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Note

Gel chromatography of the iron(III)–Tiron complex

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Gel chromatography is a technique for separating and characterizing solute compounds according to their molecular dimensions in the solution state, and is used particularly in biological and polymer chemistry. The unique abilities of gel chromatography, however, have not so far been actively applied in the chemistry of metal complexes. A few papers have dealt with some hydrophilic or hydrophobic metal complexes, such as the metal–EDTA complexes[1,2] or the metal–β-diketonato complexes[3–7]. Most of the metal complexes used as the test samples involved simple proportions of metal to ligand, such as 1:1, 1:2 or 1:3. In an aqueous solution containing a metal and a ligand, however, various species of complex are generally possible by varying the conditions, such as pH and the metal and ligand concentrations in the solution. Such complex systems, have so far not been widely studied in gel chromatography.

In this work, the gel chromatographic behaviour of the iron(III)–Tiron complex used as a model metal–ligand system was examined at various pH values of the aqueous media. Tiron is colourless in aqueous solution, whereas it forms strongly coloured complexes with some metal ions and is therefore useful in spectrophotometric metal analysis. A test for iron(III) using Tiron was reported by Yoe and Jones[8] to be so sensitive that a positive result is obtained in the presence of fluoride, phosphate, tartrate, citrate, oxalate and other masking agents. It is well known that the colour of the iron(III)–Tiron system is dependent on the pH of the solution, being blue at pH 1–4, purple at pH 5–7 and red at pH 8. A photometric study of this system by Harvey and Manning[9] revealed a blue 1:1, a purple 1:2 and a red 1:3 complex species.

EXPERIMENTAL

Reagents

All of the chemicals were of analytical-reagent grade (Wako, Osaka, Japan, or Kanto, Tokyo, Japan). Tiron (disodium salt) (Dojin Labs., Kumamoto, Japan) was dissolved in redistilled water to give a 0.1 *M* solution. The stock solution of iron(III) was prepared at a concentration of 0.2 *M* in 0.1 *M* nitric acid.

Column

Sephadex G-15 gel (Pharmacia, Uppsala, Sweden) was swollen overnight in

the solution to be used as the eluent, then placed in a Pyrex column of I.D. 9 mm. A 6.97-g amount (in the dry state) of the gel was placed into the column. The height of the gel bed, after being conditioned with the eluent to be used, was 39.5 cm. The solvent regain, S_r, of the Sephadex G-15 gel, measured by means of the equilibration method[10] using Blue Dextran 2000 (Pharmacia) as a reference material, was almost constant at 1.5 ml per gram of dry gel in the pH range of the eluents of 4–9. On this account, once having been prepared the column was used throughout the experiments without repacking, although eluents with various pH values were used. The column void volume was determined to be 7.87 ml from the measurement of the elution volume for Blue Dextran 2000, and the volume of the internal solvent of the gel was estimated to be 10.45 ml from the S_r value and the amount of gel packed in the column.

Eluents

The eluents were 0.05 *M* sodium nitrate solutions buffered at pH 4, 5 or 6 with sodium acetate and hydrochloric acid, at pH 7 or 8 with disodium hydrogen phosphate and potassium dihydrogen phosphate, or at pH 9 with disodium hydrogen phosphate and sodium hydroxide. The preliminary experiments using these eluents resulted in irreversible dissociation of the solute complex in the chromatographic process. In order to avoid this problem, eluents containing Tiron were required. Tiron was therefore added to each eluent to a final concentration of 0.001 *M*.

Procedure

Portions of the stock solution of iron(III) were diluted with the solution to be used as the eluent in order to obtain test sample solutions containing iron(III) in the range $0.1 \cdot 10^{-3}$–$5.0 \cdot 10^{-3}$ *M*. This concentration range was selected so as to be able to prepare the sample solutions with both sub- and super-stoichiometric proportions for complexation to give 1:1, 1:2 and 1:3 species. A 0.2-ml portion of each sample solution was placed into the column and elution was carried out at an eluent flow-rate of 0.08–0.13 ml/min with the aid of a peristaltic pump. The column was thermostated at 25.0 ± 0.1°. A Hitachi (Tokyo, Japan) EPU-2 spectrophotometer with a flow cell made from a quartz capillary (effective light path *ca.* 1 mm) was used as a detector, and the absorbance at a wavelength of 520, 560 or 620 nm was recorded. An optical drop counter was used to measure the flow-rate of the eluent and the elution volume of the complex. The eluate corresponding to an elution band fraction was collected and its absorption spectrum recorded in the visible region.

RESULTS AND DISCUSSION

The iron(III)–Tiron complex gave an elution curve without excessive skewness in every elution system. The elution volume observed with an eluent was hardly dependent on the iron(III) content of the sample solution. The elution volume, however, depended strongly on the pH of the eluent. The elution curves obtained with the eluents of pH 8, 6, 5 and 4 are shown in Fig. 1. The elution volume *versus* pH relationship presented by Fig. 2 shows that the elution volume decreases with an increase in the pH of the eluent and approaches constancy at pH 8.

It was observed that the colour of the solute band migrating along the column

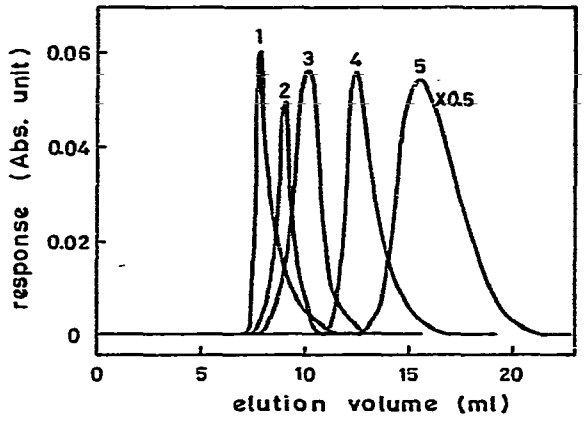

Fig. 1. Elution curves recorded for the iron(III)–Tiron complex. Column, Sephadex G-15, 39.5 cm × 9 mm I.D., 25.0 ± 0.1°; iron content of the sample solution, 0.001 *M*; eluent, 0.05 *M* sodium nitrate + 0.001 *M* Tiron, 0.08–0.13 ml/min. Peaks for Blue Dextran 2000: 1 = at pH 9, 620 nm; 2 = at pH 8, 520 nm; 3 = at pH 6, 560 nm; 4 = at pH 5, 620 nm; 5 = at pH 4, 620 nm.

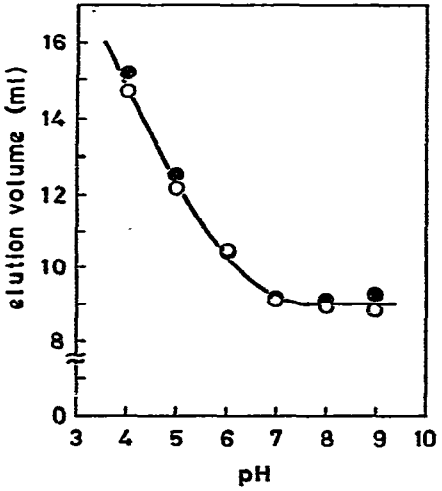

Fig. 2. Elution volume of the iron(III)–Tiron complex *versus* pH of the eluent. Iron content of the sample solution: ○, $5 \cdot 10^{-3}$ *M*; ●, $1 \cdot 10^{-4}$ *M*. Other conditions as in Fig. 1.

changed during the chromatographic process. For example, with the eluent of pH 4, the solute band at the top of the column was blue, and changed gradually to purple as it migrated down the column. The colour change began initially at the edge of the band and extended gradually to the whole of the band. Finally, at the column outlet, the band was reddish purple. On the other hand, with the eluent of pH 9, the band maintained a red colour. The absorption maximum wavelengths observed for the test sample solutions and for the solute band fractions of column effluent are given in Table I.

Referring to the photometric study of the iron(III)–Tiron system by Harvey

TABLE I

ABSORPTION MAXIMUM WAVELENGTHS OF SAMPLE SOLUTION AND ELUATE

The iron content of each sample solution was $2.5 \cdot 10^{-4}$ *M* and the Tiron content of the eluent was $1 \cdot 10^{-3}$ *M*.

pH	$\lambda_{max.,\ sample}$ (nm)	$\lambda_{max.,\ eluate}$ (nm)
4	680	585
5	610	560
6	560	550
7	545	495
8	525	483
9	490	480

and Manning[9], it is implied that the colour change in the chromatographic process results from partial transformation of the complex, *e.g.*, from a 1:1 to a 1:2 or from a 1:2 to a 1:3 species. Some sample solutions injected into the column contained excess of iron(III) relative to the Tiron content, *i.e.*, there was a sub-stoichiometric relationship between iron(III) and Tiron for the complexation. The solute injected into the column is diluted during the elution process owing to band spreading, so that the iron concentration in the band decreases gradually. As the eluent used contains Tiron, the concentration of Tiron relative to iron increases gradually with the migration of the band through the column, and finally approaches an excess. The predominant species in the migrating band is thus governed by the pH of the eluent. When the molecular dimensions of the species increase in the order 1:1 < 1:2 < 1:3 species, it would be expected on the basis of the molecular sieve effect that the elution volume of the complex would decrease with an increase in the pH of the eluent. The results in Fig. 2 support this suggestion.

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