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## SEPARATION OF CHLORO COMPLEXES OF METALS BY MICRO-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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### SUMMARY

The separation of chloro complexes of metals has been carried out using micro columns and a strong acidic eluent. The micro column was packed with silica gel, particle size 10  $\mu\text{m}$ , impregnated with methyltri-*n*-octylammonium chloride. Three or four chloro complexes of iron, nickel, copper, tin, antimony, mercury, lead and bismuth were separated by using 1–8 *N* hydrochloric acid with or without a small amount of perchloric acid as eluent. The analysis time is *ca.* 20 min, and the *H* value is *ca.* 0.2 mm. Quantitative determination between 1 ng and 1  $\mu\text{g}$  is possible.

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### INTRODUCTION

For the separation of chloro complexes of metals, ion-exchange chromatography has been used<sup>1,2</sup>. A strong anion-exchange resin, made with polystyrene gel, and hydrochloric acid have generally been used as column support and eluent in column liquid chromatography. Hydrochloric acid serves not only to separate the metals but also to form the chloro complexes of metals that might show UV adsorption to some extent<sup>3,4</sup>.

As hydrochloric acid is very reactive with stainless steel at pH values less than 2, the modern commercially available liquid chromatographs cannot be used directly for the separation of chloro complexes. Seymour and Fritz<sup>1</sup> were first to use forced-flow of hydrochloric acid in anion-exchange chromatography by using the pressure of a helium cylinder and also automatic UV detection of eluted elements. They used a resin with 150–200 mesh. Horwitz and Bloomquist<sup>5</sup> demonstrated high-speed radiochemical separation of metal ions by using a column packed with controlled porosity supports (CSP), particle diameter 14–34  $\mu\text{m}$ . Their system was as follows: a short column (3–5 cm  $\times$  2.8 mm I.D.) was packed with CSP coated with quaternary ammonium chloride; eluate fractions were dried under heat lamps and their activities were counted with an end-window proportional counter. Horwitz and co-workers<sup>6,7</sup> subsequently used di-(2-ethylhexyl)phosphoric acid in dodecane as the stationary phase. The system proposed was designed specially for radiochemical use, and chromatograms were obtained by plotting the activity of eluate fraction.

Micro-high-performance liquid chromatography (HPLC), as proposed by Ishii *et al.*<sup>8</sup>, has several advantages in the separation of chloro complexes of metals. First it needs only a very small amount of eluate for each run, a few hundred microlitres. Second, the dilution of the sample by eluate is much less than with ordinary liquid chromatography (column diameter *ca.* 2–4 mm). Third, the system is easy to modify.

So we attempted to apply micro-HPLC to the separation of chloro complexes of metals. We examined the UV absorption spectra of the complexes in the range 200–300 nm and used a UV or visible detector in the on-column mode, which gives a sensitivity higher than that previously reported<sup>1</sup>. Most commercial anion-exchange resins for HPLC are limited in use to pH values greater than 2. We tested some of these resins in strongly acidic conditions, but they did not give good results. Trialkylamines and their salts have often been used in the extraction of metal chloro complexes from strongly acidic solution<sup>9,10</sup>. They have also been impregnated on silica gel for thin-layer chromatography<sup>11</sup> and on supports for liquid chromatography<sup>5</sup>. So we prepared our own anion exchanger by using alkylamines for micro-HPLC.

## EXPERIMENTAL

### *Anion exchanger*

Capriquat®, methyltri-*n*-octylammonium chloride (Dojin, Kumamoto, Japan), was coated on silica gel LiChrosorb, 10  $\mu\text{m}$ , (E. Merck, Darmstadt, G.F.R.). The procedure for making this anion exchanger was as follows: LiChrosorb Si 60 was immersed in 6 *N* HCl at *ca.* 80°C for 2 h, washed with distilled water until neutral and then dried. A 0.5-ml volume of 1% Capriquat–acetonitrile solution was added to 0.085 g of the dried LiChrosorb. Then 2 ml of acetonitrile were added. The suspended solution was sonicated and dried under an infrared lamp (100 W). To obtain a uniform coating, the silica gel coated with alkylamine was wetted with 2 ml of acetonitrile, sonicated and dried under the infrared lamp. This process was repeated three times.

### *Liquid chromatographic system*

The anion-exchange supports were slurry-packed<sup>8</sup> into the PTFE micro column (7–20 cm  $\times$  0.5 mm I.D.), with water as solvent. The chromatographic system used was essentially as described in ref. 8. As the hydrochloric acid used as eluent was a strong acid, it was contained in PTFE tubing (3 m  $\times$  0.5 mm I.D.). This eluent was fed into the column by a micro-syringe type pump in which distilled water was kept. Platinum–iridium tubing, 0.3 mm I.D. and 0.6 mm O.D., was used for injection and connection parts instead of stainless-steel tubing. A micro quartz cell (quartz tubing, 10 mm  $\times$  0.3 mm I.D., 0.8 mm O.D.) was directly connected to the column end. A slit for the quartz cell, 1.5  $\times$  0.3 mm, was used in the UV cell housing. The detectors were UVIDEC 100-II and 100-II-W for UV and visible, respectively (Japan Spectroscopic, Hachioji, Japan). For step-gradient elution, two microfeeders were used in which different eluents were kept, and the operational procedure was as follows: after the first eluent had been run, the connection at the injection port was disconnected by hand and connected to second eluent. All standard solutions used were arranged as follows: each metal chloride, guaranteed grade, was dissolved in 8 *N* HCl.

## RESULTS AND DISCUSSION

*UV detection of chloro complexes*

The UV-visible spectra of chloro complexes of several metals, e.g. thallium, mercury, lead and bismuth, have been examined by Rogers *et al.*<sup>3</sup>. Although they recorded the spectra in the range 210–420 nm, absorption maxima below 220 nm were not well defined. We surveyed the absorption spectra in UV region, especially in the range 200–230 nm, for metal chloro complexes in 8 N HCl. The spectra of chloro complexes of manganese(II), cobalt(II), nickel(II) and lead(II) show maxima at 209–213 nm. The extinction coefficients of manganese(II), cobalt(II), nickel(II) and cadmium(II) in 8 N HCl are  $5.6 \cdot 10^2$  (209 nm),  $1.2 \cdot 10^3$  (212 nm),  $5.3 \cdot 10^2$  (211 nm) and  $5.5 \cdot 10^2$  (209 nm), respectively. In these measurements 8 N HCl was taken as reference solution. The spectra of chloro complexes of copper(II), tin(IV), antimony(III) and mercury(II) in 8 N HCl also show a shoulder peak at 210 nm, and their extinction coefficients at *ca.* 210 nm are nearly same as above. Chloro complexes of tin(IV), antimony(III) and mercury(II) show maxima at 220–228 nm.

Therefore, we could detect many metal chloro complexes if we set the wavelength of UV detector at 210–220 nm.

*k' vs. concentration of HCl*

The effect of the concentration of HCl in the mobile phase on capacity ratios ( $k'$ ) is shown in Fig. 1. The relationships shown correspond, to some extent, to that

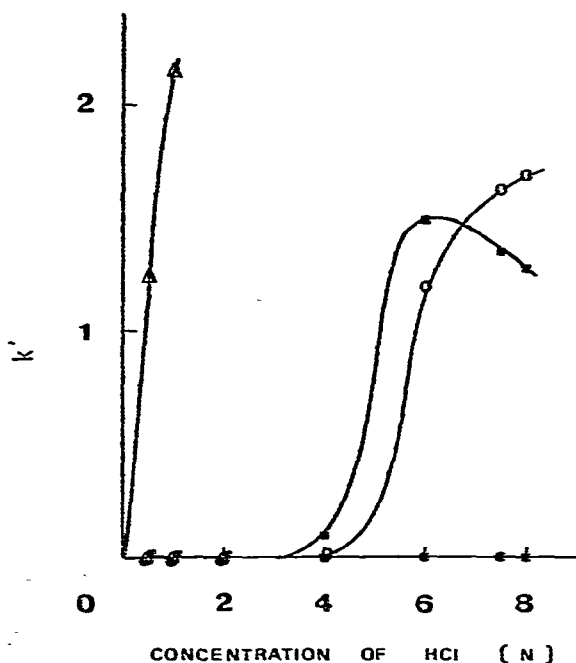


Fig. 1. Relationships between  $k'$  values and concentration of HCl. Column, 11.8 cm  $\times$  0.5 mm I.D., packed with our own support; injection volume, 0.13  $\mu$ l of 0.1 M metal chloride–8 N hydrochloric acid solution; mobile phases, different normality of hydrochloric acid saturated with Capriquat; flow-rate, 4.1  $\mu$ l/min; detection wavelength, 400 or 700 nm. Samples:  $\Delta$  iron(III);  $\circ$  cobalt(II);  $\star$  nickel(II);  $\blacksquare$  copper(II).

between  $R_F$  values and the normality of HCl for thin-layer chromatography in which silica gel impregnated with Amberlite LA-1 was used<sup>9</sup>. The relationships shown in Fig. 1 also correspond basically to that between the normality of HCl and percentage of chloro complexes extracted by tri-*n*-octylamine-xylene solution from HCl solution<sup>10,11</sup>. Therefore, we were able to select the appropriate separation conditions for multi-elements, such as normality of HCl as mobile phase.

For checking the stability of liquid phase, 7.5 *N* HCl, which was not saturated with Capriquat, was passed through the column for 20 h. Although the retention volume of the chloro complex of cobalt(II) was unchanged, there was a small (20%) increase in the  $H$  value. As the mobile phase was saturated with Capriquat in most experiments, the liquid phase was stable enough for repeated analysis.

The height equivalent to a theoretical plate ( $H$ ) is sensitive to the injection volume, so it is necessary to keep the latter to less than 0.3  $\mu$ l. With the chloro complexes, however, up to 0.5  $\mu$ g of the element can be injected without any appreciable effect on  $H$ .

Two typical chromatograms are shown in Figs. 2 and 3. The calculated  $H$  values are *ca.* 0.17–0.27 mm. These values are reasonable in the present strongly acidic system.

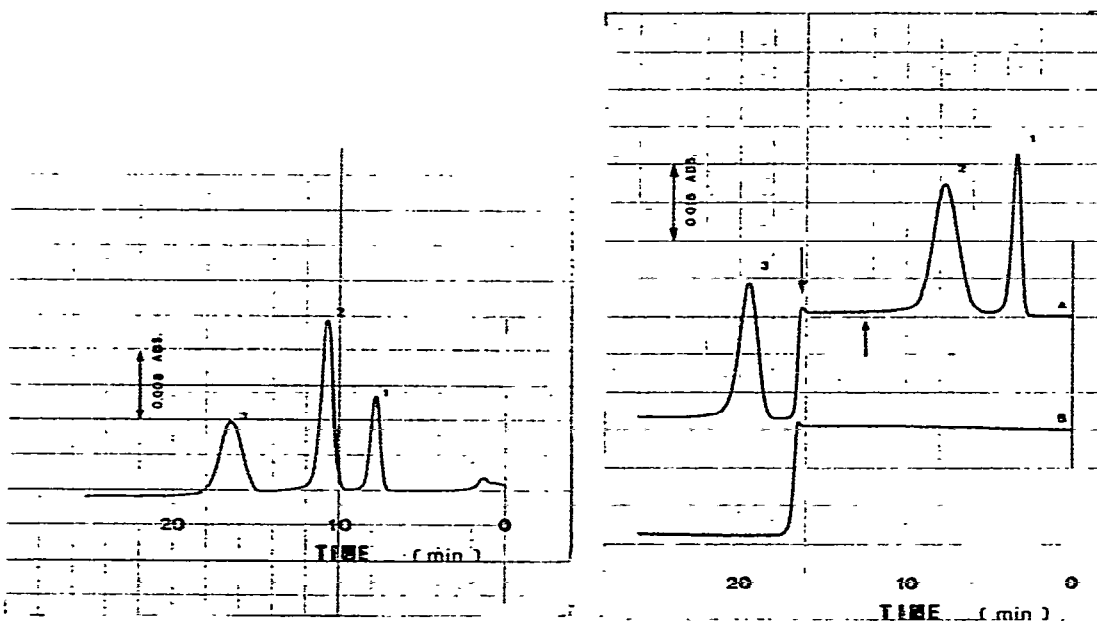


Fig. 2. Chromatogram of chloro complexes of (1) nickel(II), (2) copper(II) and (3) cobalt(II). Column, 13.8 cm  $\times$  0.5 mm I.D., packed our own support; mobile phase 7.5 *N* hydrochloric acid saturated with Capriquat; flow-rate, 4.1  $\mu$ l/min; injection volume, nickel (190 ng), copper (100 ng) and cobalt (96 ng); detection wavelength, 220 nm.

Fig. 3. Chromatogram of chloro complexes of (1) lead(II), (2) antimony(III) and (3) tin(IV). Column, same as Fig. 2 except that the length was 12.5 cm; mobile phase, 0.02 *N* perchloric acid in 8 *N* hydrochloric acid solution at 4.1  $\mu$ l/min, followed by 0.25 *N* perchloric acid in 8 *N* hydrochloric acid solution at 8.3  $\mu$ l/min. Sign (†) shows when the second eluent was introduced into the column and sign (‡) shows when it reached the end of column. Injection volume, lead (54 ng), antimony (63. ng) and tin (62 ng); detection wavelength, 215 nm; (B) was the baseline in same conditions.

The mutual or multiple separation of iron(III), cobalt(II), nickel(II), copper(II), lead(II) and bismuth(III) is possible by using HCl as eluent in an isocratic or step-gradient mode. But the chloro complexes of tin(IV), antimony(III) and mercury(II) are retained too long when HCl is used as eluent. Therefore, in order to reduce  $k'$  values, perchloric acid is added to the HCl in the separation system<sup>1</sup>. Addition of perchloric acid is also helpful in the separation of bismuth(III).

A ghost peak appeared in some cases, generally only when perchloric acid was present in the eluent and a step-gradient mode was used for the separation of tin(IV) and mercury(II). To solve this problem, we tried first washing the injection port well, and second adding ascorbic acid or hydroxylamine to the eluent to prevent oxidation. These tactics were not completely successful. However, if the column was washed after each run with eluent containing a slightly greater concentration of perchloric acid than in the original eluent, the ghost peak did not appear in the following run.

Calibration curves for copper, mercury and lead are shown in Fig. 4. Chloro complexes of elements can be determined quantitatively from 1–10 ng to 1  $\mu$ g by the present chromatographic method. Use of the loop injection method might lead to smaller deviations of each point from the calibration curve. The lower limit of determination in Fig. 4 is about one-thousandth of those reported in refs. 1 and 2.

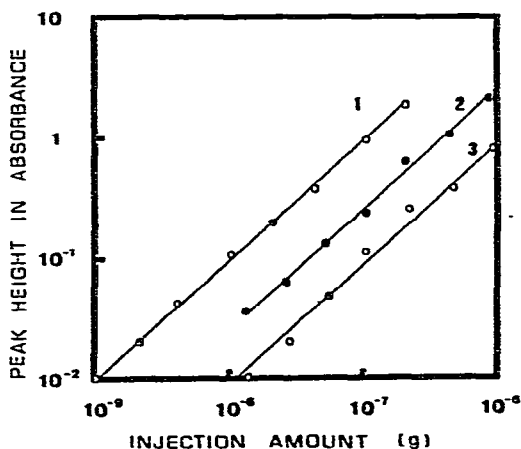


Fig. 4. Calibration curves for (1) mercury, (2) copper and (3) lead. Column, same as Fig. 2; mobile phase, 7.5 *N* hydrochloric acid for copper and 0.1 *N* perchloric acid in 8 *N* hydrochloric acid solution for mercury and lead, both saturated with Capriquat; detector wavelength, 210 nm for copper and 225 nm for mercury and lead.

PTFE micro-bore packed columns<sup>8</sup> have the advantage of being suitable for use with a strongly acidic eluent, because such a small volume of eluent is required. The drawbacks of the present system are that it is not fully automatic and that leakage of the eluent sometimes occurs. This would be overcome if we use a modified LC system, such as the one proposed by Horwitz *et al.*<sup>6</sup> Despite these drawbacks, the present system is a very useful and it could be readily adapted to most laboratories. A rapid analysis of chloro complexes is possible in keeping their chemical forms if appropriate separation conditions are selected.

By spreading alkylamines on the inner surface of the glass capillary tubing, we

could obtain an open-butular micro capillary column. Separation of metal chloro complexes on such a column might be a good method for ultra micro analysis<sup>12</sup>. If a strong anion-exchange resin made from polystyrene gel become available, it might be more durable than our home-made supports impregnated with Capriquat.

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