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## GEL CHROMATOGRAPHY OF ACETYLACETONE AND ITS METAL(II, III) COMPLEXES IN THE MERCKOGEL OR-2000-TETRAHYDROFURAN SYSTEM

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### SUMMARY

The gel chromatographic behaviour of acetylacetone and its metal(II, III) complexes was studied, using the polyvinyl acetate gel (Merckogel OR-2000)-tetrahydrofuran system. Seven metal complexes, *viz.*, cobalt(III), iron(III), chromium(III), aluminium(III), copper(II), nickel(II) and beryllium(II), of acetylacetone were investigated. Experiments with some *n*-alkanes were also carried out for comparison.

The elution characteristics, *viz.*,  $K_{av}$ , HETP and skew ratio, were obtained with high precision with the aid of an on-line data processing system. The correlation between  $K_{av}$  values and molar volumes of the solutes is discussed.

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### INTRODUCTION

Gel chromatography has a unique separation mechanism based on differences in the molecular sizes of the sample components. It has become a powerful method especially for the separation and characterization of high-molecular-weight substances.

In comparison with other liquid chromatographic methods based mainly on chemical or physical interactions in the column system, gel chromatographic separations, without any of those interactions (so-called secondary effects), can be carried out under such mild conditions that any labile compounds do not suffer from undesirable influences. These attractive features will expand the applications of this method not only to high- but also to low-molecular-weight substances, especially metal-containing compounds.

The application of this method to metal-containing substances has been reported. Most of the studies described<sup>1-6</sup> were carried out using Sephadex in aqueous media.

Metal complexes that are insoluble in aqueous media are important in analytical chemistry, where the formation of such complexes is frequently utilized for the separation and determination of some metal ions. It is expected that the combination of the advantages of gel chromatography with the usefulness of the metal complexes will expand their analytical utility further.

However, gel chromatographic studies of such complexes have rarely been made. Nevertheless, a few workers have reported on some neutral metal chelates in organic media. Yamamoto *et al.*<sup>7</sup> investigated tris(acetylacetonato)chromium(III) and -cobalt(III) using some organic solvent-polystyrene gel systems. We have previously studied<sup>8</sup> tris(acetylacetonato)chromium(III) in the polyvinyl acetate gel (Merckogel OR-2000)-chloroform system. In order to expand the utility of gel chromatography to such metal compounds, more systematic fundamental studies must be carried out.

Separations in gel chromatography result from the preferential diffusion of small solute molecules into the porous gel structure with the exclusion of large molecules. These solutes are characterized by the equation<sup>9,10</sup>

$$V_e = V_0 + K_d V_l \quad (1)$$

The elution volume,  $V_e$ , is equal to the sum of the void (interstitial) volume,  $V_0$ , and a fractional volume,  $V_l$ , which is related to the solvent volume taken up by the gel beads.  $K_d$  is normally derived from the above equation and is similar to a distribution coefficient.

The void volume,  $V_0$ , is assumed to be the peak elution volume of a sufficiently high-molecular-weight compound. However, the exact determination of the volume  $V_l$  is very difficult.

Eqn. 1 shows that for the chromatographic determination of  $V_l$  it is necessary to use a reference material for which  $K_d$  is unity. In general, *n*-pentane or acetone is selected as such a reference material for gel chromatography using organic solvents. However, in the treatment of low-molecular-weight molecules, it is not safe to assume that the  $K_d$  value of these reference materials is unity because their molecules are too large.

The following equation was derived by Laurent and Killander<sup>11</sup>:

$$V_e = V_0 + K_{iv} V_x \quad (2)$$

where  $V_x$  is the volume of the gel phase and  $K_{iv}$  corresponds to a distribution coefficient of solute between the interstitial solution phase and the swollen gel phase. The volume  $V_x$  is equal to the sum of the volume of the gel matrix,  $V_g$ , and the volume  $V_l$ , and can easily be determined by the equation

$$V_x = V_l + V_0 \quad (3)$$

where  $V_l$  is the total volume of the column system (bed volume) and is a measurable parameter. Consequently,  $K_{iv}$  is a more appropriate parameter than  $K_d$  for the expression of the elution characteristics of small molecules such as metal complexes.

In this work,  $K_{iv}$  values of acetylacetonone (AA) and its metal complexes,  $M(AA)_n$ , were measured in the Merckogel OR-2000-tetrahydrofuran (THF) system. Acetylacetonone is a typical  $\beta$ -diketone that is well known as an important chelating agent in analytical chemistry. The metal complexes studied were  $Co(AA)_3$ ,  $Fe(AA)_3$ ,  $Cr(AA)_3$ ,  $Al(AA)_3$ ,  $Cu(AA)_2$ ,  $Ni(AA)_2$  and  $Be(AA)_2$ . In addition, the  $K_{iv}$  values of some *n*-alkanes were also obtained for comparison.

## EXPERIMENTAL

*Materials*

Merckogel OR-2000 (E. Merck, Darmstadt, G.F.R.) was washed with re-distilled water, acetone and methanol (with *ca.* 50 ml per gram of gel in each case), in that order. The gel was dried under reduced pressure and further over silica gel for 3 days. The resulting solid mass was then ground to a fine material using an agate mortar and then finally sieved to obtain 200–300 mesh fractions.

The metal(II, III) acetylacetonates, except for  $\text{Be}(\text{AA})_2$ , were prepared and purified as reported elsewhere<sup>12,13</sup>.  $\text{Be}(\text{AA})_2$  was reagent-grade material (Dojindo Co., Kumamoto, Japan).

According to the C, H elemental analyses for the materials finally applied in this work, all of the complexes, except that of Ni(II), were anhydrous; the Ni(II) complex was a dihydrate, *i.e.*,  $\text{Ni}(\text{AA})_2 \cdot 2\text{H}_2\text{O}$ .

Acetylacetonone was purified by washing the reagent-grade material with 1 *M* ammonia solution and distillation after drying over anhydrous sodium sulphate.

Polystyrene, used as a reference material to measure the column void volume,  $V_0$ , was monodisperse polystyrene standard (Pressure Chemical Co., Pittsburgh, Pa., U.S.A.), with mol. wt. 200,000 and  $M_w/M_n < 1.06$  (Batch No. 1c).

THF was purified by re-distillation of the reagent-grade material (Wako, Osaka, Japan) over iron(II) sulphate at 66.0°.

*Apparatus*

The apparatus used is shown schematically in Fig. 1. As acetylacetonone has a high reactivity with metals, all parts that come into contact with liquid are made not of metal but of PTFE or Pyrex in order to avoid undesirable influences, except for the pump, which is made of stainless steel.

The column consists of 100-cm sections of 5-mm I.D. Pyrex tubing with a water-jacket. The column is kept at  $25.00 \pm 0.02^\circ$  by a Haake Model FK-2 constant-temperature circulator. The Jasco Model FLC-350 pumping system (Japan Spectroscopic Co., Tokyo, Japan) is a syringe-type high-pressure pump. The sample injection

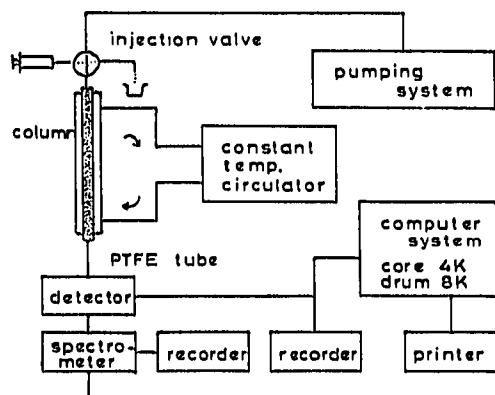


Fig. 1. Schematic diagram of the chromatographic system.

valve (Jeol, Tokyo, Japan) is calibrated to 40- $\mu$ l. A refractometric detector (Laboratory Data Control, Riviera Beach, Fla., U.S.A.) is used, and the detection signal is fed to a JEC-5 computer system (Jeol) for on-line data processing. The effluent flowing out the detector is fed to a Jasco Model UVIDEC-I spectrophotometer with micro-flow cells made of PTFE (8  $\mu$ l).

The details of the present system and the data processing software will be published elsewhere.

### Procedure

Merckogel OR-2000, after being swollen in THF for at least 24 h, was packed into the column using a packing reservoir supplying the solvent at a flow-rate of 0.20 ml/min. After the gel had settled, the solvent was further pumped through the column at a flow-rate of 0.20 ml/min for 24 h so as to ensure complete setting of the gel bed under normal operating conditions, and this flow-rate was maintained throughout the experiments.

Sample solutions were prepared by dissolving the mixture of a metal complex or *n*-alkane and polystyrene in 5 ml of THF, as shown in Table I. Polystyrene acts as an internal reference. A 40- $\mu$ l portion of a sample solution was fed into the column by the injection valve.

TABLE I  
COMPOSITION OF SAMPLE SOLUTIONS

No.	Sample	Amount taken (mg*)	Polystyrene added (mg*)
1	Co(AA) <sub>3</sub>	15.35	8.70
2	Fe(AA) <sub>3</sub>	15.91	6.82
3	Cr(AA) <sub>3</sub>	15.04	6.11
4	Al(AA) <sub>3</sub>	15.40	7.19
5	Cu(AA) <sub>2</sub>	10.75	7.13
6	Ni(AA) <sub>2</sub> ·2H <sub>2</sub> O	13.90	6.30
7	Be(AA) <sub>2</sub>	14.64	6.87
8	Acetylacetone	29.80	7.00
9	<i>n</i> -Hexadecane	58.50	6.30
10	<i>n</i> -Heptane	100 $\mu$ l	6.36
11	<i>n</i> -Pentane	50 $\mu$ l	6.80

\* In 5 ml of solution.

UV absorption profiles of the effluent were recorded on the spectrophotometer in order to identify the species being eluted.

The computer printed out the data on the chromatographic behaviour of the sample components each time the elution of all of the components of an injected sample was completed. Each experiment on a sample was carried out in triplicate.

### RESULTS AND DISCUSSION

The elution volumes of the sample components increased in the following order: polystyrene, *n*-hexadecane, *n*-heptane, Al(AA)<sub>3</sub>, Cr(AA)<sub>3</sub>, Co(AA)<sub>3</sub>, Fe(AA)<sub>3</sub>,

*n*-pentane, Ni(AA)<sub>2</sub>, Cu(AA)<sub>2</sub>, Be(AA)<sub>2</sub>, acetylacetonone. Some examples of the chromatograms obtained are shown in Fig. 2.

According to the measurements of the gel bed volume,  $V_r$ , and the elution volume of polystyrene, the void volume,  $V_0$ , and the gel phase volume,  $V_x$ , were calculated by eqn. 3 as  $V_r = 19.63$ ,  $V_0 = 6.82$  and  $V_x = 12.81$  ml.

These values are based on the assumption that the  $K_d$  and  $K_{av}$  values for polystyrene are zero, and these are the results corrected for the dead volume (0.24 ml), which is increased by the internal volume of the tubing and the detector.

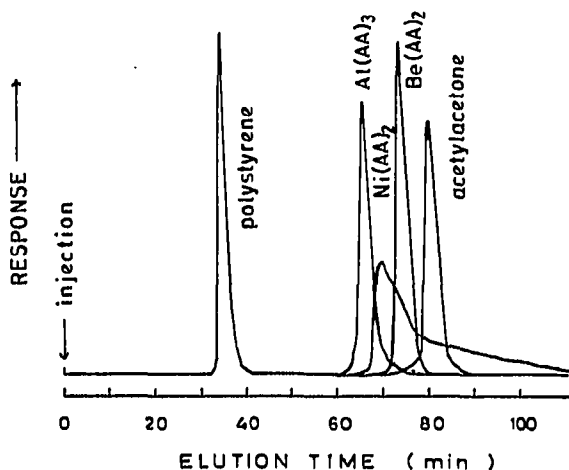


Fig. 2. Chromatograms of acetylacetonone and some of its metal complexes. Polystyrene is added as the reference material in each sample solution. Merckogel OR-2000 column, 100 cm  $\times$  5 mm I.D., THF solvent at 0.20 ml/min, 25.0°.

The distribution coefficients,  $K_{av}$ , of the solutes were obtained by means of eqn. 2 and are listed together with the skew ratio,  $R$ , and HETP in Table II. The reproducibility of the  $K_{av}$  values is satisfactory. The relative standard deviation in each case is not more than 0.7%. The values shown in Table II are the average values of triplicate measurements.

The skew ratio is a convenient parameter for expressing the shapes of elution peaks, and is defined as the ratio of the magnitude of the slopes of the trailing and leading edges at their points of inflection<sup>14</sup>; thus, when the peak is symmetrical, the skew ratio is unity; however, when the leading slope is steeper than the trailing slope, the skew ratio is less than unity. According to the results for the skew ratios, the complexes of the trivalent metals, except iron, show symmetrical peaks. The value for Fe(AA)<sub>3</sub> is 0.58, which indicates a skewed peak. However, the HETP of Fe(AA)<sub>3</sub> is less than those for the other complexes and therefore it can be said that Fe(AA)<sub>3</sub> gives a sharp peak with a somewhat trailing shape.

Of the complexes of divalent metals, Be(AA)<sub>2</sub> gives a sharp and symmetrical peak, but the complexes of copper and nickel had small skew ratios. The complex of nickel gave a particularly distorted elution peak, as shown in Fig. 2. The UV absorption profile of the trailing fraction of the effluent was different from the profile of the

TABLE II

OBSERVED VALUES OF DISTRIBUTION COEFFICIENT,  $K_{av}$ , HEIGHT EQUIVALENT TO A THEORETICAL PLATE, HETP, AND SKEW RATIO,  $R$ Column: Merckogel OR-2000, 100 cm  $\times$  5 mm I.D., 25.0°. Solvent: THF, 0.20 ml/min.

No.	Substance	$K_{av}$	HETP (mm)	$R$
1	Co(AA) <sub>3</sub>	0.523	0.36	0.97
2	Fe(AA) <sub>3</sub>	0.536	0.19	0.58
3	Cr(AA) <sub>3</sub>	0.517	0.32	0.97
4	Al(AA) <sub>3</sub>	0.497	0.32	0.89
5	Cu(AA) <sub>2</sub>	0.571	0.22	0.58
6	Ni(AA) <sub>2</sub>	0.557	—	0.28
7	Be(AA) <sub>2</sub>	0.620	0.28	0.99
8	Acetylacetone	0.714	0.17	0.77
9	<i>n</i> -Hexadecane	0.252	0.22	0.97
10	<i>n</i> -Heptane	0.464	0.17	0.94
11	<i>n</i> -Pentane	0.539	0.16	0.95

original solution of nickel acetylacetonate. Nickel acetylacetonate is well known as a labile complex, and decomposition may occur in the column.

Be(AA)<sub>2</sub> is tetrahedral and the central metal atom is surrounded by the ligands. Therefore, the complex can be assumed to be a spherical molecule. The bis(acetylacetonato) complexes of copper and nickel, however, are square-planar. The differences in the elution behaviour of these complexes must be also related to stereochemical effects. In addition, the nickel complex used in this work is a dihydrated crystal, whereas the other complexes are anhydrous.

According to the principles of gel chromatography, a larger solute molecule is expected to have a smaller  $K_{av}$  value. It is not easy to measure the exact sizes of the solute molecules. Therefore, the molar volume of each solute, which is known, is adopted in the following discussion.

Irving<sup>15</sup> showed that the molar volumes of M(AA)<sub>3</sub> in organic solvents (Al(AA)<sub>3</sub>, 271; Cr(AA)<sub>3</sub>, 267; Fe(AA)<sub>3</sub>, 269; Co(AA)<sub>3</sub>, 261) are not very different from those for the solid state. The molar volume of Be(AA)<sub>2</sub> is calculated to be 184 when the empirical relationship between the molar volume of acetylacetonate,  $V_{AA}$ , and that of its metal complex,  $V_{M(AA)_n}$ , expressed by the equation<sup>15,16</sup>  $V_{M(AA)_n}/V_{AA} = 0.9n$ , is used. Wakabayashi *et al.*<sup>16</sup> showed that the partition behaviour of acetylacetonate can be explained satisfactorily by assuming that the molar volume of acetylacetonate is 102\*.

The molar volumes of *n*-pentane, *n*-heptane and *n*-hexadecane are 116, 147 and 295, respectively<sup>17</sup>. The correlations between the  $K_{av}$  values and the molar volumes,  $V$ , of the solutes are clearly shown in Fig. 3.

Comparing the  $V$  values for the *n*-alkanes (group A) with the  $K_{av}$  values, the order of decreasing molar volume is the same as that for increasing  $K_{av}$  values. A similar correlation is also obtained for acetylacetonate and its metal complexes (group B).

However, it seems that the elution behaviour of the two groups is different.

\* From density measurements in our laboratory, the molar volume of acetylacetonate is calculated to be 103.4 at 25.0°.

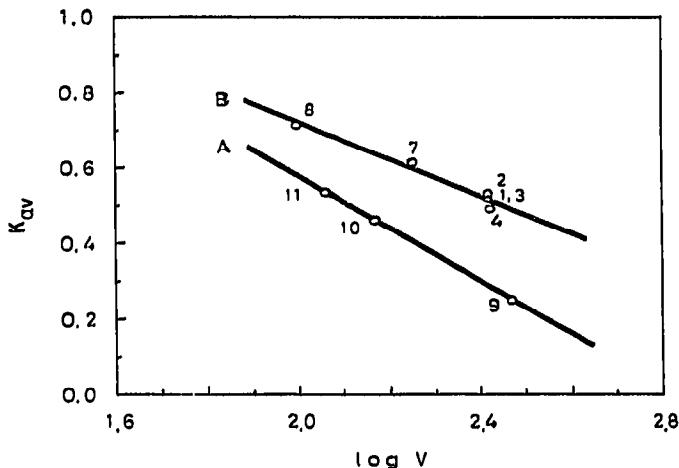


Fig. 3. Correlation between  $K_{av}$  and molar volume,  $V$ . A,  $n$ -Alkanes; B, acetylacetonone and its metal complexes. The numbers correspond to those in Table II.

For example, the molar volume of  $n$ -heptane is apparently less than that of  $M(AA)_3$ , but the  $K_{av}$  value of the former is not greater than that of the latter.

Considering that  $n$ -alkanes are inert compounds, the elution behaviour of the group B compounds can be explained not only by the sieving effect but also by some interactions between the solute and gel phase and also by the difference in the geometrical structures of the molecules. For the elucidation of the chromatographic behaviour of these compounds, it will be necessary to investigate the effects of solvents and gels more extensively, and such studies will be published elsewhere.

From a practical point of view, the  $K_{av}$  and HETP results indicate that the separation of some of these metal complexes will be successful under more appropriate conditions, e.g., lower flow-rate of the solvent and longer column length.

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