7. Elution counts are abnormally high for the compounds having a molecular volume greater than 150. In the low molecular weight region, elution behavior is similar to that of esters and ketones. Abnormal lag in high molecular volume region will come from adsorption on the cross-linked polystyrene gel. Chlorides are also complicated as shown in FIGURE 12 and TABLE 8. Mono- and dichlorides seem to follow the same rule as esters and ketones. On the other hand, polychlorides and chlorides containing aromatic rings show adsorption effects on the cross-linked polystyrene gel.

Other compounds containing aromatic rings, such as esters and alcohols, are eluted behind the corresponding aliphatic ones, as shown in FIGURES 1-3, 5 and 6. This behavior is also explained by the same idea. Consequently, these compounds exhibit both solvation and adsorption effects.

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