

High Performance Ion-exchange Chromatography of Cobalt(III) Complexes of Charge +3

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A search for a microanalytical method for separation and detection of isomers of cobalt(III) complex ions of charge +3 has led to a HPLC investigation. It is shown that a column material TSK gel SP-5PW with sulfopropyl functional groups, gives HPLC ion exchange results which are comparable with those obtained on macro scale with SP Sephadex chromatography. Retention volumes are related to outer-sphere association constants, and it is noted that this technique provides an easy way to obtain information on outer-sphere association. The temperature dependence of the degree of separation has been rationalized in terms of the difference between the enthalpies of reaction for the ion exchange and for the ion association.

Ion exchange chromatography (IEC) on SP-Sephadex has been used extensively for the separation of geometric isomers, diastereoisomers and enantiomers of coordination compounds.¹ These separations are generally time consuming, and may take days if long columns are required to achieve a complete separation. Reversed phase high performance liquid chromatography (RP-HPLC) on C18 columns has recently been shown to be very successful in separating complexes of charge 0, 1 and 2,²⁻⁵ and the efficiency and speed of the separations are superior to those obtained by normal IEC. RP-HPLC has not, however, been satisfactory in separating trivalent ions.⁴ With the reagents reported, rather poor separations have been achieved, since the high positive charge results in broad bands. It thus appears that for cationic coordination compounds in general, it is hard to benefit from the advantageous speed of RP-HPLC, and it was thought that it might be better to use an ion-exchange material containing sulfopropyl functional groups, as in SP Sephadex C 25 which is widely used to separate isomers of trivalent coordination compounds. Our recent attempts to isolate as many as possible of the eight isomers in the bis[(2-aminoethyl)(3-aminopropyl)sulfide]cobalt(III) system have led us to try an ion exchange column of this nature which has already been used extensively for protein separations.⁶ The effectiveness of a HPIEC column in separating isomers and closely related cations of charge +3 is demonstrated in this paper, and a quantitative rationalization which makes it possible to investigate measurements of outer-sphere association constants and their temperature dependence is given.

EXPERIMENTAL

Materials. The following cobalt(III) complexes were prepared by literature methods: 2 Co(en)₃Cl₃, NaCl, 6 H₂O, (en=1,2-ethanediamine),⁷ Co(tn)₃Cl₃, H₂O, (tn=1,3-

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propanediamine),⁸ $\text{Co}(\text{daes})_2\text{Cl}_3$, H_2O , (daes=di(2-aminoethyl)sulfide)⁹ and $\text{Co}(\text{dien})_2(\text{NO}_3)_3$ (dien=1,4,7-triazaheptane).¹⁰ The preparation and characterization of the isomers of $\text{Co}(\text{aeaps})_2\text{Cl}_3$ [aeaps=(2-aminoethyl)(3-aminopropyl)sulfide] will be reported.¹¹ Sodium di- μ -(*R,R*)-tartrato(4-)-diantimonate(III) monohydrate (BDH) was of reagent grade while all other reagents were of analytical grade.

Apparatus and columns. A Waters HPLC system (M6000 A solvent delivery unit, M481 variable-wavelength detector, U6K universal injector) was employed. Chromatograms were recorded on an OmniScribe recorder from Houston Instruments. HPIEC was carried out with a TSKgel SP-5PW column from Toyo Soda, Japan, and a Protein Pak SP-5PW column from Waters. Both had the dimensions 75 mm \times 7.5 mm ID. The number of effective plates in each column was measured to be more than 1400 at 25 °C and their performance was found to be identical. The ion-exchange material, TSKgel SP-5PW, employed in both columns contains approximately 0.12 mequiv anions/ml (as $-\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$) in particles with a diameter of 10 μm and an average pore diameter of approximately 1000 Å.⁶

Solvents and sample preparation. The eluent solutions were prepared using distilled deionized water. The following eluents have proved to have general applicability: 0.30 M citrate buffer (19.8 g citric acid monohydrate and 49.2 g trisodium citrate dissolved to 1 l solution, pH measured to 4.9), 0.25 M sodium di- μ -(*R,R*)-tartrato(4-)-diantimonate(III) (154.4 g dihydrate dissolved to 1 l solution) and 0.30 M sodium sulfate (42.6 g anhydrous salt per l, pH was adjusted to pH 4.0 with sulfuric acid). Solutions were filtered and degassed before use by passage through an all-glass filter unit fitted with a 0.45 μm membrane (Millipore MF type membrane).

Solutions were generally prepared by dissolving the pure compounds in 0.01 M HCl and filtering the solutions through a Millex HV₄ filter unit (Durapore 0.45 μm) prior to injection. Crude products, etc., containing strongly retained compounds (e.g. traces of highly charged polynuclear complexes) were purified by prechromatography on a small column filled with SP-Sephadex C25 (15 mm \times 5 mm ID) using the same eluent as in the HPLC experiments. The eluate from this column was diluted 1:1 prior to injection with distilled water and filtered through a Millex HV₄ filter unit. The anionic complex $\text{Co}(\text{edta})^-$ as the potassium salt¹², was used as a marker for the void volume.

Temperature control. In order to carry out HPLC at different temperatures, the column and inlet were immersed in a thermostat bath. Temperature control was achieved by electrical heating (50 °C) and by cooling with ice (0 °C). The column was not thermostated when used at room temperature (21–23 °C).

Analytical procedure. The column was washed initially with distilled deionized water (20 ml, 0.5 ml/min) and then equilibrated with the eluent (30 ml, 0.5 ml/min). All separations were made with isocratic elution. Samples of 2–150 μl (Hamilton syringe 25 μl and 250 μl) were injected into the injector assembly unit. Chromatograms were recorded from 0–50 min with full scale absorbance in the range 0.01–2.0 at different wavelengths depending upon the complexes.

RESULTS

Separation of different 3+ ions. The separation of tris(1,2-ethanediamine)cobalt(III) ions and hexaamminecobalt(III) ions obtained by elution with 0.3 M sodium sulfate (pH 4) is shown in Fig. 1. The separation is achieved in less than 20 min with a flow rate of 1.0 ml/min. This is of course much faster than a conventional separation on Sephadex. The eluent concentration was varied and the optimum concentration was found to be 1.5–2 times that used in Sephadex chromatography. The capacity of the column was tested by injection of increasing amounts of the complexes. Band shapes were ideal up to the point of over-loading, corresponding to 500 nmol of trivalent complex. However, at levels 300 nmol per column, a decrease in the degree of separation was observed.

Effect of column temperature variations. The separation of the four cations $\text{Co}(\text{en})_3^{3+}$, $\text{Co}(\text{tn})_3^{3+}$, *trans*-5,5-*u-fac*- $\text{Co}(\text{aeaps})_2^{3+}$ and *trans*-5,5-*s-fac*- $\text{Co}(\text{aeaps})_2^{3+}$ (see below), with

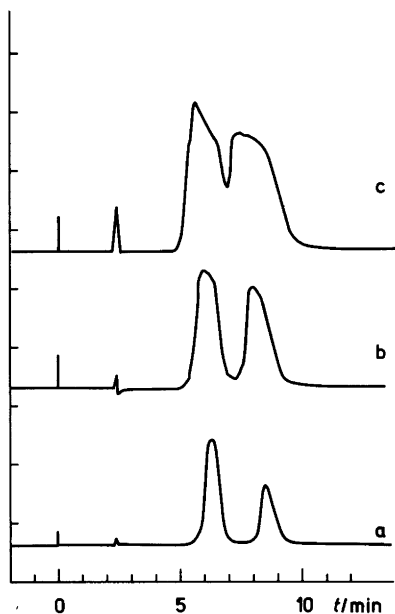


Fig. 1. Chromatograms for the separation of Co(en)_3^{3+} and $\text{Co(NH}_3)_6^{3+}$. Eluent 0.30 M Na_2SO_4 , pH 4.4; flow-rate 1.0 ml/min, chart speed 0.5 cm/min, column temperature 22 °C. (a) 40 nmol (4 μl) of each cation, (b) 160 nmol (16 μl) and (c) 640 nmol (64 μl) of each complex. Monitor wavelength 450 nm, ordinate division is 0.1 absorbance.

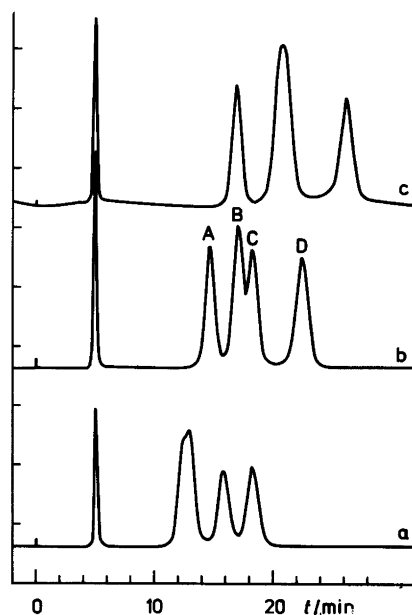


Fig. 2. Separation of the cations A: Co(en)_3^{3+} , B: Co(tn)_3^{3+} , C: *trans*-5,5-*u-fac*- Co(aeaps)_2^{3+} , and D: *trans*-5,5-*s-fac*- Co(aeaps)_2^{3+} . Eluent 0.30 M Na_2SO_4 , pH 4.3 with flow-rate 0.5 ml/min. Detection at 250 nm, ordinate division 0.05 A. 12, 6, 8 and 12 nmol of each cation in 10 μl applied with Co(edta)^- as marker. Column temperature in (a), (b) and (c) were 0, 22 and 46 °C, respectively.

sulfate eluent is shown in Fig. 2 and they demonstrate the important influence of column temperature. By chance, the best separation of this mixture is achieved at room temperature, but separations of other complexes have been found to require either higher or lower temperature. The number of effective plates varies with temperature. For this particular column we have found the number of effective plates to be 1400, 2000 and 2600 at 0, 22 and 50 °C, respectively.

Separation of geometric isomers. Separation of geometric isomers is probably the most important application of SP-Sephadex chromatography. The chromatogram in Fig. 3a shows the separation of a mixture of four geometric isomers of Co(aeaps)_2^{3+} . A total of eight geometric isomers are possible (see Fig. 4) and four of these have been isolated and characterized.¹¹ The isolation on a preparative scale involved fractional crystallization and IEC on SP-Sephadex. HPIEC has been used as an analytical tool to check isomeric purity and to obtain information about the composition of the crude product from the synthesis. For this system, 0.3 M sodium sulfate proved to be unsatisfactory since two isomers overlapped heavily at 0 °C, 22 °C and 50 °C. Instead, a sodium citrate buffer was found to be efficient, as show in Fig. 3a. Artificially made mixtures of the four isolated geometric isomers were easily analyzed. Fig. 5 shows the chromatogram of the crude product. The four

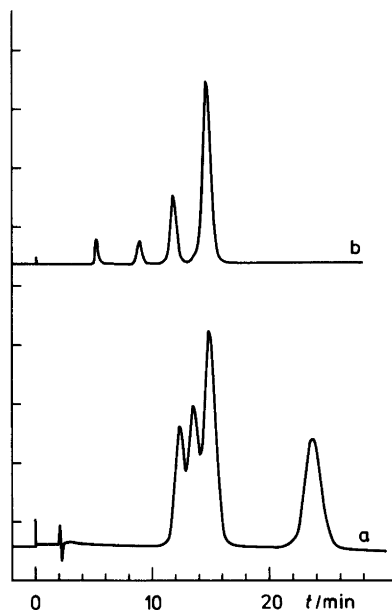


Fig. 3. a. The separation of an artificial mixture of $\text{Co}(\text{aeaps})_2^{3+}$ isomers. 25 μl containing *trans*-5,6-*u-fac*- (16 μmol), *trans*-5,5-*u-fac*- (16 μmol), *trans*-6,6-*u-fac*- (25 μmol), and *trans*-5,5-*s-fac*- $\text{Co}(\text{aeaps})_2^{3+}$ (20 μmol) and eluted in that order. Eluent 0.30 M sodium citrate/ citric acid pH 4.9, flow-rate 1.0 ml/min, column temperature 22 °C. Detection at 250 nm, ordinate division 0.05 A. b. Chromatogram of a crude product containing the three geometric isomers of $\text{Co}(\text{dien})_2^{3+}$. Chromatographic conditions as in Fig. 2b. The isomers are eluted in the order *s-facial*, *u-facial*, and last, the *meridional*.

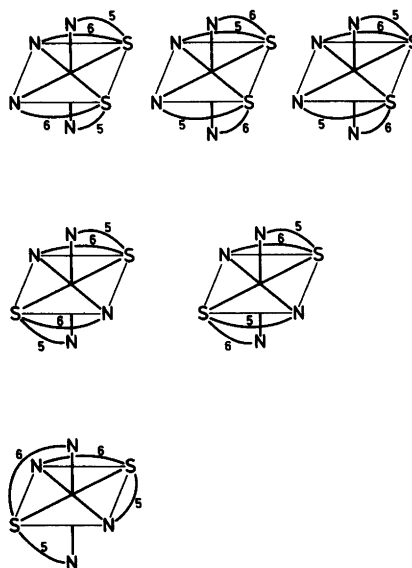


Fig. 4. Diastereoisomers of bis((2-aminoethyl)(3-aminopropyl) sulfide)cobalt(III), where 5 and 6 shows the position of five- and six-membered rings, respectively. The first four have been characterized and they are *trans*-6,6-*u-fac*-, *trans*-5,5-*u-fac*-, *trans*-5,6-*u-fac*- and *trans*-5,5-*s-fac*- $\text{Co}(\text{aeaps})_2^{3+}$. The meridional figure represents three diastereoisomers.

isolated isomers dominated, but by increasing the sensitivity of the detector, three more very weak bands were detected, indicating that all the possible isomers may be formed.

The separation of the three isomers of the $\text{Co}(\text{dien})_2^{3+}$ was possible using sulfate eluent, as shown in Fig. 3b.

Enantiomeric separations. The resolution of enantiomers by SP-Sephadex IEC with the use of an optically active eluent is very important on a preparative scale. HPIEC may prove very useful as an analytical tool for checking enantiomeric purity, provided the separation is complete. The resolution of tris(1,2-ethanediamine)cobalt(III) ions obtained using 0.26 M sodium di- μ -(*R,R*)-tartrato(4-)-diantimonate(III) (sodium antimonyl tartrate) as eluent is shown in Fig. 6. A very slight contamination with the undesired enantiomer can clearly be detected with this method. Fig. 6 also shows the separation of Λ and Δ *trans*-6,6-*u-fac*- $\text{Co}(\text{aeaps})_2^{3+}$ by the same eluent. These two separations are representative of those which have been performed on SP Sephadex and which can be achieved on an analytical scale by HPIEC.

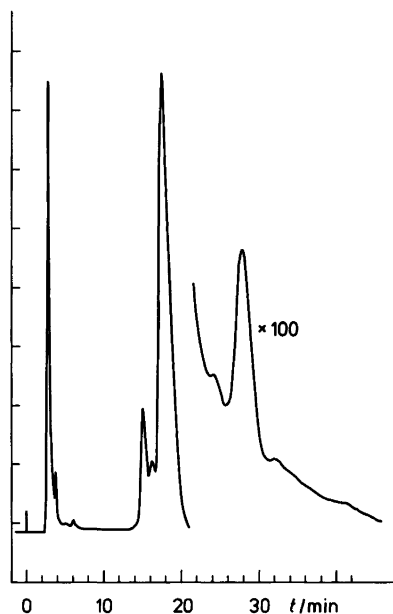


Fig. 5. Chromatogram of crude product from synthesis of $\text{Co}(\text{aeaps})_2^{3+}$. Eluting conditions as discussed for Fig. 3 except column temperature which was 50°C . $\text{KCo}(\text{edta})$ was added to mark the front. The first eluted isomers are III, II and then I of fig. 4, while isomer IV gives the dominating peak observed after increasing the sensitivity.

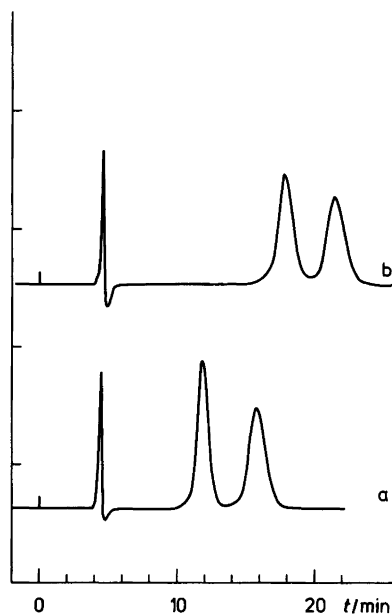
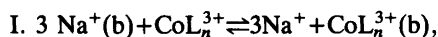


Fig. 6. Separation of enantiomers. Eluent 0.26 M "sodium antimonyl tartrate", flow-rate 0.5 ml/min , column temperature 22°C . (a): $10\ \mu\text{l}$ with 129 nmol of $\text{Co}(\text{en})_3^{3+}$. Detection at 469 nm ordinate division 0.005 A . The $\Delta(+)_589$ enantiomer is eluted first. (b) $10\ \mu\text{l}$ with 57 nmol of *trans*- $6,6\text{-u-fac-Co}(\text{aeaps})_2^{3+}$. Detection at 500 nm . The $\Delta(+)_589$ enantiomer has the smaller retention time.

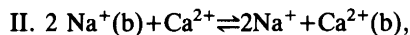
DISCUSSION

High-performance ion-exchange chromatography (HPIEC) on columns of the strong cation exchanger SP-5PW has proved to be very suitable for the rapid (10–30 min) analysis of mixtures of multivalent complexes. The SP-5PW ion exchange material contains sulfopropyl groups bound to a rigid matrix of G5000 PW, which is a hydrophilic polymer-based material with high porosity employed in high performance gel filtration.⁶ The gel is stable in normal aqueous salt buffer solutions (1 M) in the pH-range 2 to 12. The speed and the separation efficiency are much better than for conventional IEC. However, the general methodology is quite analogous to that employed with SP-Sephadex, which means that most of the experience obtained with SP-Sephadex columns is applicable to this new type of HPLC column. However, the ease and reproducibility of HPIEC will soon reverse this situation, since pilot experiments can be carried out conveniently with HPLC equipment. The following section considers some relationships which are relevant in the design of new separations.

Basic considerations. In order to be able to make use of ion association and of its temperature variation, it may be worthwhile to consider the effect in some detail. The pure ion-exchange reaction is



where the ions with (b) are bound to the macromolecular polymer. This reaction is expected¹³ to have positive enthalpy and entropy changes analogous to those for the reaction



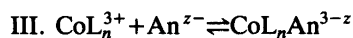
$$(\Delta H_{\text{II}}^{\circ} = 6.4 \text{ kJ mol}^{-1}, \Delta S_{\text{II}}^{\circ} = 38 \text{ J K}^{-1} \text{ mol}^{-1})$$

which has been investigated on Dowex ion exchanger.¹⁴ The entropy change in I is predicted to be positive since a net release of ions occurs, and in dilute aqueous solutions this provides the driving force for the reaction (negative $\Delta G_{\text{I}}^{\circ}$). Thus, at low ionic strength, the free energy change of I is dominated by the entropy term:

$$\Delta G_{\text{I}}^{\circ} = \Delta H_{\text{I}}^{\circ} - T\Delta S_{\text{I}}^{\circ}.$$

This explains why highly charged ions preferably are bound to the column.

Outer-sphere complex formation with the anion An^{z-}



is also expected to occur with a free energy change dominated by the entropy term at low ionic strength. Outer-sphere complex formation has been studied extensively and a theoretical model based on electrostatic interactions has been developed¹⁵ and extended.¹⁶ *E.g.* has equilibrium data for the process



been obtained by spectrophotometry at several temperatures.¹⁷ From these measurements the following parameters have been calculated by extrapolation to zero ionic strength at 25 °C:

$$\Delta G_{\text{IV}}^{\circ} = -19.1 \text{ kJ/mol}, \Delta H_{\text{IV}}^{\circ} = 1.7 \text{ kJ/mol}, \Delta S_{\text{IV}}^{\circ} = 70 \text{ J K}^{-1} \text{ mol}^{-1}.$$

ΔH for this type of reaction is known to remain relatively constant with increasing ionic strength, whereas ΔG decreases.¹⁸

The electrostatic model for such outer-sphere complex formation or ion-pair association predicts an equilibrium constant^{15,16,18,19}

$$K = \frac{4\pi N_A a^3 \exp(b)}{3000}, \text{ where } b = \frac{z_+ z_- e^2}{DkTa}$$

N_A is Avogadro's number, a is the sum of the radii of the two ions, z_+ and z_- are the charges of the ions, e is the charge of an electron, D is the dielectric constant of the medium, k is Boltzmann's constant and T is the temperature. This simple model explains the observation that two geometric isomers have different association constants, if the effective

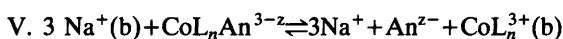
contact distance between cation and anion is smaller for one part than for the other. The enthalpy of reaction for ion association varies with the distance according to

$$\Delta H^{\circ} = -RTb\left(1 + \frac{d \ln D}{d \ln T}\right).$$

For water $d \ln D/d \ln T$ is -1.37 and consequently ΔH° is positive and inversely proportional to the sum of the ionic radii.¹⁹

It has been suggested that hexaamminecobalt(III) complexes may bind phosphate ions and sulfate ions in a more specific way, such that three hydrogen atoms from nitrogens spanning an octahedral trigonal face form hydrogen bonds to three oxygen atoms from the anion.^{10,20} This proposal is possibly useful but it is not necessary to invoke it to explain the gross effect. However, when the cation and the anion have been forced into contact by nonspecific electrostatic forces it is quite possible that different geometric isomers may have slightly different association constants for just this reason. Different values for the pure ion-exchange equilibrium constants for geometric isomers may also originate in slightly different hydrogen bonding arrangements.

Separations in general. The over-all equilibrium important in the column chromatography is



The reaction V is a sum of I and -III and thus the free energy change and the enthalpy changes of the reaction are

$$\Delta G_V^{\circ} = \Delta G_I^{\circ} - \Delta G_{III}^{\circ} \text{ and } \Delta H_V^{\circ} = \Delta H_I^{\circ} - \Delta H_{III}^{\circ}.$$

A description of V is thus possible if the free energy and enthalpy changes for the reactions I and III are known. From the experiments reproduced in Fig. 2 it is possible to deduce that $\Delta H_I^{\circ} > \Delta H_{III}^{\circ}$ since the equilibrium constant for the process V

$$K_V = \exp(-\Delta G_V^{\circ}/RT)$$

has a temperature dependence dictated by ΔH_V° according to

$$\frac{d \ln K_V}{dT} = \frac{\Delta H_V^{\circ}}{RT^2}$$

Separations of geometric isomers or of related complexes of identical charge can therefore often be improved by changing the column temperature.

Resolution of a Racemate. When enantiomers are separated on an SP column this is solely due to a difference in ΔG_{III} -values for the pair since only a chiral eluent will produce a resolution. However, chiral column materials have been used successfully in resolutions of enantiomers²⁰ and in such columns there are contributions to ΔG_V from ΔG_I . In our case, it is possible to conclude that the influence of the temperature on the resolution should be low because ΔH_{III} small and nearly the same for the two diastereomeric systems. This has also been observed experimentally. Therefore when a separation of enantiomers is unsatisfactory it is advisable to try other concentrations of the chiral eluent or change to another eluent.

REFERENCES

1. Yoshikawa, Y. and Yamasaki, K. *Coord. Chem. Rev.* 28 (1979) 205.
2. Warner, B.D. and Legg, I.J. *Inorg. Chem.* 20 (1981) 1625.
3. Isied, S.S., Lyon, J., Vassilian, A. and Worosila, G. *J. Chromatogr.* 262 (1983) 537.
4. Buckingham, D.A., Clark, C.R., Deva, M.M. and Tasker, R.F. *J. Chromatogr.* 262 (1983) 219.
5. Buckingham, D.A., Clark, C.R. and Tasker, R.F. *Inorg. Chem.* 22 (1983) 2772.
6. Kato, Y., Nakamura, K. and Hashimoto, T. *J. Chromatogr.* 294 (1984) 207.
7. Meisenheimer, J., Angermann, L. and Holsten, H. *Justus Liebigs Ann. Chem.* 438 (1924) 269.
8. Bailar, J.C. and Work, J.B. *J. Am. Chem. Soc.* 68 (1946) 232.
9. Searle, G.H. and Larsen, E. *Acta Chem. Scand. A* 30 (1976) 143.
10. Keene, F.R. and Searle, G. *Inorg. Chem.* 11 (1972) 148.
11. Bjerrum, M.J., Lair, T. and Larsen, E. *Inorg. Chem.* *In press.*
12. Kirschner, S. *Inorg. Synth.* 5 (1957) 186.
13. Helfferich, F. *Ion Exchange*, McGraw-Hill, New York 1962, p. 166.
14. Jensen, B.S. and Jensen, H. *Risø Nat. Lab.*, DK-4000 Roskilde. *Private communication.*
15. Bjerrum, N. K. *Dan. Vidensk. Selsk. Mat.-Fys. Medd.* 7 (1926) No. 9.
16. Fuoss, R.M. *J. Am. Chem. Soc.* 78 (1958) 5059.
17. Posey, F.A. and Taube, H. *J. Am. Chem. Soc.* 78 (1956) 15.
18. Bjerrum, J. *Proc. 3rd. Symp. Coord. Chem. 2, Debracen, Hungary 1970*, Akademiai Kiado 1971.
19. Prue, J.E. *J. Chem. Educ.* 46 (1969) 12.
20. Mason, S.F. *Molecular Optical Activity and the Chiral Discriminations*, Cambridge Univ. Press, London, New York 1982.
21. Okamoto, Y., Honda, S., Yashima, E. and Yuki, H. *Chem. Lett.* (1983) 1221.
22. Rossotti, F.J.C. and Rossotti, H. *The Determination of Stability Constants*, McGraw-Hill, New York 1961.

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