

Short Communication

Retention behavior of salicylidene-glycinatoaluminium(III) in reversed-phase high-performance liquid chromatography

Nobuo Uehara*, Sachie Iso and Yoshio Shijo

Department of Applied Chemistry, Faculty of Engineering, Utsunomiya University, Ishii-cho, Utsunomiya 321 (Japan)

(First received May 27th, 1991; revised manuscript received July 23rd, 1991)

ABSTRACT

The retention behavior of salicylidene-glycinatoaluminium(III) was investigated. The capacity factor of the complex decreased with increasing concentration of ionic solutes and increasing water content in the eluent. The capacity factor of the complex was also smaller when an end-capped octadecylsilane column was used than when a non-end-capped octadecylsilane column was used. The complex was tightly retained with a silica gel column, but it was not retained with a polymer gel column. The complex was retained through the electrostatic interaction with the silanol groups of silica gel. It was found that the high-performance liquid chromatographic system was highly selective for aluminium(III).

INTRODUCTION

Silica-based materials have been used extensively in high-performance liquid chromatographic (HPLC) studies because of their chemical stability, rigidity and availability [1–4]. In HPLC separations using silica-based columns, residual silanol groups on the surface of packing materials often affect the retention behavior of analytes [5–7]. Silanol groups also affect the separation of metal complexes [8,9].

In a previous paper [10], we reported the determination of gallium using HPLC with salicylidene-glycine and mentioned the peculiar retention behavior of salicylidene-glycinatoaluminium(III). The retention behavior of the aluminium(III) complex did not obey the ordinary reversed-phase mode. We suggested that the behavior was due to the effect of the silanol groups. In this paper, this peculiar retention behavior of the aluminium(III) complex was investi-

gated. For this purpose, the effect of the addition of various solutes in the eluent was examined. The effect of acetonitrile content and various column materials was also investigated.

EXPERIMENTAL

Reagents and apparatus

Bissalicylideneethylenediamine (BSED) was synthesized from salicylaldehyde and ethylenediamine using the method reported by Freeman and White [11]. The crude product was recrystallized from benzene, then identified by mass spectrometric analysis and elemental analysis. The BSED solution ($1 \cdot 10^{-3} M$) was prepared by dissolving a fixed amount of BSED in acetonitrile. The standard solution of aluminium(III) ($1000 \mu\text{g ml}^{-1}$) was prepared by dissolving potassium aluminium sulphate in 0.05 M sulphuric acid. Analytical reagent-grade acetonitrile

was distilled and filtered through a membrane filter (pore size $0.45\ \mu\text{m}$). Water was purified with a Milli-Q system after being distilled and deionized. Other reagents used were of analytical reagent-grade.

The HPLC system consisted of a Shimadzu LC-6A pump (Kyoto, Japan), a Shimadzu RF-535 fluorometric detector, a Rheodyne 7125 loop injector equipped with a $20\text{-}\mu\text{l}$ sample loop and a Shimadzu R-1231 recorder. Four Merck LiChro-CART columns ($125\ \text{mm} \times 4\ \text{mm}$ I.D.) filled with different kinds of silica-based materials were used: (1) LiChrosorb RP-18 [$7\text{-}\mu\text{m}$ irregularly shaped silica gel bonded with octadecylsilane (ODS)]; (2) LiChrospher RP-18 ($5\text{-}\mu\text{m}$ spherical silica gel bonded with ODS); (3) LiChrospher RP-18(e) (end-capped LiChrospher RP-18); (4) LiChrospher Si-60 ($5\text{-}\mu\text{m}$ spherical silica gel). A Tosoh TSK gel Octadecyl 4PW column ($150\ \text{mm} \times 4.6\ \text{mm}$ I.D.) (Tokyo, Japan) filled with $7\text{-}\mu\text{m}$ hydrophilic polymer gel bonded with octadecyl groups was also used.

Procedure

To an acidic solution containing $1.0\ \mu\text{g}$ of aluminium(III) ion in a 25-ml volumetric flask, $1.0\ \text{ml}$ of $0.5\ \text{M}$ glycine solution, $1.0\ \text{ml}$ of $1.0 \cdot 10^{-2}\ \text{M}$ BSED solution and $2.0\ \text{ml}$ of $2\ \text{M}$ acetate buffer (pH 3.5) were added, and the solution was diluted to the mark with water. The solution was heated at 60°C for 10 min. After cooling, an aliquot ($20\ \mu\text{l}$) of the solution was injected into the HPLC system.

The eluent was acetonitrile-water (50:50, v/v) containing a solute to be examined. The pH of the eluent was adjusted to 3.5 by the addition of dilute hydrochloric acid prior to the addition of acetonitrile. The flow-rate was $0.8\ \text{ml}\ \text{min}^{-1}$. The temperature of the eluent was kept at $20^\circ\text{C} \pm 1^\circ\text{C}$. The excitation and emission wavelengths of the fluorometric detector were set at 350 and 455 nm, respectively.

RESULTS AND DISCUSSION

Salicylidene-glycine formed from salicylaldehyde and glycine reacts with aluminium(III) to form a fluorescent complex [12]. Although the aluminium(III) complex gave maximum fluorescent intensity at pH 4.0 in a water-acetonitrile mixture, the best chromatograms were obtained at 3.5 (both

eluent and sample). It has been reported [12] that the composition of the aluminium(III) complex is the metal to ligand in a ratio of 1:1, and that the salicylidene-glycine acted as a diprotic acid. Therefore the aluminium(III) complex has a positive charge.

Cationic complexes are retained with ODS stationary phases through two mechanisms: the first is the hydrophobic interaction with alkyl chains bonded onto silica; the second is the ion-exchange interaction with unreacted silanol groups. If ion-exchange interaction is more dominant than hydrophobic interaction, in retaining the cationic complex, the following predictions are introduced.

(a) With increasing cation concentration in the mobile phase, the retention of the cationic complex decreases because the cation competes with the complex.

(b) With increasing acetonitrile content in the mobile phase, the retention of the complex increases, because the dielectric constant of the mobile phase decreases.

(c) With increasing content of silanol groups, the retention of complex increases.

Fig. 1 shows the chromatograms of the aluminium(III) complex in various concentrations of

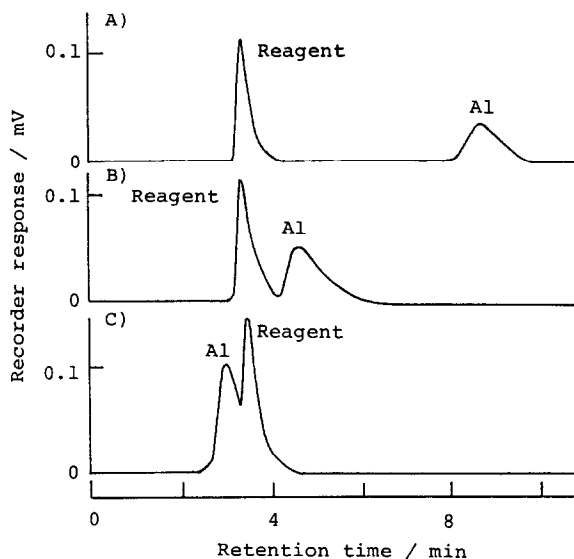


Fig. 1. Chromatograms of the aluminium complex with various sodium acetate concentrations of the eluents. (A) $0\ \text{M}$; (B) $0.33 \cdot 10^{-3}\ \text{M}$; (C) $1.0 \cdot 10^{-3}\ \text{M}$. Eluent: acetonitrile-water (50:50, v/v), pH 3.5; column: LiChrosorb RP-18.

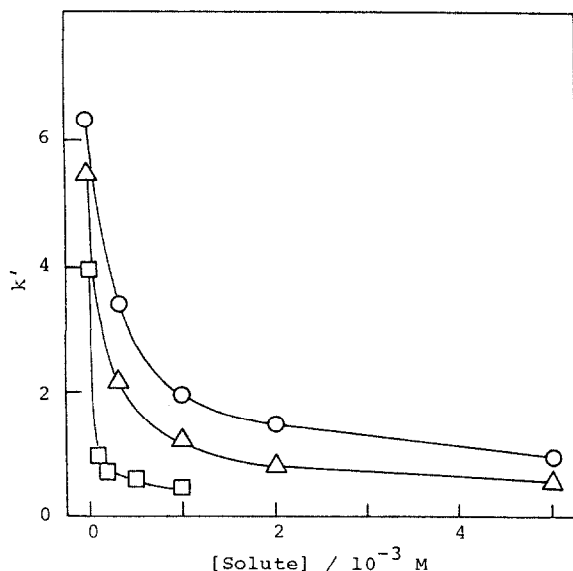


Fig. 2. Effect of ammonium salt concentration on the capacity factor. ○ = Ammonium chloride; △ = trimethylamine hydrochloride; □ = tetrabutylammonium bromide. Other conditions are the same as those in Fig. 1.

sodium acetate used as buffer for the eluent. Increasing the concentration of sodium acetate decreased the retention time of the aluminium(III) complex. Other sodium carboxylates such as sodium acetate, sodium propionate and sodium *n*-butylate gave similar effects to the retention of the aluminium(III) complex. In this case, the sodium ion was the competing cation, so that the number of carbons in the carboxylates did not influence the retention. Fig. 2 shows the results of the addition of amine salts in the eluent. The effects of trimethylamine hydrochloride and tetraethylammonium chloride were not very large compared with that of ammonium chloride. As the total carbon number of the solutes used increased, the effect of the solute increased, because alkyl amines interacted with the stationary phase through hydrophobic interaction. Tetrabutylammonium bromide, which has sixteen carbons, was the most effective reagent in this work. Inorganic salts gave similar results to those of ammonium salts. The divalent salts were more effective than the monovalent salts. Non-ionic solutes gave no effects and zwitter-ionic solutes slightly affected the retention behavior.

We examined the effect of acetonitrile content in

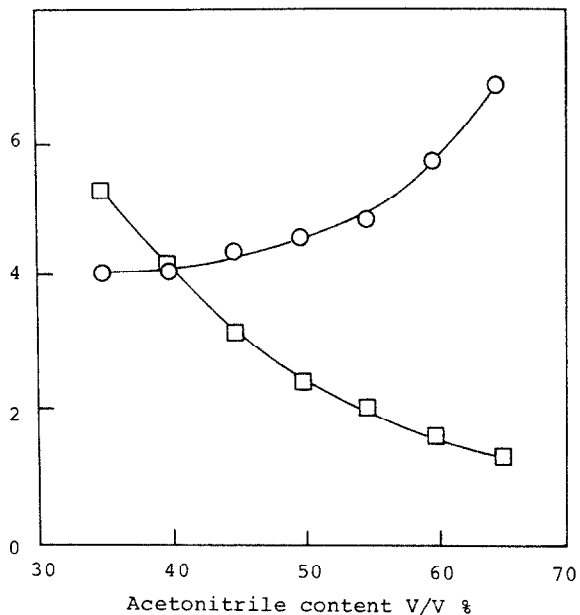


Fig. 3. Effect of acetonitrile content on the capacity factors of reagent and aluminium complex. □ = Reagent; ○ = aluminium complex. Other conditions are the same as those in Fig. 1.

the eluent upon the capacity factor of the aluminium(III) complex. Although the capacity factor of the ligand decreased, the capacity factor of the aluminium(III) complex increased with increasing acetonitrile content, as shown in Fig. 3, which was consistent with the prediction.

There are innumerable silanol groups on the surface of silica-based materials, which act as a cation-exchange site. We used various HPLC columns in order to examine the effect of silanol groups on the retention of the aluminium(III) complex. Fig. 4 shows that the aluminium(III) complex eluted faster with the end-capped ODS column [LiChrospher RP-18(e)] than with the non-end-capped ODS column (LiChrospher RP-18). Moreover, the aluminium(III) complex was little retained on a polymer gel column (TSK gel Octadecyl 4PW) which has no silanol groups. The results show that silanol groups play an important role in retaining the aluminium(III) complex. A silica gel column (LiChrospher Si-60) did not give the peak of the aluminium(III) complex. The complex was tightly retained on the silica gel surface, but the complex was eluted when a more water-rich eluent was used.

The results obtained above support the theory

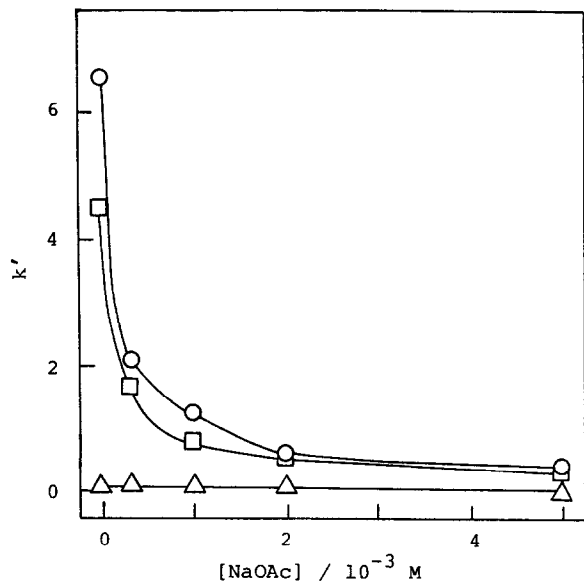


Fig. 4. Effect of column materials on the capacity factor. ○ = LiChrospher RP-18; □ = LiChrospher RP-18(e); △ = TSK gel Octadecyl 4PW. Other conditions are the same as those in Fig. 1.

that salicylidene-glycinatoaluminium(III) was retained on a silica-based column through electrostatic interaction with silanol groups. The electrostatic interaction of the residual silanol groups on the ODS column was investigated by several workers [13,14]. In their cases, however, hydrophobic interaction with the stationary phase was still the dominant force in retaining the complexes. The retention of metal complexes only through ion-exchange interaction has not been reported as far as we

know. We found that in the new metal complex system ion-exchange reaction with silanol groups played the dominant role in the separation of cationic complexes in the aqueous reversed-phase mode.

The HPLC system was quite selective for the aluminium(III) ion. No transitional metal ion gave a peak on the chromatogram. Nor did the alkaline earth metal ions or the indium ion give a peak. Only the peak of gallium ion appeared near the solvent front in the chromatogram. The gallium(III) complex was rarely retained on the column. The application of this finding to the determination of aluminium will be considered in further work.

REFERENCES

- 1 R. Rosett, *Analysis*, 15 (1987) 1.
- 2 I. B. Brenner, G. A. Jones, A. E. Watson and T. W. Steele, *Chem. Geol.*, 45 (1984) 135.
- 3 R. E. Majors, H. G. Barth and C. H. Lochmuler, *Anal. Chem.*, 54 (1982) 323R.
- 4 R. E. Majors, H. G. Barth and C. H. Lochmuler, *Anal. Chem.*, 56 (1984) 300R.
- 5 Y. Ohtsu, Y. Shiojima, T. Okumura, J. Koyama, K. Nakamura and O. Nakata, *J. Chromatogr.*, 481 (1989) 147.
- 6 J. Nawrocki and B. Buszewski *J. Chromatogr.*, 449 (1988) 1.
- 7 T. Welsh, H. Frank and G. Vigh, *J. Chromatogr.*, 506 (1990) 97.
- 8 M. C. Gill, Y. T. Shih and D. W. Carr, *Talanta*, 36 (1989) 293.
- 9 M. D. Palmieri and J. S. Fritz, *Anal. Chem.*, 60 (1988) 2244.
- 10 N. Uehara, M. Kasahara and Y. Shijo, *Anal. Sci.*, 6 (1990) 469.
- 11 D. C. Freeman, Jr. and C. E. White, *J. Am. Chem. Soc.*, 78 (1956) 2678.
- 12 K. Watanabe and I. Aoki, *Bunseki Kagaku*, 35 (1986) 487.
- 13 J. Miura, *Anal. Chem.*, 62 (1990) 1424.
- 14 N. Suzuki, T. Takeda and K. Saitoh, *Chromatographia*, 22 (1986) 43.