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## THIN-LAYER CHROMATOGRAPHY OF VARIOUS $\beta$ -DIKETONES AND THEIR METAL COMPLEXES ON STYRAGEL 60A

MASAMI SHIBUKAWA\*, KOICHI OGUMA and ROKURO KURODA\*\*

*Laboratory for Analytical Chemistry, Faculty of Engineering, University of Chiba, Yayoi-cho, Chiba (Japan)*

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### SUMMARY

The gel chromatographic behaviours of six  $\beta$ -diketones and their metal complexes, particularly chromium complexes, have been investigated by thin-layer chromatography in dioxan and benzene media at  $25 \pm 1^\circ$ . The effects of several development conditions on the  $R_F$  values are not marked, the systems yielding very reproducible  $R_F$  values for  $\beta$ -diketones and their complexes. For both eluents, each  $\beta$ -diketone exhibits a lower  $R_F$  value than its chromium complex, which suggests that the sieving effect may be a dominant factor for the chromatographic process. A plot of  $\log(1/R_F - 1)$  vs.  $K_{av}$ , obtained by a precise column method yields a straight line with a slope of unity, indicating that the thin-layer chromatographic elution proceeds under conditions very close to the precise column chromatography.

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### INTRODUCTION

Gel chromatography permits the separation of solute compounds largely according to their molecular sizes and is being used increasingly as a means of determination of the molecular weight and size of solute species.

The application of gel chromatography to metal complexes has recently been reported. Several workers have discussed the gel chromatographic behaviour of neutral metal chelates in organic solvent media, from the viewpoint of mutual interactions between the metal chelate, eluent and gel matrix. Thus, Saitoh and co-workers<sup>1-3</sup> reported a series of investigations of the gel chromatographic behaviour of  $\beta$ -diketones and their metal chelates, using various organic solvents as eluents. Yamamoto and co-workers<sup>4,5</sup> also investigated tris(acetylacetonato)-aluminium(III), -chromium(III) and -cobalt(III) in various organic solvent-polystyrene gel systems.

Thin-layer gel chromatography (TLGC), as well as the more usual types of

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\* Present address: Department of Chemistry, Faculty of Science, Tokyo Metropolitan University, Fukazawa, Setagaya-Ku, Tokyo, Japan.

\*\* To whom correspondence should be addressed.

thin-layer chromatography, has the obvious advantages of simplicity, rapidity, high sensitivity and multiple sample application and has been utilized extensively for the separation and/or estimation of macromolecular substances in aqueous media. The use of the technique in volatile organic solvents, however, is limited because of the lack of development apparatus which gives reproducible results. Recently, we devised a development apparatus for TLGC, which can be easily handled and gives highly reproducible results even with volatile organic solvent systems<sup>6</sup>.

We attempted to apply TLGC to metal complexes. In the present work, the TLGC behaviour of six  $\beta$ -diketones and their metal chelates, selected as model compounds, and the effects of several development conditions were investigated. The relationship between the  $R_F$  values obtained and the distribution coefficients measured with the column method<sup>3</sup> was also examined. The chromatographic system investigated consisted of Styragel 60A, polystyrene-divinylbenzene copolymer, and *p*-dioxan or benzene as eluent.

## EXPERIMENTAL\*

### Materials

The  $\beta$ -diketones and their metal chelates used are listed in Table I. HAA was washed with 1 *N* ammonia solution and then purified by distillation. Other  $\beta$ -diketones, except HTFA, were recrystallized from appropriate solvents. The chromium(III) chelates, except for Cr(DBM)<sub>3</sub> and Cr(TFA)<sub>3</sub>, were prepared by analogous methods to that for Cr(AA)<sub>3</sub><sup>7</sup>, while Cr(DBM)<sub>3</sub><sup>8</sup> and Cr(TFA)<sub>3</sub><sup>9</sup> were prepared according to respective methods by Charles. Co(AA)<sub>3</sub> and Al(AA)<sub>3</sub> were prepared

TABLE I

### $\beta$ -DIKETONES AND THEIR METAL COMPLEXES STUDIED

The  $\beta$ -diketones have the following general formula (keto form):  $R_1\text{-COCH}_2\text{CO-R}_2$ . HTFA and Be(AA) were reagent-grade materials.

Compound	Abbreviation	$R_1$	$R_2$	C (%)		H (%)	
				Calc.	Found	Calc.	Found
Acetylacetone	HAA	CH <sub>3</sub>	CH <sub>3</sub>				
Trifluoroacetylacetone	HTFA	CH <sub>3</sub>	CF <sub>3</sub>				
Benzoylacetone	HBA	C <sub>6</sub> H <sub>5</sub>	CH <sub>3</sub>				
Benzoyltrifluoroacetone	HBFA	C <sub>6</sub> H <sub>5</sub>	CF <sub>3</sub>				
Thenoyltrifluoroacetone	HTTA	C <sub>4</sub> H <sub>3</sub> S	CF <sub>3</sub>				
Dibenzoylmethane	HDBM	C <sub>6</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>5</sub>				
Tris(acetylacetonato)chromium(III)	Cr(AA) <sub>3</sub>			51.58	51.73	6.06	6.12
Tris(trifluoroacetylacetonato)chromium(III)	Cr(TFA) <sub>3</sub>			35.24	35.36	2.37	2.30
Tris(benzoylacetato)chromium(III)	Cr(BA) <sub>3</sub>			67.28	68.25	5.08	5.26
Tris(benzoyltrifluoroacetato)chromium(III)	Cr(BFA) <sub>3</sub>			51.66	52.18	2.60	2.74
Tris(thenoyltrifluoroacetato)chromium(III)	Cr(TTA) <sub>3</sub>			40.29	40.67	1.69	1.81
Tris(dibenzoylmethanato)chromium(III)	Cr(DBM) <sub>3</sub>			74.89	74.67	4.61	4.69
Tris(acetylacetonato)aluminium(III)	Al(AA) <sub>3</sub>			55.55	55.22	6.53	6.66
Tris(acetylacetonato)cobalt(III)	Co(AA) <sub>3</sub>			50.57	51.04	5.94	5.98
Bis(acetylacetonato)beryllium(II)	Be(AA) <sub>2</sub>			57.96	58.07	6.81	6.95

\* For abbreviations, see Table I.

according to Bryant and Fernelius<sup>10</sup> and Young<sup>11</sup>, respectively. The composition of each product, after purification by recrystallization, was confirmed by carbon and hydrogen elemental analyses. HTFA and Be(AA)<sub>2</sub> were reagent-grade materials (Dojindo Co., Kumamoto, Japan).

Reagent-grade *p*-dioxan and benzene were used after purification involving chemical treatment, drying and distillation. The purity of each final material was tested by UV absorption spectrometry and gas chromatography.

Styragel 60A (polystyrene-divinylbenzene copolymer, particle size <37  $\mu$ m; Waters Assoc., Milford, Mass., U.S.A.) was used after washing with methanol and drying at 60°.

Standard polystyrene (M.W.  $2 \cdot 10^5$ ; Pressure Chem., Pittsburgh, Pa., U.S.A.), which can be regarded as excluded completely from the network of Styragel 60A, was used as an internal standard.  $R_F$  values are then calculated by

$$R_F = d_s/d_{ps}$$

where  $d_s$  and  $d_{ps}$  are the distances travelled by the sample and the standard polystyrene, respectively.

A preliminary experiment in the benzene system showed that the zone of standard polystyrene was distorted (leading) at concentrations above 0.6% (w/v) at a migration rate of *ca.* 40 mm/h. In the *p*-dioxan system, such distortions were never observed in the range examined, 0.2–1.0%. Based on these observations, we adopted the concentrations of standard polystyrene of 0.4–0.5% and migration rates of less than 40 mm/h in the benzene system.

### Procedure

A gel suspension was prepared by thoroughly mixing 7.4 g of Styragel 60A and 20 ml of the eluent solvent, *i.e.*, *p*-dioxan or benzene, and the gel allowed to swell overnight. The gel slurry was spread on glass plates (20  $\times$  20 cm) with a conventional applicator (Yamato Scientific, Tokyo, Japan). Two uniform gel layer plates, 250  $\mu$ m in thickness, could be prepared from each batch of the slurried gel. Thicker gel layer plates were prepared by using greater quantities of the slurried gel, the above mixing ratio of gel to eluent solvent being kept constant. Both sides of the gel layer were then scraped off for about 5 mm in width. Before the gel layer became completely dry, the plate was set up in the development apparatus previously reported<sup>6</sup>. The development chamber was then allowed to incline at a desired angle to conduct a preliminary development for at least 14 h before each run. This pre-equilibration process was necessary for obtaining reproducible results.

For application of samples, the development chamber was mounted horizontally, and 1- $\mu$ l portions of the sample solutions containing standard polystyrene as an internal standard were applied on the gel plate with micropipettes (Drummond, Broomall, Pa., U.S.A.) as a series of spots *ca.* 2 cm apart in a line 3 cm from the edge of the plate. When development had proceeded for *ca.* 100 mm [1-(2-pyridylazo)-2-naphthol was used as a marker] the gel plate was taken out of the chamber, and a paper replica was prepared by using a sheet of silicone-treated filter-paper impregnated with the eluent, as reported previously<sup>6</sup>.

The  $\beta$ -diketones and their metal chelates were detected on the paper replicas as dark spots under UV light. Standard polystyrene was visualized as red spots on paper replicas by spraying with 20% (w/w) antimony pentachloride in carbon tetrachloride and heating gently over a hot plate.

For chemical confirmation of the nature of the sample after development, the individual spots were scraped off from the plate and subjected to extraction with the eluent solvent. After removal of the gel grains by filtration, the extracted material was examined by UV spectrophotometry.

Throughout this work, development was carried out at  $25 \pm 1^\circ$ .

## RESULTS AND DISCUSSION

### *The effects of experimental conditions on $R_F$ values*

The effects of the development rate and the thickness of the gel layer on  $R_F$  values in the *p*-dioxan system were examined. On a gel layer of fixed thickness (250  $\mu\text{m}$ ),  $R_F$  values were measured for HBA, HDBM,  $\text{Cr}(\text{TFA})_3$ ,  $\text{Cr}(\text{DBM})_3$  and  $\text{Cr}(\text{TTA})_3$  at various development rates (10–50 mm/h), which were represented by the migration rates of standard polystyrene. The development rate was controlled by inclining the development chamber at an appropriate angle. The results obtained are given in Table II. The  $R_F$  values for the same compounds as described above were also measured on gel layers of different thickness (250–750  $\mu\text{m}$ ) keeping the development rate constant at 30 mm/h and the results obtained are listed in Table III. As can be seen from these tables, the  $R_F$  values are independent of the development rate and the thickness of the gel layer under the conditions examined.

TABLE II  
EFFECT OF DEVELOPMENT RATE ON  $R_F$  VALUES  
Eluent *p*-dioxan; gel layer thickness, 250  $\mu\text{m}$ .

Development rate (mm/h) [angle ( $^\circ$ )]	$R_F$				
	HBA	HDBM	$\text{Cr}(\text{TFA})_3$	$\text{Cr}(\text{DBM})_3$	$\text{Cr}(\text{TTA})_3$
10 [10]	0.60	0.62	0.76	0.78	0.79
20 [20]	0.58	0.61	0.76	0.77	0.81
30 [30]	0.60	0.61	0.77	0.77	0.80
40 [40]	0.61	0.62	0.76	0.77	0.79
50 [50]	0.61	0.62	0.78	0.77	0.80
av.	0.60	0.62	0.77	0.77	0.80

TABLE III  
EFFECT OF GEL LAYER THICKNESS ON  $R_F$  VALUES  
Eluent, *p*-dioxan.

Gel layer thickness ( $\mu\text{m}$ ) [angle ( $^\circ$ )]	$R_F$				
	HBA	HDBM	$\text{Cr}(\text{TFA})_3$	$\text{Cr}(\text{DBM})_3$	$\text{Cr}(\text{TTA})_3$
250 [30]	0.61	0.62	0.76	0.77	0.79
500 [40]	0.59	0.61	0.75	0.76	0.82
750 [40]	0.59	0.60	0.74	0.75	0.81
av.	0.60	0.61	0.75	0.76	0.81

The effect of the concentration of sample applied was examined for  $\beta$ -diketones and their chromium(III) chelates in the *p*-dioxan system. The  $R_F$  values were found to be independent of the sample concentration within the tested ranges shown in Table IV.

TABLE IV

 $R_F$  VALUES OF  $\beta$ -DIKETONES AND THEIR METAL COMPLEXESEluent, *p*-dioxan.

Compound	Concn. ( $\times 10^{-2}$ M)	$R_F$	S.D.
HBA	0.92-16.3	0.60	0.02
HDBM	0.99-13.0	0.61	0.01
HBFA	7.25-12.1	0.64	0.02
HTTA	5.69-10.7	0.66	0.02
Cr(AA) <sub>3</sub>	0.46- 5.93	0.65	0.02
Cr(BA) <sub>3</sub>	0.07- 1.05	0.73	0.02
Cr(TFA) <sub>3</sub>	0.16- 2.21	0.76	0.02
Cr(DBM) <sub>3</sub>	0.06- 0.21	0.77	0.02
Cr(BFA) <sub>3</sub>	0.14- 0.74	0.79	0.02
Cr(TTA) <sub>3</sub>	0.04- 0.65	0.80	0.01
Be(AA) <sub>2</sub>	1.79-14.9	0.67	0.01
Co(AA) <sub>3</sub>	1.06	0.66	0.01
Al(AA) <sub>3</sub>	2.33	0.69	0.02

$R_F$  values for  $\beta$ -diketones and their metal chelates in the *p*-dioxan system, based on the means from 8-16 determinations under various conditions described above, are summarized in Table IV, together with the standard deviations. Similarly,  $R_F$  values obtained in the benzene system (means from 5-10 determinations) are listed in Table V. These results show that the  $R_F$  values obtained in this work are quite reproducible.

TABLE V

 $R_F$  VALUES OF  $\beta$ -DIKETONES AND THEIR METAL COMPLEXES

Eluent, benzene.

Compound	Concn. ( $\times 10^{-2}$ M)	$R_F$	S.D.
HBA	3.07	0.62	0.01
HDBM	2.92	0.65	0.02
HBFA	7.26	0.69	0.02
HTTA	5.64	0.70	0.02
Cr(AA) <sub>3</sub>	1.41	0.75	0.02
Cr(BA) <sub>3</sub>	0.59	0.80	0.01
Cr(TFA) <sub>3</sub>	0.89	0.78	0.02
Cr(DBM) <sub>3</sub>	0.17	0.81	0.03
Cr(BFA) <sub>3</sub>	0.59	0.82	0.02
Cr(TTA) <sub>3</sub>	0.52	0.83	0.01
Be(AA) <sub>2</sub>	6.08	0.66	0.03
Co(AA) <sub>3</sub>	1.39	0.75	0.01
Al(AA) <sub>3</sub>	5.13	0.76	0.01

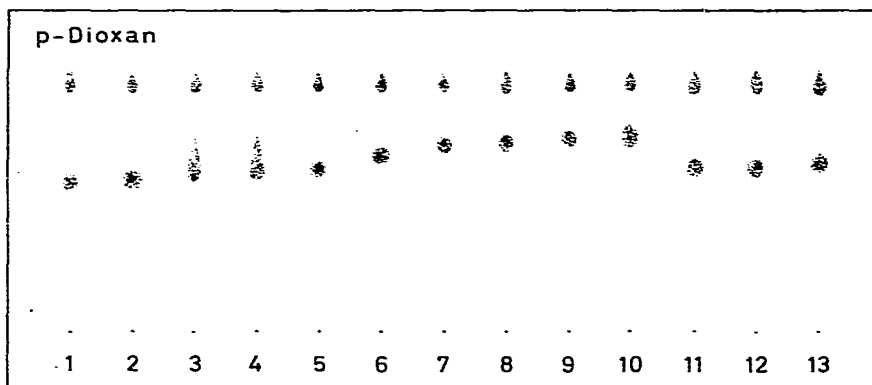


Fig. 1. Chromatogram of  $\beta$ -diketones and their metal complexes. Eluent, *p*-dioxan. Spots: 1 = HBA; 2 = HDBM; 3 = HBFA; 4 = HTTA; 5 = Cr(AA)<sub>3</sub>; 6 = Cr(BA)<sub>3</sub>; 7 = Cr(TFA)<sub>3</sub>; 8 = Cr(DBM)<sub>3</sub>; 9 = Cr(BFA)<sub>3</sub>; 10 = Cr(TTA)<sub>3</sub>; 11 = Be(AA)<sub>2</sub>; 12 = Co(AA)<sub>3</sub>; 13 = Al(AA)<sub>3</sub>.

*TLGC behaviour of  $\beta$ -diketones and their metal chelates*

Chromatograms obtained for  $\beta$ -diketones and their metal chelates in *p*-dioxan and in benzene are shown in Figs. 1 and 2, respectively. These chromatograms show that the present method gives compact and round zones without any distortion. HAA and HTFA could not be detected, probably because they vaporized rapidly during the development and/or the detection process.

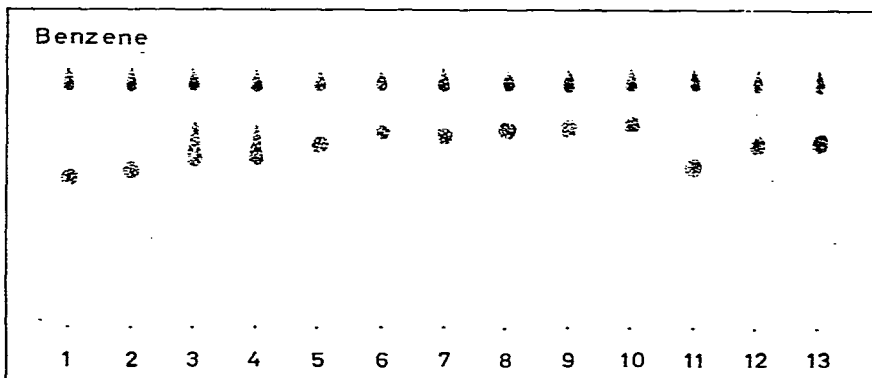


Fig. 2. Chromatogram of  $\beta$ -diketones and their metal complexes. Eluent, benzene. Spots as in Fig. 1.

HBFA and HTTA gave diffuse spots (leading), in contrast to the other compounds. According to the UV spectra of HTTA after development, such specific behaviour seems to be caused by the conversion into the hydrated form owing to the reaction with water present in the eluent<sup>12</sup>. The water was probably introduced from the air in the development chamber. HTTA hydrate appears to have a larger size than non-hydrated HTTA, *i.e.*, the keto or enol form, so that the spot may have been distorted. The behaviour of HBFA can also be explained in a similar manner.

In either *p*-dioxan or benzene, each  $\beta$ -diketone has a lower  $R_F$  value than its chromium(III) chelate (see Tables IV and V), which indicates that the sieving effect is a dominant factor in the separation mechanism. HBA and HDBM were separated from their own chromium(III) chelates.

Four metal acetylacetonates exhibited similar  $R_F$  values in *p*-dioxan, while in benzene Be(AA)<sub>2</sub> gave a lower  $R_F$  value than the other metal(III) acetylacetonates, Al(AA)<sub>3</sub>, Co(AA)<sub>3</sub> and Cr(AA)<sub>3</sub>, and was separated from the M(AA)<sub>3</sub> group. So far, the details of this solvent effect have not been clarified.

#### Correlation between $R_F$ values and distribution coefficients

According to the equation of Martin and Synge<sup>13</sup>

$$\alpha = \frac{A_m}{A_s} \left( \frac{1}{R_F} - 1 \right)$$

the  $R_F$  value depends on two factors: the distribution coefficient,  $\alpha$ , and the ratio of the cross-sectional areas of the mobile and stationary phases,  $A_m/A_s$ . In thin-layer chromatography (TLC) it is generally difficult to maintain constant the  $A_m/A_s$  factor, so that good reproducibility of  $R_F$  values in TLC cannot be expected<sup>14</sup>. The  $R_F$  values obtained in this work, however, were so reproducible independent of the development rate and the thickness of gel layer (see Tables II and III) that the  $A_m/A_s$  factor in this method should be considered as actually constant.

Suzuki *et al.*<sup>3</sup> have reported the precise distribution coefficients,  $K_{av}$ , of  $\beta$ -diketones and their chromium(III) chelates in the system *p*-dioxan–Poragel 60A (the previous trade name of Styragel 60A) using a column method,  $K_{av}$  being derived from the following equation<sup>15</sup>

$$K_{av} = (V_e - V_o)/V_x$$

where  $V_e$ ,  $V_o$  and  $V_x$  are the elution volume of the solute, the volume of mobile phase and the volume of the swollen gel phase in column, respectively.

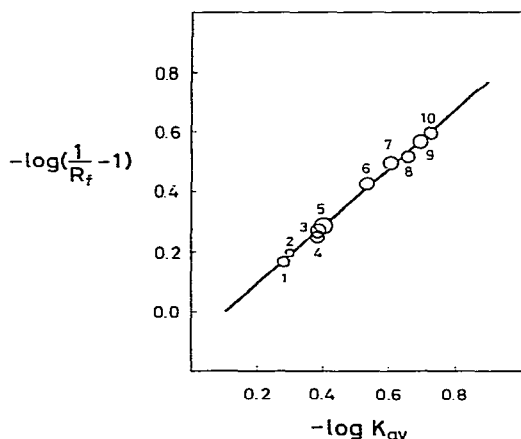


Fig. 3. Relation between  $K_{av}$  and  $R_F$ . 1 = HBA; 2 = HDBM; 3 = Cr(AA)<sub>3</sub>; 4 = HBFA; 5 = HTTA; 6 = Cr(BA)<sub>3</sub>; 7 = Cr(TFA)<sub>3</sub>; 8 = Cr(DBM)<sub>3</sub>; 9 = Cr(BFA)<sub>3</sub>; 10 = Cr(TTA)<sub>3</sub>. The diameter of each circle represents the 95% confidence limit of  $\log(1/R_F - 1)$ .

By use of the  $K_{av}$  values of ref. 3 and the values in the present work, the relationship between  $\log K_{av}$  and  $\log (1/R_F - 1)$  could be approximated by a straight line as shown in Fig. 3. The regression equation is

$$\log \left( \frac{1}{R_F} - 1 \right) = 0.97 \log K_{av} + 0.10$$

The slope is almost unity and the correlation coefficient,  $r$ , is 0.996. The results obtained reveal that the development in the present method was achieved under conditions very close to the precise column chromatography, and TLGC can be expected to give a simple and useful method for obtaining the distribution coefficient.

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