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Peak Splitting for Co(III) and Al(III) Complexes in High Performance Liquid Chromatography by Linkage Isomerism of Unsymmetric o,o'-Dihydroxyazobenzene Derivatives

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Peak splitting, which was found in reversed phase chromatography of Co(III) and Al(III) complexes with unsymmetric dihydroxyazobenzene derivatives, is ascribed to linkage isomerism by ^{1}H NMR spectroscopy. Both isomerization and dissociation reactions take place in the column in contrast to normal phase chromatography.

High performance liquid chromatography(HPLC) of metal chelates has widely been used as a tool for simultaneous determination of metal ions.1) Properties of the reagents suitable to HPLC coupled with spectrophotometry, which is the most convenient and versatile detecting system, have been discussed from various points of view such as thermodynamic stability, dissociation kinetics and spectroscopic characteristics of the complexes. An unsymmetric bidentate ligand possibly forms geometrical isomers, fac and mer, with a metal ion of octahedral configuration.2) To avoid unfavorable peak splitting by the isomerism, symmetric bidentate ligands have generally been used as a pre-column derivatization reagent.1) In the case of chromogenic tridentate ligands, formation of geometrical isomers need not be considered, since they adopt planar conformation to have only a mer configuration.

Recently, Hoshino and others have constructed a reversed phase HPLC-spectrophotometric determination system for Al, Co, V, and Fe, using tridentate ligands of o,o'-dihydroxyazobenzene(DHAB) derivatives.3) We have found unfavorable peak splitting for Co(III) and Al(III) complexes with unsymmetric DHAB derivatives under certain conditions. Similar splitting had also been reported by Pfitzner and Schweppe in thin layer chromatography of the same complexes using silica gel as stationary phase.4) They suggested the possibility of linkage isomerism at an azo group; unsymmetric derivatives have two coordination modes depending on which one of two nonequivalent N atoms is used in complexation together with two phenolate O atoms. No experimental evidence, however, has been given. In this paper, we have studied chromatographic behavior of Co(III), Al(III) and Fe(III) complexes with 2,2'-dihydroxyazobenzene(**1**) and 2,4,2'-trihydroxyazobenzene(**2**) as a function of solvent composition and counter ion concentration. Two of three linkage isomers of Co(III) complexes were fractionated by normal phase chromatography and structurally characterized by ^{1}H NMR spectroscopy.

Solutions of Co(II), Al(III) and Fe(III) salts were reacted with the azo compounds at pH 6. Molar ratio method has indicated that 1:2 complexes are exclusively formed in the presence of excess reagents. Inertness against a substitution reaction with ethylenediamine-N,N,N',N'-tetraacetate(Na_2H_2edta) suggests the formation of Co(III) complexes. Sample solutions(10^{-2} cm^3; C_M=4x10^{-5}; C_L=2x10^{-4} mol dm^{-3}) were injected on the column (TSK-GEL ODS-120T) thermostated at 25 °C and the eluent(water/acetonitrile: 40/60 to 20/80 for **1** ; 55/45 to 30/70 for **2**) containing 1.0x10^{-4} mol dm^{-3} Na_2H_2edta, 5x10^{-3} mol dm^{-3} acetate buffer(pH 6), and varying concentration(up to 5x10^{-2} mol dm^{-3}) of tetrabutylammonium bromide(TBABr) was pumped at a flow-rate of 0.8 cm^3 min^{-1}. The elution was monitored with a multichannel photodiode-array detector(JASCO MULTI330).

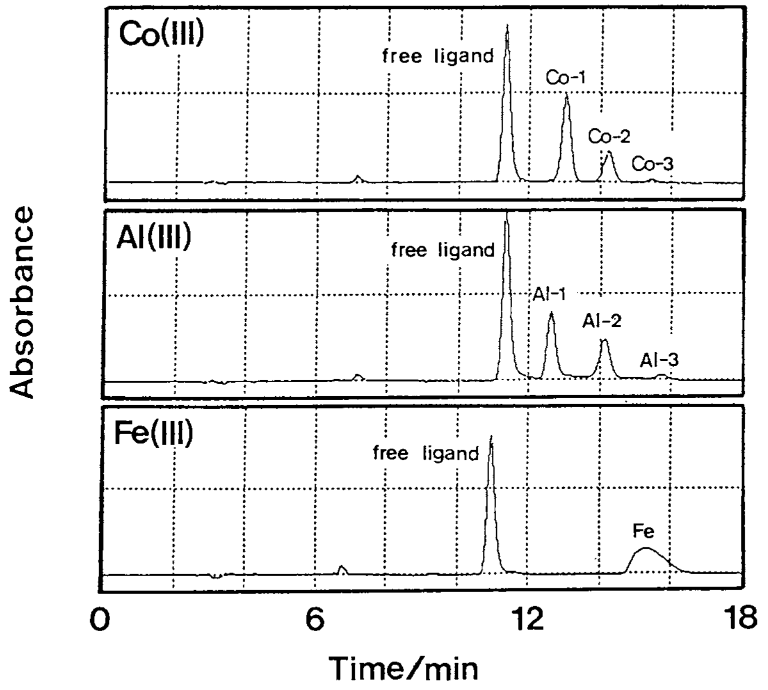

Fig. 1. Chromatograms of Co(III), Al(III) and Fe(III) complexes with **2**. Column: TSK-GEL ODS-120T; Mobile phase: water/acetonitrile(45/55), 2x10^{-2} mol dm^{-3} TBABr. Other conditions are described in the text. Ordinate is maximum absorbance between 350-600 nm.

Decrease in acetonitrile content or increase in TBABr concentration enhances the retention of both the reagents and their metal complexes. Only one peak was observed for any metal with **1** or for Fe with **2**, whereas three peaks for Co and Al with **2** along with increase in retention(Fig. 1). The ratios of peak area for each fraction named Co-1 to -3 and Al-1 to -3 are 20:10:1 and 10:7:1, respectively. When each fraction of Co(III) complexes was injected again in the chromatographic system, a single peak was observed at the same elution time. For Al(III), it was the case just after separation, but other peaks gradually grew on standing and a chromatogram with three peaks of the original ratio and one peak of a free ligand was obtained after 6 hours. All these findings can be explained by linkage isomerism. Isomerization is faster than time scale of chromatography for Fe(III) but not for Co(III) or Al(III). Pfitzner and Schweppe found two peaks even for 1:1 complexes of labile ions such as Cu(II) or 1:1 complexes of other divalent metals of lower stability such as Ni(II).[4)] In normal phase chromatography, isomerization as well as dissociation are suppressed in the column. In reversed phase chromatography, only one peak was found for Cu(II), whereas none for Ni(II). Both isomerization and dissociation reactions can take place in this chromatographic system as in an aqueous solution.

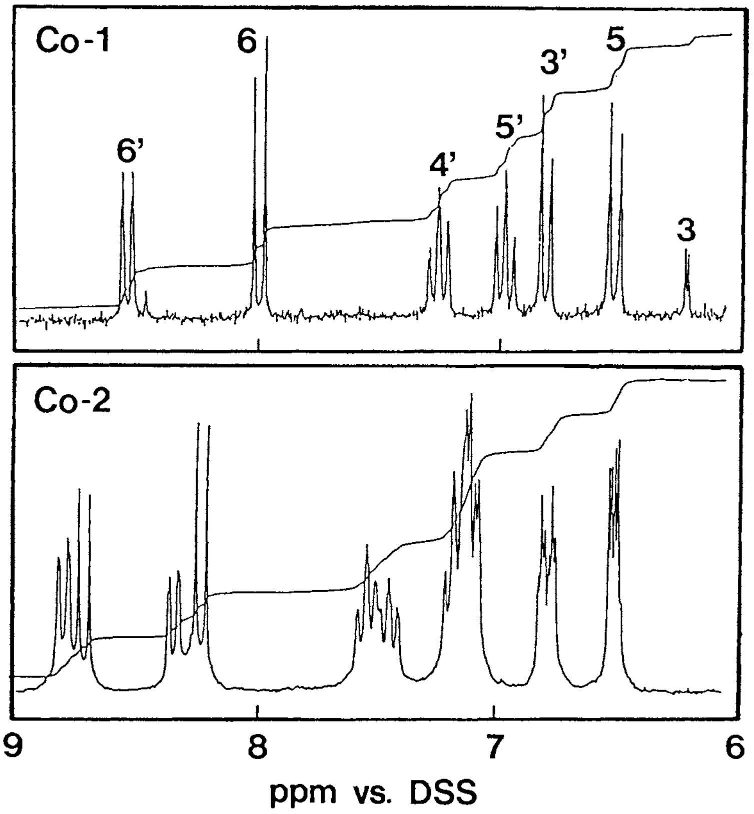

Fig. 2. 200 MHz ^{1}H NMR spectra of fraction Co-1 in D_2O and Co-2 in $(CD_3)_2SO$.

Two major Co(III) isomers of three were fractionated by normal phase chromatography using silica gel(Wakogel C-100; 30 mm i.d. x 350 mm) as stationary phase and $CH_3COOC_2H_5/CH_3OH/CH_3COOH$(97/2/1) as mobile phase. After evaporation, a fraction Co-1 was dissolved in D_2O, whereas Co-2 in $(CD_3)_2SO$, and ^1H NMR spectra were recorded at 200 MHz using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an external standard. Each signal of Co-1 was assigned as given in Fig. 2, by the conventional method. Deuteration diminishes the peak for the 3- proton as reported for 4-(2-pyridylazo)resorcinol(PAR) complexes.5) The spectrum for Co-2 has twice as many peaks as that for Co-1. This clearly indicates that two ligands of the complex in the fraction Co-2 have different coordination modes. To identify the structures of the complexes in the fractions Co-1 and Co-3, which respectively have two ligands of the same coordination mode, an X-ray crystallographic study is now in progress.

In conclusion, unsymmetric DHAB derivatives are generally not suited for pre-column derivatization reagent because of unfavorable peak splitting. If these reagents are dared to be used, chromatographic conditions must carefully be settled.

References

1) B. R. Willeford and H. Veening, J. Chromatogr., 251, 61 (1982); J. W. O'Laughlin, J. Liquid. Chromatorg., 7, 127 (1984); G. Nickless, J. Chromatogr., 313, 129 (1985); A. R. Timerbaev, O. M. Petrukhin, and Yu. A. Zolotov., Fresenius Z. Anal. Chem., 327, 87 (1987); B. Steinbrech, J. Liquid. Chromatogr., 10, 1 (1987).

2) P. C. Uden, I. E. Bigley, and F. H. Walters, Anal. Chim. Acta, 100, 555 (1978); T. J. Janjic, Z. Lj. Tesic, G. N. Vuckovic, and M. B. Celap, J. Chromatogr., 404, 307 (1987); D. E. Henderson, S. J. Saltzman, P. C. Uden, and Z. Cheng, Polyhedron, 7, 369 (1988).

3) H. Hoshino and T. Yotsuyanagi, Chem. Lett., 1984, 1445; H. Hoshino, K. Nakano, and T. Yotsuyanagi, Analyst(London), 115, 133 (1990).

4) H. Pfitzner, Angew. Chem., Int. Ed. Engl., 11, 312 (1972); H. Pfitzner and H. Schweppe, Z. Anal. Chem., 268, 337 (1974).

5) K. Mochizuki, T. Ito, and M. Fujimoto, Bull. Chem. Soc. Jpn., 52, 441 (1979).

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