CHROM. 14,664

SOLVENT DEPENDENCE OF GEL CHROMATOGRAPHIC RETENTION OF LOW-MOLECULAR-WEIGHT COMPOUNDS ON POLYSTYRENE–DIVINYLBENZENE GEL

KOICHI SAITOH*, ETSUO OZAKI and NOBUO SUZUKI

Department of Chemistry, Faculty of Science, Tohoku University, Sendai, Miyagi 980 (Japan)

SUMMARY

Chromatographic distribution coefficients, K_{av}, of small solute molecules on a polystyrene–divinylbenzene gel were measured with five different solvents, *viz.*, benzene, toluene, chlorobenzene, *o*-dichlorobenzene and tetrahydrofuran. Various types of compounds, such as alkanes, ethers, esters, ketones and alcohols, were selected as model solutes. The K_{av} values are well correlated with the molar volumes, V_m, of compounds within every homologous series. The retention sequence depends on the solvent used; in toluene it is alkanes < ethers < esters < ketones < alcohols, even if the molar volumes of the compounds are identical. The reverse sequence, except for alcohols, is observed in tetrahydrofuran, chlorobenzene and *o*-dichlorobenzene. In benzene, compounds other than alcohols share an identical relationship between K_{av} and log V_m. The solvent effects on the retention behaviour are well explained for the compounds other than alcohols by introducing a partition concept into gel chromatography.

INTRODUCTION

The solvent systems used in gel chromatography are relatively simple compared with those used in adsorption and partition chromatography. Binary or more complex solvent mixtures are requently required in the latter two techniques, whereas single component solvents are generally satisfactory in gel chromatography. This is one of the practical merits of gel chromatography as a laboratory technique with respect to the preparation, recovery and re-preparation of eluents. An important problem is the selection of a suitable solvent for the eluent.

Most reported gel chromatographic experiments involve the use of only a few limited kinds of solvents as eluents, in contrast to the variety of solvents used in adsorption and partition chromatographies. This suggests that little attention has been paid to solvent dependence in gel chromatography. However, the importance of the solvent effects in gel chromatography was pointed out in several studies with Sephadex LH-20 gel[1,2] and polystyrene–divinylbenzene gel[3,4].

There are two directions in which investigations of solvent effects in gel chromatography are focused. One is to find the optimum solvent with which pure steric

exclusion process can be realized. When the chromatographic process is governed only by the steric exclusion effect, *i.e.*, free from any additional effects (so-called "secondary effects"), the determination of molecular weight or size of a sample can be made with accuracy. The other direction is to clarify the solvent dependence of additional effects concurring with the steric exclusion process, in order to utilize these effects actively to improve the resolution of the compounds to be separated. With much attention to both of these aspects, we have made systematic investigations of solvent dependence in the gel chromatographic behaviour of small molecules. Various alkanes and metal chelates of β-diketones were selected as model compounds, and the retention behaviour of these compounds was investigated in various organic solvent systems on poly(vinyl acetate) gels[5-8] or polystyrene gels[9,10]. One of the important results was that the secondary effects in gel chromatography could be interpreted by introducing the solubility concept into the chromatographic process[11].

This paper describes a systematic study of the retention behaviour of small molecules on a polystyrene gel with several solvents. The compounds investigated include alkanes, ethers, esters, ketones and alcohols. Four aromatic solvents, *viz.*, benzene, toluene, chlorobenzene and *o*-dichlorobenzene, and tetrahydrofuran were selected as eluents.

EXPERIMENTAL

Apparatus

A JASCO (Japan Spectroscopic Co., Tokyo, Japan) FLC-350 high-performance liquid chromatograph was used with a Laboratory Data Control (Riviera Beach, FL, U.S.A.) Model 1107L refractometric detector. A glass column (100 cm × 5 mm I.D.) was used with a constant-temperature water-jacket. A sample injection valve with a sample loop of 40-μl capacity was arranged in the flow system between the pump and the column.

Materials and reagents

Six *n*-alkanes, viz., pentane, hexane, octane, decane, dodecane and hexadecane, were standard-grade materials (Standard Kit NP A-1, Tokyo Chemical Industry, Tokyo, Japan). All other compounds used as solutes (samples) were reagent-grade materials purchased from several sources. They were diethyl, dipropyl, dibutyl and dihexyl ethers, ethyl, butyl and isoamyl acetates, acetone, methyl ethyl, diethyl, methyl isobutyl and dibutyl ketones and ethyl, propyl, butyl, hexyl, isoamyl, octyl and decyl alcohols.

Benzene, toluene, chlorobenzene (CB), *o*-dichlorobenzene (DCB) and tetrahydrofuran (THF) were carefully distilled prior to use as eluents.

The column packing material was Styragel 60A (<37 μm) (Waters Assoc., Milford, MA, U.S.A.) (cross-linked polystyrene). The gel beads, after being swollen overnight in the solvent to be used, were packed into the column by the slurry packing procedure.

Procedure

The column was thermostated at 25 ± 0.1°C by circulating water through the column jacket from a constant-temperature water bath. The solvent flow-rates were adjusted to 1.0 ml/min.

Sample solutions were prepared by dissolving the compound of interest in the same solvent as the eluent. Sample concentrations were selected between 10 and 30 mg/ml so as to obtain a suitable response from the detector. The sample injection volume was 40 μl. The measurement of the retention time of each sample in a given column system was carried out at least in triplicate.

The dead volume between the sample injection valve and the detector was estimated by injecting an air bubble into the flow system from which the column had been omitted.

In order to determine the column void volume, mono disperse polystyrene standard with of molecular weight 200,000 (Pressure Chemical Co., Pittsburg, PA, U.S.A.) was used as a reference sample.

RESULTS AND DISCUSSION

In order to characterize a solute substance in gel chromatography, the distribution coefficient. K_d, derived from the following equation is frequently used:

$$V_e = V_0 + K_d V_i \tag{1}$$

where V_e. V_0 and V_i are the elution volume of the solute, the volume of the interstitial solvent (column void volume) and the volume of the solvent imbibed in the pores of the gel (pore volume). respectively. The solvent in the pores corresponds to the stationary phase in the column, and the gel matrix polymer is simply regarded as supporting material. Therefore, K_d is apparently independent of the physical properties of the gel matrix polymer.

When chromatographic behaviour is governed not only by pure steric exclusion but also by additional effects due to the interactions among the solute, solvent and gel matrix polymer, the whole gel phase. that is, the gel in the swollen state with the solvent, has to be taken into account as the practical stationary phase. Therefore, in this work, the distribution coefficient, K_{av}, derived from eqn. 2 was used.

$$V_e = V_0 + K_{av} V_x \tag{2}$$

where V_x is the volume of the gel swollen by the solvent (gel phase). By introducing the total column volume, V_t ($= V_0 + V_x$), eqn. 2 can be rearranged to give

$$K_{av} = (V_e - V_0)/(V_t - V_0) \tag{3}$$

In this work, the V_0 value of the Styragel 60A column conditioned with a given solvent was assumed to be equal to the V_e value of the monodisperse polystyrene standard of molecular weight 200,000, which was regarded as a large molecule enough to be completely excluded from the gel network. The V_t value was determined to be 19.24 ml in any solvent system. The value of V_e for a compound was calculated from the elution time and the solvent flow-rate. The K_{av} values were thus determined for a series of alkanes, ethers, esters, ketones and alcohols, on Styragel 60A with benzene, toluene, CB, DCB and THF. The results are summarized in Table I together with molar volumes, V_m, and solubility parameters, δ, of the compounds. The solvents are arranged in this table in increasing order of their δ values.

TABLE I

K_{av} VALUES OF LOW-MOLECULAR-WEIGHT COMPOUNDS ON STYRAGEL 60A WITH DIFFERENT SOLVENTS

Compound	V_m*	δ*	K_{av}**				
			Toluene ($\delta = 8.9$)	THF ($\delta = 9.1$)	Benzene ($\delta = 9.2$)	CB ($\delta = 9.5$)	DCB ($\delta = 10.0$)
Pentane	116.6	7.0	0.467	0.441	0.518	0.487	0.554
Hexane	131.6	7.3	0.433	0.417	0.480	0.455	0.526
Octane	163.5	7.6	0.384	0.363	0.426	0.399	0.458
Decane	194.9 §		0.328	–	0.373	0.341	0.407
Dodecane	228.6	7.9	0.293	0.283	0.335	0.311	0.362
Hexadecane	295***	8.0***	0.235	0.230	0.264	0.244	0.289
Diethyl ether	104.8	7.4	0.510	0.469	0.537	0.485	0.545
Dipropyl ether	138.8 §		0.440	0.407	0.466	0.428	0.488
Dibutyl ether	169.0 §		0.385	0.359	0.411	0.378	0.435
Dihexyl ether	234.8 §		0.299	0.280	0.324	0.296	0.344
Ethyl acetate	98.5	9.1	0.544	0.465	0.545	0.493	0.561
Butyl acetate	132.5	8.5	0.466	0.407	0.474	0.428	0.487
Isoamyl acetate	148.8	7.8	0.436	0.373	0.443	0.403	0.460
Acetone	74.0	9.0	0.611	0.487	0.608	0.533	0.598
Methyl ethyl ketone	90.1	9.3	0.565	0.467	0.568	0.496	0.558
Diethyl ketone	106.4	8.8	0.525	0.449	0.532	0.464	0.519
Methyl isobutyl ketone	125.8***	8.5***	0.477	0.409	0.490	0.428	0.479
Diisobutyl ketone	177.1	7.8	0.385	0.331	0.401	0.354	0.403
Ethyl alcohol	58.5	12.7	0.713	0.429	0.718	0.662	0.773
Propyl alcohol	75.2	11.9	0.645	0.405	0.658	0.606	0.701
Butyl alcohol	91.5	11.4	0.594	0.380	0.617	0.563	0.647
Isoamyl alcohol	108.9 §		0.548	–	0.577	0.521	0.602
Hexyl alcohol	124.8 §		0.508	0.338	0.540	0.483	0.562
Octyl alcohol	157.7	10.3	0.440	0.298	0.468	0.426	0.489
Decyl alcohol	190.5 §		0.392	0.267	0.416	0.373	0.432

* Data from ref. 12, except where indicated otherwise. (a) From ref. 15.
** The relative standard deviation in each instance is less than 0.7%.
*** Data from ref. 15.
§ Data calculated from molecular weight and density.

The gel chromatographic behavior of a solute is, in general, closely correlated with the size of the solute molecule. For large molecules such as polymers, molecular weight or chain length is frequently used as an effective size parameter for discussing the solute retention in gel chromatography, whereas for small molecules, such as those of molecular weight less than 1000, V_m is superior to the above two parameters[13]. The relationships between K_{av} and log V_m prepared from the data in Table I are shown in Fig. 1a–e. It is obvious that the K_{av} versus log V_m plot is approximately linear within every homologous series of compounds, and also that the arrangement of such plots for five different series of compounds depends on the solvent used. Even
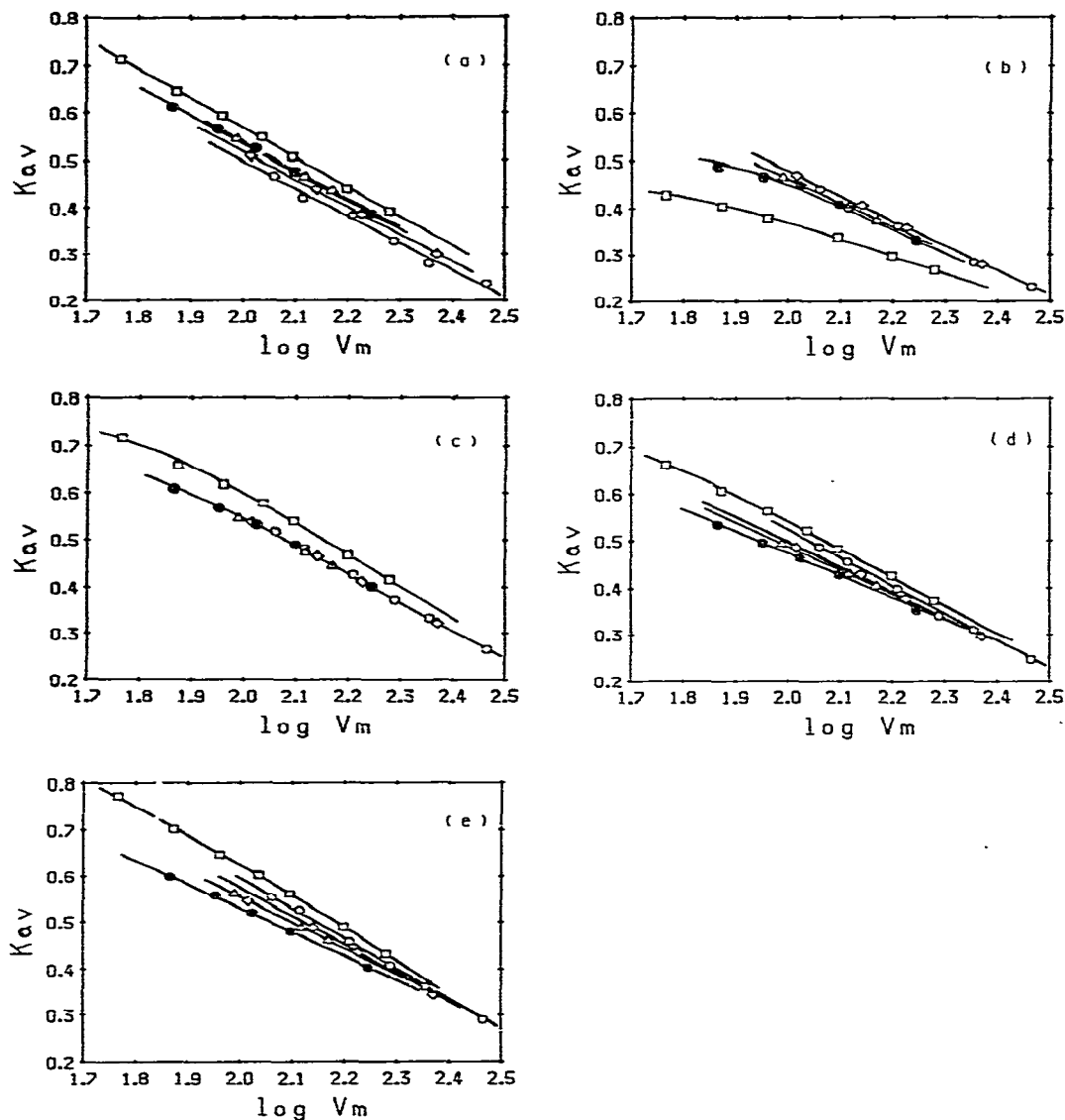
Fig. 1. Relationship between K_{av} values and molar volume. V_m, for alkanes (○), ethers (◇), esters (△), ketones (●) and alcohols (□) in toluene (a), THF (b), benzene (c), CB (d) and DCB (e) systems. Column: Styragel 60A; 25°C.

if the compounds with equal V_m values are not identical, the K_{av} value tends to increase in the following orders of compounds:

    (a) in toluene, alkanes < ethers < esters < ketones < alcohols;

    (b) in THF, alcohols < ketones < esters < ethers ≈ alkanes;

    (c) in benzene, ketones = esters = ethers = alkanes < alcohols;

    (d) in CB or DCB, ketones < esters < ethers < alkanes < alcohols.

These facts can not be explained simply in terms of steric exclusion. The solute distribution process has to be discussed, taking additional effects into consideration.

Solute distribution in gel chromatography was previously discussed on the assumption that practical gel chromatography was based on a combination of steric exclusion and partition effects[11]. On such an assumption, the V_e value of a solute S is expressed as

$$V_e = V_0 + K_{part.} K_{size} V_x \tag{4}$$

where $K_{part.}$ is the partition coefficient, defined as the concentration of S in the gel phase divided by the concentration of S in the solvent phase. The effective volume of the gel phase to the solute partition is not V_x, but rather only a certain fraction, K_{size}, of V_x is available for S owing to the steric exclusion effect. According to the theoretical treatment by Laurent and Killander[14], K_{size} is expressed by the equation

$$K_{size} = \exp[-\pi L (r_r + r_s)^2] \tag{5}$$

where r_s is the radius of the solute molecule, r_r is the radius of the polymer chain of gel matrix and L is the concentration of the polymer chain in the gel phase. Both r_r and L are constant in a combination of the gel matrix and a solvent. If the molecular dimensions (r_s) of various compounds are identical, the compounds have the same K_{size} value. It is obvious from eqn. 4 that solute retention on the basis of pure steric exclusion can be realized under the condition $K_{part} = 1$. In other words, even if the molecular dimensions of different compounds are identical, the V_e values of the compounds can be made to differ by controlling the $K_{part.}$ fractions so as to be different to each other.

According to the solubility parameter theory[15], the partition of S between the solvent and the gel phases is given by the solubility parameters, δ, of S, the solvent and the gel phase (indicated by subscripts s, o and x, respectively), the molar volume of S, $V_{m,s}$, the gas constant, R, and temperature, T:

$$\log K_{part.}^x = V_{m,s}[(\delta_0 - \delta_s)^2 - (\delta_x - \delta_s)^2]/2.3RT \tag{6}$$

where $K_{part.}^x$ is the partition coefficient defined as the molar fraction of S in the gel phase divided by that in the solvent phase. $K_{part.}$ in eqn. 4 is related to $K_{part.}^x$ by the equation

$$K_{part.} = K_{part.}^x (V_{m,o}/\bar{V}_{m,x}) \tag{7}$$

where $V_{m,o}$ and $\bar{V}_{m,x}$ are the molar volume of the solvent and the average molar volume of the gel phase. As the gel phase consists of gel matrix polymer substance and solvent, δ_x is, in general, not equal to the δ value of the gel matrix polymer, δ_g, but to the δ of the mixture of the above two components, approximately expressed by

$$\delta_x = \varphi_g \delta_g + (1 - \varphi_g)\delta_0 \tag{8}$$

where φ_g is the volume fraction of the gel-matrix polymer in the gel phase.

From eqns. 2, 4 and 6–8, the following equation[11] is derived:

$$\log K_{av} = \log K_{size} + V_{m,s}[2(\delta_s - \delta_0)(\delta_g - \delta_0)\varphi_g - (\delta_g - \delta_0)^2\varphi_g^2]/2.3RT +$$
$$\log (V_{m,o}/\bar{V}_{m,x}) \tag{9}$$

The last term of the above equation is constant in a given solvent system.

We assume here that different compounds have the same K_{size} value in a given gel chromatographic column if the V_m values of the compounds are identical. When two different compounds, S_1 and S_2, with molar volumes equal to $V_{m,s}$ are chromatographed in a column, the relative retention, R_{s_1/s_2}, of S_1, compared with S_2, is given by the following equation:

$$\log R_{s_1/s_2} = \log (K_{av,s_1}/K_{av,s_2})$$
$$= \log (K_{part.,s_1}/K_{part.,s_2})$$
$$= 2V_{m,s} (\delta_{s_1} - \delta_{s_2}) (\delta_g - \delta_0)\varphi_g/2.3RT \tag{10}$$

It is difficult to evaluate the fraction of $K_{part.}$ directly in the experimentally available distribution coefficient, K_{av}. However, by comparing the K_{av} values of two compounds, the contribution of the partition effect in gel chromatography can be discussed quantitatively by using known or measurable physical properties of the solvent, gel and solute, as shown in eqn. 10. Eqn. 10 predicts that R_{s_1/s_2} depends on the solvent used. Log R_{s_1/s_2} decreases with increase in δ_0, provided that δ_{s_1} is larger than δ_{s_2}. As φ_g, in general, depends on the solvent[11,16], log R_{s_1/s_2} does not always bear in a linear relationship to δ_0. Under the condition $\delta_0 = \delta_g$, log R_{s_1/s_2} is always zero, which means the absence of the partition effect from the solute distribution process in the column.

We assume here an imaginary alkane, ester, ketone and alcohol with V_m values equal to 100, a value selected for convenience. The K_{av} values of these imaginary
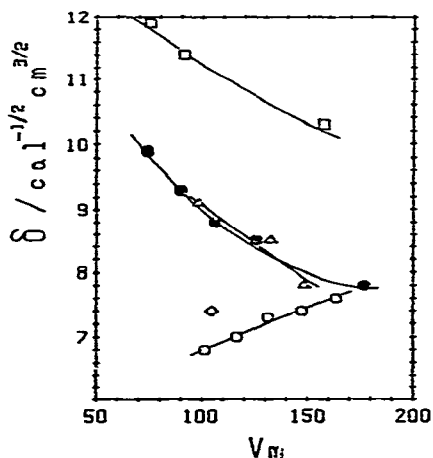

Fig. 2. Relationship between solubility parameter, δ, and molar volume, V_m, for alkanes (O), ether (◇), esters (△), ketones (●) and alcohols (□). The data[12] for butane ($V_m = 101.4$, $\delta = 6.8$) and heptane ($V_m = 147.4$, $\delta = 7.4$) are used in addition to those in Table I.

TABLE II

ESTIMATED VALUES OF δ AND K_{av} FOR IMAGINARY COMPOUNDS WITH $V_m = 100$

Compound	δ	$K_{av}*$				
		Toluene	THF	Benzene	CB	DCB
Alkane	6.8	0.50	0.48	0.54	0.52	0.60
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
Ether	7.4	0.52	0.48	0.54	0.50	0.57
		(1.04)	(1.00)	(1.00)	(0.96)	(0.95)
Ester	9.1	0.54	0.46	0.54	0.49	0.56
		(1.08)	(0.96)	(1.00)	(0.94)	(0.93)
Ketone	9.0	0.54	0.45	0.54	0.47	0.53
		(1.08)	(0.94)	(1.00)	(0.90)	(0.88)
Alcohol	11.2	0.57	0.37	0.59	0.54	0.62
		(1.14)	(0.77)	(1.09)	(1.04)	(1.03)

* Values in parentheses are relative retentions. R, taking the K_{av} value of the alkane as unity.

compounds are estimated by reading off the corresponding values at log $V_m = 2.0$ from the K_{av} versus log V_m plots shown in Fig. 1. A rough estimate of the δ values of these imaginary compounds is made from the δ versus V_m plots shown in Fig. 2. The results of these estimations are summarized in Table II.

When the relative retention, R, is calculated for each compound, taking the K_{av} value of the imaginary alkane as unity, the relationship between log R and δ_0 of the solvent prepared for each compound is as shown in Fig. 3. The log R value of every compound tends to decrease with increase in δ_0. The plots for compounds other than alcohols intersect each other in benzene ($\delta_0 = 9.2$). This suggests that the δ_g value of
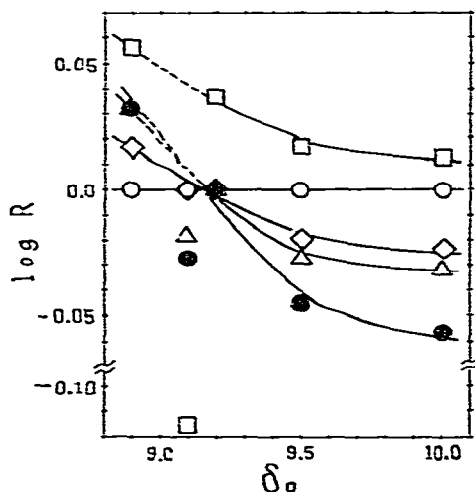

Fig. 3. Relationship between the relative retention, R, of an imaginary compound with $V_m = 100$ and solubility parameter, δ_0, of solvent. The K_{av} value of an imaginary alkane is taken as unity. Compounds: ○, alkane; ◇, ether; △, ester; ●, ketone; □ alcohol.

the gel matrix polymer used (Styragel 60A) is about 9.2. The δ value range for polystyrene is 9.1–9.4[12]. The imaginary alcohol shows log R values larger than zero in all solvents except THF. In contrast, the log R value of this compound observed in THF is the smallest of all.

The relationship between log R and δ_s for the imaginary compounds is shown in Fig. 4. With the exception of alcohol, an increasing tendency of log R with increasing δ_s is observed in solvents with δ_0 smaller than δ_g ($=9.2$), such as toluene. The reverse tendency is observed in solvents with δ_0 larger than δ_g, such as CB ($\delta_0 = 9.5$) and DCB ($\delta_0 = 10.0$). A similar tendency to the latter is also observed in THF, although the δ value of THF ($\delta_0 = 9.1$) is close to δ_g. In benzene ($\delta_0 = 9.2$), log R is always zero.
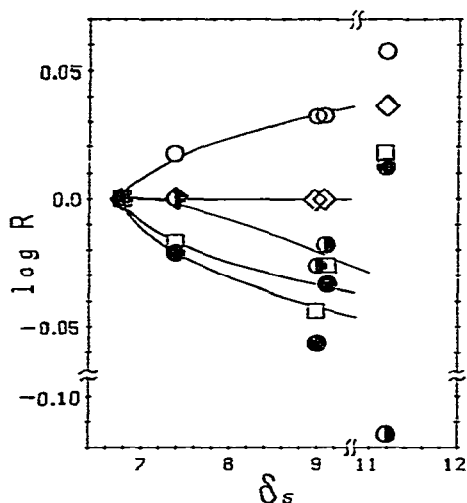

Fig. 4. Relationship between the relative retention, R, and solubility parameter, δ_s, for imaginary compounds with $V_m = 100$ in toluene (O), THF (◑), benzene (◇), CB (▢) and DCB (◕).

These facts are consistent with the prediction made from eqn. 10. The poor linearity of each plot is presumably caused by errors in the estimations of δ_s and R.

The retention behaviour of alcohols exhibited in Figs. 1a–e, 3 and 4 is so anomalous that the behaviour can no longer be explained on the basis of eqn. 9. Alcohols, compared with other compounds, show relatively large K_{av} values in all solvents other than THF. The interaction between the alcohol molecule and the π-electrons on the benzene ring in polystyrene is presumably one of the factors that lead to the high retention of alcohols. However, no clear evidence has been obtained. On the other hand, alcohols have very small K_{av} value in THF (see Fig. 1b). This is considered to be a result of the increase in the effective molecular size of alcohols due to solvation[17].

It is concluded that the solvent effect in gel chromatography on polystyrene gel can be interpreted by the theory in which the solubility concept is introduced. Benzene is a suitable solvent for achieving a pure size exclusion process for alkanes, ethers, esters and ketones in a Styragel 60A column. So-called secondary effects in gel chromatography can be controlled by using a carefully selected solvent.

REFERENCES

1 C. A. Streuli, *J. Chromatogr.*, 56 (1971) 225.
2 M. Wilk, J. Rochlitz and H. Bende, *J. Chromatogr.*, 24 (1966) 414.
3 J. G. Bergman, L. J. Duffy and R. B. Stevenson, *Anal. Chem.*, 43 (1971) 131.
4 H. Noda, K. Saitoh and N. Suzuki, *J. Chromatogr.*, 168 (1979) 250.
5 K. Saitoh and N. Suzuki, *J. Chromatogr.*, 109 (1975) 333.
6 K. Saitoh and N. Suzuki, *J. Chromatogr.*, 111 (1975) 29.
7 N. Suzuki and K. Saitoh, *Bull. Chem. Soc. Jap.*, 50 (1977) 2907.
8 K. Saitoh and N. Suzuki, *Anal. Chem.*, 52 (1980) 30.
9 N. Suzuki, K. Saitoh and M. Shibukawa, *J. Chromatogr.*, 138 (1977) 79.
10 E. Ozaki, K. Saitoh and N. Suzuki, *J. Chromatogr.*, 177 (1979) 122.
11 K. Saitoh and N. Suzuki, *Bull. Chem. Soc. Jap.*, 51 (1978) 116.
12 A. F. M. Barton, *Chem. Rev.*, 75 (1975) 731.
13 W. B. Smith and A. Kollmansberger, *J. Phys. Chem.*, 67 (1965) 4157.
14 T. C. Laurent and J. Killander, *J. Chromatogr.*, 14 (1964) 317.
15 J. H. Hildebrand and R. S. Scott, *The Solubility of Nonelectrolytes*, Dover, New York, 1964.
16 K. Saitoh, T. Ozawa and N. Suzuki, *J. Chromatogr.*, 124 (1976) 231.
17 J. G. Hendrickson and J. C. Moore, *J. Polym. Sci., Part A-1*, (1966) 167.