Journal of Chromatography, 92 (1974) 291–297
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 7257

DISTRIBUTION COEFFICIENTS OF ACETYLACETONE AND TRIS(ACETYLACETONATO)CHROMIUM(III) IN THE MERCKOGEL OR-2000–CHLOROFORM SYSTEM*

KOICHI SAITOH, MIEKO SATOH and NOBUO SUZUKI**
Department of Chemistry, Faculty of Science, Tohoku University, Sendai (Japan)

(Received December 27th, 1973)

SUMMARY

In order to clarify the mechanism of the separation of species containing metals by gel chromatography, the influence of column temperature and amount of sample on the distribution coefficient was studied. Acetylacetone and tris(acetylacetonato)-chromium(III) were selected as model compounds, and a chromatographic system consisting of Merckogel OR-2000 and chloroform was used. The distribution coefficient was influenced neither by the amount of sample nor by temperature, and the elution peak was symmetrical. It was concluded that the contribution of adsorption or partition was negligible and the sieving effect was the dominant factor in the separation mechanism.

A new technique of packing, the "closed packing method", was adopted successfully. It was a simpler and more reliable method than other methods for the column system used in the present work.

INTRODUCTION

Gel chromatography, which is based mainly on the differences in the geometrical dimensions of chemical species, has become a powerful method for separation and characterization. In comparison with other liquid chromatographic methods based on chemical or physical interactions between solute molecules and stationary substances in the chromatographic system, gel chromatographic separations can be carried out under mild conditions such that the solutes do not suffer from undesirable chemical or physical influences.

Most studies on gel chromatography reported previously were concerned with biochemically interesting water-soluble polymers, *e.g.*, proteins and sugars, and only a few have dealt with organic solvents and were also limited to high-molecular-weight synthesized polymers, *e.g.*, polystyrene and polyethylene, and were car-

---

[*] Presented at the Analytical Chemistry Symposium held by the Japan Society for Analytical Chemistry at Muroran Industrial College, Muroran, Japan, July 26th, 1973.

[**] To whom correspondence should be addressed.

ried out mainly in order to estimate the molecular weight distribution. A few applications to low-molecular-weight compounds, especially metal-containing species, have been reported, *e.g.*, involving molybdate[1], molybdophosphoric acid[2], polymeric iron(III) hydroxides[3], iron(III) nitrate[4], alkali metal ions[5,6], alkaline earth metal ions[7-11] and Mg–EDTA chelate[12]. These studies were carried out using Sephadex in aqueous media. Recently, the gel chromatography of neutral metal chelates in organic solvent media has been investigated[13], and the application of gel chromatography to such compounds will be further extended.

The theoretical basis of gel chromatography has been dealt with in many papers[14-17]. Separations by gel chromatography result from the different rates of diffusion of the solute molecules into the gel structure. Such solute molecules are characterized by the equation

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

where the elution volume, V_e, is equal to the sum of the interstitial (void) volume, V_0, and a fraction of V_i, which is the solvent volume acquired by the gel beads (pore volume); K_d is the distribution coefficient, which is equal to the ratio of the accessible pore volume to the total pore volume:

$$K_d = V_{i,\mathrm{acc}}/V_i \tag{2}$$

and assumes values between zero (representing complete exclusion from the gel interior) and unity (representing complete penetration).

When only the molecular sieve mechanism is operative, K_d does not exceed unity, but when the adsorption or partition mechanism occurs to a significant extent, K_d exceeds unity. Furthermore, in the molecular sieve mechanism, K_d is almost independent of temperature, while with other mechanisms a dependence of K_d on temperature may be observed. In addition, with a large contribution from adsorption, the value of K_d is influenced by the amount of solute fed into the column and the elution curve is asymmetrical with tailing. Adsorption or partition effects have been reported, not only for metal ions on Sephadex in aqueous media[4-11], but also for aromatic compounds on Sephadex LH-20[18-20] using organic solvents.

For the purpose of the practical application of gel chromatography to metal chelate compounds, it is necessary to elucidate the mechanism of the separation of such compounds. We have attempted to study the chromatographic behaviour of β-diketone chelates, and in the present work the gel chromatographic behaviour of acetylacetone chelates and the free ligand and the effects of the amount of sample applied to the column and the operating temperature were investigated, using tris(acetylacetonato)chromium(III), $Cr(AA)_3$, as a model compound. Acetylacetone (AA) is a typical β-diketone, and its chromium(III) chelate is very stable and soluble in common organic solvents. The chromatographic system investigated consisted of Merckogel OR-2000, which is a copolymer of polyvinyl acetate, with chloroform as the eluting solvent. According to our previous work, chloroform is an effective organic solvent in liquid–liquid partition[21,22].

EXPERIMENTAL

Materials

Merckogel OR-2000 (E. Merck, Darmstadt, G.F.R.) was washed with redistilled water (*ca.* 20 ml per gram of gel) and dried overnight at 40°. The gel was then ground to fines using an agate mortar and dried further over silica gel for a week.

$Cr(AA)_3$ was prepared and purified as described elsewhere[23]. The product was identified by elemental analysis.

Acetylacetone was washed with 1 N ammonia solution in order to remove acetic acid and then purified by distillation.

Polystyrene (mol. wt. 10,000; $\bar{M}_w/\bar{M}_n$ 1.06) and acetone purified in the usual manner were used as standard materials to measure the values of V_0 and V_t.

Chloroform used as the eluent was reagent-grade material (Wako, Osaka, Japan) or was purified in the usual manner, *i.e.*, by washing it with concentrated sulphuric acid, water, 10% sodium hydroxide solution and water, in that order, then drying it over calcium chloride and finally distilling it at 61.1°.

The apparatus used in this work was constructed by the authors. As acetylacetone shows a high reactivity towards metals, all parts that came into contact with it were made not of metals but of PTFE or Pyrex. The solvent reservoir temperature was controlled. A JEOL Model P-2705S constant flow pump was used. Sample injectors were obtained from JEOL and the sampling volume was calibrated as 124 and 223 μl. The column temperature was regulated to an accuracy of ± 0.02° with a Haake Model FT thermostat. A Hitachi Model EPU-2A spectrophotometer with a hand-made flow cell (light path 1 mm; inner volume across the light beam *ca.* 4 μl) was used as a detector.

Closed column packing method

When a heavy solvent such as chloroform was used, it was difficult to pack the gel beads into the column homogeneously because they floated, even when recom-

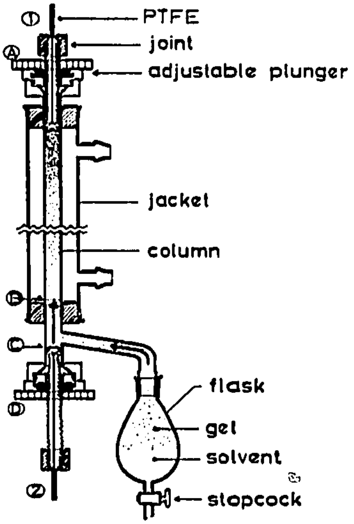

Fig. 1. Column assembly for the "closed packing method".

mended methods were used[24,25]. In this work, a new technique, the "closed packing method", was adopted. The column assembly is shown in Fig. 1, and the procedure was as follows.

Weighed gel and the required amount of solvent were placed in the flask and the gel was swollen overnight in the vessel. The flask was then connected to the side-arm of the column, the lower adjustable plunger set initially at position C and solvent was made to flow into the column through the lower tube (2). After the interior of the assembly had filled completely with solvent, the gel moved slowly and continuously upwards from the flask into the column owing to the difference in the specific gravities and was packed homogeneously in the column. After all of the gel had been transferred to the column, the adjustable plunger was shifted to position B by turning the screw D. Solvent was made to flow continuously through the column overnight so as to settle the gel bed completely, and finally the position of the plunger was set. When recovering the gel from the column, the reverse process to packing was carried out, *i.e.*, the assembly was turned upside down and solvent was supplied from tube (1) after shifting back the plunger to position A. All of the gel should be recovered into the original flask. By using this method, the packing of the gel was carried out very easily, and the loss of gel in the procedure was negligible.

The column parameters used in the present work are given in Table I.

TABLE I

COLUMN PARAMETERS

Parameter	*Value*
Length	47 cm
Diameter	8 mm
Bed volume	23.6 ml
Gel packed*	4.301 g
Interstitial volume, V_0**	9.83 ml
Internal volume, V_i***	7.37 ml

* Weight of dry gel.

** Calculated from V_e for polystyrene.

*** Value taking K_d of acetone as unity.

Procedure

A chloroform solution of $Cr(AA)_3$ and AA, which contained a certain amount of polystyrene as an internal standard, was applied as a test sample. A 124- or 223-μl portion of the sample was injected into the column with a loop injector. The sample was eluted with chloroform at a constant flow-rate of *ca.* 0.3 ml/min. Spectrophotometric detection was carried out at a wavelength of 265 nm and a band width of 2 nm. The column temperature was set at 5, 10, 15, 20 and 25°. Each measurement was repeated at least four times.

RESULTS AND DISCUSSION

An example of a chromatogram obtained at 25° using reagent-grade chloroform is shown in Fig. 2. Polystyrene (PS), $Cr(AA)_3$ and AA were clearly separated from each other.

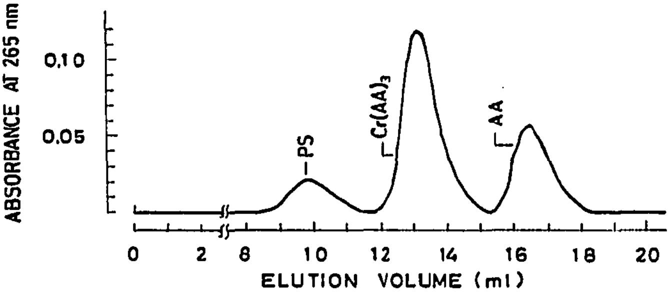

Fig. 2. Chromatogram of mixture of polystyrene (PS), $Cr(AA)_3$ and AA. The amounts injected into the column were: PS, 132 μg; $Cr(AA)_3$, 38.9 μg; and AA, 10.7 μg. Merckogel OR-2000; column, 47 cm × 8 mm I.D.; eluting solvent, reagent-grade chloroform; flow-rate, 0.33 ml/min; temperature, 25°.

The distribution coefficients, K_d, of $Cr(AA)_3$ and AA were measured at different column temperatures and amounts injected. The values were calculated using eqn. 1, with the assumption that V_0 and $V_i + V_0$ are equal to the elution volumes of polystyrene and acetone, respectively. The correlation of K_d with the amount of sample injected at 25° is shown in Fig. 3. The distribution coefficients were found to be independent of the amount of sample injected within the tested range. The same results were found at other column temperatures.

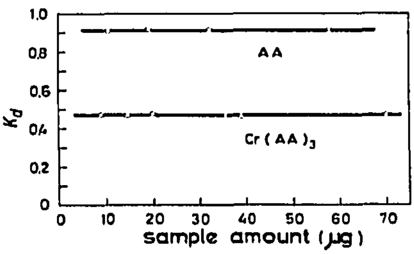

Fig. 3. Dependence of K_d on amount of sample injected into the column. Column conditions as in Fig. 2. The K_d values are relative values, taking K_d of acetone as unity.

The distribution coefficients measured at various column temperatures are listed in Table II, in which each value given is the average of the several values ob-

TABLE II

DISTRIBUTION COEFFICIENTS, K_d, OF $Cr(AA)_3$ AND AA AT VARIOUS COLUMN TEMPERATURES

Column temperature (°C)	*K_d**	
	$Cr(AA)_3$	*AA*
5	$0.461 \pm 0.003_5$	$0.918 \pm 0.006_6$
10	$0.470 \pm 0.004_7$	$0.925 \pm 0.015_1$
15	$0.467 \pm 0.008_1$	$0.917 \pm 0.013_2$
20	$0.462 \pm 0.010_9$	$0.909 \pm 0.015_3$
25	$0.469 \pm 0.006_0$	$0.911 \pm 0.008_8$

* Relative values, taking K_d of acetone as unity; the figures show 95% confidence limits.

tained at a particular temperature. The value of K_d was found to be independent of the column temperature under the conditions examined.

The chloroform used in the above experiments was reagent-grade material, which contained ethanol as a stabilizer. The concentration of ethanol was determined to be 1.06% by gas chromatography. In order to examine the influence of chloroform of different purities, similar experiments were carried out using ethanol-free chloroform. Chloroform was applied immediately after purification in order to avoid any decomposition. The results were similar to those obtained when using reagent-grade solvent, *i.e.*, the value of K_d was independent of both the amount of sample and temperature. The mean values of the distribution coefficient, K_d, of AA and $Cr(AA)_3$ calculated from all of the data obtained are listed in Table III, and it can be seen that none of them exceeded unity. The values obtained by using the purified solvent were slightly lower than those obtained by using reagent-grade material. The separation mechanism is the same, however, when either purified or reagent-grade chloroform is used, as no influence of the amount of sample or column temperature was observed in either instance.

TABLE III

DISTRIBUTION COEFFICIENTS, K_d, OF $Cr(AA)_3$ AND AA ON MERCKOGEL OR-2000 USING REAGENT-GRADE AND PURIFIED CHLOROFORM AS ELUTING SOLVENT

Compound	K_d*	
	Reagent-grade $CHCl_3$	*Purified* $CHCl_3$
$Cr(AA)_3$	$0.466 \pm 0.002_7$	$0.462 \pm 0.009_6$
AA	$0.916 \pm 0.004_3$	$0.855 \pm 0.037_4$

* Relative values, taking K_d of acetone as unity; the figures show 95% confidence limits.

CONCLUSION

The gel chromatographic behaviour of $Cr(AA)_3$ and AA on Merckogel OR-2000 in chloroform medium can be summarized as follows. The distribution coefficients are independent of both the amount of sample and column temperature. The contributions of both adsorption and partition to the separation mechanism are negligible, *i.e.*, the separation was due only to the sieve effect. The "closed packing method" was evidently a successful method of column packing.

REFERENCES

1 C. A. Streuli and L. B. Rogers, *Anal. Chem.*, 40 (1968) 653.
2 N. Yoza, H. Matsumoto and S. Ohashi, *Anal. Chim. Acta*, 54 (1971) 538.
3 R. A. Henry and L. B. Rogers, *Separ. Sci.*, 3 (1968) 11.
4 C. H. Lochmuller and L. B. Rogers, *Anal. Chem.*, 41 (1969) 173.
5 H. Ortner and H. Spitzy, *Z. Anal. Chem.*, 238 (1968) 167.
6 H. Ortner and H. Spitzy, *Z. Anal. Chem.*, 238 (1968) 251.
7 D. Saunders and R. L. Pecsok, *Anal. Chem.*, 40 (1968) 44.
8 B. Z. Egan, *J. Chromatogr.*, 34 (1968) 382.

9 N. Yoza and S. Ohashi, *J. Chromatogr.*, 41 (1969) 429.
10 Y. Ueno, N. Yoza and S. Ohashi, *J. Chromatogr.*, 52 (1970) 321.
11 N. Yoza, T. Ogata and S. Ohashi, *J. Chromatogr.*, 52 (1970) 329.
12 N. Yoza, T. Ogata, Y. Ueno and S. Ohashi, *Bull. Chem. Soc. Jap.*, 41 (1968) 2550.
13 Y. Yamamoto, M. Yamamoto, S. Ebisui, T. Takagi, T. Hashimoto and M. Izuhara, *Anal. Lett.*, 6 (1973) 451.
14 R. L. Steere and G. K. Ackers, *Nature (London)*, 196 (1962) 475.
15 A. Polson, *Biochim. Biophys. Acta*, 50 (1961) 565.
16 G. H. Lathe and C. R. J. Ruthven, *Biochem. J.*, 62 (1956) 665.
17 J. Porath, *Pure Appl. Chem.*, 6 (1963) 233.
18 W. Wilk, J. Pochlitz and H. Bende, *J. Chromatogr.*, 24 (1966) 414.
19 C. A. Streuli, *J. Chromatogr.*, 56 (1971) 219.
20 S. Mori and T. Takeuchi, *J. Chromatogr.*, 50 (1970) 419.
21 T. Wakahayashi, S. Oki, T. Omori and N. Suzuki, *J. Inorg. Nucl. Chem.*, 26 (1964) 2255.
22 T. Omori, T. Wakahayashi, S. Oki and N. Suzuki, *J. Inorg. Nucl. Chem.*, 26 (1964) 2265.
23 W. C. Fernerius and J. E. Branch, *Inorg. Syn.*, 5 (1957) 130.
24 P. Flodin, *J. Chromatogr.*, 5 (1961) 103.
25 R. Widén and K.-E. Eriksson, *J. Chromatogr.*, 15 (1964) 429.