

CHROM. 8313

SOLVENT EFFECTS IN THE GEL CHROMATOGRAPHY OF *n*-ALKANES ON MERCKOGEL OR-2000

KOICHI SAITOH and NOBUO SUZUKI*

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

(Received March 14th, 1975)

SUMMARY

Solvent effects in the elution behaviour of small solute molecules in gel chromatography have been investigated. Four *n*-alkanes, *viz.*, *n*-pentane, *n*-heptane, *n*-decane and *n*-hexadecane, were selected as the model compounds. The chromatography was carried out using a polyvinyl acetate gel (Merckogel OR-2000) and ten solvent systems were used.

The elution parameter, K_{av} , of a solute clearly depends on the solvents, and the upper exclusion limit of the column system also seems to be influenced by the solvents. These solvent effects on the K_{av} values are discussed by using a modified Ogston-Laurent equation.

INTRODUCTION

Gel chromatography is a technique for separating and characterizing solute molecules according to their size in solution, large solute molecules being eluted earlier than smaller molecules. In most instances, gel chromatographic behaviour has been explained in terms of steric exclusion or sieving effects in the gel phase.

However, some other effects in the gel phase, the so-called secondary effects, have occasionally been observed. For example, when aromatic compounds are eluted with an organic solvent, they are not always eluted in the order expected from their molar volumes¹. These phenomena have been discussed from the viewpoint of resonance energy^{2,3} or mutual interactions between the gel, solvent and solute⁴. It is very important that the solvent is one of the components that comprise the column system, and the solvent occasionally affects the elution behaviour of a solute molecule.

We have studied the chromatography of metal complexes using metal- β -diketonates selected as model compounds^{5,6}. In previous work on the chromatography of some metal(II, III) acetylacetonate complexes and *n*-alkanes using the Merckogel OR-2000-tetrahydrofuran (THF) system⁷, the elution behaviour of the metal complexes seems to be different from those of the alkanes.

* To whom all correspondence should be addressed.

In order to elucidate the chromatographic behaviour of these compounds, further systematic studies in many solvent systems are necessary. In this work, the gel chromatography of some alkanes, *viz.*, *n*-pentane, *n*-heptane, *n*-decane and *n*-hexadecane, which are regarded as compounds inert to the gel matrix, has been carried out in systems of Merckogel OR-2000 with various organic solvents, *viz.*, chloroform, benzene, toluene, *p*-dioxane, ethyl acetate, *n*-butyl acetate, acetone, methyl ethyl ketone and methanol. The solvent effects, including previous data for THF⁷, on K_{av} values are discussed.

EXPERIMENTAL

The apparatus and experimental procedures were the same as those used in the previous work⁷, except for the solvent systems and sample materials.

The solvents, except for chloroform, were used after purification by appropriate chemical treatment, drying and distillation. The purity of each final material was checked by UV absorption spectrometry or gas chromatography.

As it is well known that purified chloroform is unstable, the reagent-grade material (Wako, Osaka, Japan) was used without further purification, and the amount of ethanol contained as a stabilizer was determined by gas chromatography to be 1.05%.

All four *n*-alkanes were of reagent grade (Wako). The sample solutions were prepared by dissolving each *n*-alkane in 5 ml of the solvent used as the eluent. The amounts of C₅, C₇, C₁₀ and C₁₆ *n*-alkanes taken were 100 μ l, 100 μ l, 60 mg and 60 mg, respectively. A 7-mg amount of standard polystyrene (mol. wt. 200,000; Pressure Chemical Co., Pittsburgh, Pa., U.S.A.) was added to each sample solution, except for methanol, as an internal reference material. With methanol, 10 mg of methylated blue dextran was added to each sample solution. The methylated blue dextran was prepared as reported elsewhere⁸, by the methylation of blue dextran 2000 (Pharmacia, Uppsala, Sweden) with dimethyl sulphate. The final product was soluble in methanol.

A 40- μ l portion of a sample solution was fed into the column with the aid of injection valve.

All of the elution data were obtained by a computerized on-line data processing system.

The following chromatographic conditions were used throughout the experiments: Merckogel OR-2000 column, 100 cm \times 5 mm I.D. (bed volume = 19.63 ml); column temperature, 25.0 \pm 0.02 $^\circ$; solvent flow-rate, 0.20 ml/min; detector, refractomonitor (Laboratory Data Control, Riviera Beach, Fla., U.S.A.).

RESULTS AND DISCUSSION

The parameter K_{av} is frequently adopted for the characterization of a solute molecule in gel chromatographic elution. It is normally derived from the following equation and corresponds to the distribution coefficient of the solute between the interstitial solution phase and the swollen gel phase:

$$V_e = V_0 + K_{av} V_x \quad (1)$$

where V_e is the elution volume of the solute and V_0 and V_x are the void (interstitial) volume and swollen gel phase volume, respectively.

By the introduction of the total volume of the gel bed, V_t , ($V_t = V_0 + V_x$), eqn. 1 can be rearranged to

$$K_{av} = \frac{V_e - V_0}{V_x} = \frac{V_e - V_0}{V_t - V_0} \quad (2)$$

The K_{av} values of an *n*-alkane in various solvent systems were calculated according to eqn. 2 by measurement of the elution volume, V_e , and the void volume, V_0 , which is assumed to be equal to the elution volume of an internal reference material contained in the sample solution, *i.e.*, methylated blue dextran in the methanol system and polystyrene in the other solvent systems.

The reproducibility of the K_{av} values is satisfactory, the relative standard deviation in each instance being not more than 0.7%. The K_{av} values are listed in Table I. The data for tetrahydrofuran are taken from previous work⁷.

As the detector used is based on the difference in refractive indices of the solute and solvent, reliable data cannot be given for some combinations of solute and solvent, and therefore some data are absent from Table I.

TABLE I

 K_{av} VALUES OF *n*-ALKANES IN VARIOUS SOLVENT SYSTEMS

No.	Solvent system	K_{av}			
		<i>n</i> -Hexadecane	<i>n</i> -Decane	<i>n</i> -Heptane	<i>n</i> -Pentane
1	Chloroform	0.240	0.366	0.455	0.525
2	Benzene	0.228	0.353	0.444	0.518
3	Toluene	0.182	0.300	0.390	0.466
4	<i>p</i> -Dioxane	0.350	0.493	0.593	0.659
5	Tetrahydrofuran*	0.252	—**	0.464	0.539
6	Ethyl acetate	0.334	0.451	0.526	0.591
7	Acetone	0.415	0.517	0.583	0.606***
8	Methyl ethyl ketone	0.322	0.445	0.526	0.584
9	<i>n</i> -Butyl acetate	0.257	0.382	—**	0.536
10	Methanol	0.555	0.612	0.642	0.660

* Reported in ref. 7.

** Not measurable owing to low sensitivity of detection.

*** Value for *n*-hexane.

The relation between K_{av} and the molar volume of a solute, V , which is one of the parameters that describe the molecular size, is shown empirically by the equation

$$\log V = a K_{av} + b \quad (3)$$

where a and b are arbitrary constants. Such relationships applied to this work are shown graphically in Fig. 1.

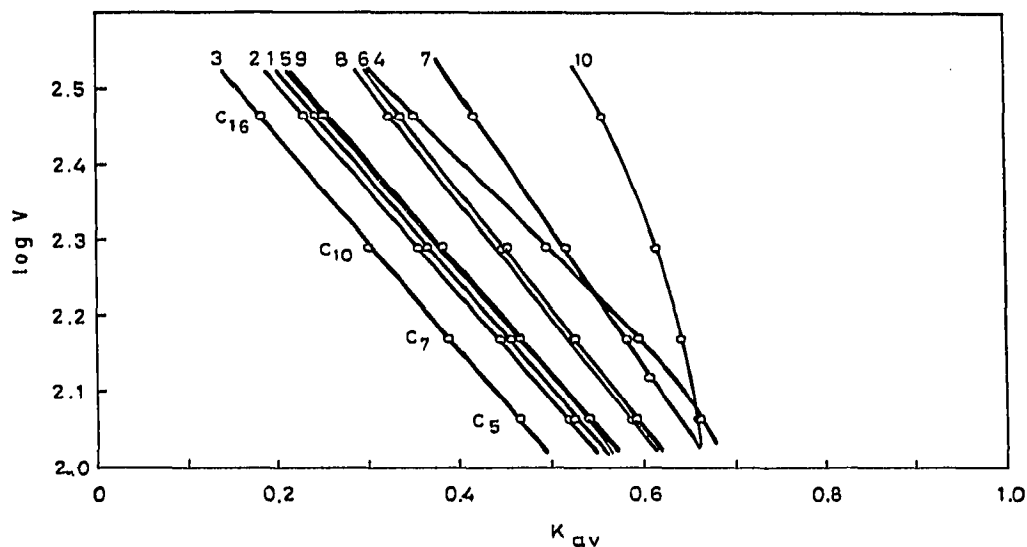


Fig. 1. Relationship between molar volumes of *n*-alkanes, V , and K_{av} values in various solvent systems. C_{16} , C_{10} , C_7 and C_5 are the abbreviations for *n*-hexadecane, *n*-decane, *n*-heptane and *n*-pentane, respectively. Column: Merckogel OR-2000, 25.0°. Solvents 1-10 as in Table I.

The slopes of the lines that correspond to a in eqn. 3 seem not to depend on the solvents, except for the *p*-dioxane (4) and methanol (10) systems. The value of b in eqn. 3 is related to the upper exclusion limit of the column system, and can be evaluated from the intercept on the $\log V$ axis of the extrapolated line in Fig. 1. According to the b values obtained, it is suggested that the molar volumes at the upper exclusion limit increase in the following order of solvents: toluene < benzene < chloroform < THF < *n*-butyl acetate < *p*-dioxane < methyl ethyl ketone < ethyl acetate < acetone < methanol. It is interesting that the K_{av} value of a solute and the upper exclusion limit depend so much on the solvents.

Laurent and Killander⁹ designed a physical model of the gel network, based on the assumption that the polymer chains of the gel are straight rigid rods that are infinitely long and distributed at random in the gel. They applied Ogston's theoretical treatment concerning the distribution of spaces in a random network of straight fibres¹⁰ to a chromatographic model and finally derived the equation

$$K_{av} = \exp [-\pi L (r_r + r_s)^2] \quad (4)$$

where L is the concentration of the rods in the solution, expressed as centimetres of rod per millilitre of solution, r_s is the radius of a spherical solute molecule and r_r is the radius of the rod.

In order to apply the above equation to the present situation, the following assumptions were made for simplification:

- (1) r_r is constant independent of the solvent;
- (2) the equation can be applied to *n*-alkanes even though they are not spherical

molecules, and then r_s corresponds to the radius of the equivalent sphere of such a molecule;

(3) r_s is constant independent of the solvent;

(4) consequently, some solvent effects are reflected only in the parameter L .

If the practical gel network can be approximated to one based on the Ogston-Laurent model, eqn. 4 can be substituted into eqn. 1 without any modification. However, in practice, it is difficult to make the approximation that the gel used in the present work consists of infinitely long rigid rods, because of its densely cross-linking structure. In addition, the effective volume of the solvent that contributes to a chromatographic separation may be less than the net volume of a solvent in the swollen gel phase, owing to some solvent-gel interactions, *e.g.*, solvation.

Therefore, all of the swollen gel phase will not be available to act as the stationary phase and K_{uv} defined by eqn. 4 will be available for some fraction of the volume of the gel phase, V_s . Consequently, eqn. 4 is amended by introducing a correction factor, β , to give

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

When β is unity, eqn. 5 corresponds to eqn. 4.

Eqn. 5 is modified to

$$\ln K_{uv} = \ln \beta - \pi L (r_r + r_s)^2 \quad (6)$$

Introducing $K_{uv,0}$, which is the K_{uv} value for another solute, S_0 , taken as a reference, K_{uv} can then be related to $K_{uv,0}$ by the equation

$$-\ln K_{uv} + \ln \beta = \frac{(r_r + r_s)^2}{(r_r + r_{s0})^2} (-\ln K_{uv,0} + \ln \beta) \quad (7)$$

where r_{s0} is the radius of the solute S_0 .

If β is independent of the solvent, it is expected that the plot of $-\ln K_{uv}$ versus $-\ln K_{uv,0}$ for various solvent systems will be a straight line. The slope of the line is governed by the sizes of the solute molecules:

$$\text{Slope} = (r_r + r_s)^2 / (r_r + r_{s0})^2 \quad (8)$$

When the reference solute is maintained as S_0 and different solutes are taken as S , all of the plots for the various solutes are expected to cross at a point corresponding to

$$\ln K_{uv} = \ln \beta, \ln K_{uv,0} = \ln \beta$$

In Fig. 2, such plots are shown, *n*-decane being taken arbitrarily as S_0 . The K_{uv} value for *n*-decane in THF, which is absent from Table I, was evaluated as 0.380 from the relationship between $\log V$ and K_{uv} shown in Fig. 1. Fig. 2 shows that the relationship described by eqn. 7 is valid for the solvent systems used, except for *p*-dioxane (4) and methanol (10).

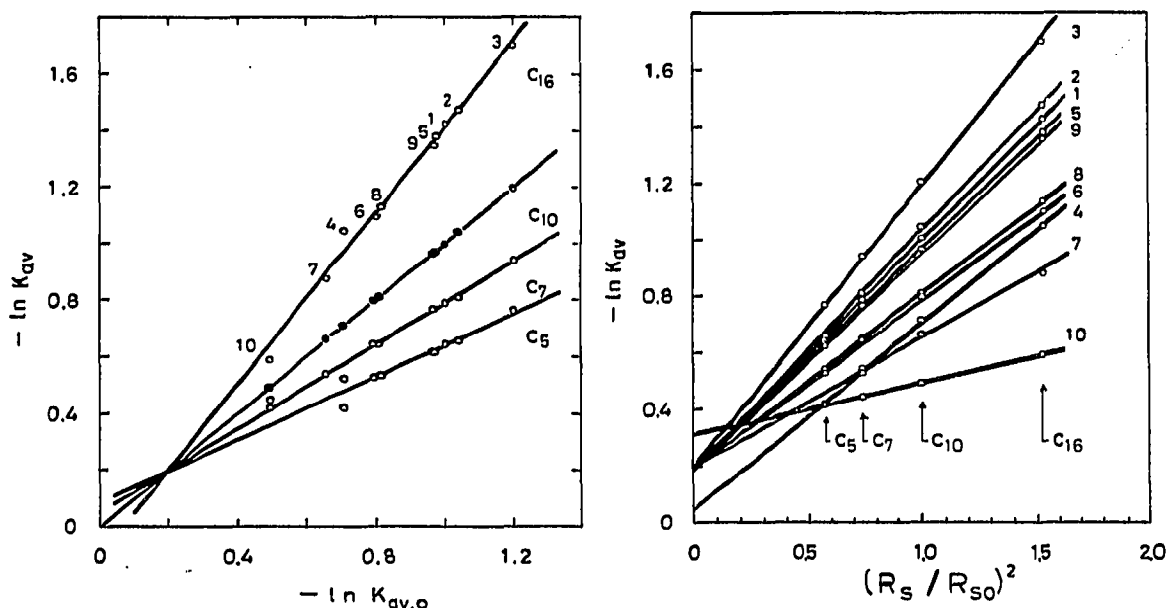


Fig. 2. Correlation of K_{uv} values for n -alkanes with those of n -decane, $K_{uv,0}$. n -Alkanes as in Fig. 1; solvents as in Table I.

Fig. 3. $-\ln K_{uv}$ versus $(R_s/R_{s0})^2$ relationships for n -alkanes in various solvent systems. n -Decane (C_{10}) is taken as S_0 . n -Alkanes as in Fig. 1; solvents as in Table I.

The plots cross at the point (0.19, 0.19), with a deviation of ± 0.02 , which corresponds to a β value of 0.83. However, the β values in the p -dioxane and methanol systems are expected to be very different.

According to the assumptions made, some solvent effects on K_{uv} are reflected only in the value of L . If the exact values of r_r for n -alkanes are known, the value of L in a solvent system can be evaluated by plotting $(-\ln K_{uv})^2$ versus r_s . However, the determination of r_s is not easy.

In this discussion, a practical parameter, $R_s (= r_r + r_s)$ is adopted. As r has been assumed to be constant, R_s is due only to the size of a solute molecule S .

By the introduction of the parameter R_s , eqn. 5 is modified to give

$$\begin{aligned} \ln K_{uv} &= -\ln \beta + \pi L R_s^2 \\ &= -\ln \beta + \pi L R_{s0}^2 (R_s/R_{s0})^2 \end{aligned} \quad (9)$$

Further, by the introduction of $\alpha_{s0} (= \pi L R_{s0}^2)$,

$$-\ln K_{uv} = -\ln \beta + \alpha_{s0} (R_s/R_{s0})^2 \quad (10)$$

where R_{s0} corresponds to the R_s value of the solute S_0 .

According to the relationship described by eqns. 7 and 8, the value of $(R_s/R_{s0})^2$ is given by the slope of the plot of $-\ln K_{uv}$ versus $-\ln K_{uv,0}$.

When n -decane is arbitrarily taken as S_0 , the values of $(R_s/R_{s0})^2$ for alkanes

taken as S are evaluated from the slopes of the plots shown in Fig. 2. The values are 1.52 ± 0.03 , 1.00, 0.74 ± 0.01 and 0.57 ± 0.02 for the C₁₆, C₁₀, C₇ and C₅ *n*-alkanes, respectively. Using these values, the relationship described by eqn. 10 can be shown graphically by plotting $-\ln K_{uv}$ versus $(R_s/R_{s0})^2$, and such plots for various solvent systems are shown in Fig. 3.

The values of α_{s0} and $-\ln \beta$ correspond to the slope and $-\ln K_{uv}$ intercept, respectively for each plot. The observed values of α_{s0} and β in various solvent systems are listed in Table II.

It is obvious that the values of β in *p*-dioxane and methanol are very different from the others, and α_{s0} is very dependent on the solvent. It is interesting that the molar volume at the upper exclusion limit already estimated for respective solvents decreases with increase in α_{s0} .

TABLE II
OBSERVED VALUES OF α_{s0} AND β IN VARIOUS SOLVENT SYSTEMS

No.	Solvent system	α_{s0} *	β
1	Chloroform	0.82 ± 0.02	0.84 ± 0.02
2	Benzene	0.86 ± 0.03	0.84 ± 0.02
3	Toluene	0.98 ± 0.03	0.81 ± 0.03
4	<i>p</i> -Dioxane	0.67 ± 0.01	0.96 ± 0.01
5	Tetrahydrofuran	0.79 ± 0.03	0.84 ± 0.02
6	Ethyl acetate	0.59 ± 0.03	0.82 ± 0.03
7	Acetone	0.43 ± 0.07	0.79 ± 0.06
8	Methyl ethyl ketone	0.62 ± 0.01	0.84 ± 0.01
9	<i>n</i> -Butyl acetate	0.77 ± 0.07	0.83 ± 0.06
10	Methanol	0.18 ± 0.01	0.73 ± 0.01

* Values, taking *n*-decane as S₀, in arbitrary units. The figures show 95% confidence limits.

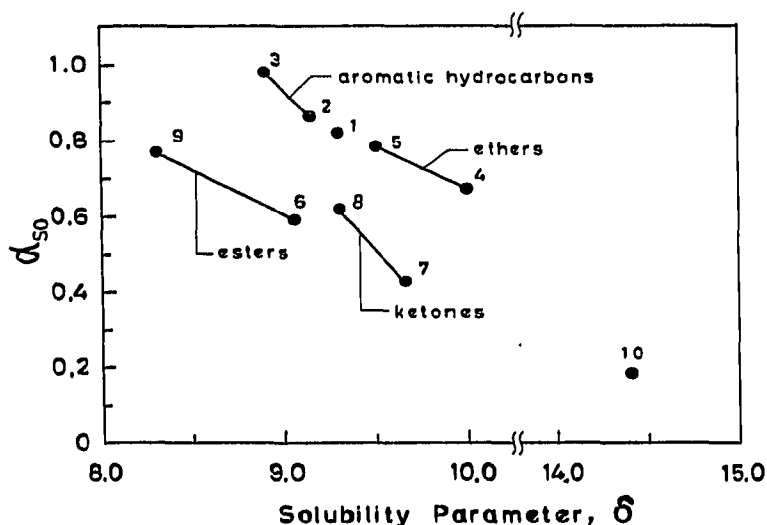


Fig. 4. Correlation between α_{s0} values and solubility parameters, δ , of solvents. *n*-Decane is taken as S₀.

In order to make the solvent effects clearer, it is necessary to clarify the correlation between α_{s0} and some of the physical properties of the solvents. Fig. 4 shows the correlation between α_{s0} and the solubility parameters, δ , of solvents. The solubility parameter is defined in regular solution theory¹¹, and it is well known to be very useful for the prediction of the affinity and solubility in solute-solvent systems. The parameter has been applied successfully in solvent extraction studies^{12,13}.

For each homologous pair of solvents coupled by a solid line in Fig. 4, it is evident that the larger the solubility parameter of the solvent the smaller is α_{s0} . As α_{s0} is proportional to the concentration of the rod, L , the smaller the value of α_{s0} the lower one would expect the concentration of the gel matrix in the swollen gel phase to be.

The above discussion has been carried out on the basis of assumptions that r_r and r_s are independent of the solvent and only the parameter L is dependent on the solvent. However, in practice, the radius of the rod r_r , and the radius of a solute, r_s , may be influenced by the solvent. In addition, some solute-gel interactions may occasionally be important. Therefore, the parameter L may in practice include some factors that are attributable to such solvent effects. Consequently, the parameter α_{s0} includes all of the solvent effects in the column system.

It is to be expected that the details of the contributions of the solvents to such parameters will be revealed to a considerable extent by investigations of solvent-gel interactions, and we are currently undertaking such studies.

REFERENCES

- 1 M. Wilk, J. Rochlitz and H. Bende, *J. Chromatogr.*, 24 (1966) 414.
- 2 C. A. Streuli, *J. Chromatogr.*, 56 (1971) 219.
- 3 C. A. Streuli, *J. Chromatogr.*, 56 (1971) 225.
- 4 J. G. Bergmann, L. J. Duffy and R. B. Stevenson, *Anal. Chem.*, 43 (1971) 131.
- 5 K. Saitoh and N. Suzuki, *J. Chromatogr.*, 92 (1974) 371.
- 6 K. Saitoh, M. Satoh and N. Suzuki, *J. Chromatogr.*, 92 (1974) 291.
- 7 K. Saitoh and N. Suzuki, *J. Chromatogr.*, 109 (1975) 333.
- 8 T. Takeuchi and S. Mori, *Gel Chromatography*, Kodansha, Tokyo, 1972, p. 259.
- 9 T. C. Laurent and J. Killander, *J. Chromatogr.*, 14 (1964) 317.
- 10 A. G. Ogston, *Trans. Faraday Soc.*, 54 (1958) 1754.
- 11 J. H. Hildebrand and R. L. Scott, *The Solubility of Non-electrolytes*, Dover, New York, 1964.
- 12 N. Suzuki, K. Akiba and H. Asano, *Anal. Chim. Acta*, 52 (1970) 115.
- 13 N. Suzuki and K. Akiba, *J. Inorg. Nucl. Chem.*, 33 (1971) 1169.