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EMPIRICAL APPROACH TO THE UNIVERSAL CALIBRATION OF ISOMERS OF PHTHALATE ESTERS AND SIMILAR COMPOUNDS IN SIZE EXCLUSION CHROMATOGRAPHY

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SUMMARY

Assuming geometrical shapes of molecules in solution, new universal size parameters were introduced and a universal calibration graph for three phthalate ester isomers (ortho-, iso- and tere-) was constructed. The projected area of a molecule on a projection plane which is parallel to the plane of the phenyl group of the molecule is one of the new parameters. The product of the components for the X axis and the Y axis of the projection on the XY plane and the Z axis of the projection on the XZ plane is the other parameter. This approach was applied to trimesic and benzoic acid esters, *n*-alkylbenzenes, *n*-hydrocarbons, oligostyrenes and oligoethylenes, and good correlations were observed.

INTRODUCTION

Size exclusion chromatography (SEC) (or gel permeation chromatography, GPC) is widely used for calculating average molecular weights of polymers and measuring the sizes or molecular weights of molecules (especially low-molecular-weight organic compounds). Recent developments in high-performance liquid chromatography have accelerated the advancement of techniques in SEC, especially as regards separations of low-molecular-weight compounds such as oligomers. In an SEC analysis the elution volume can be related to the molecular weight or the size of the sample by calibrating the column system with standard samples of known molecular weights or sizes.

The basic separation mechanism in SEC is probably based on the hydrodynamic volume of the molecule in solution[1], and this factor has been used in formulating the calculation of average molecular weights of various polymers. The hydrodynamic volume corresponds in nature to the actual molecular volume occupied by the solute molecule in solution. Unfortunately, the technique needed for determining the hydrodynamic volume or the actual volume of small molecules, such as oligomers and low-molecular-weight compounds, in solution is impracticable. Therefore, from the practical standpoint of setting up a standard basis, the molar volume (defined as molecular weight divided by density) has usually been applied[2–4].

Knowing the observed SEC molar volume, one is able to estimate the molecular weight of an unknown sample, provided one knows its density and chemical type.

A single equation may be obtained when the elution volume is plotted against the logarithm of the molar volumes of molecules of the same chemical type, such as a series of oligomers obtained from the product of an addition reaction. However, this equation may not describe the elution behaviour in SEC of other solute molecules of different chemical types. Organic functional groups exert a systematic influence[3]. Corrections needed for various molecules resulted in the establishment of experimentally obtained effective molar volumes[2,4]. Another approach for the universal calibration of small molecules is the concept of "effective chain length"[5], for which one needs an experimentally obtained "effective carbon number". Both approaches are useful for molecules for which effective molar volumes or effective carbon numbers are obtained experimentally.

Size parameters other than molar volume or chain length must be introduced for the universal calibration of low-molecular-weight substances. One cannot ignore the geometrical shape of a molecule in solution. The elution volumes of a rod-shaped molecule and a spherical molecule which have the same volume are not necessarily the same. The mean external length better characterizes partitioning in specific systems[6]. In order to obtain this parameter, one must know the steric structure of the molecule in solution. In this work, attempts have been made to obtain a universal parameter by assuming the geometrical shapes of molecules in solution. A common calibration graph for phthalate ester isomers (esters of ortho-, iso- and terephthalic acids) was constructed and this approach was applied to other compounds.

EXPERIMENTAL

Samples

Esters of orthophthalic, isophthalic, terephthalic, trimesic and benzoic acids with primary alcohols from methanol to *n*-octadecanol were prepared. These acids and phthalic anhydride were subjected to reaction with thionyl chloride by refluxing a mixture of 5 g of acid, 25 g of thionyl chloride and 0.1 ml of triethylamine for 12 h. The resulting acid chlorides were purified by recrystallization from *n*-hexane, except benzoyl chloride, which was distilled. A 50-mg amount of acid chloride was subjected to reaction with 0.1 ml of an appropriate alcohol in a test-tube by heating for several minutes. A few drops of triethylamine were added. A 10-ml volume of chloroform was added to the reaction mixtures and the solutions were used as sample solutions for SEC analysis. In addition to these esters, alkylbenzenes from benzene to *n*-heptadecylbenzene, *n*-hydrocarbons from *n*-pentane to *n*-hexatriacontane, oligostyrenes up to n (number of monomer units) $= 12$ and oligoethylene glycols up to $n = 13$ were examined.

SEC apparatus and measurements

A JASCO (Japan Spectroscopic Co., Hachioji, Tokyo, Japan) Trirotar high-performance liquid chromatograph was used with a Uvidec-100 variable-wavelength UV detector (JASCO) and an SE-11 differential refractometric detector (Showa Denko, Minato-ku, Tokyo, Japan).

Two different combinations of columns and eluents were prepared. In system I, two Shodex A801 high-performance SEC columns (50 cm × 8 mm I.D.) (Showa Denko) packed with polystyrene gel of exclusion limit 1000 molecular weight as polystyrene were used. The eluent was chloroform at a flow-rate of 1.1 ml/min, and the temperature was ambient. Volumes of 5 μl of sample solutions of the esters and alkylbenzenes were injected. The UV detector was set at 254 nm and 0.32 a.u.f.s. System II included four Shodex A802 SEC columns and tetrahydrofuran as the eluent at a flow-rate of 1.0 ml/min. A differential refractometer was used as detector attenuation at ×8. Samples (except the esters) were dissolved in tetrahydrofuran to give 0.1% solutions and 0.1-ml volumes of the solutions were injected.

RESULTS AND DISCUSSION

Elution volumes of some esters and alkylbenzenes determined by system I are given in Table I. By plotting the logarithm of the molecular weights and elution volumes of three isomeric phthalate esters, one readily obtains three different parallel lines. A single line for these isomers was not obtained even if molar volumes, calculated by dividing molecular weight by density, were plotted instead of molecular weights. Similarly, different calibration graphs were obtained for the other esters and alkylbenzenes. Neither molecular weight nor molar volume can be used satisfactorily as a molecular size parameter for the universal calibration of these

TABLE I

ELUTION VOLUMES OF ESTERS OF ISOMERIC PHTHALIC, BENZOIC AND TRIMESIC ACIDS AND ALKYLBENZENES WITH CHLOROFORM AS ELUENT

n	*Elution volume (ml)**					
	AB	*B*	*OP*	*IP*	*TP*	*T*
0	31.3	27.4				
1	30.4	26.7	24.1	23.7	23.2	22.1
2	29.4	25.6	22.9	22.5	22.0	20.8
3	28.5	25.0	22.2	21.8	21.3	20.0
4	27.6	24.4	21.6	21.1	20.8	19.5
5		23.8	21.0	20.5	20.2	19.0
6	26.5		20.6	20.1	19.8	18.6
7	25.5		20.2	19.7	19.4	18.3
8	24.8	22.5	19.9	19.4	19.1	17.9
9	24.3					
10	23.8	21.7	19.3	18.8	18.5	17.3
11	23.4	21.4	19.1	18.4	18.3	17.2
12	23.0	21.1	18.8	18.3	18.0	16.9
13	22.6					
14			18.45			16.4
16			18.1			16.2
17	21.3					
18		19.7	17.8	17.2	16.9	15.9

* AB = Alkylbenzenes, $C_6H_5(CH_2)_nH$; B = benzoates, $C_6H_5COO(CH_2)_nH$; OP = orthophthalates, $C_6H_4[COO(CH_2)_nH]_2$; IP = isophthalates; TP = terephthalates; T = trimesic acid esters, $C_6H_3[COO(CH_2)_nH]_3$.

materials, because neither molecular weight nor molar volume represents the true molecular volume occupied by a molecule in solution or a parameter proportional to the steric structure of a molecule in solution.

The effective molar volume as defined by Smith and Kollmansberger[2] and re-calculated experimental molar volumes as elucidated by Lambert[4,7] were the experimentally obtained values that were calculated on the basis of the molar volumes of *n*-hydrocarbons. Their concepts for the interpretation of the effective size of a molecule in solution were probably the same as that for the effective chain length defined by Hendrickson and Moore[5]. In the technique based on effective chain length, *n*-hydrocarbons were used as the standard for calculation and the "effective carbon numbers" for various molecules were experimentally determined or corrected by correlating the extended chain lengths and carbon numbers with the elution volumes. The values of the effective carbon number for the phenyl group of alkylbenzenes measured in system II on the basis of *n*-hydrocarbons were 2.85, 2.35 and 2.25 units for benzene, toluene and ethylbenzene, respectively, compared with 3.52, 3.08, and 2.84 units as reported in the literature[5]. By correlating these values with elution volumes in systems I and II for alkylbenzenes, the effective carbon numbers for the isomeric phthalate and benzoate esters were calculated. The residual effective carbon number of each ester, which was obtained by subtracting the effective carbon number of alkyl group(s) from the total effective carbon number, which can be assumed to be constant for members of a homologous series, varied with the number of monomer units present. For example, the value for orthophthalate esters changed from 9.8 for the methyl ester to 10.2 for the butyl ester, that for isophthalate esters from 10.7 for the methyl ester to 12.2 for the butyl ester and that for terephthalate esters from 11.6 for the methyl ester to 13.4 for the butyl ester. Although the value for benzoic acid esters remained constant at 7.2 from the methyl to the butyl ester, it increased from 7.3 for the pentyl ester to 8.4 for the dodecyl ester. These results indicate that the effective carbon number of these esters cannot be calculated by definition.

The magnitude of the association between the molecules and that of their association with the solvent or the gel will be almost identical among the isomeric phthalate esters, and the solute molecules appearing at the same elution volume may possess identical molecular dimensions in solution. With this assumption, a new universal molecular size parameter which can represent the relationship for the elution volumes of several molecules as a single curve is proposed. When a series of molecules are of spherical shape, the diameter or a radius can be used as the molecular size parameter. However, with rigid-rod molecules, the possibility of permeation of the molecules into the gel pores might be different, depending on whether the molecular axis (the axis of revolution) of the solute molecule is parallel to or at right-angles to the inlet of a gel pore. Actually, it is necessary to average the molecular length at the inlet of a gel pore in one particular direction over all possible directions.

With this consideration, the molecular forms of the isomeric phthalate esters in solution at the time of their permeation into the gel pores must be assumed. Fig. 1 is a projection chart of diamyl isophthalate to the projection plane which is parallel to the plane of the phenyl group. The projected area is the product of lengths *a* and *b*. This area may be used to formulate a general expression for the partitioning of

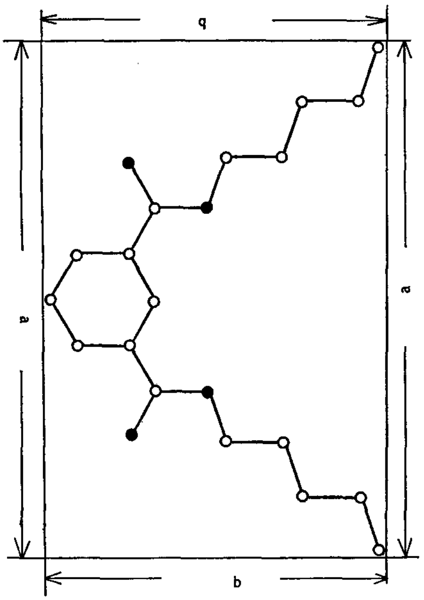

Fig. 1. Illustration of average configuration at the time of permeation of a molecule into a gel pore. The molecule is diamyl isophthalate.

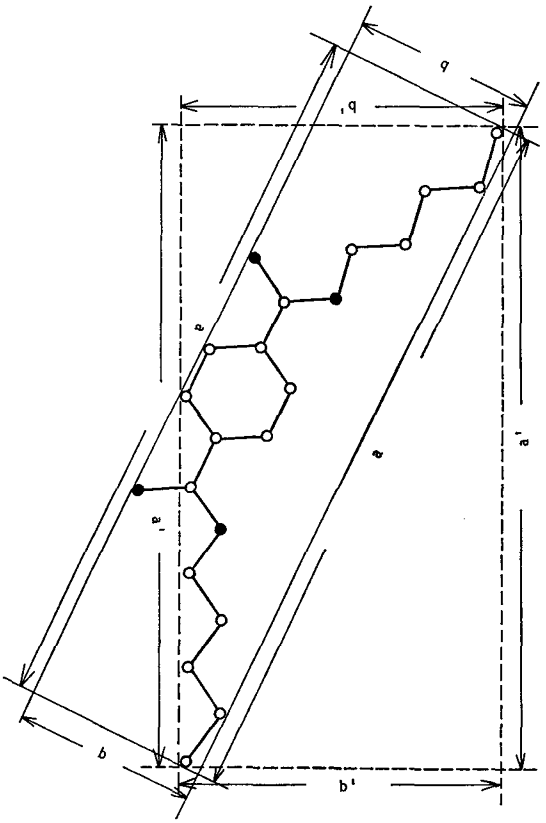

Fig. 2. Projection chart of diamyl terephthalate to the plane parallel to the planar phenyl group. Closed circles indicate oxygen atoms and open circles carbon atoms.

ester molecules between solvent outside and inside the gel pores. The closed circles indicate oxygen atoms and the open circles carbon atoms. The covalent radius of hydrogen was included and the Van der Waals radius of hydrogen was neglected in the calculation of the projected area. Two different conformations were assumed for the two ester groups in the *meta* position, but it was decided to prefer the form shown in Fig. 1. The projection of orthophthalate esters was formulated in a similar manner.

Fig. 2 shows the projection chart of diamyl terephthalate and two extreme values for length and breadth are illustrated. Actually, the arithmetic means of lengths *a* and *a'*, and *b* and *b'*, were used to calculate the projected area. The conformation of terephthalic acid esters was assumed to be identical with that of isophthalate and orthophthalate esters. Plots of the logarithm of the projected areas and the elution volumes for the isomeric phthalate esters gave a single line, as shown in Fig. 3. The projected areas for benzoic and trimesic acid esters were calculated in a similar manner, but the correlation of the areas and the elution volumes deviated from the single line for the above isomers (see Fig. 3).

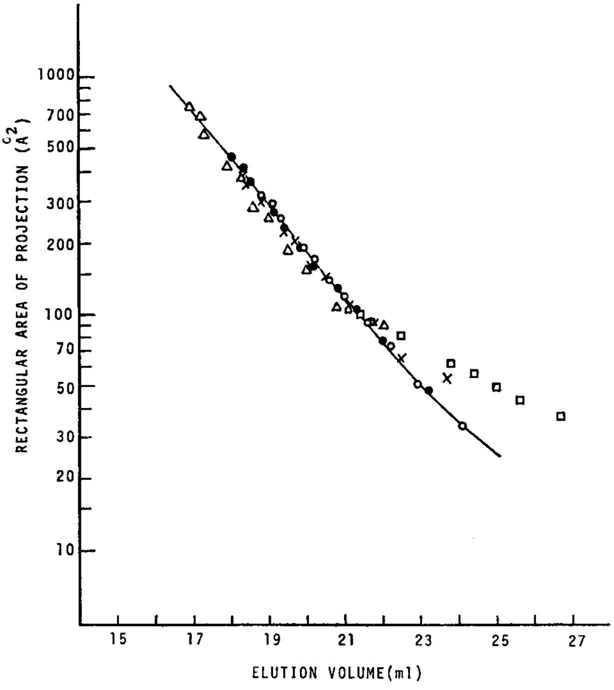

Fig. 3. Relationships between the logarithm of the projected rectangular area and the elution volume. ○, Orthophthalic acid; ×, isophthalic acid; ●, terephthalic acid; □, benzoic acid; △, trimesic acid.

This projected area of a molecule can be used as a molecular size parameter that represents the molecular dimensions at the time of its permeation into a gel pore. However, when the residence of a molecule in a gel pore is taken into account, volume will be a better size parameter than area. First, the projection of a molecular model of *n*-hydrocarbons that were distributed over rectangular coordinates on *XY*, *XZ* and *YZ* planes was considered. The C–C linkage at the terminus of *n*-hydrocarbons (C_1–C_2) was placed parallel to both the *X* axis and the *XY* plane, and then the remainder of the carbon atoms were distributed according to the C–C bond angle and length. The plane represented by the C–C linkage should be parallel to the *XY* plane.

Fig. 4 illustrates the basic configuration on the coordinates. The solid line shows the projection chart of *n*-hexane on the *XY* plane. The products of the components for the *X* axis and the *Y* axis of the projection on the *XY* plane and the *Z* axis of the projection on the *XZ* plane will correspond to the size parameter. The components of the *X*, *Y* and *Z* axes for *n*-hexane were 6.5, 4.6 and 1.9 Å, respectively, and those for *n*-eicosane (C_{20}) were 21.2, 14.4 and 1.9 Å, respectively.

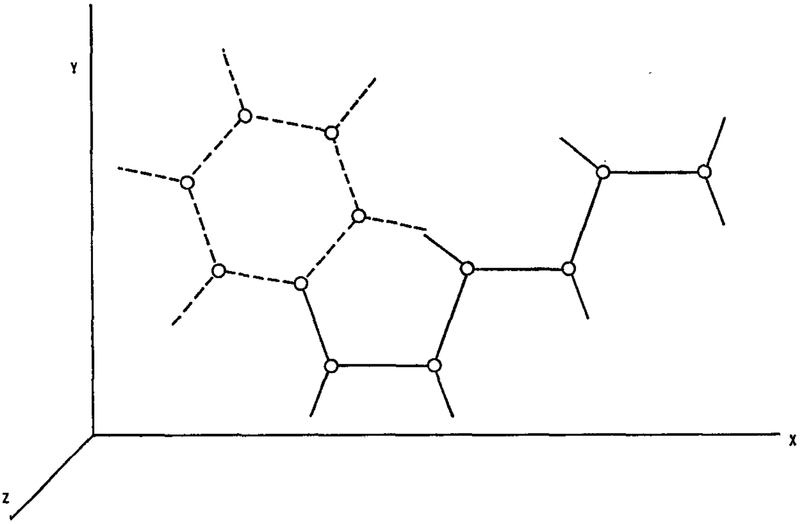

Fig. 4. Illustration of basic configurations of molecules on rectangular coordinates. The solid lines are a projection chart of *n*-hexane on the *XY* plane. The broken lines are a phenyl group.

In oligoethylene glycols, two carbon atoms in the terminal monomer unit of the chain (C_1 and C_2 with $n = 1$) were placed over the rectangular coordinates in a similar manner to *n*-hydrocarbons, and then two oxygen atoms at the terminus and in the chain were attached to the carbon atoms. Finally, the chain was extended according to the rule of the bond angle and length. The components of *X*, *Y* and *Z* for diethylene glycol were 6.7, 5.5 and 1.9 Å, respectively, and those for pentaethylene glycol ($n = 5$) were 15.1, 12.0 and 1.9 Å, respectively. In alkylbenzenes, the alkyl group was first distributed in a similar manner to *n*-hydrocarbons and then the phenyl group was located. Two extreme configurations for the face of a phenyl group are visualized: one is parallel to the *XY* plane (a broken line in Fig. 4) and the other at right-angles to it. The actual configuration is probably intermediate between the two. The component of the *Z* axis was between 1.9 Å (if a phenyl group is parallel to the *XY* plane) and 4.2 Å (vertical), and from a comparison with the data for *n*-hydrocarbons the value was 2.45 Å. The component of the *Y* axis from toluene to *n*-hexylbenzene was 4.9 Å and the values increased from *n*-heptylbenzene onwards. For example, the components of the *X*, *Y* and *Z* axes for *n*-hexylbenzene were 8.0, 4.9 and 2.45 Å, respectively, and those for *n*-decylbenzene were 12.1, 7.6 and 2.45 Å, respectively. The configuration of oligostyrenes that possess a terminal *n*-butyl group may be equivalent to that of polysubstituted *n*-hydrocarbons with phenyl groups. The component of the *Z* axis was 4.6 Å for the dimer and 5.2 Å for the trimer and higher polymers. The components of the *X* and *Y* axes for oligostyrenes were equivalent to *n*-hydrocarbons that possess the same number of carbon atoms other than in the phenyl groups. For example, the components of the *X* and *Y* axes for oligostyrene octamer ($n = 8$) were 21.2 and 14.4 Å, respectively, and these values were identical with those for *n*-eicosane (C_{20}).

The rectangular volume, which was made up by superposition of the three projection charts on the *XY*, *YZ* and *XZ* planes, calculated from the product of the three components of the *X*, *Y* and *Z* axes, shows a good correlation with the elution volume, except for the oligoethylene glycols below $n = 3$, as shown in Fig. 5. Fig. 6, which shows the relationships for molecular weight *versus* elution volume and effective chain length[5] *versus* elution volume for the same samples, is shown for comparison purposes.

Assuming two ester groups of phthalate esters (*o*-, *m*- and *p*-) distribute in the same or the opposite directions against the plane of the phenyl group, the average

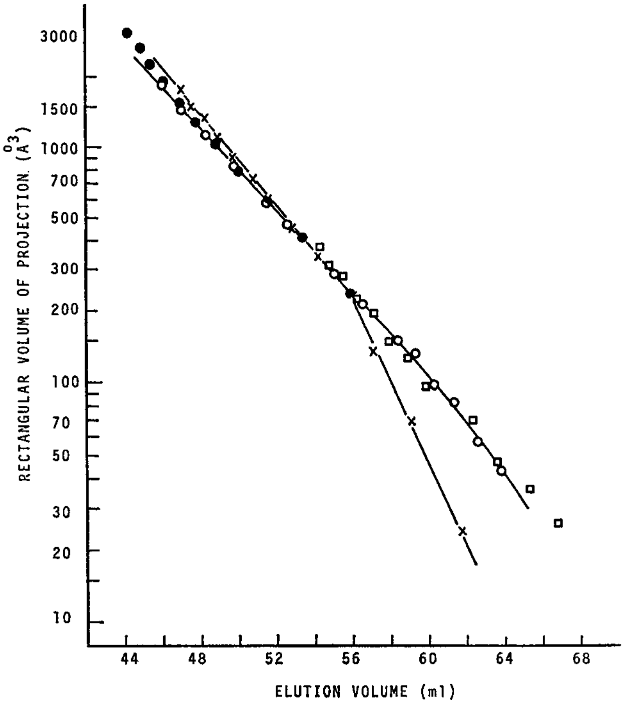

Fig. 5. Rectangular volume *versus* elution volume for non-ester solutes. ○, *n*-Alkane; ●, polystyrene; ×, polyethylene glycol; □, alkylbenzene.

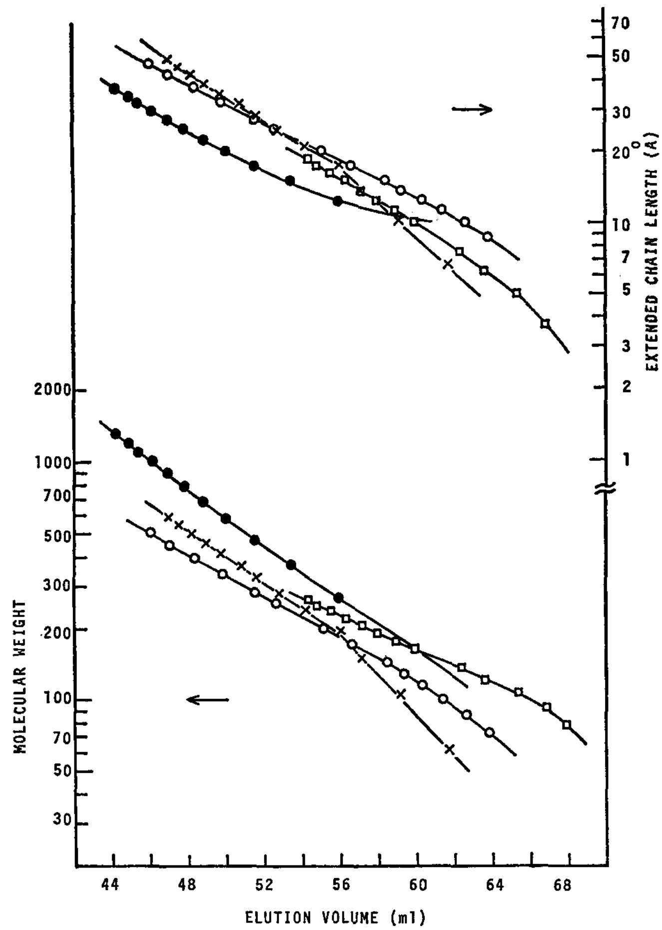

Fig. 6. Relationships between molecular weight or effective chain length and elution volume for the same samples as in Fig. 5. Symbols as in Fig. 5.

volume of projection was calculated. In Fig. 7, the orientation of one ester group in the same (broken line) and in the opposite direction (full line) to the other is illustrated. Steric arrangements of amyl isophthalate are projected in the *XY* and the *XZ* planes. Fig. 8 shows the correlation of the rectangular volume of projection to the elution volume for these esters.

Hjertén[8] derived an equation for the relationship between the distribution coefficients and the parameters characterizing the solute and the gel bed using thermodynamics. The equation is simplified for isochemical solutes as

$$-\log K = C_1V + C_2A + C_3$$

where K, V and A are the distribution coefficient, the partial molal volume and the molecular area of a solute, respectively. C_1, C_2 and C_3 are constants corresponding

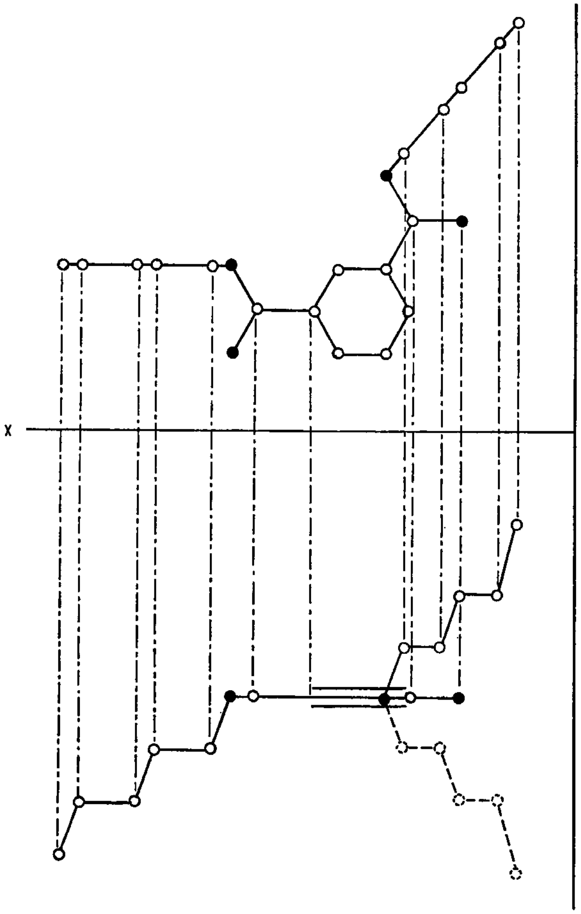

Fig. 7. Illustration of the orientation of two ester groups of phthalate esters against the plane of the phenyl group. Amyl isophthalate is projected.

to the differences in the pressure, the interfacial tension and the sum of the electrical potential and the adsorption energy for a solute distributed between the gel and the mobile phase, respectively. Hjertén obtained an equation for K as a function of the molecular weights, M, of solutes by assuming $V = \text{constant}\cdot M$ and $A = \text{constant}\cdot M$ or $A = \text{constant}\cdot M^{2/3}$, and then observed that the equation correlated well with the experimental results. Although his model and ours are based on different assumptions, it might be possible to correlate the projected volume, V_p, and the projected area, A_p, of our model with this equation if V_p and A_p are associated with molecular weight, M, as

$$V_p = C_4M^a$$

and

$$A_p = C_5M^b$$

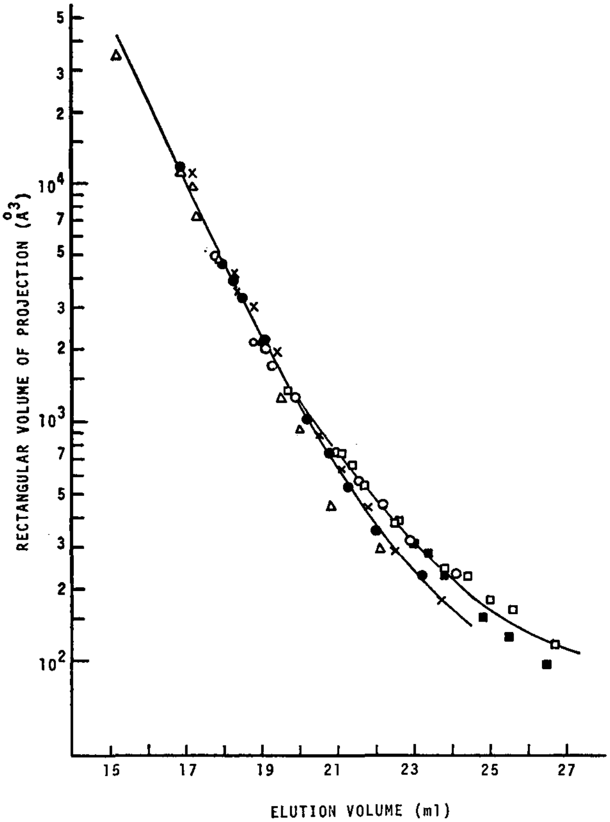

Fig. 8. Rectangular volume *versus* elution volume for ester solutes. ○, Orthophthalic acid; ×, isophthalic acid; ●, terephthalic acid; △, trimesic acid; □, benzoic acid; ■, alkylbenzene.

where C_4, C_5, a and b are constants for a homologous series. With this consideration, the exponents a and b were calculated as follows: values of a for orthophthalates and benzoates were about 2.4 and those for isophthalates and terephthalates were 3.5; values of b for ortho-, iso- and terephthalates were about 2.2, and that for benzoates was 1.5. These results suggest that V_p and A_p would be better parameters than molecular weight for the correlation of molecular size with the distribution coefficient among the solutes of different homologous series.

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