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Determination of Ethylenediaminetetraacetic Acid and Nitrilotriacetic Acid as Their Iron(III)-Complexes by Reversed Phase High Performance Liquid Chromatography

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The iron(III) complexes with ethylenediaminetetraacetic acid (EDTA, H_4edta) and nitrilotriacetic acid (NTA, H_3nta) were separated by the ion-association reversed phase high performance liquid chromatography. The retention behavior of iron(III)–nta complex on the column (4.6 mm i.d., 25 cm long) packed with ZORBAX-ODS was seriously affected by the pH of eluent composed of phosphate buffer solution containing tetrabutylammonium bromide and acetonitrile. A mixture of edta and nta in a synthetic sample solution containing the commonly present metal ions was converted to the corresponding iron(III) complexes and determined successfully by the present method.

The importance of analysis of naturally occurring and pollutant chelating agents has been claimed[1] and a method was proposed in which the copper(II) complexes of amine-*N*-carboxylic acids, such as EDTA and NTA, were separated by liquid chromatography and detected by atomic absorption spectrometry. The detection sensitivity was enough, but the separation of the chelates on a weak anion exchange resin column was insufficient. Recently, reversed phase high performance liquid chromatography (RPLC) was successfully applied to the separation of edta complexes of bismuth(III), iron(III), and copper(II).[2]

This paper describes a simple RPLC method for determining iron(III)–edta and –nta complexes. The conversion of free and complexed edta and nta to iron(III) complexes is made quantitatively by heating a sample solution in the presence of excess iron(III).[3] The excess iron(III) is removed by a cation exchange resin column and the effluent is directly subjected to the analysis by RPLC.

Experimental

Apparatus. The liquid chromatograph employed was a Shimadzu product, type LC-3A, equipped with a UV-detector, model SPD-2A (195—350 nm), and a data processor Chromatopac C-R1A. A prepacked ODS column (ZORBAX, Du Pont) was consisted of a stainless steel tube, 4.6 mm i.d. and 25 cm long. A Shimadzu spectrophotometer, UV-240, was used for measurements of absorption spectra.

Reagents. All chemicals used were of analytical reagent grade. Dotite reagents, Na_2H_2edta·$2H_2O$ and H_3nta were used. A 10^{-2} M (1 M = 1 mol dm^{-3}) edta stock solution was prepared by dissolving dried reagent (80 °C, 2 h) in deionized water. A 10^{-2} M nta stock solution was prepared by dissolving dried acid (110 °C, 2 h) in sodium hydroxide solution, the pH of the final solution being around 8. Ammonium iron(III) sulfate dodecahydrate was dissolved into acidified water to give 10^{-2} M solution (pH = 1). Tetrabutylammonium bromide (TBAB) was obtained from Wako Pure Chemicals.

Ion Exchange Resin. The sodium-form Dowex 50W-X8 (100—200 mesh) resin was packed in a column, 1 cm i.d., 23 cm long, in a 1-cm bed.

Preparation of Mobile Phase. To a one-liter flask containing 500 ml of redistilled water, were added 1.15 g of ammonium dihydrogenphosphate, 17.5 ml of 10^{-2} M TBAB solution, 50 ml of acetonitrile, and 6 ml of 1.67 M phosphoric acid. The mixture was filled up to the mark with redistilled water. The pH of the final solution was 2.5. The solution was then filtered through a membrane filter of 0.45 μm pore size.

Procedure. The sample solution containing edta and nta was taken into a 50-ml volumetric flask and 2.5 ml of 0.05 M sulfuric acid, 1 ml of 0.01 M iron(III) solution, and 3 ml of sodium formate were added successively. When the sample contained metal ions, it was heated in the presence of excess iron(III) before the addition of formate. The mixture was made up to the volume with water, the pH of the final solution being around 3. A part of the solution was passed through the sodium-form resin column at the flow rate of 2—3 ml min^{-1}. The first 10-ml portion of effluent was discarded and the next 5-ml portion was collected. The test solution thus obtained was filtered with the use of MILLEX-SR (Millipore Co.) unit with 0.5 μm membrane filter. A 10-μl portion of the filtrate was injected with a 25-μl syringe to the column. Ion-assosiation chromatography with organic modifier was applied. Tetrabutylammonium (TBA) ion was used as the counter ion and acetonitrile as the organic modifier in the phosphate buffer solution. Chromatographic conditions were summerised in Table 1. The wavelength was fixed to the absorption maximum of iron(III)–edta complex.

Table 1. Chromatographic conditions

Column: ZORBAX-ODS (4.6 × 250 mm)
Mobile phase: 0.02 M phosphate buffer solution (pH 2.5)
0.175 mM TBAB
5% acetonitrile
Flow rate: 1 ml min^{-1}
Pressure: 95 kg cm^{-2}
UV-detection: 255 nm
Injection size: 10 μl

Results and Discussion

Effect of pH. The pH of mobile phase was adjusted with the use of phosphate buffer solution. The retention time and peak area of iron(III)–nta complex were drastically influenced by the pH values,

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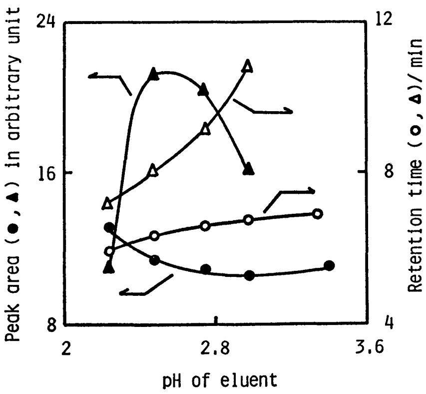

Fig. 1. Effects of pH on retention time (○, △) and peak area (●, ▲).
(○, ●): 20.1 μM iron(III)–edta, (△, ▲): 81.3 μM iron(III)–nta. Conditions except pH of eluent are the same as in Table 1.

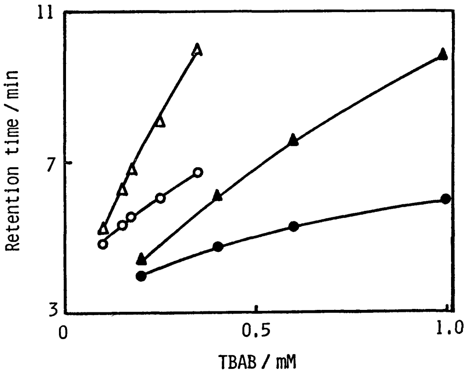

Fig. 2. Effect of concentrations of TBAB and acetonitrile.
Retention time of 20.1 μM iron(III)–edta: (○) 5% acetonitrile, (●) 10% acetonitrile; retention time of 81.3 μM iron(III)–nta: (△) 5% acetonitrile, (▲) 10% acetonitrile. The conditions are the same as in Table 1.

compared with those of iron(III)–edta complex (Fig. 1). The peak width of iron(III)–nta complex broadened with increasing pH, and pH>3.5, the peak was no longer detectable.

Effect of Tetrabutylammonium Ion and Acetonitrile. The retention time of both compounds increased with increasing concentration of TBA ion and decreased with increasing concentration of acetonitrile (Fig. 2). The peak shape of iron(III)–nta complex was also extremely affected by the concentrations of TBA ion and acetonitrile. The distorted peak was observed when the phosphate buffer mobile phase contained 0.35 mM TBA ion and 5% acetonitrile, or 1.0 mM TBA ion and 10% acetonitrile. The combained use of 0.175 mM TBA ion and 5% acetonitrile were pertinent. A typical chromatogram was shown in Fig. 3.

Calibration Curve. The calibration curves of edta and nta were linear over the concentration range 2—20 μM and 6—50 μM, respectively, while the curve for nta did not pass through the origin. The relative standard deviations ($n=5$) were in the range of 2.0 to 1.7% for edta and 7.3 to 3.1% for nta, respectively.

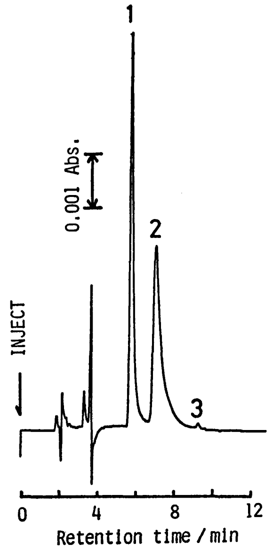

Fig. 3. Typical chromatogram.
(1) 20.1 μM iron(III)–edta, (2) 50.8 μM iron(III)–nta, (3) unknown peak, column: ZORBAX-ODS, Eluent: 0.02 M phosphate buffer solution containing 0.175 mM TBAB and 5% acetonitrile (pH 2.5). Flow rate: 1 ml min^{-1}, temperature: ambient, injection size: 10 μl, UV-detection: 255 nm

TABLE 2. EFFECT OF METAL(II) IONS

[edta]/μM			[nta]/μM		
Taken	Found	Error(%)	Taken	Found	Error(%)
10.1	9.9	−2.0	25.4	25.5	+0.4
12.1	12.1	0	30.5	30.2	−1.0
14.1	14.1	0	35.6	35.0	−1.7

Mg, Ca each in 1 mM, Co, Cd, Zn, Ni, and Cu each in 0.1 mM.

Effect of Diverse Ions. The quantitative conversion of free edta and nta or the complexes with metal ions commonly present was carried out by heating sample solution in the presence of excess iron(III).[3] The excess iron(III) and metal ions were removed by the cation exchange resin column, and hence the present method was essentially free from interference of metal ions (Table 2).

Among the anions studied, a high concentration of nitrate ion gave the peak which had almost the same retention time as iron(III)–nta and caused the positive error (Table 3). In the presence of considerable amounts of phosphate, white precipitate formed by the addition of excess iron(III). However, the precipitate being removed by the sodium form resin column, no interference was observed. The anionic surfactant, dodecylbenzenesulfonate is expected to block TBA cation in the mobile phase, but no effect was found.

Absorption Spectra of Iron(III)–edta and –nta. The spectrum of iron(III)–nta complex in the formate buffer solution was different from that in the phosphate buffer solution, and both spectra were different from that in the dilute sulfuric acid solution (Fig. 4). The mixed ligand complexes of iron(III)–nta with formate or phosphate were postulated. The spectra of iron-

Table 3. Effect of foreign anions

Anions		12.1 μM edta		30.5 μM nta	
added as	Concn(mM)	Found(μM)	Error(%)	Found(μM)	Error(%)
$NaNO_3$	0.1	12.3	+1.7	30.3	−0.7
	0.5	12.0	−0.8	30.1	−1.3
	1.0	12.4	+2.5	31.6	+3.6
	2.0	12.2	+0.8	37.0	+21.3
NaCl	2.0	11.9	−1.7	31.0	+1.6
$NH_4H_2PO_4$	2.0	11.9	−1.7	29.7	−2.6
DBS[a]	0.4	12.0	−0.8	30.9	+1.3

a) Sodium dodecylbenzenesulfonate.

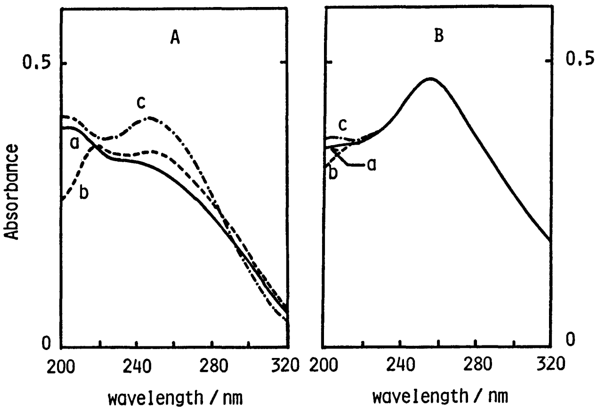

Fig. 4. Absorption spectra of iron(III)–nta and iron(III)–edta.
Spectra of (A) 50 μM iron(III)–nta and (B) 50 μM iron(III)–edta: (a) in dilute sulfuric acid, pH 3.0; (b) in 0.05 M HCOOH–0.01 M HCOONa, pH 2.9; (c) in 0.02 M phosphate buffer solution, pH 2.5.

(III)–edta complex in the varying media coincided each other (Fig. 4). The TBA ion and acetonitrile gave no effect on the absorption spectra. The absorption profiles of iron(III)–edta and –nta in the mobile phase were obtained by plotting each peak area against wavelength, the peak areas having been determined at different wavelength. The profiles were in close agreement with the absorption spectra in the phosphate buffer solution.

Separation of Iron(III)–edta and –nta by Means of an Open Column. Both complexes were found to be retained by the anion exchange resin and eluted with formate buffer solution. The chromatogram obtained on a 1-cm open column packed with sulfate-form Dowex 1-X4 (100—200 mesh) was shown in Fig. 5. The eluate was fractionated and analysed by the present method. Evidently both chelates are negatively charged.

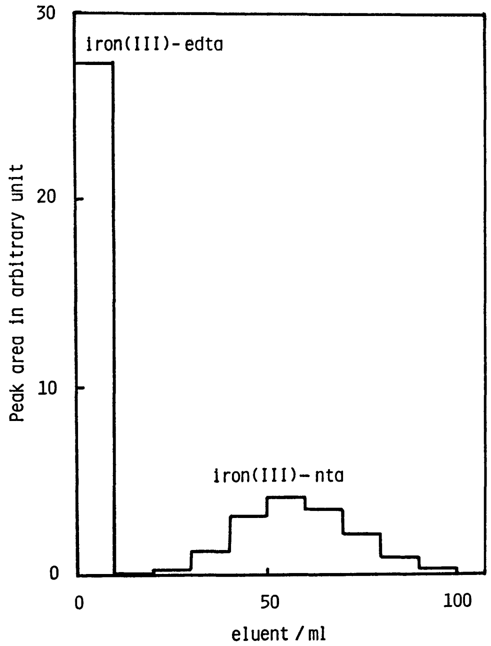

Fig. 5. Separation of iron(III)–nta and –edta by use of a sulfate-form anion exchange resin column.
The mixture of 0.5 μmole iron(III)–edta and 1.0 μmole iron(III)–nta was retained on the column from aqueous solution and eluted by formate buffer solution (0.05 M HCOOH–0.01 M HCOONa).

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