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CHROMATOGRAPHIC SEPARATION OF ISOTHIOCYANATO COMPLEXES OF CHROMIUM(III) BY USE OF A SEPHADEX GEL COLUMN

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SUMMARY

The separation of inert successive complexes of chromium(III) with thiocyanate was achieved by adsorption chromatography using Sephadex gels. The separation may be due to the hydrophobic interaction of thiocyanate groups in the complexes with the gel matrix. The more the complex species contains thiocyanate as ligand, the later it emerges from the column. The geometric isomers of bis- and tris(isothiocyanato)chromium(III) could be separated.

INTRODUCTION

Gel chromatography using Sephadex gels has been employed as a means of separation of inorganic compounds[1–3]. Sephadex gels are composed of dextran cross-linked by epichlorohydrin, and adsorption, partition, and/or ion exchange participate in the separation of inorganic compounds.

It has been shown in the previous papers[4,5] that borate and vanadate(V) are adsorbed selectively on Sephadex G-25 gel at different pH ranges and desorbed reversibly with acidic solutions. This characteristic was utilized for the spectrophotometric determination of boron and vanadium in natural waters and rocks. Oxo-anions of boron, vanadium(V)[6], molybdenum(VI)[7] and tungsten(VI)[8] are considered to form chelate complexes with hydroxyl groups of glucose units in the gel matrix. On the other hand, simple inorganic anions such as iodide, perchlorate and thiocyanate are adsorbed on to the gel, mainly because of hydrophobic interactions between the solutes and the gel matrix[9,10]. Kura *et al.*[11] have separated some metal ions in the thiocyanate media by use of Sephadex G-15 gel as an anion exchanger in the thiocyanate form.

Inert successive complexes relating to these anions mentioned above may have different affinities with the gel matrix. If a long time is taken to reach complexation equilibrium at room temperature, each successive complex can be separated by column chromatography. In this study, the chromium(III)–isothiocyanato complex system was investigated.

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EXPERIMENTAL

Chemicals

All the reagents used were of analytical-reagent grade.

$Cr(ClO_4)_3 \cdot 9H_2O$ and $K_3[Cr(NCS)_6] \cdot 4H_2O$ were prepared by the methods of Weinland and Enograber[12] and Roester[13], respectively.

The mixture of successive isothiocyanato complexes of chromium(III) was prepared by heating 50 ml of solution containing $Cr(ClO_4)_3 \cdot 9H_2O$ (1.28 g) and potassium thiocyanate (0.73 g) at 95°C for 3 h. After the solution was cooled, $Cr(ClO_4)_3 \cdot 9H_2O$ (0.26 g) and $K_3[Cr(NCS)_6] \cdot 4H_2O$ (0.30 g) were added to the solution. The solution was stored in a refrigerator and the precipitate of potassium perchlorate was removed by filtration.

Sephadex G-10, G-15 and G-25 (Medium) gels (Pharmacia, Uppsala, Sweden) were used.

Elution procedure

In a glass column (45 × 1.0 cm I.D.), a gel suspended in water was packed as described in the literature[14]. The sample solution (1 ml) was loaded on to the top of the column, and then eluted. The effluent was collected with an automatic fraction collector. The chromium content in each fraction was determined by atomic-absorption spectrophotometry with a Nippon Jarrell-Ash Model AA-781 instrument.

Another column techhique was employed for the preparation of solutions containing the complex species corresponding to bands V–VIII in Fig. 1. A poly(ethylene chloride) column (50 × 0.8 cm I.D.) was packed with 23 ml of Sephadex G-15 gel; 2 ml of the sample solution were loaded and then eluted with 0.1 *M* perchloric acid solution. After the complex species corresponding to band IV in Fig. 1 had been eluted from the column, the column was cut and four portions of purple bands were taken out. Then, each complex species was eluted with 0.1 *M* perchloric acid solution.

Determination of ratios of thiocyanate to chromium(III)

The value of the SCN/Cr ratio of each complex separated was determined by analysis of chromium and thiocyanate. Chromium was determined by atomic-absorption spectrophotometry. Thiocyanate was determined absorption spectrophotometrically as iron(III) complexes after decomposition of the complexes by sodium hydroxide solution[15].

Measurement of distribution ratio for identification of the geometric isomers

To 25 ml of solution containing the chromium complex corresponding to band III or IV in Fig. 1 and various amounts of perchloric acid, 1 g of AG 50W-X8 (H^+, 100–200 mesh) cation-exchange resin was added. After equilibration at 20°C (15 min), the resin was allowed to settle and then the absorbance of the supernatant solution was measured at 300 nm with a Hitachi Model EPS-2U. The distribution ratio, *D*, of the chromium complex species was calculated by

$$D = \frac{\text{mmol Cr(III) adsorbed/g of air-dried resin}}{\text{mmol Cr(III)/ml of solution}}$$

Measurement of distribution coefficient

To investigate the adsorption of complex species on Sephadex G-10, G-15 and G-25 gels, the distribution coefficient, K_d or K_{av}, was measured at 20°C by a column technique for the chromium species corresponding to bands–IV, and by a batch technique for those corresponding to bands V–VII in Fig. 1 and $[Cr(NCS)_6]^{3-}$ complex species.

For the column technique, the value of K_d or K_{av} is given by

$$K_d = \frac{V_e - V_0}{V_i} \text{ or } K_{av} = \frac{V_e - V_0}{V_t}$$

where V_e, V_0, V_i, and V_t represent the elution void, inner and total bed volumes, respectively[14]. The values of V_0 were determined using sodium polyphosphate for the Sephadex G-10 column[16] and Blue Dextran 2000 (Pharmacia) for the Sephadex G-15 and G-25 columns. V_i is defined as the product of the water regain, W_r, and the weight of dry gel, m; $V_i = W_r \cdot m$. The values of W_r were 1.27 and 2.34 ml/g of dry gel for Sephadex G-15 and G-25, respectively (separate test with Blue Dextran 2000).

For the batch technique, K_d is given by

$$K_d = [(C_i - C_f)V/C_f m\ W_r] + 1$$

when m g of dry gel is added to V ml of solution. C_i and C_f are the initial concentration of the complex species and the concentration of the complex species in the equilibrated solution, respectively. These values were determined by atomic-absorption spectrophotometry or by absorbance measurements at 300 nm.

The value of each parameter is shown in the caption to each figure.

RESULTS AND DISCUSSION

Separation of successive isothiocyanato complexes of chromium(III)

Fig. 1 shows a typical elution curve for the complex species obtained on the Sephadex G-15 gel column using 0.2 *M* sodium perchlorate solution as eluent. In order to prevent the complexes from being hydrolysed, the pH of the eluent was maintained at 2 by addition of perchloric acid. The concentration scales of some bands are different from one another. It can be seen that the chromium species were separated into eight fractions. The elution profiles of Blue Dextran 2000 and thiocyanate ion obtained in separate tests are also shown. The chromium species were all more strongly adsorbed on the gel than thiocyanate ion. As expected, the different affinity of each isothiocyanato complex for the gel matrix facilitates their separation.

Identification of chromium(III) complex species

The ratios of thiocyanate to chromium in the portions of bands I–VIII were tabulated as integers (Table I). The separated species were considered to be successive complexes, $[Cr(NCS)_n(H_2O)_{6-n}]^{(3-n)+}$ (n = 0–6). Band VIII was a mixture of n = 5 and 6. The more the complex species contains thiocyanate as ligand, the stronger is the interaction with the gel matrix.

The complex species of n = 0–2 have already been separated by cation-ex-

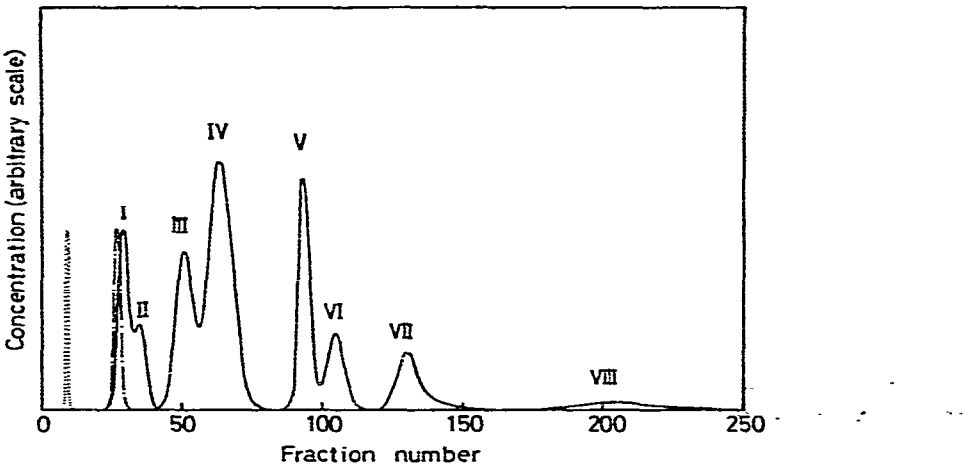

Fig. 1. Chromatographic separation of successive isothiocyanato complexes of chromium(III). Gel: Sephadex G-15, 8 g. Column: 33 × 1.0 cm I.D. Eluent: 0.2 *M* $NaClO_4$ (pH 2). Flow rate: 40 ml/h. Fraction volume: 1–80, 1 ml; 81–110, 5 ml; 111–250, 20 ml. ·····, Blue Dextran 2000; — · —, free thiocyanate ion.

TABLE I

ANALYSIS OF SCN TO Cr RATIO OF EACH BAND IN ELUTION CURVE

Elution band	I	II	III	IV	V	VI	VII	VIII
SCN/Cr ratio	0	1	2	2	3	3	4	5–6

change chromatography[17]. The first two complex species were eluted at the positions of bands I and II shown in Fig. 1, whereas the bis(isothiocyanato) complex obtained as one band by the cation-exchange chromatography was separated into two bands, III and IV, in this study.

The cation-exchange equilibrium for *m*-positive charged species is expressed in the logarithmic form:

$$\log D = \log K + m \log [H^+]_R - m \log [H^+]$$

where K and the subscript R refer to the ion-exchange equilibrium constant between *m*-positive charged species and a proton, and to the resin phase, respectively. At low loading $[H^+]_R$ is almost unchanged, hence the charge of the species can be obtained from the slope of a plot of log D *versus* log $[H^+]$. The loading analysis on the cation-exchange resin for the complex species corresponding to bands III and IV shows that both species are mono-positively charged ions, and the species of band III has a lower affinity for the cation-exchange resin (Fig. 2). As has already been found by King and Walter[18] and by Mori *et al.*[19], a *cis*-isomer is more strongly adsorbed on to cation-exchange resins than the corresponding *trans*-isomer, owing to its relatively larger dipole moment. These results show that the species of band III is *trans*-bis(isothiocyanato)chromium(III) and that of band IV is the *cis*-isomer. Hougen *et*

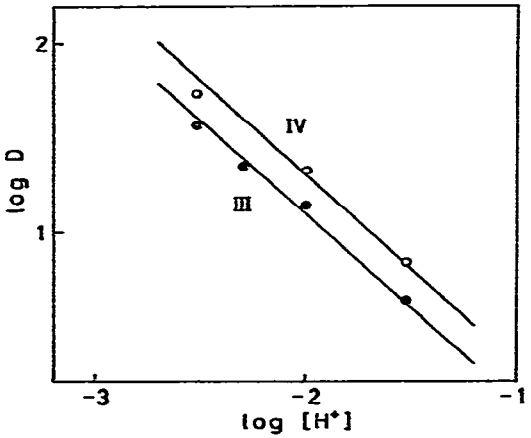

Fig. 2. Determination of the ionic charge of the complexes of SCN/Cr = 2 (III and IV in Fig. 1). Solution: 25 ml ($HClO_4$). Resin: AG 50W-X8 (H^+, 100–200 mesh). 1 g. Time for equilibration: 15 min.

al.[20] have separated the geometric isomers using a cation-exchange resin column of length 240 cm and have found that the *trans*-isomer is more easily eluted than the *cis*. The absorption spectra of the separated isomers were measured. In the ultraviolet region their spectra are in fair agreement with ours, whereas they are not in the visible region. From the experimental evidence of our study, it is concluded that the visible absorption spectrum of the "*cis*-isomer" reported by Hougen *et al.* is that of the *trans*-isomer, and *vice versa*.

The complex species of bands V and VI are geometric isomers, and may be *mer*- and *fac*-tris(isothiocyanato)chromium(III), respectively, from the viewpoint of the dipole moment of the two complexes. The absorption spectra for each complex species will be described elsewhere in detail.

Effect of background electrolytes on the adsorption of complex species

The affinity of the complex species for Sephadex G-15 was measured in 0.2 *M* solutions of the sodium salts of thiocyanate, perchlorate, nitrate, chloride and sulphate. The results are shown in Fig. 3. The affinity of the complex species for the gel increases in the order $SCN^- < ClO_4^- < NO_3^- < Cl^- < SO_4^{2-}$. The order is consistent with the reverse order of affinity of these anions for the gel. The values of K_{av} for these anions are 1.54, 1.45, 0.74, 0.54 and 0.20, respectively[10]. There may be a competitive reaction between the complexes and these anions. Kura *et al.*[11] have pointed out the similar adsorption characteristics of thiocyanate on the G-15 gel and have suggested that the anions which interact more strongly with the gel cause a greater decrease in the amount of thiocyanate ion adsorbed. It can be seen that the adsorbability of complex species with higher ligand numbers is more strongly influenced by the presence of various kinds of electrolytes (Fig. 3). Thiocyanate groups in the complex species may significantly participate in the adsorption.

The effect of the concentration of sodium perchlorate on K_d values was examined over the concentration range 0.1–1 *M*, the pH of the solution being maintained at 2 (Fig. 4). The complex species of $n = 0$–2 were hardly affected in the presence of sodium perchlorate in various concentrations. For those of $n = 3$–6, the

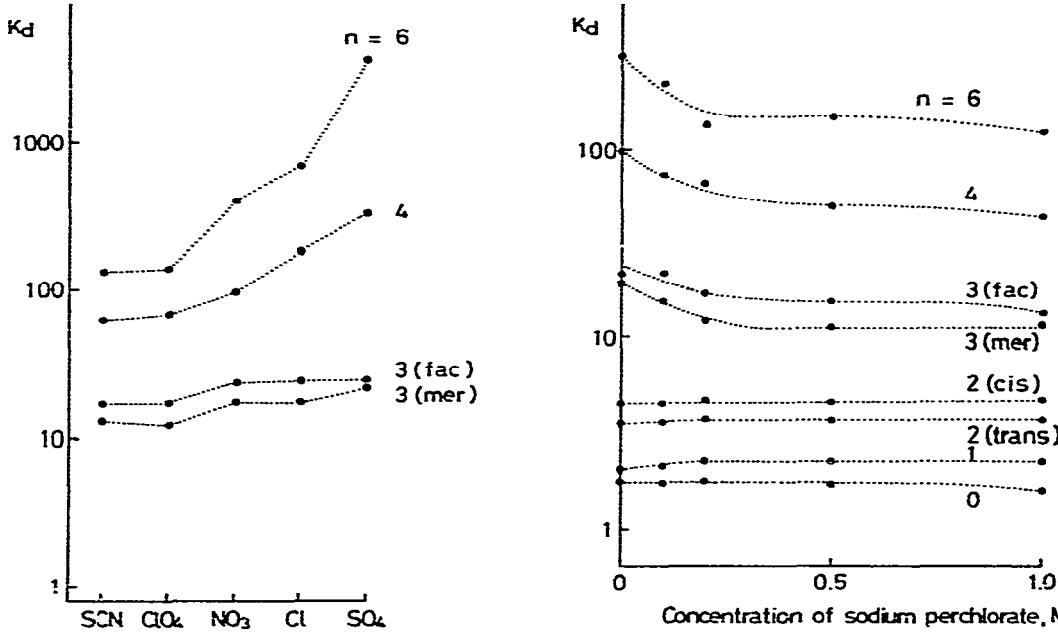

Fig. 3. Effects of co-existing electrolytes on K_d values for the successive complexes, $[Cr(NCS)_n(H_2O)_{6-n}]^{(3-n)+}$ (n = 3–6). Solution: 25 ml (sodium salt, 0.2 M, pH 2). Gel: Sephadex G-15 (W_r = 1.27 ml/g dry gel), 0.5 g. Time for equilibration: 30 min.

Fig. 4. Effects of the concentration of sodium perchlorate on K_d values for the successive complexes. n = 0–2: the column method. Gel: Sephadex G-15. Column: 50 × 1.0 cm I.D., V_0 = 13.0 ml, V_i = 15.2 ml. Flow-rate: 14 ml/h. n = 3–6: the batch method. Solution: 25 ml (pH 2). Gel: Sephadex G-15 (W_r = 1.27 ml/g dry gel), 0.5 g. Time for equilibration: 30 min.

degree of affinity for the gel decreased on increasing the concentration of perchlorate. It can be seen that thiocyanate or perchlorate solution as eluent is efficient for the separation of the respective complex species, but the gradient-elution method may not be effective for the present system.

Effect of the degree of crosslinking of the gel on the adsorption of complex species

The difference of cross-linking of the dextran gel affects the adsorption behaviour of the complex species. Fig. 5 shows the K_{av} values of the complex species (n = 0–2) on Sephadex G-10, G-15 and G-25 gels. The K_{av} values are plotted as a function of the concentration of sodium perchlorate. Because it was difficult to obtain the value of V_i for Sephadex G-10, the comparison was carried out using the K_{av} values. All the complex species show the strongest affinity for Sephadex G-10. The Sephadex G-10 system was most influenced by the presence of sodium perchlorate. At lower concentrations of the salt, the ion-exchange mechanism may be present. The separation factors for the bis(isothiocyanato) isomers are 1.07, 1.25 and 1.51 for Sephadex G-25, G-15 and G-10, respectively, and therefore the separation of the isomers may be efficiently carried out by use of a Sephadex G-10 column.

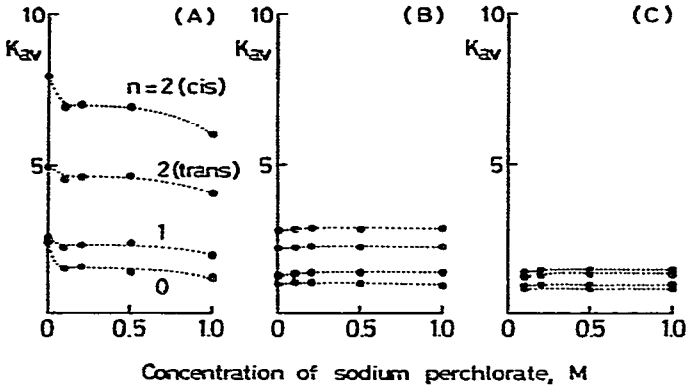

Fig. 5. Effects of the degree of cross-linking on K_{av} values for the cationic complexes. (A) Gel: Sephadex G-10, V_0 = 13.2 ml, V_t = 35.7 ml. (B) Gel: Sephadex G-15, V_0 = 13.0 ml, V_t = 38.1 ml. (C) Gel: Sephadex G-25, V_0 = 15.1 ml, V_t = 37.4 ml.

Comparison with other methods

Bjerrum has investigated the chromium(III)–isothiocyanato complex system by the precipitate-formation and solvent-extraction methods and has reported stability constants of the successive complexes[21]. However, he failed to separate mono- and bis(isothiocyanato) complexes and also pentakis- and hexakis(isothiocyanato) complexes. The cation-exchange method made it possible to separate the cationic complex species[17,20]; bis(isothiocyanato) complexes are eluted with 0.15 *M* perchloric acid, mono(isothiocyanato) complex witn 1 *M* perchloric acid and hexa-aqua species with 5 *M* perchloric acid. As anionic complexes are so strongly adsorbed by polystyrene-type anion-exchange resins, they cannot be eluted. Kaufman and Keyes[22] have succeeded for the tetrakis(isothiocyanato) complex with the cellulose anion exchanger and 1 *M* perchloric acid as eluent. However, pentakis- and hexakis(isothiocyanato) complexes could not be eluted without the decomposition occurring.

The present method makes it possible to separate successive complexes of n = 0–4 by use of a single column under mild conditions and to separate geometric isomers of a tris(isothiocyanato) complex for the first time. Complex species with various charges are separated from one another without concentration-gradient elution. However, the later the complexes are eluted, the more the samples are diluted. It is convenient, in this case, to use the poly(ethylene chloride) tube method, as mentioned in the Experimental section. Alternatively, it can be seen from Figs. 3 and 5 that the selection of gel and eluent may give a relatively rapid and complete separation method for the complexes concerned. Complete separation of complexes of n = 0–2 was accomplished by use of the Sephadex G-10 gel column (Fig. 6a). Group separation of the successive complexes can be almost completely and rapidly separated by a combination of the Sephadex G-25 gel column and 0.2 *M* sodium chloride–sodium perchlorate solution as eluent (Fig. 6b).

The present method may be utilized to separate preparatively many inert complexes containing thiocyanate as ligand, and may be available for investigation of complexation equilibrium in solution.

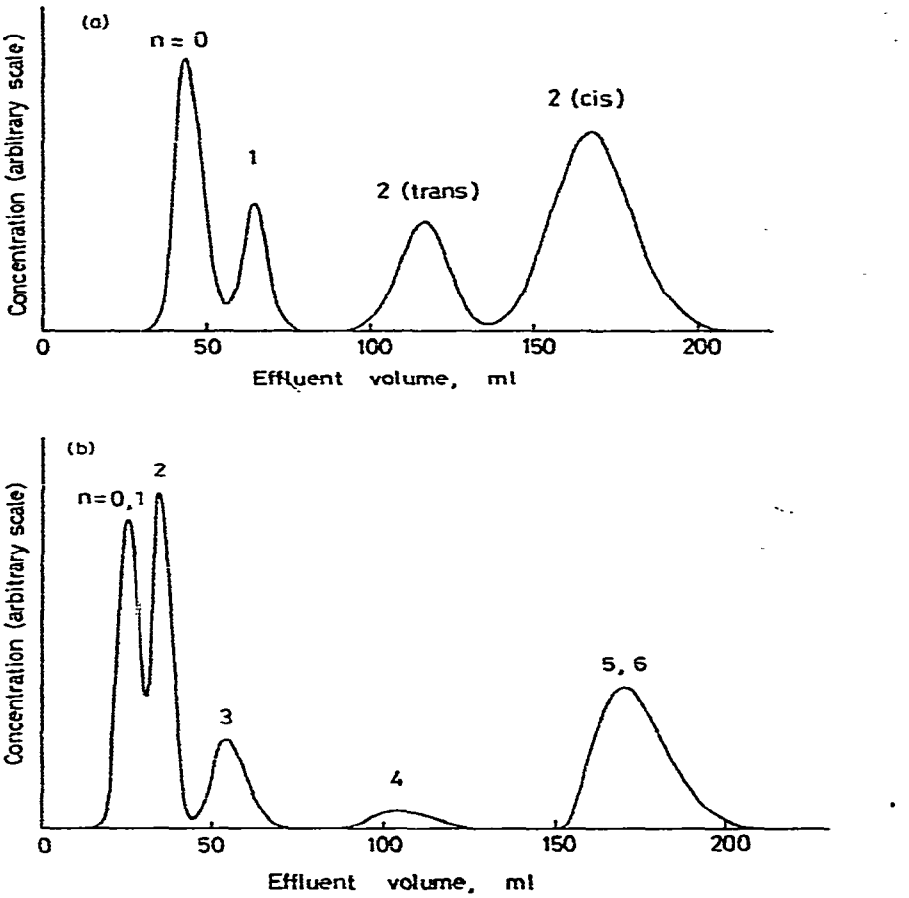

Fig. 6. (a) Chromatographic separation of the cationic isothiocyanato complexes of chromium(III). Gel: Sephadex G-10. Column: 46 × 1.0 cm I.D. Eluent: 0.1 *M* $HClO_4$. Flow-rate: 19 ml/h. (b), Chromatographic group separation of successive complexes. Gel: Sephadex G-25 (Fine). Column: 41 × 1.0 cm I.D. Eluent: 0–125 ml, 0.2 *M* NaCl (pH 2); 125–350 ml; 0.2 *M* $NaClO_4$ (pH 2). Flow-rate: 39 ml/h.

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