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# Retention Behavior of Iron(III) Chelates with Amine-*N*-carboxylic Acids on Silica-gel and Octadecyl-silica Columns

Akihiro Yamaguchi, Atsuko Toda, Kunio Ohzeki,* and Tomihito Kambara
*Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060*

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The separation of iron(III)–edta, –methyl-edta, –dtpa, and –cdta was investigated by high performance liquid chromatography on silica-gel (ZORBAX SIL) and octadecyl-silica (ZORBAX ODS) columns. The chelates were retained on both columns from phosphate buffer mobile phase of pH 3, while the baseline separation was not achieved on the ODS column. The degree of increase in retention caused by the addition of tetrabutylammonium bromide to the eluent was greater on the ODS column than on the silica-gel column, and well separated chelates were eluted in the order, iron(III)–edta, –methyl-edta, –dtpa, and –cdta on both columns.

The application of reversed-phase high performance liquid chromatography (RPHPLC) to analysis of metal chelates has become of interest in recent years.[1,2] The mobile phase containing hydrophobic counter ions has been used to enhance retention and resolution of charged metal complexes on alkyl-bonded silica gel[1] and silica-gel columns.[3]

We have recently shown[4] that iron(III) complexes with EDTA and the analogous compounds were retained from phosphate buffer mobile phase on a silica-gel column, and that the retention increased either with increasing buffer concentration or with increasing amount of sodium sulfate added to the eluent. The purpose of this paper is to show the effect of a tetraalkylammonium ion on the retention behavior of the chelates on the silica-gel column, and to compare the results with those observed on the octadecyl-silica column.

## Experimental

*Apparatus.* A Shimadzu Model LC-3A chromatograph equipped with a Model SIL-1A syringe injector and a Model SPD-2A variable wavelength UV-detector was used in combination with a Chromatopac C-R1A recorder. The prepacked columns of ZORBAX SIL and ZORBAX ODS, (DuPont Co.) were used. Both columns were 25 cm × 4.6 mm i.d. A Horiba Model F-7C pH meter was used.

*Reagents.* The chelating agents were obtained from Dojin Laboratory. The compounds and their abbreviations are disodium salt of ethylenediamine-*N,N,N′,N′*-tetraacetic acid (EDTA), 1,2-propanediamine-*N,N,N′,N′*-tetraacetic acid (Methyl-EDTA), diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid (DTPA), and 1,2-cyclohexanediamine-*N,N,N′,N′*-tetraacetic acid (CDTA). Inorganic salts used were of analytical reagent grade. Iron(III) standard solution was prepared from ammonium iron(III) sulfate dodecahydrate. Tetrabutylammonium bromide (TBAB) and chromatographic grade acetonitrile were obtained from Wako Pure Chemicals Co. Deionized and redistilled water was used.

*Procedure.* Sample solutions were prepared by mixing standard iron(III) and chelating agent solutions in the stoichiometric ratio. The final concentration of each chelate was 20 μmol dm$^{-3}$. The 10-μl portions were injected. All experiments were carried out at room temperature. The capacity factors were calculated in the usual way.[5] The retention time of a nonretained component was evaluated from the first detectable peak on the chromatogram.

## Results and Discussion

*Effect of TBAB on Silica-gel Column.* The retention of iron(III)–edta, –methyl-edta, –dtpa, and –cdta complexes on the silica-gel column was remarkably enhanced by the addition of TBAB to the phosphate buffer mobile phase. In the presence of 0.1 mmol dm$^{-3}$ TBAB in 2.3 mmol dm$^{-3}$ phosphate buffer eluent of pH 3, the retention time increased from 3.4 to 12.7 min for iron(III)–edta, and 12.6 to 107 min for iron(III)–cdta complexes, respectively, at a flow rate of 1 ml min$^{-1}$. The capacity factor of each chelate increased with increasing concentration of TBAB in the mobile phase, as generally occurs in reversed-phase chromatography. Among mechanisms which were reviewed[6] to describe the effect of lipophilic alkyl ions in RPHPLC the ion-exchange model[7] seems to be most familiar.

*Effect of Electrolyte.* The effect of electrolyte added to the phosphate buffer mobile phase was studied in the

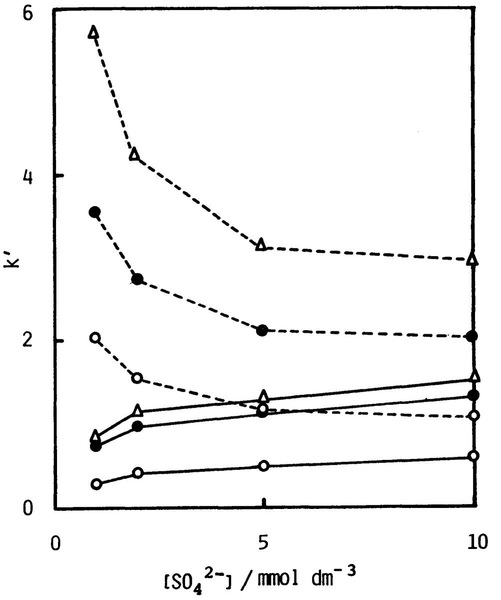

Fig. 1. Effect of sodium sulfate concentration in 2.3 mmol dm$^{-3}$ phosphate buffer eluent (pH 3) on the capacity factor in the absence (solid line) and in the presence (dashed line) of 0.1 mmol dm$^{-3}$ TBAB. Column: ZORBAX SIL, 25 cm × 4.6 mm i.d.; sample: (○) Fe$^{III}$–edta, (●) Fe$^{III}$–methyl-edta, (△) Fe$^{III}$–dtpa.

TABLE 1. THE CAPACITY FACTORS OF IRON(III) CHELATES ON ZORBAX SIL AND ODS

| | ZORBAX SIL | | | ZORBAX ODS | | |
|---|---|---|---|---|---|---|
| | $k'_1$ | $k'_2$ | $k'_2/k'_1$ | $k'_1$ | $k'_2$ | $k'_2/k'_1$ |
| $Fe^{III}$-edta | 0.20 | 0.46 | 2.3 | 0.13 | 1.78 | 13.7 |
| $Fe^{III}$-methyl-edta | 0.35 | 0.68 | 1.9 | 0.29 | 2.57 | 8.9 |
| $Fe^{III}$-dtpa | 0.44 | 0.88 | 2.0 | 0.29 | 3.28 | 11.3 |
| $Fe^{III}$-cdta | 1.11 | 1.72 | 1.5 | 2.26 | 12.31 | 5.4 |

Eluent: 0.012 mol dm$^{-3}$ phosphate buffer solution, 5% (V/V) acetonitrile, pH 3. $k'_1$: Capacity factor in the absence of TBAB. $k'_2$: Capacity factor in the presence of 0.1 mmol dm$^{-3}$ TBAB.

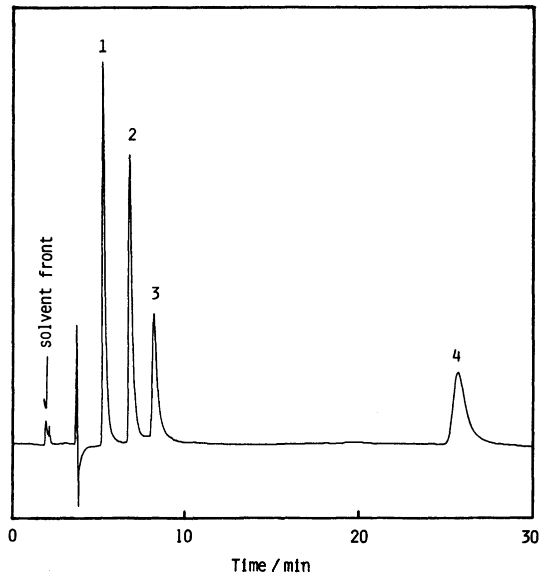

Fig. 2. Separation of iron(III) chelates on ZORBAX ODS in the presence of TBAB. Eluent: 12.0 mmol dm$^{-3}$ phosphate buffer solution containing 0.1 mmol dm$^{-3}$ TBAB and 5% (V/V) acetonitrile, pH 3; flow rate: 1 ml min$^{-1}$; detection at 260 nm, 0.01 a.u.f.s.; sample: 10 μl containing each chelate in 20 μmol dm$^{-3}$; peak 1, $Fe^{III}$-edta; peak 2, $Fe^{III}$-methyl-edta; peak 3, $Fe^{III}$-dtpa; peak 4, $Fe^{III}$-cdta.

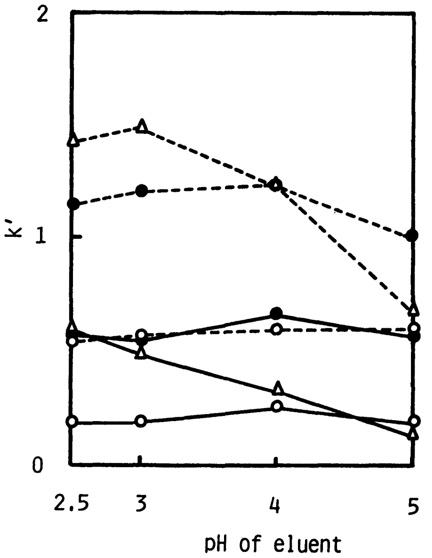

Fig. 3. Effect of pH on the capacity factor. Column: ZORBAX ODS (solid line), ZORBAX SIL (dashed line); eluent: phosphate buffer solution containing 10 mmol dm$^{-3}$ sodium sulfate; sample: (○) $Fe^{III}$-edta, (●) $Fe^{III}$-methyl-edta, (△) $Fe^{III}$-dtpa.

presence and absence of TBAB (Fig. 1). In the absence of TBAB, the retention of each complex increased gradually with increasing concentration of sodium sulfate in the mobile phase. The increase in retention is caused by the salting-out effect[8] of the electrolyte, the hydrophobic interaction[4] between the solute and stationary phase being facilitated. On the other hand, in the presence of TBAB, the retention decreased with increasing concentration of sodium sulfate, and reached almost constant value for each chelate in the concentration range of 5 to 10 mmol dm$^{-3}$ of sodium sulfate. It is clearly concluded that the retention observed in the presence of TBAB is governed by at least two factors, namely, ion-exchange and salting-out effects. With increasing concentration of electrolyte in the eluent, the retention due to ion-exchange decreases and the retention caused by salting-out effect becomes predominant.

*Separation of Chelates.* The capacity factors of the chelates on silica-gel and ODS columns obtained by using mobile phase of the same composition were summarized in Table 1. The acetonitrile was used to reduce the retention time. The degree of increase in retention

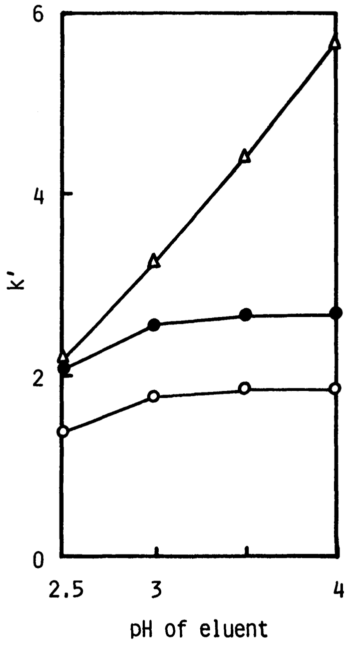

Fig. 4. Effect of pH on the capacity factor in the presence of TBAB. Column: ZORBAX ODS; eluent: phosphate buffer solution containing 0.1 mmol dm$^{-3}$ TBAB and 5% acetonitrile; sample: (○) $Fe^{III}$-edta, (●) $Fe^{III}$-methyl-edta, (△) $Fe^{III}$-dtpa.

caused by the addition of TBAB to the mobile phase was much greater on the ODS column than on the silica-gel column. The results suggested that the hydrophobic tetrabutylammonium ion is more feasibly adsorbed on the hydrocarbonaceous silica gel than on the original silica gel. The typical chromatogram was shown in Fig. 2. In the absence of TBAB, baseline separation of the iron(III) chelates were not achieved on the ODS column, even though the retention and resolution of the chelates were enhanced with increasing buffer concentration. The iron(III)–dtpa complex eluted between EDTA and Methyl–EDTA complexes on the ODS column, while the compound was eluted after the Methyl–EDTA complex on the silica-gel column. In the presence of TBAB, the chelates were well separated and eluted in the following order, iron(III)–edta, –methyl-edta, –dtpa, and –cdta on both columns. The elution sequence depends on the hydrophobicity of the chelates[4] and the pH of mobile phase as described below.

*The Effect of Eluent pH.* The effect of eluent pH was examined on the silica-gel and alkyl-modified silica-gel columns. On both columns, the retention of iron(III)–dtpa decreased with increasing pH of the mobile phase (Fig. 3). The DTPA complex has two carboxyl groups which do not participate in the complexation.[9] The dissociation of one or two uncoordinated carboxyl groups will reduce the hydrophobicity of the complex. The retention of DTPA complex begins to decrease at lower pH on the ODS column than on the silica-gel column. The silanol groups on the silica-gel surface will suppress the dissociation of carboxyl groups. The effect of pH on the ODS column was also studied in the presence of TBAB (Fig. 4). The retention of iron(III)–dtpa increased with increasing pH, which suggested also the dissociation of uncoordinated carboxyl groups.

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