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Note

Gel chromatography of *n*-alkanes on a polystyrene gel with various organic solvents

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Gel chromatography is fractionally used in the fields of polymer chemistry and biological chemistry. Recent developments of column-packing materials have made the method applicable to compounds of low molecular weight (mol. wt. several hundreds). Most of the gel chromatographic experiments reported in the literature involved the use of only a few kinds of solvent as eluents, in contrast to the variety of eluents being used in adsorption and partition chromatography. This fact suggests that little attention has been paid to solvent dependence in gel chromatography. A few papers have dealt with this problem, using Sephadex LH-20^{1,2}, polystyrene gels^{3,4} or isodecylmethacrylate-divinylbenzene gel⁵.

We have systematically investigated solvent dependence in the gel chromatographic behaviour of *n*-alkanes or metal β -diketonato complexes with a poly(vinyl acetate) gel⁶⁻¹⁰.

This paper describes the gel chromatographic elution data for low-molecular-weight *n*-alkanes on a polystyrene gel with different organic solvents.

EXPERIMENTAL

Chromatographic system

A JASCO Model LCP-350 syringe-type pump (Japan Spectroscopic, Tokyo, Japan) was used to deliver a solvent to a 100 cm \times 5 mm I.D. glass column packed with Styragel 60A ($-37 \mu\text{m}$ in the dry state) (Water Assoc., Milford, Mass., U.S.A.). The columns used with different solvents were individually prepared by the slurry packing method. Samples were introduced into the solvent stream via rotary injection valve with a sample loop of 40- μl capacity. A Laboratory Data Control Model 1107L refractometer (Riviera Beach, Fla., U.S.A.) was used as the detector.

Reagents

The solvents listed in Table I were used after purification of their corresponding reagent-grade materials by appropriate chemical treatment, drying, and distillation. *n*-Alkanes (C₅-C₁₆) were standard-grade materials available for gas chromatographic analysis (Standard-Kit NP A-1, Tokyo Chemical Industry, Tokyo,

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TABLE I

K_{av} VALUES OF *n*-ALKANES ON STYRAGEL 60A WITH VARIOUS ORGANIC SOLVENTS AT 25°

Column	$K_{av} \times 10^3$													
	V_0 (ml)	V_x (ml)	N_c^{**} V_m^{***}	5 115	6 131	7 146	8 163	9 179	10 195	12 228	13 244	14 260	15 276	16 293
I	6.12	13.11		430	401	376	356	333	—	—	—	—	—	—
II	6.12	13.11		—	442	—	372	—	328	—	278	—	—	238
III	6.20	13.03		—	439	—	—	—	343	—	—	237	—	—
IV	5.43	13.80		—	445	—	389	—	341	—	290	—	—	244
V	6.68	12.55		—	526	—	462	—	407	360	—	318	—	—
VI	7.00	12.23		514	499	—	—	—	—	409	—	381	—	358
VII	7.03	12.20		551	545	—	—	—	—	—	487	—	469	460
VIII	5.71	13.52		606	586	—	555	—	520	—	476	—	—	—
IX	9.82	9.41		—	—	—	—	735	750	845	—	917	—	992

* I = Tetrahydrofuran; II = toluene; III = benzene; IV = chlorobenzene; V = 1,2-dichlorobenzene; VI = butyl acetate; VII = ethyl acetate; VIII = 1,2-dichloroethane; IX = acetone.

** Numbers of carbon atoms in a molecule of *n*-alkane.

*** Molar volume (ml/mol) at 20°

Japan). Mono-disperse polystyrene standards of mol.wt. 10,000 and 200,000 (Pressure Chemical, Pittsburgh, Pa., U.S.A.) were used for the determination of the column void volume.

Procedure

A sample solution of an *n*-alkane was prepared at a concentration of *ca.* 0.7% (w/w) by dissolving it in the solvent used as an eluent. The chromatographic experiments were carried out under the following conditions: column temperature, 25°; solvent flow-rate, 0.30 ml/min; volume of the sample solution injected into the column, 40 μ l. A minimum of three runs were done for each sample.

RESULTS AND DISCUSSION

No compounds gave an elution curve with excessive asymmetric shape. A solute in gel chromatography is characterized by the distribution coefficient, K_{av} , derived by Laurent and Killander¹¹.

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

where V_e , V_0 , and V_x are the elution volume of the solute, the column void volume, and the volume of the gel phase, respectively. In the present work, the V_0 value of a given column was assumed to be equal to the V_e value of mono-disperse polystyrene of mol.wt. either 10,000 or 200,000. The V_x values were calculated from the total column volume (bed volume), V_t , and V_0 , thus $V_x = V_t - V_0$. Prior to the calculation of K_{av} , a correction was made for the dead volume related to the tubing and fitting around the column. The K_{av} values of *n*-alkanes are given in Table I, as are the data for the column parameters, V_0 and V_x .

The gel chromatographic behaviour of solute substances is, in general, closely

related to their molecular dimensions. For large molecules such as polymers, the molecular weight is frequently used as a size parameter for a solute in gel chromatography, whereas for small molecules such as those with mol.wts. less than 1000, the molar volume is an appropriate size parameter¹².

The V_m values for *n*-alkanes calculated from the molecular weight and density are given in Table I. The relationship of K_{av} and $\log V_m$ for *n*-alkanes prepared on the basis of the present data is shown in Fig. 1, and appears to be solvent-dependent. In each solvent system, except for the acetone system, K_{av} decreases as V_m increases. This fact implies that the molecular sieve effect contributes predominantly to the solute distribution mechanism in the column. The relationship of K_{av} to $\log V_m$ is not always linear. Slightly convex plots are obtained from the systems with butyl acetate, ethyl acetate, and 1,2-dichloroethane. Compared with most of the solvents that show a linear relationship between K_{av} and $\log V_m$, these three lead to relatively large K_{av} values for a particular solute. The slope of the plot in the 1,2-dichlorobenzene system is remarkably steep.

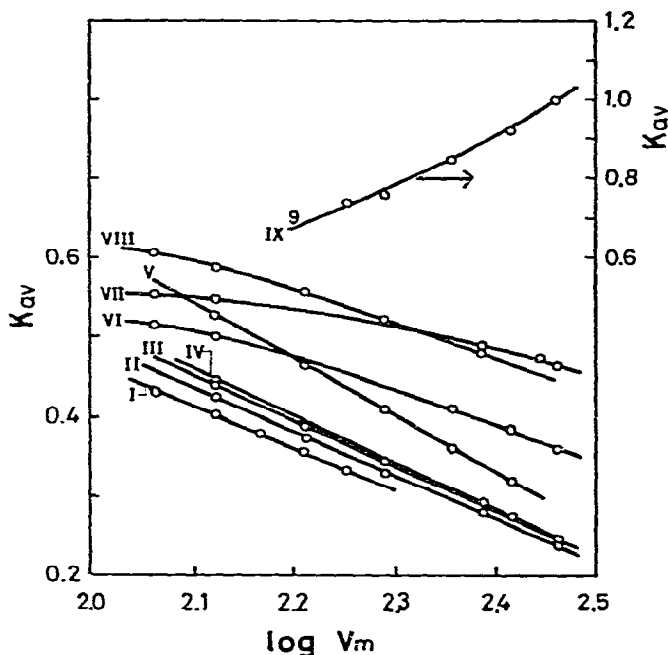


Fig. 1. Relationship between K_{av} values and molar volume, V_m , for *n*-alkanes in various solvent systems. Column: Styragel 60A, 25°. Solvents I-IX as in Table I.

When acetone is used as eluent, K_{av} increases with an increase in V_m . The separation mechanism in the acetone system cannot be explained solely in terms of the molecular sieve effect. Some interactions among solute, solvent, and gel should be taken into account.

The solvent effect in gel chromatography has been quantitatively discussed in terms of the usual solution theory¹⁰. When the solubility parameters of the solvent, δ , and the gel matrix, δ_g , are equal, the solute distribution mechanism can be

explained in terms of the molecular sieve effect. However, the difference between δ and δ_g causes the so-called secondary effect in gel chromatography, and results in an increase in the K_{av} value of a solute. The validity of this argument was examined with the experimental data for metal acetylacetonato complexes on a poly(vinyl acetate) gel¹⁰.

Some physical properties of the solvents used in this work are shown in Table II. The solvents that give lower K_{av} versus $\log V_m$ plots in Fig. 1 have δ values close to the δ_g value of polystyrene gel ($= 9.1$, ref. 13). Most of the solvents that give relatively large values of K_{av} for a solute have δ values far from the δ_g value.

TABLE II
PHYSICAL PROPERTIES OF SOLVENT AT 25°

Solvent*	Solubility parameter ($\text{cal}^{1/2}/\text{cm}^{3/2}$) (ref. 14)	Dipole moment (Debye units) (ref. 15)	Dielectric constant (ref. 15)
I	9.1	1.75	7.58
II	8.9	0.31	2.379
III	9.2	0	2.275
IV	9.5	1.54	5.621
V	10.0	2.27**	9.93
VI	8.5	1.84***	5.01 [§]
VII	9.1	1.88	6.02
VIII	9.8	1.86	10.36
IX	9.9	2.69 [§]	20.70

* Solvent numbers as in Table I.

** At 24°.

*** At 22°.

§ At 20°.

As shown in Table II, acetone is the most polar of all the solvents investigated. It is reasonable to suggest that the low affinity of acetone for polystyrene gel causes a secondary effect sufficiently large that the molecular sieve effect is no longer predominant. The V_0 value in the acetone system is very large (Table I), even larger than V_x . This fact may indicate that the V_e value of mono-disperse polystyrene cannot be assumed to be equal to V_0 , because of the retention due to the solute-gel interaction. Reference material other than polystyrene should have been used for determining the value of V_0 , especially in the acetone system.

1,2-Dichlorobenzene gives "normal" relationship between K_{av} and $\log V_m$, despite large values of the solubility parameter and the dipole moment. Some specific interactions, such as the π - π electron interaction, between gel and solvent may contribute to the suppression of undesirable solute-gel interactions.

Solvent effects in gel chromatography should be more systematically examined. The elution behavior of various kinds of compounds in different solvent systems will be discussed in a subsequent paper.

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