SHORT COMMUNICATION

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF IRON(III) CHELATES WITH AMINOCARBOXYLIC ACIDS ON SILICAGEL COLUMN

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The iron(III)-complexes of EDTA, Methyl-EDTA, DTPA, and CyDTA were separated on a silicagel column (ZORBAX SIL) using phosphate buffer solution (pH 3) as the mobile phase. The retention of the chelates was enhanced with increasing concentration of the buffer solution and with increasing amount of sodium sulfate added to the eluent. However, the retention was reduced in the presence of acetonitrile. The chelates were eluted in the order: iron(III)-EDTA, -Methyl-EDTA, -DTPA, -CyDTA.

High performance liquid chromatography (HPLC) has been appreciated as the useful method for the separation and determination of metal chelates. The reversed phase systems using chemically bonded silicagel as the stationary phase have been widely used. The separation of EDTA-chelates of bismuth(III), iron(III), and copper(II) was successfully carried out on an octadecyl silica (ODS) column by means of so-called ion-association chromatography. On the other hand, so far, the use of silicagel column without hydrocarbonaceous bonded phase has been limited to the separation of neutral chelates in normal phase chromatography.

EXPERIMENTAL

Apparatus: The high performance liquid chromatograph used was a Shimadzu product, Model LC-3A, equipped with a Model SIL 1A syringe injector, a Model SPD-2A variable wavelength UV detector, and a Chromatopac C-R1A recorder. A silicagel column (ZORBAX SIL, Dupont, 4.6 mm i.d. and 25 cm long) was used throughout the study. A Horiba Model F-7LC pH meter was used.

Reagents: The chelating agents were obtained from Dojindo Co. The symbols used in this study for the reagents are as follows: EDTA, (ethylenedinitrilo)-tetraacetic acid; Methyl-EDTA, (1,2-propylenedinitrilo)tetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; CyDTA, (1,2-cyclohexylenedinitrilo)tetraacetic acid. Acetonitrile of HPLC grade was obtained from Wako Pure Chemicals Co. Ammonium iron(III) sulfate dodecahydrate, ammonium dihydrogenphosphate, and all other chemicals used were of analytical reagent grade. Sample solutions were prepared by mixing standard iron(III) and chelating agent solutions in the stoichiometric ratio. The concentration of each chelate was 20 µmol dm⁻³ and the pH of sample solution was around 3.1.

All experiments were carried out at room temperature.
RESULTS AND DISCUSSION

At first, pure water was used as a mobile phase. Every chelates were eluted without retention. By changing the eluent to an aqueous phosphate buffer solution (pH 3), however, well resolved peaks were obtained. The capacity factor of each complex increased with increasing concentration of the buffer solution (Fig. 1). The complexes were eluted in the order iron(III)-EDTA, -Methyl-EDTA, -DTPA, -CyDTA. With the use of $1.2 \times 10^{-2}$ mol dm$^{-3}$ phosphate buffer eluent, the retention time of each chelate was 4.9, 7.3, 7.9, and 30.1 min, respectively, at a flow rate of 1 ml min$^{-1}$. The increase in retention was also observed with the addition of sodium sulfate to the eluent. In the presence of $10^{-2}$ mol dm$^{-3}$ sodium sulfate in the phosphate buffer eluent of $2 \times 10^{-3}$ mol dm$^{-3}$, the retention time increased from 3.4 to 5.3 min for iron(III)-EDTA and 12.6 to 26.8 min for the CyDTA complex. It was clearly concluded that these chelates are strongly retained on the silicagel column with increasing concentration of electrolyte in the eluent. The chelating agents have the hydrophobic moiety, namely, ethylene, propylene, two ethylene, and cyclohexylene groups, respectively, while the complex forming properties are essentially invariant. The retention order of the chelates depends primarily on the interaction of these groups with silicagel surface. The effect of pH of the phosphate buffer eluent was examined in the presence of $10^{-2}$ mol dm$^{-3}$ sodium sulfate to keep a constant ionic strength. No significant change of capacity factor, except DTPA complex, was observed in the pH range 2.5 - 5.0 studied (Fig. 2). Above pH 3, the retention of DTPA complex decreased with increasing pH. At pH 5, the peak appeared between those of EDTA and Methyl-EDTA complexes. The ligand DTPA has two carboxyl groups which do not take part in the complexation with iron(III). The dissociation of these carboxyl groups will reduce the hydrophobicity of the complex. The addition of acetonitrile to the eluent resulted in

Fig. 1. Effect of phosphate buffer concentration (pH 3).

Fig. 2. Effect of pH. The figures have the same meanings as in Fig. 1.
the decrease of capacity factor of every chelates (Fig. 3). Outstanding effect was observed on the retention of iron(III)-CyDTA complex which has the highest hydrophobicity among the complexes studied. In the presence of 1.5% acetonitrile in the phosphate buffer eluent of 1.2 x 10^{-2} mol dm^{-3}, the retention time of CyDTA chelate decreased from 30.1 to 13.1 min.

The typical chromatogram obtained under the best conditions examined was shown in Fig. 4. The validity of silicagel column for the separation of iron(III)-EDTA and analogous complexes was demonstrated.

Fig. 3. Effect of acetonitrile concentration. Eluent: 1.2 x 10^{-2} mol dm^{-3} phosphate buffer solution (pH 3). The figures have the same meanings as in Fig. 1.

Fig. 4. Separation of iron(III)-complexes on a ZORBAX SIL column. The concentration of each chelate was 20 µmol dm^{-3}. Mobile phase: 1.2 x 10^{-2} mol dm^{-3} H_3PO_4/H_2PO_4^-, 1.5% CH_3CN, pH 3. Flow rate: 1 ml min^{-1}. Detection: 0.01 a.u.f.s., 260 nm. Injection size: 10 µl.

REFERENCES

Keyword phrases

HPLC separation on silicagel column; iron(III)-EDTA, -Methyl-EDTA, -DTPA, -CyDTA.

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