

Complete chromatographic separation of aquachromium(III)–isothiocyanato successive complexes including geometric isomers using cross-linked dextran and poly(vinyl alcohol) gel columns

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Abstract

The successive isothiocyanato complexes of chromium(III) including the geometric isomers, $[\text{Cr}(\text{NCS})_n(\text{OH}_2)_{6-n}]^{(3-n)+}$ ($n=0-6$), were completely separated for the first time by using gel chromatography. Together with a cation exchanger, Toyopearl HW-40 was mainly employed to separate the $n=0-3$ complexes and Sephadex G-25 for the $n=3-6$ complexes. The adsorption mechanism of the complexes on the gels was discussed on the basis of the thermodynamic functions obtained from the temperature dependence of the K_{av} values of the complexes. The adsorption could be interpreted in terms of the difference in the degree of the water structure breaking by the hydrophobic ions in the bulk and gel phases, and the hydrogen bonding between the inert H_2O ligand of the complexes and hydrophilic groups in the gel matrix.

1. Introduction

The complexes of Cr(III) are known to be inert in substitution reactions. The rate of substitution of ligands is very slow; the rate constant of exchange of hydrated water is about $10^{-6}-10^{-7} \text{ s}^{-1}$ at 25°C. Therefore, the complexation equi-

librium of Cr(III) with ligands at high temperature can be fixed at room temperature, and the successive complexes in the equilibrated solution can be isolated. In the chromium(III)–isothiocyanato complex system, seven successive complexes, represented by the general formula, $[\text{Cr}(\text{NCS})_n(\text{OH}_2)_{6-n}]^{(3-n)+}$ ($n=0-6$), are present. There are three pairs of geometric isomers: *trans* and *cis* forms for the $n=2$ and 4 complexes, and *mer* and *fac* forms for the $n=3$ complex. Therefore, ten kinds of octahedral complexes are present, as shown in Fig. 1.

In 1921, Bjerrum [1,2] reported that seven

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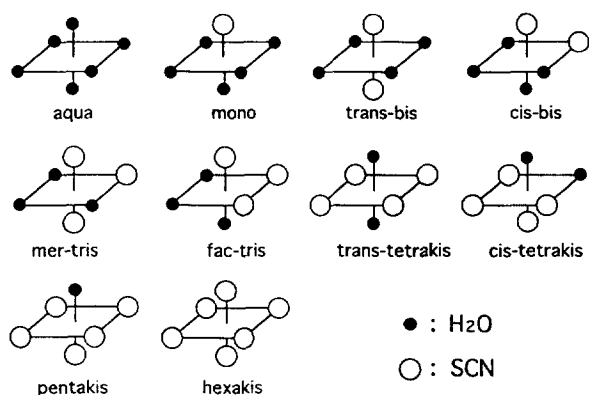


Fig. 1. All of the successive isothiocyanato complexes of chromium(III) including geometric isomers.

successive complexes were separated, and their equilibrium concentrations and successive stability constants were determined. The successive complexes were separated by precipitation and extraction, but the geometric isomers were not included. The cationic complex ions, including the geometric isomers, were separated by using a Dowex 50 cation-exchange column, the absorption spectra of the $n = 2$ isomeric complexes were observed and the equilibria and the kinetics of the cationic complex ions were investigated [3–6]. The anionic complex ions including geometric isomers were separated using alumina [7] and an anion exchanger, Selectacel DEAE-cellulose [8]; the latter separated the $n = 4$ geometric isomers. The $n = 0$ –6 complexes were separated by a combination of the cation- and anion-exchange and the extraction methods, but the extraction of the $n = 3$ complex with diethyl ether was impossible owing to the contamination of other chromium species, and the eluents employed were acetone–water mixed solvents containing perchloric acid in high concentration [9].

Sephadex gels are based on dextran, a linear polysaccharide consisting of glucose units with 1,6- α -glucoside bonds. Epichlorohydrin causes 1,3-glyceride–ether bonds to form between the dextran chains. The matrix of Toyopearl gels is produced from the polymerization of vinyl alcohol monomers, cross-linked with epichloro-

hydrin. These gels have been mainly employed to separate macromolecules such as proteins. Gel chromatography using Sephadex gels can be applied to the aquachromium(III)–isothiocyanato complex system, and the complexes, including the geometric isomers of the $n = 3$ complexes, were separated. However, the separation of the $n = 5$ and 6 complexes and the *trans*–*cis* $n = 4$ complex pair could not be achieved [11]. Preliminary experiments have revealed that Toyopearl gel shows a greater affinity than Sephadex gel to the isothiocyanato complexes. In this study, the chromatographic behaviours of the complexes on Sephadex and Toyopearl gels were investigated in detail to separate all the complexes including geometric isomers completely in a short time and under conditions as mild as possible. The mechanism of the adsorption of the complexes on the gels is discussed on the basis of thermodynamic functions obtained from the temperature dependence of the K_{av} values of the complexes.

2. Experimental

2.1. Reagents

All the reagents used were of analytical-reagent grade.

2.2. Preparation of sample solution containing the successive Cr(III)–NCS complexes

The mixture of the successive isothiocyanato complexes of chromium(III) was prepared by heating 25 cm³ of a solution [0.05 mol dm⁻³ Cr(ClO₄)₃ + 0.3 mol dm⁻³ NaSCN (0.01 mol dm⁻³ HClO₄) for $n = 0$ to 3, or 0.05 mol dm⁻³ Cr(ClO₄)₃ + 1.5 mol dm⁻³ NaSCN (0.01 mol dm⁻³ HClO₄) for $n = 3$ to 6] at 50°C for over 2 days, and stored in a refrigerator.

Cr(ClO₄)₃·9H₂O was prepared following the Weinland and Engraber method [12].

Na₃[Cr(NCS)₆]·4H₂O was prepared following the Roester method [13]. The peak position of the $n = 6$ complex was confirmed with this sample.

2.3. Chromatographic separation

The flow diagrams of the designed separation systems I and II are shown in Fig. 2. Sephadex G-25 (Superfine) gel (Pharmacia, Uppsala, Sweden) or Toyopearl HW-40 (Fine) gel (Toso, Tokyo, Japan) was used with the cation exchanger AG 50W-X4 (200–400 mesh) (Bio-Rad, Richmond, CA, USA).

To separate the $n = 0$ –3 complexes and SCN^- , three columns were used: AG 50W-X4, 5 cm \times 1 cm I.D., (a) for the $n = 0$ and 1 complexes, and Toyopearl HW-40, 1 cm I.D., one 8 cm long (b) for the *mer* and *fac* $n = 3$ complexes and the other 3 cm long (c) for SCN^- and the *trans* and *cis* $n = 2$ complexes. To separate the $n = 3$ –6 complexes and SCN^- , three columns were also needed: Sephadex G-25, 1 cm I.D., one 100 cm long (α) for the $n = 5$ and 6 complexes and one

40 cm long (β) for the *mer* and *fac* $n = 3$ complexes and the *trans* and *cis* $n = 4$ complexes, and AG 50W-X4, 5 cm \times 1 cm I.D., (γ) for SCN^- and the $n = 0, 1$ and 2 complexes.

Two kinds of eluents were used: $E_{0.2}$, 0.2 mol dm^{-3} NaClO_4 (0.01 mol dm^{-3} HClO_4); and E_1 , 1 mol dm^{-3} NaClO_4 (0.01 mol dm^{-3} HClO_4). In order to prevent the hydrolysis of the Cr(III) complexes, the pH of the eluent was maintained at 2; when the pH was lower, the supporting net of the column was damaged.

The eluent was pumped with a DM2M-1024 Sanuki pump at a flow-rate of 1.25 $\text{cm}^3 \text{min}^{-1}$. The absorbance of the effluent was observed at 240 nm for the $n = 1$ –6 complexes and 570 nm for the $n = 0$ complex with a spectrophotometer (Hitachi, Model 100-20) attached with a micro-black flow-through cell (Nippon Quartz Glass). The Sephadex gel columns were kept at 0°C with

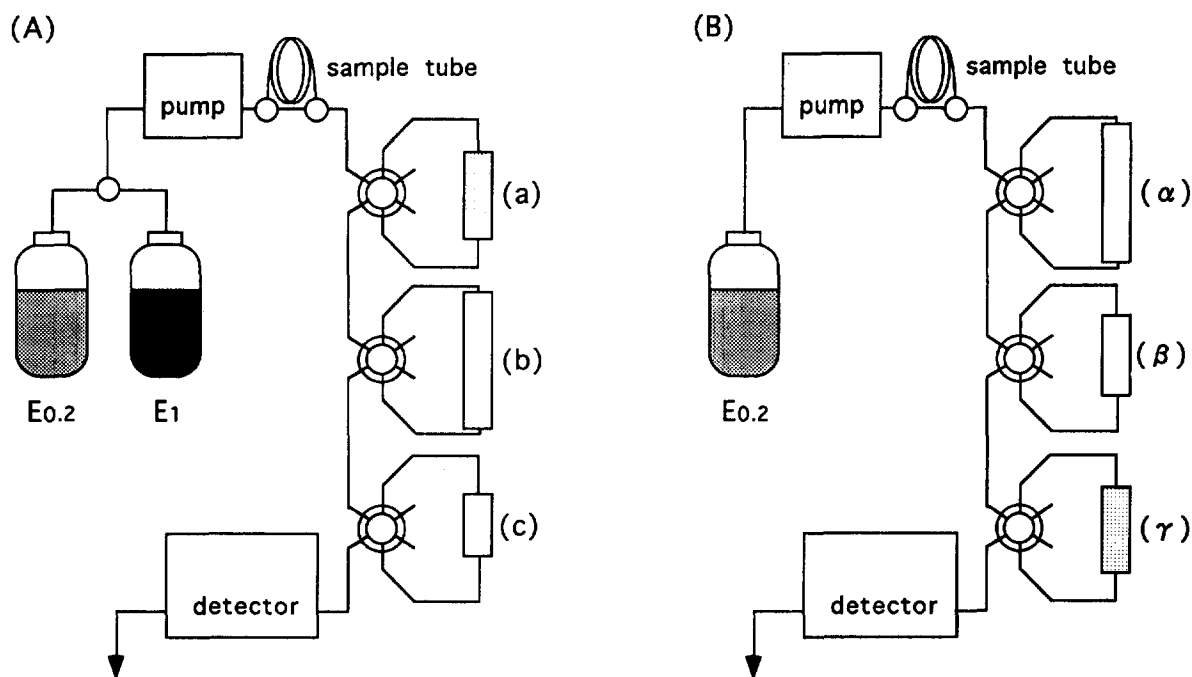


Fig. 2. Flow diagrams of separation systems. (A) Separation system I for the $n = 0$ –3 complexes. (B) Separation system II for the $n = 3$ –6 complexes. Eluent: $E_{0.2}$, 0.2 mol dm^{-3} NaClO_4 (0.01 mol dm^{-3} HClO_4); E_1 , 1 mol dm^{-3} NaClO_4 (0.01 mol dm^{-3} HClO_4). Column: (a) AG 50W-X4 (5 cm \times 1 cm I.D.); (b) Toyopearl HW-40 (8 cm \times 1 cm I.D.); (c) Toyopearl HW-40 (3 cm \times 1 cm I.D.); (α) Sephadex G-25 (100 cm \times 1 cm I.D.); (β) Sephadex G-25 (40 cm \times 1 cm I.D.); (γ) AG 50W-X4 (5 cm \times 1 cm I.D.).

a thermostat bath (Advantec Lab Cool LC-80F and Advantec Immersion Circulator LH-2000).

2.4. Distribution measurements of the successive complexes

The K_{av} value, defined by the following equation, was calculated:

$$K_{av} = (V_c - V_0)/(V_t - V_0)$$

where V_c , V_0 and V_t are the elution volume of the sample, the void volume outside the gel and the total column volume, respectively. The elution volume of Blue Dextran 2000 (Pharmacia, Uppsala, Sweden) was used as V_0 . A Toyopearl HW-40 column (7 cm × 1 cm I.D.) and a Sephadex G-25 column (40 cm × 1 cm I.D.) were used to measure the K_{av} values for the complexes and SCN^- . The K_{av} values at five temperatures, 278, 283, 288, 293 and 298 K, were obtained.

Especially for the Toyopearl HW-40 system, a batch technique was applied for the $n = 3$ –6 complexes. For the $n = 3$ and 4 complexes, a 10-cm³ sample solution mixture containing 0.01 mmol of the complex, 2 mmol of $NaClO_4$, 0.1 mmol of $HClO_4$ and 0.50 g of the Toyopearl gel was stirred for 30 min. A 15-cm³ mixture of a sample solution with 0.10 g of the gel for the $n = 5$ complex system and one with 20 cm³ of the sample solution and 0.10 g of the gel for the $n = 6$ complex system were employed for distribution measurements. After equilibration, the absorbances of the bulk solutions were measured at 300 nm. From the initial and final absorbance values, the distribution ratio, D , was calculated. D is defined as

$$D = [\text{solute}]_g / [\text{solute}]_b$$

where $[\text{solute}]_g$ and $[\text{solute}]_b$ are the concentrations of solute in the gel and bulk phases in (mol)/(g: mass of dry gel) and (mol)/(dm³: volume of bulk solution), respectively. In order to relate D to K_{av} , D was divided by the ratio of the total column volume to the dry-basis mass of the packed gel measured in a separate test.

3. Results and discussion

3.1. Chromatographic separation of Cr–NCS successive complexes

The K_{av} values increased with increasing SCN number in the complexes, while the geometric isomers with the same number of the ligand had different K_{av} values. They were hardly influenced by the co-existing electrolytes in the available concentration range [11]. Therefore, neither concentration-gradient elution nor concentration-stepwise elution could be applied. The temperature dependence of the K_{av} values for Toyopearl HW-40 and Sephadex G-25 is shown in Fig. 3. The K_{av} values for Toyopearl HW-40 were greater than those for Sephadex G-25. The adsorption was exothermic: the K_{av} values increased with decrease in temperature. The adsorbability of the respective complexes depended on the temperature in different ways. In the Sephadex gel system, the K_{av} values of the *trans* and *cis* geometric isomers of the $n = 4$ complex, and the $n = 5$ and 6 complexes were similar to one another. However, at lower temperature, the K_{av} values diverged more from each other.

As it was impossible to separate all of the complexes using only one column packed with one kind of gel, two separation systems consisting of a Dowex 50 cation exchanger, one with Sephadex G-25 and the other with Toyopearl HW-40 columns, were designed, as shown in Fig. 2. To make the separation more efficient, columns with different lengths were used, but each column could be bypassed with a six-way rotary valve, if necessary. To prevent photosubstitution of the complexes the columns were shielded from light [14].

Separation system I is shown in Fig. 2A. Toyopearl HW-40, which has greater K_{av} values for the isothiocyanato complex species, was employed for the separation of free SCN^- and the $n = 0$ –2 complexes. Since the K_{av} values of the $n = 0$ –2 complexes and SCN^- were similar, as shown in Fig. 3A, a long column was required to separate them, which resulted in a long separation time and in dilution of the samples.

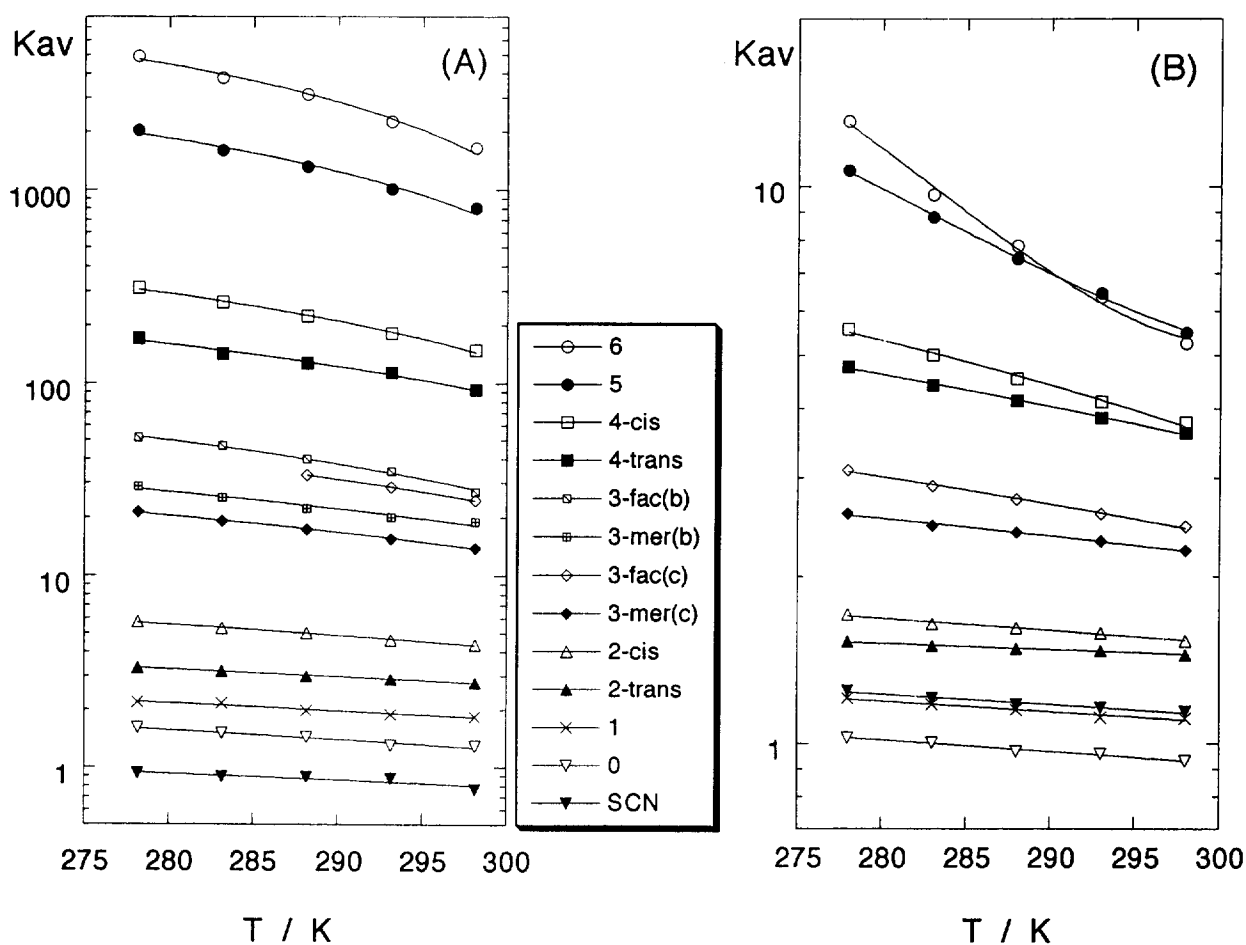


Fig. 3. Temperature dependence of K_{av} values of the successive chromium(III) complexes for (A) Toyopearl HW-40 and (B) Sephadex G-25. (b) Measured by the batch method; (c) measured by the column method.

The cation-exchange column was attached in front of the Toyopearl gel columns to trap the $n=0$ and 1 complexes, which have charges of +3 and +2, respectively, and they were eluted stepwise in a separate elution process. A typical elution curve is shown in Fig. 4A. Initially, the SCN^- was eluted, followed by the *trans* and *cis* isomers of the $n=2$ complex. Then, columns a and c were bypassed, and the *mer* and *fac* isomers of the $n=3$ complex in column b were eluted successively. Finally, from column a, the $n=1$ complex was eluted using eluent $E_{0,2}$ and the $n=0$ complex was eluted with the eluent E_1 .

Separation system II for complexes of higher

SCN^- number is shown in Fig. 2B. Sephadex G-25, which has smaller K_{av} values for the isothiocyanato complex species, was employed (Fig. 3B). The temperatures of columns α and β were maintained at $0^\circ C$; the K_{av} values of the *trans* and *cis* geometric isomer of the $n=4$ complex and the $n=5$ and 6 complexes were different enough to be distinguishable. Since the SCN^- and the $n=0-2$ complexes could not be separated with the Sephadex G-25 column, the cation-exchange column (γ) was connected at the outlet of the gel columns to remove the cationic species of chromium(III). It was possible to separate the $n=0, 1$ and 2 complexes

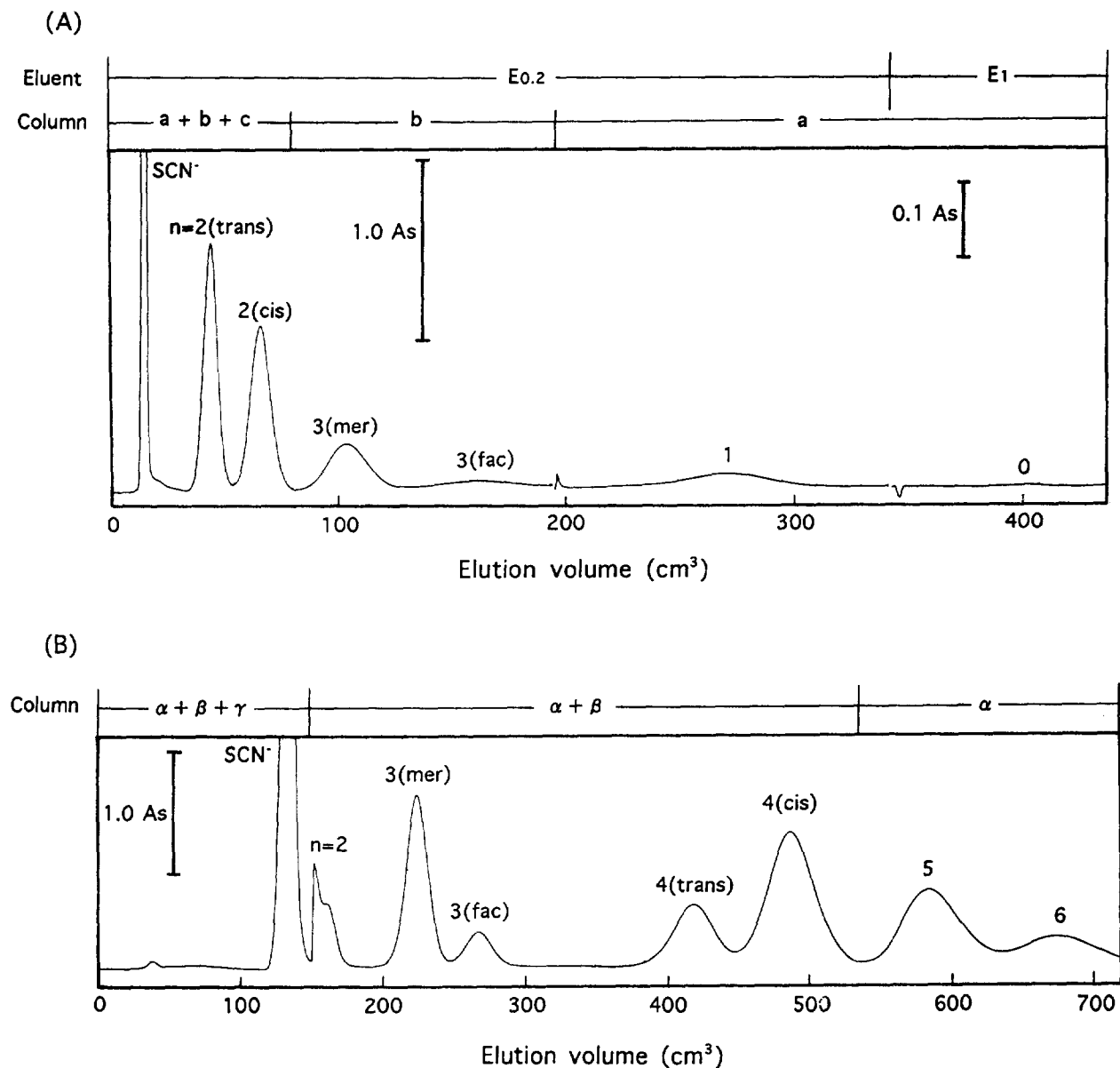


Fig. 4. Chromatographic separation of the successive Cr-NCS complexes including geometric isomers. (A) Elution curve for the $n = 0-3$ complexes and SCN^- using separation system I. (B) Elution curve for the successive Cr-NCS complexes using separation system II. Wavelength: 240 nm for free SCN^- and the $n = 1-6$ complexes and 570 nm for the $n = 0$ complex.

using the longer cation-exchange column, but this system was not efficient enough to separate them within a short time. A typical elution curve is shown in Fig. 4B. When SCN^- was first eluted and the $n = 0$ and 1 complexes were trapped by

column γ , the column was bypassed. The $n = 2$ complexes were eluted followed by the *mer* and *fac* isomers of the $n = 3$ complex and the *trans* and *cis* isomers of the $n = 4$ complex. Finally, column β was bypassed and the $n = 5$ and 6

complexes were eluted from column α . Ten kinds of inert successive octahedral complexes were obtained for the first time in this study.

3.2. Identification

The identification of the successive complexes and the geometric isomers was based on the literature [3,6–8,11]. The elution order of all the complexes was roughly identical with that in the references. For the $n=2$ geometric isomers, Hougen et al. [6] explained that the *trans* isomer was more easily eluted from the cation-exchange column, and also eluted from the gel column. It is assumed that the less polar species are eluted more easily. In a similar way, for the isomeric $n=3$ and 4 complex pairs, the more easily eluted were the *mer* and *trans* isomer. Although the Raman spectra are available to identify the geometric isomers [15], a high concentration of the isolated sample is required and, therefore, the Raman spectra have not yet been measured. As both the sulfur and the nitrogen in thiocyanate are donor atoms, the binding isomers are possible. However, it was reported that the isomerization constant for the mono complex, $[\text{Cr-NCS}]/[\text{Cr-SCN}]$, was very large and the Cr-SCN complex was hardly formed [16].

3.3. Adsorption of Cr-NCS successive complexes on gels

The K_{av} value corresponds to the equilibrium constant based on partitioning of the solute between the gel phase and the bulk phase outside the gel particles. The thermodynamic functions can be obtained by the equation

$$-RT \ln K_{av} = \Delta G$$

$$\Delta G = \Delta H^\circ - T \Delta S^\circ$$

where ΔG denotes the free energy change, ΔH° the standard enthalpy change, ΔS° the standard entropy change, required to transfer 1 mol of the solute from the bulk to the gel phase, R the gas constant and T absolute temperature. When the plot of $\Delta G/T$ against $1/T$ (van't Hoff plot) is linear, ΔH° can be obtained from the slope and

ΔS° from the intercept. For Toyopearl HW-40, the K_{av} values of the *mer* and *fac* $n=3$ complexes were measured by both the column and batch methods, and the values obtained from respective methods were similar to each other.

As shown in Table 1, the values of ΔH° and ΔS° obtained were negative: they were different from those of the solutes with alkyl groups, such as aliphatic alcohols or tetraalkylammonium ions [17–19], and the adsorption was enthalpic, similar to that of inorganic solutes such as SCN^- , Cl^- or Na^+ [18]. The thermodynamic functions, ΔH° and ΔS° , depend on the number of ligands n , as shown in Table 1. The contributions of ΔH° and ΔS° to the overall free energy change were partially compensated in each case with similar proportions, but the relationships between the functions and the number of the ligands were not linear. The linear free energy relationships (LFER) between ΔH° values for Sephadex G-25 and those for Toyopearl HW-40 were found to hold for the $n=0-3$ and $n=3-6$ complexes (Fig. 5). The adsorption of the complexes for Sephadex G-25 and for Toyopearl HW-40 are regarded as identical, and two kinds of interactions may be present.

In general, the bulk water forms the so-called "iceberg structure". In aqueous solution, solutes change the water structure depending on their structure-making or -breaking properties [20]. The Cr-NCS complexes are regarded as structure breakers. In the bulk phase the complexes break the water structure. On the other hand, hydrophobic alkyl groups and hydrophilic hydroxyl groups are present in the gel matrices. The gel phase is regarded as one kind of mixed solvent like an aqueous methanol solution, and therefore the water structure is partly broken: methanol is known to be a water structure breaker. As a result, the insertion of the complex in the gel phase affects the structure breaking of water to a small extent. The degree of water structure breaking in the Toyopearl gel phase is expected to be much larger than that in the Sephadex gel phase. The N atom of SCN^- coordinates to Cr, and the hydrophobic S is directed towards the outside of the complex. The hydrophobicity, or structure-breaking property,

Table 1
Thermodynamic functions for the successive chromium(III) complexes

Complex ^a	Toyopearl HW-40			Sephadex G-25		
	ΔG° (kJ mol ⁻¹)	ΔH° (kJ mol ⁻¹)	ΔS° (J mol ⁻¹ K ⁻¹)	ΔG° (kJ mol ⁻¹)	ΔH° (kJ mol ⁻¹)	ΔS° (J mol ⁻¹ K ⁻¹)
$n = 0$	-0.631	-7.96	-24.6	-0.181	-3.33	-7.37
$n = 1$	-1.51	-5.98	-15.0	-0.260	-3.46	-10.9
$n = 2$ <i>trans</i>	-2.52	-6.24	-12.4	-0.916	-1.85	-3.11
<i>cis</i>	-3.65	-9.62	-19.4	-1.06	-3.67	-8.76
$n = 3$ <i>mer</i> (c)	-6.52	-14.4	-26.8	-1.98	-5.22	-10.9
<i>mer</i> (b)	-7.30	-16.5	-31.2			
$n = 3$ <i>fac</i> (c)	-7.91	-21.5	-46.0	-2.23	-8.02	-19.4
<i>fac</i> (b)	-8.17	-22.0	-45.8			
$n = 4$ <i>trans</i>	-11.2	-20.7	-32.0	-3.18	-9.61	-21.6
<i>cis</i>	-12.4	-25.5	-44.0	-3.29	-13.4	-33.8
$n = 5$	-16.6	-31.6	-50.4	-4.22	-22.6	-61.6
$n = 6$	-18.3	-37.3	-63.5	-4.11	-28.5	-81.7

^a (c) Measured by the column method; (b) measured by the batch method.

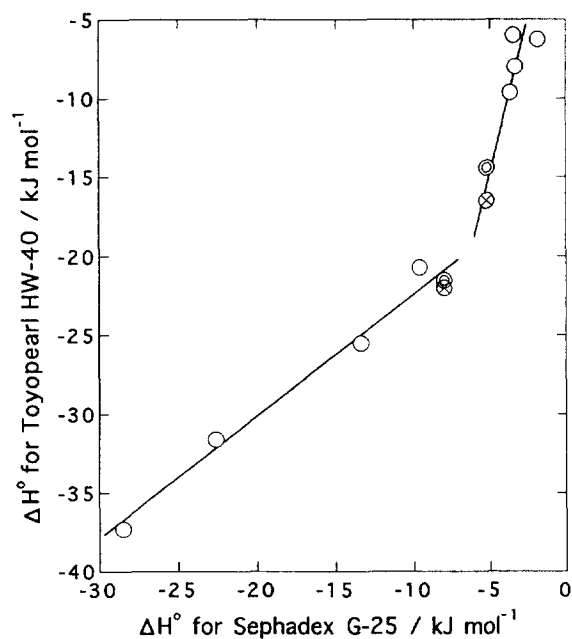


Fig. 5. Linear free energy relationships between the ΔH° values for Sephadex G-25 and Toyopearl HW-40. \circ = By the column method; \otimes = by the batch method.

of the complex is expected to be greater than that of free SCN^- , and this increases with increasing number of the hydrophobic ligands in the complex. The contribution of increasing n to the ΔH° value corresponded to about -7 kJ mol⁻¹ for both Toyopearl HW-40 and Sephadex G-25. The geometric isomers have different structure-breaking properties owing to their polarity. The effect will be greater for more polarized species, and therefore the *cis* or *fac* isomers have greater K_{av} values than the *trans* or *mer* isomers.

On the other hand, the aqua complex and the mono complex, which has many hydrophilic H_2O in the first hydration sphere, have unexpectedly large K_{av} values. There are many hydroxyl groups in the gel matrices forming hydrogen bonds with the H_2O in the complex. Although coordinated water molecules in labile complexes also interact with the hydroxyl groups by hydrogen bonding, they cannot contribute to such a strong adsorption. In contrast, the H_2O in the Cr(III) complex is inert and the interaction between the stationary hydroxyl group in the gel matrices and the inert, H_2O in the complex will play an important role in this adsorption.

The interaction concerned with SCN increased and that concerned with H_2O decreased, with

increasing SCN ligand number. Therefore, the ΔH° value depended on n as shown in Table 1. Two LFERs shown in Fig. 5 will be ascribed to different contributions of respective interactions between Toyopearl HW-40 and Sephadex G-25.

4. Conclusion

The most important result of our study is that all ten kinds of successive octahedral complexes including geometric isomers were obtained for the first time. The combination of Toyopearl HW-40 and Sephadex G-25 gels and AG 50W-X4 cation exchanger made it possible to separate them completely. As a result, the absorption spectra of all the complexes could be obtained. In addition, our separation system can afford a simple, rapid method for the investigation of the whole equilibrium and the kinetics on the aquachromium(III)–isothiocyanato complex system. Results on these topics will be presented elsewhere in detail.

The mechanism of the adsorption of the complexes on the gel phase could be interpreted in terms of hydration structure of complexes in bulk and gel phases and hydrogen bonding between the inert H₂O ligand of the complexes and hydrophilic groups in the gel matrix.

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