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# Association between cobalt(III) coordination compounds and anions measured by capillary electrophoresis

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## Abstract

This study reports an analysis of the association between various cobalt(III) coordination compounds and the anions perchlorate and sulphate using capillary electrophoresis. Apart from determining the association constants, it is shown that activity correction is necessary for accurate determination of association constants using CE. It is further shown, that the electric field has no significant influence on the results. The constants obtained after activity corrections for the association of cobalt(III) coordination compounds of charge +1 or +2 with either  $\text{ClO}_4^-$  or  $\text{SO}_4^{2-}$  agree well with values obtained in other studies. For cobalt coordination compounds of charge +3 deviations occur, probably due to binding of more than one ligand. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Association constants; Activity coefficients; Coordination compounds; Inorganic anions; Cobalt; Inorganic cations

## 1. Introduction

The majority of studies using capillary electrophoresis (CE) is on analysis of organic compounds and biomolecules. This includes both separation of mixtures of such compounds as well as analysis of substrate–ligand complexes using different modes of affinity capillary electrophoresis. For a review see for example Busch et al. [1]. Much less attention has been devoted to the implementation of CE as a tool for analysis in inorganic systems even though electrophoresis has been applied for separation of charged inorganic coordination compounds as well as for studying complex ion equilibria as long ago as in 1951 [2].

We have undertaken this study in order to explore the use of CE for the separation of classic inorganic

coordination compounds and for the analysis of formation of outersphere coordination of those species, using coordination compounds of cobalt(III) as model compounds. Cobalt(III) coordination compounds are, compared to biomolecules, small spherical species with a very simple charge distribution. This means, that their behaviour in an electric field will be rather well defined. Therefore, besides providing information concerning separations of inorganic compounds, those molecules can serve as models for some of the factors involved in the separation and analysis of more complex species such as proteins and nucleic acids by CE.

The measured or apparent mobility,  $\mu_{\text{app}}$ , of a molecule in a CE capillary consists of two contributions:

$$\mu_{\text{app}} = \mu_e + \mu_{e_0} \quad (1)$$

where  $\mu_e$  is the part of the apparent mobility that is due to the electrophoretic mobility and  $\mu_{e_0}$  is the part

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of the apparent mobility that is due to the electroosmotic flow in the capillary. The contribution from the electroosmotic flow can be experimentally eliminated or measured and subtracted from the apparent mobility thereby giving information of the electrophoretic mobility.

The ability to separate inorganic charge compounds by CE depends primarily on differences in charge and size of the compounds. The electrophoretic mobility of each compound is given by Stokes law:

$$\mu_e = \frac{zE}{6\pi\eta r} \quad (2)$$

where  $z$  is the ionic charge,  $\eta$  is the viscosity of the solution and  $r$  is the Stokes radius of the ion (the radius that determines the resistance to flow).

The association constant  $K$  between a cationic cobalt coordination compound  $[\text{Co}(\text{L})_n]^{m+}$  and an anion  $\text{A}^{k-}$  can be expressed by

$$K = \frac{[\text{Co}(\text{L})_n\text{A}^{m-k}]}{[\text{Co}(\text{L})_n]^{m+}[\text{A}^{k-}]} \quad (3)$$

where  $[\text{Co}(\text{L})_n\text{A}^{m-k}]$  represents the concentration of the association complex between the anion and the cobalt(III) innersphere complex. The electrophoretic mobility of the free and the associated compound, respectively will be different mainly because of the different ionic charges.

Since association is a dynamic process the effective electrophoretic mobility,  $\mu_{\text{eff}}$ , of the compound will depend on, for how long an anion is associated relative to the time where it is in the unassociated state. This can be expressed by

$$\mu_{\text{eff}} = \frac{[\text{Co}(\text{L})_n]^{m+}}{[\text{Co}(\text{L})_n]^{m+} + [\text{Co}(\text{L})_n\text{A}^{m-k}]} \mu_{\text{M}} + \frac{[\text{Co}(\text{L})_n\text{A}^{m-k}]}{[\text{Co}(\text{L})_n]^{m+} + [\text{Co}(\text{L})_n\text{A}^{m-k}]} \mu_{\text{MA}} \quad (4)$$

in a simple system where only 1:1 association takes place. The two fractions represent the mole fractions of unassociated complex and the mole fractions of associated complex at a given time.  $\mu_{\text{M}}$  is the electrophoretic mobility of the unassociated coordination compound, and  $\mu_{\text{MA}}$  is the mobility of the associated compound. Those two equations leads to

the following general expression [3] for the estimation of association constants by ACE:

$$\mu_{\text{eff}} = \frac{\mu_{\text{M}} + \mu_{\text{MA}}K[\text{A}^{k-}]}{1 + K[\text{A}^{k-}]} \quad (5)$$

If both  $\mu_{\text{M}}$  and  $\mu_{\text{MA}}$  are known,  $K$  can be determined at any anion concentration. In a simple 1:1 association system  $\mu_{\text{M}}$  can be determined by extrapolation to infinite low concentrations of  $\text{A}^{k-}$ , while  $\mu_{\text{MA}}$  will be the limiting mobility at high concentrations of  $\text{A}^{k-}$ . Often  $\mu_{\text{MA}}$  cannot be determined because of practical reasons such as solubility of  $\text{A}^{k-}$  or joule heating in the capillary due to high conductivity of the buffer. Alternatively, values of  $K$  and  $\mu_{\text{MA}}$  can be obtained by nonlinear fitting after plotting the mobility versus the anion concentration. However, such an approach contains a serious generalisation since the equilibrium constant is dependent on the ionic strength of the solution.  $K$  obtained by nonlinear fitting in this way is therefore an average value of  $K$  in the examined concentration area unless other corrections is taken into consideration.

The association constant at zero ionic strength,  $K^0$ , is related to  $K$  in a given solution by the expression

$$\log K^0 = \log \left( K \frac{\gamma_{ij}}{\gamma_i \gamma_j} \right) \quad (6)$$

where  $\gamma_i$ ,  $\gamma_j$ , and  $\gamma_{ij}$  are the activity coefficients of the coordination compound, of the anion and of the associated compound, respectively, in the solution. The activity coefficients vary strongly with the ionic strength: Generally the activity coefficients decrease when the ionic strength is increasing from 0 to 0.4, while relatively constant values are found for ionic strengths between 0.4 to 1. At higher ionic strengths an increase in the magnitude of the activity coefficients is observed. This means that in general no simple estimation of activity coefficients from the ionic strength is possible. However at rather small ionic strengths it is possible to determine the activity coefficients with a high degree of accuracy using Davies' equation [4] relating the ionic strength ( $I$ ) to the activity of the solution:

$$\log \gamma = -0.5z^2 \left( \frac{\sqrt{I}}{1 + \sqrt{I}} - 0.30I \right) \quad (7)$$

The ionic strength is expressed by

$$I = 0.5 \sum_i c_i z_i^2 \quad (8)$$

where  $c_i$  is the concentration of the ion  $i$  in the solution and  $z_i$  is the ionic charge of the same ion. Davies' equation is an empirical equation based on the activity of a large number of measured activity coefficients for various electrolytes in aqueous solution and are in general applicable for ion strengths up to 0.3 [4,5]. An ionic strength between 0 and 0.2 are typical for CE experiments, and Eq. 7 is therefore a much better choice in CE experiments than correction calculated from the generally more used Debye–Hückel theory.

The combination of Eqs. 5, 6 and 7 gives the following expression

$$\mu_{\text{eff}} = \frac{\mu_M + \mu_{MA} K^0 [A] 10^{(z_{ij}^2/z_i^2 z_j^2) ((\sqrt{I}/1 + \sqrt{I}) - 0.3I)}}{1 + K^0 [A] 10^{(z_{ij}^2/z_i^2 z_j^2) ((\sqrt{I}/1 + \sqrt{I}) - 0.3I)}} \quad (9)$$

In this study Eq. 9 has been used to determine the thermodynamic association constants between a series of cobalt(III) coordination compounds and the anions  $\text{SO}_4^{2-}$  and  $\text{ClO}_4^-$ . Furthermore, the relevance of such correction is discussed in relation to the experimental conditions. An important aim of this work has been to establish if the electric field in the capillary is affecting the value of the association constant and whether activity corrections as given by Davies' equation applies in such a system.

## 2. Experimental

### 2.1. Materials

The following cobalt(III) coordination compounds were prepared according to literature methods:  $[\text{Co}(\text{NH}_3)_6]\text{Cl}_3$  [6,7];  $\{[\text{Co}(\text{en})_3]\text{Cl}_3\}_2$ , NaCl,  $6\text{H}_2\text{O}$  (en = 1,2-ethandiamine) [8];  $[\text{Co}(\text{tn})_3]\text{Cl}_3$  (tn = 1,3-propandiamine) [9];  $[\text{Co}(\text{dien})_2](\text{NO}_3)_3$  (dien = diethylentriamine) [10];  $[\text{Co}(\text{NH}_3)_5\text{Cl}]\text{Cl}_2$  [11]; *cis*- $[\text{Co}(\text{en})_2(\text{NO}_2)_2]\text{NO}_3$  [12]; *mer*- $[\text{Co}(\text{gly})_3]$  (gly = glycine) [13], *u-fac*- $[\text{Co}(\text{daes})_2](\text{NO}_3)_3$  (daes = di(aminoethyl)sulfide) [14];  $[\text{Co}(\text{en})_2\text{Cl}_2]\text{Cl}$  [15]. All other chemicals were of analytical grade.

### 2.2. Apparatus

The separations were performed on a Hewlett-Packard 3D capillary electrophoresis apparatus (Hewlett-Packard, Waldbronn, Germany) at 40°C, using either a silica capillary (64.5 cm (56.0 cm to detection window)  $\times$  50  $\mu\text{m}$  I.D., extended lightpath) from Hewlett Packard or a BioCAP LPA coated capillary (68.5 cm (60 cm to the detection window)  $\times$  50  $\mu\text{m}$  I.D.) from Bio-Rad Labs. (Hercules, CA, USA). The capillaries were every day prior to use flushed with either 0.1 M NaOH (Hewlett Packard) (the silica capillary) or Capillary Wash Solution (the coated capillary), obtained from Bio-Rad Labs. for 2 min, followed by wash with buffer for another 2 min. All measurements were performed twice. The buffers in the inlet and outlet reservoirs were changed after each run to avoid pH changes.

### 2.3. Solvent and sample preparation

The buffers used for analysis of separation consisted of  $\text{HClO}_4$  (Merck, Darmstadt, Germany) adjusted to pH 3.1 using NaOH (Merck). The buffers for the association experiments consisted respectively of  $\text{HClO}_4$ –Tris and  $\text{H}_2\text{SO}_4$ –Tris at pH 7.4. All buffer solutions were prepared by dilution of known amount of either  $\text{HClO}_4$  (Merck) or  $\text{H}_2\text{SO}_4$  (Merck) in double-distilled water, adjusting the pH with Tris (Merck) and finally addition of water to the desired volume. Preparing the buffers this way assures a well defined solution with a fixed ionic strength, since every charged molecule of  $\text{TrisH}^+$  is counterion to  $\text{ClO}_4^-/\text{SO}_4^{2-}$ . From Eq. 8 it therefore follows that the ionic strength of the solutions of  $\text{HClO}_4$ –Tris is equal to the stoichiometric molar concentration of  $\text{HClO}_4$ , while for the solutions of  $\text{H}_2\text{SO}_4$ –Tris the ionic strength is three times the stoichiometric molar concentration of  $\text{H}_2\text{SO}_4$ .

The buffers were, prior to use, filtered using a 0.45  $\mu\text{m}$  filter purchased from Millipore (Bedford, UK). Samples were prepared by dissolving the pure compounds in the appropriate buffer and filtering the solution through a Millex HV4 filter (0.45  $\mu\text{m}$ ) (Millipore) prior to injection. Typical concentration of the coordination compounds in the sample were  $10^{-3}$  M.

## 2.4. Analytical procedure

Samples were injected on to the capillary by applying a pressure of 40 mbar for 4 s. Detection was in general performed at 200–225 nm. The electroosmotic flow through the capillary was measured by injecting either benzylic alcohol (Ferak, Berlin, Germany) or a neutral cobalt(III) coordination compounds  $\{[\text{Co}(\text{gly})_3]\}$  as flow markers. Using the silica capillary, the flow marker was added to each sample prior to measurements. For the coated capillary the electroosmotic flow was tested over time. In no cases was an electroosmotic flow mobility larger than  $1.26 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$  observed in the coated capillary. Since this value was very small compared to the electrophoretic mobility, the electrophoretic mobility was set equal to the apparent mobility. To assure that no joule heating had an effect on the mobility an Ohm's plot was obtained for the buffers of highest ionic strength. The highest voltage that still induced a linear response in current was chosen for the experiments, namely 7.5 kV for the experiments using  $\text{SO}_4^{2-}$  and 15 kV for the experiments using  $\text{ClO}_4^-$ .

## 2.5. Calculations

The apparent mobilities were calculated at each concentration of the appropriate anion from

$$\mu_{\text{app}} = \frac{lT}{t_m U} \quad (10)$$

where  $l$  is the length of the capillary from inlet to the detection window,  $T$  is the total length of the capillary,  $t_m$  is the migration time of the coordination compound and  $U$  is the applied voltage. Applying Eq. 1 makes it possible to calculate  $\mu_e$  (or more correct  $\mu_{\text{eff}}$ ) from  $\mu_{\text{app}}$  at each buffer concentration.

$\langle K \rangle$  were obtained by nonlinear fitting of  $\mu_{\text{eff}}$  versus buffer concentration to Eq. 5 and  $K^0$  by fitting of the data to Eq. 9. The results were analysed using nonlinear curve fitting. The curve fitting was performed using Origin 5.0 (Microcal Software, North Hampton, USA) and Enzfitter (Elsevier, Amsterdam, Netherlands).  $K^0$  was also determined by linear extrapolation of  $K$  determined at various ionic strengths to zero ionic strength.  $K$  at various ionic

strengths was obtained using Eq. 5 after estimation of  $\mu_{\text{ML}}$  from  $\mu_{\text{M}}$  according to Eq. 11.

## 3. Results

CE is a powerful analytic tool for separating charged inorganic compounds both by charge, size and difference in ion association. In Fig. 1 is shown the electropherograms of a separation of an artificial mixture of  $[\text{Co}(\text{NH}_3)_6]^{3+}$ ,  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  and *cis*- $[\text{Co}(\text{en})_2(\text{NO}_2)_2]^+$  using as solvent 50 mM  $\text{HClO}_4$  adjusted to pH 3.1 using NaOH. The separation is mainly due to the different charge of the ions since perchlorate only forms weak outer sphere association. The expected linear dependence of mobility on the ionic charge was also tested. The mobility values are obtained by measurements of the effective electrophoretic mobility versus  $\text{SO}_4^{2-}$  concentration and extrapolation to zero ionic strength. As can be seen from Fig. 2 proportionality exists in the range of 0–3 of ionic charge, if the compounds are approximately of the same size. The mobility of  $[\text{Co}(\text{dien})_2]^{3+}$  is somewhat smaller than it is for  $[\text{Co}(\text{NH}_3)_6]^{3+}$  (data not shown) because of the difference in the size of the compounds.

Also ions of same charge can easily be separated. Fig. 3 shows the electropherograms of a separation of an artificial mixture of three Co(III) complexes with the same charge  $[\text{Co}(\text{NH}_3)_6]^{3+}$ ,  $[\text{Co}(\text{en})_3]^{3+}$  and  $[\text{Co}(\text{daes})_2]^{3+}$  obtained in 50 mM  $\text{HClO}_4$  adjusted to pH 3.1 using NaOH. The separation here is primarily due to the different sizes of the compounds.

Separation of geometric isomers is one of the very useful applications of CE since it allows for fast analytical investigation of reaction mixtures containing geometrical isomers. The electropherogram in Fig. 4 shows the separation of a crude product containing the three geometric isomers of  $[\text{Co}(\text{dien})_2]^{3+}$ . As can be seen from the figure the three structural isomers of  $[\text{Co}(\text{dien})_2]^{3+}$  are separated during the run using  $\text{SO}_4^{2-}$  as an outersphere ligand. The separation is caused by the different association of  $\text{SO}_4^{2-}$  to the three isomers. When  $\text{ClO}_4^-$  was used instead of  $\text{SO}_4^{2-}$  as counter ion (data not shown) a much less pronounced separation was

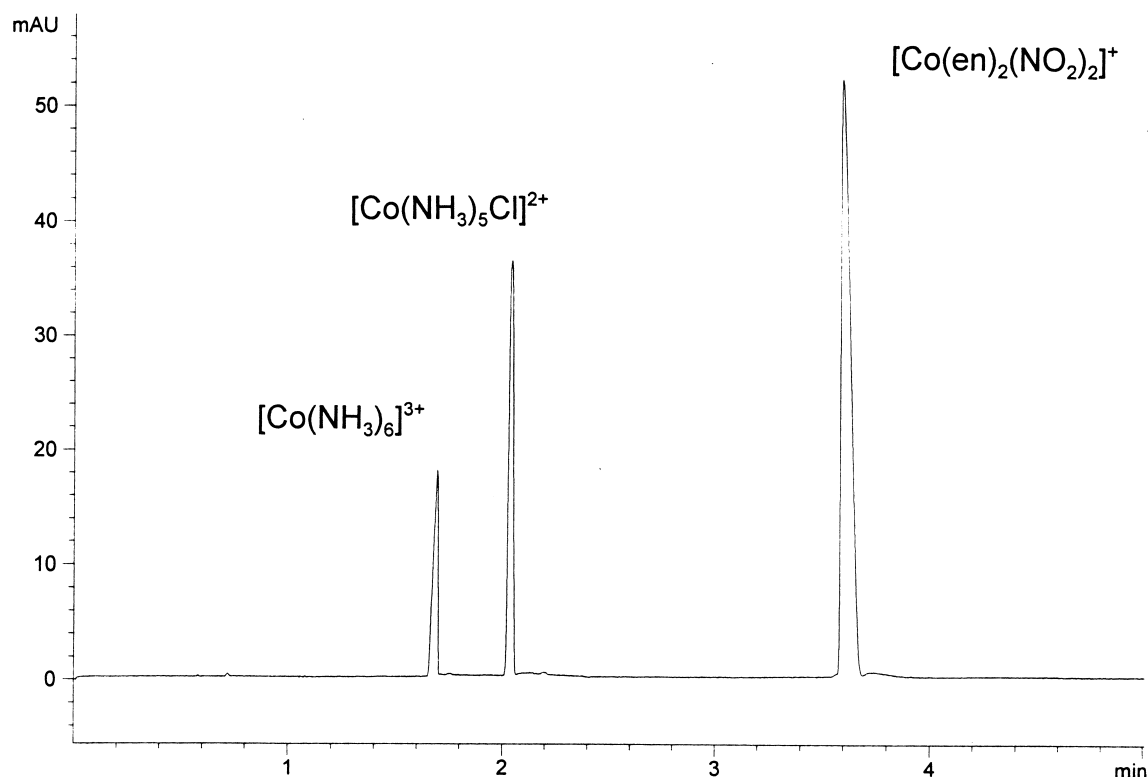


Fig. 1. Electropherogram of a mixture of  $[\text{Co}(\text{NH}_3)_6]^{3+}$ ,  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  and  $[\text{Co}(\text{en})_2(\text{NO}_2)_2]^+$  in 50 mM  $\text{HClO}_4$ , pH adjusted to 3.1 using NaOH. The concentration of each of the complexes in the sample was approximately 1 mM. A fused-silica capillary [64.5 cm (56.0 cm to the detection window)  $\times$  50  $\mu\text{m}$ , with extended lightpath from Hewlett Packard] was used and flushed prior to use with 0.1 M NaOH for 2 min. The separations were performed at 40°C applying a voltage of 15 kV. The inlet electrode was anode.

observed. The strong ion association between the different isomers of  $[\text{Co}(\text{dien})_2]^{3+}$  complexes and sulphate are clearly helping in the discrimination of the ions.

The concentration of the anion in the separation buffer has as expected a profound effect on the migration time of the various cobalt(III) complexes. In Fig. 5. is shown the electropherograms of  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  using different concentration of  $\text{SO}_4^{2-}$ . Increasing concentration of  $\text{SO}_4^{2-}$  cause a reduction in the mobility of this compound, showing an association with  $\text{SO}_4^{2-}$ . Other compounds such as  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$ , and  $[\text{Co}(\text{dien})_2]^{3+}$  have been examined in similar ways using  $\text{ClO}_4^-$  as well as  $\text{SO}_4^{2-}$  as anions.

The simplest of the investigated systems are  $[\text{Co}(\text{en})_2\text{Cl}_2]^+$  separated with  $\text{ClO}_4^-$  and

$[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  separated with  $\text{SO}_4^{2-}$ . In these systems only 1:1 association is possible, since the association product will be a neutral compound. Fig. 6 shows the mobility of  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  as a function of  $[\text{SO}_4^{2-}]$ . A value of zero is to be expected for  $\mu_{\text{MA}}$ , but a value about one third of  $\mu_{\text{M}}$  is actually found in this case. However, after activity correction using Eq. 9 the nonlinear fit is approaching zero at high ligand concentrations as seen in Fig. 7.  $\langle K \rangle$  calculated by nonlinear fitting of data to Eq. 5 and  $K^0$  calculated by nonlinear fitting of data to Eq. 9 are listed in Table 1. In the case of  $[\text{Co}(\text{en})_2\text{Cl}_2]^+$  separated with  $\text{ClO}_4^-$  both  $K^0$  and  $\langle K \rangle$  are close to the literature values because of the small activity correction in this system. However, in the case of  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  separated with  $\text{SO}_4^{2-}$  the result after activity correction is clearly the best while

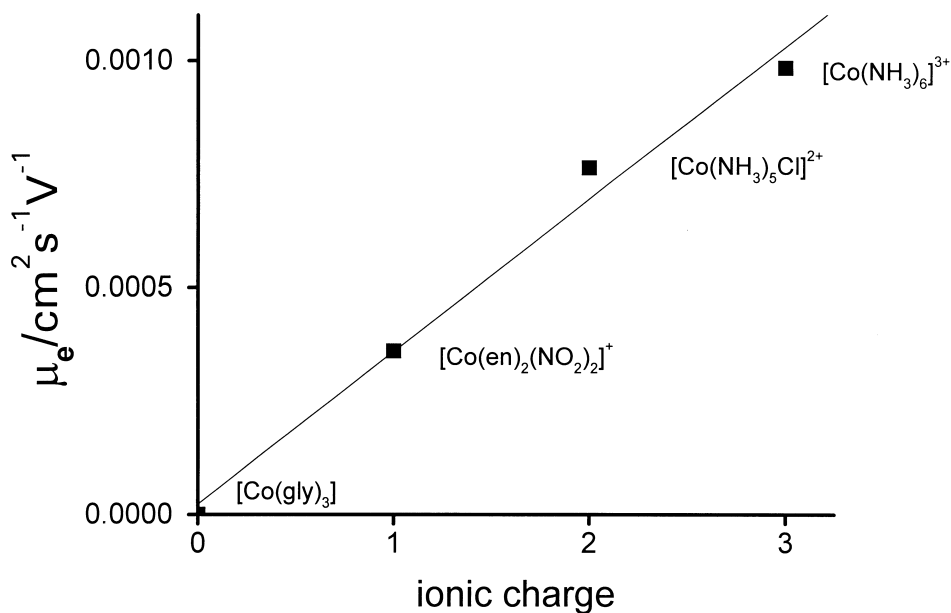


Fig. 2. Electrophoretic mobility versus the ionic charge for four Co(III) coordination compounds of ionic charge 0–3. The mobility values are obtained by measurements of the effective electrophoretic mobility versus  $\text{SO}_4^{2-}$  concentration and extrapolation to zero ionic strength using Davies' equation (see Eq. 9). A BioCAP LPA coated silica capillary [68.5 cm (60.0 cm to the detection window)  $\times$  50  $\mu\text{m}$ ] from Bio-Rad was used and flushed prior to use with capillary wash solution for 2 min. The experiments were performed at 40°C applying a voltage of 7.5 kV and with the inlet electrode as anode.

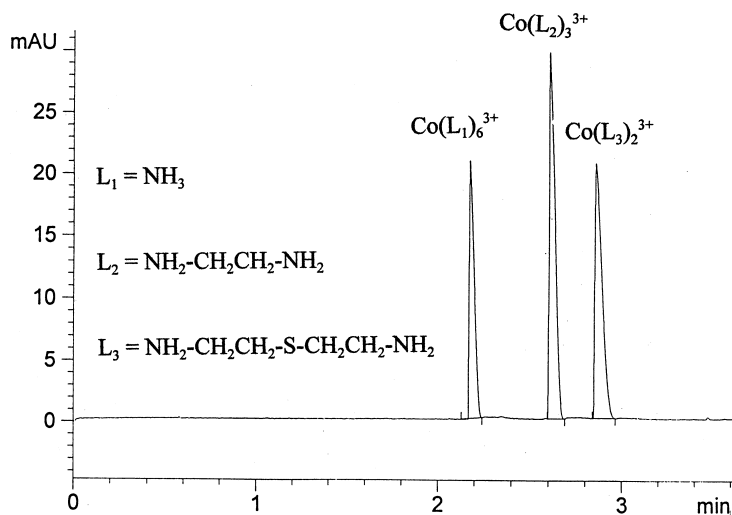


Fig. 3. Electropherogram of a mixture of 1 mM  $[\text{Co}(\text{NH}_3)_6]^{3+}$  ( $L_1 = \text{NH}_3$ ),  $[\text{Co}(\text{en})_3]^{3+}$  ( $L_2 = \text{en}$ ) and  $[\text{Co}(\text{daes})_2]^{3+}$  ( $L_3 = \text{daes}$ ) in 50 mM  $\text{HClO}_4$  pH 3.1. Data were obtained under the same experimental conditions as in Fig. 1.

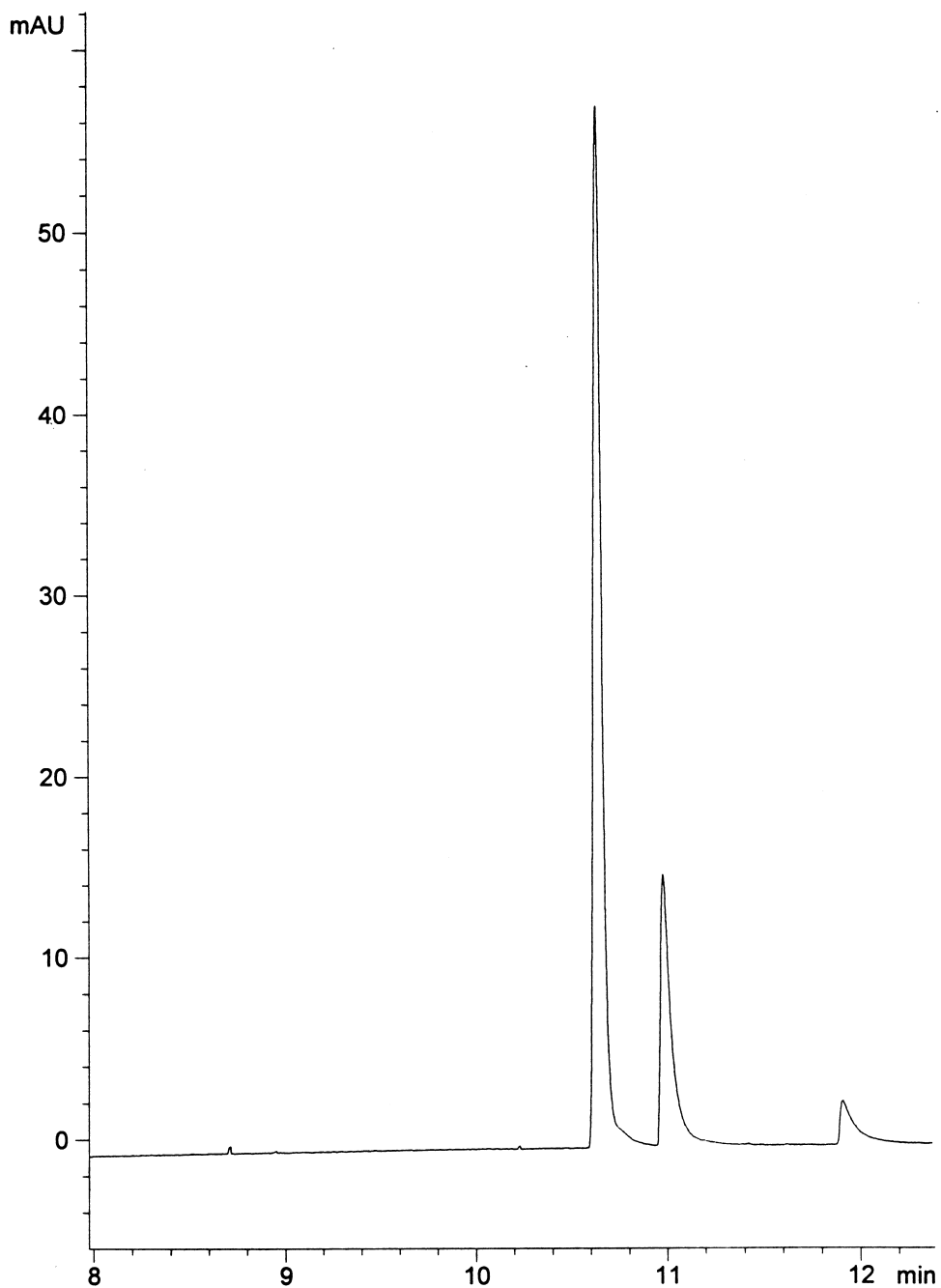


Fig. 4. Electropherogram of the three geometrical isomers of 1 mM  $[\text{Co}(\text{dien})_2]^{3+}$  in 185 mM  $\text{HClO}_4$ , 5 mM  $\text{H}_2\text{SO}_4$ , pH adjusted to 7.4 using Tris. The concentration of the complex in the sample were approximately 1 mM. A BioCAP LPA coated silica capillary [68.5 cm (60.0 cm to the detection window)  $\times$  50  $\mu\text{m}$ ] from Bio-Rad was used and flushed prior to use with capillary wash solution for 2 min. The experiments were performed at 40°C applying a voltage of 7.5 kV and with the inlet electrode as anode.

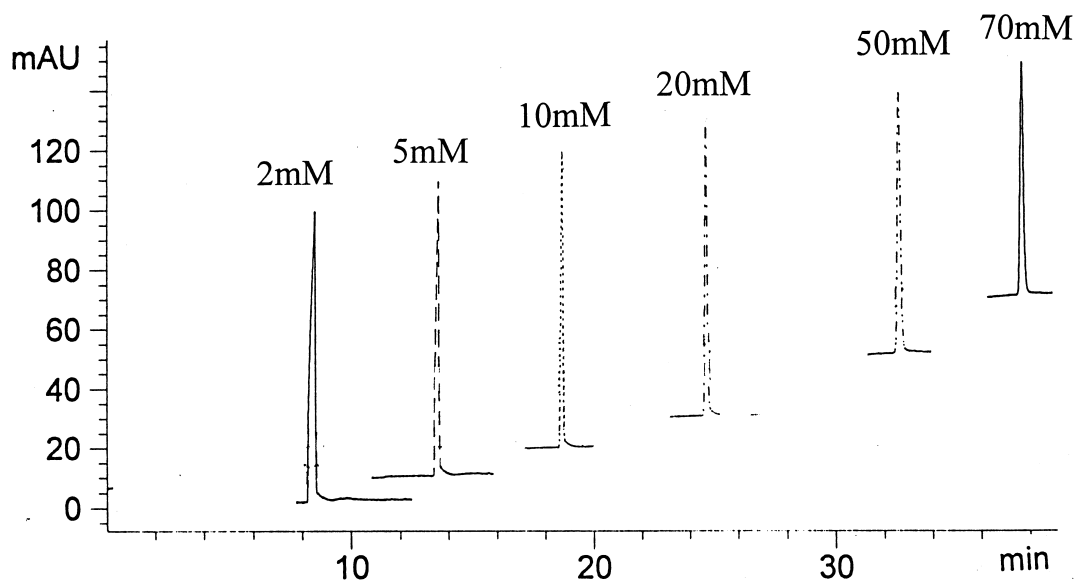


Fig. 5. Electropherograms of  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  at concentrations from 2–70 mM  $\text{H}_2\text{SO}_4$ , pH adjusted to 7.4 using Tris. The concentration of the complex in the sample was approximately 1 mM. A BioCAP LPA coated silica capillary [68.5 cm (60.0 cm to the detection window)  $\times$  50  $\mu\text{m}$ ] from Bio-Rad was used and flushed prior to use with capillary wash solution for 2 min. The experiments were performed at 40°C applying a voltage of 7.5 kV and with the inlet electrode as anode.

rather large deviations exist between  $\langle K \rangle$  and the literature values. This is a clear indication that activity correction is relevant in CE systems.

It must be expected that the association compound will behave as a dipole in the electric field in the capillary: The negatively charged anion will pre-

sumably be partly oriented towards the anode, while the positively charged coordination compound will be oriented partly towards the cathode. This means that the Stokes radius of the coordination compound will be approximately of the same size (see Eq. 1) whether or not  $\text{ClO}_4^-/\text{SO}_4^{2-}$  is attached, and the

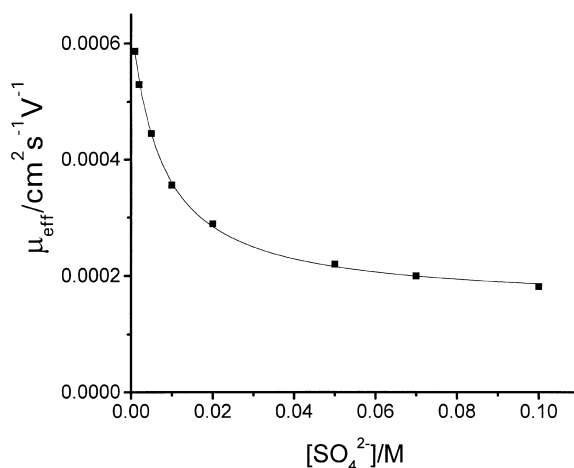


Fig. 6. Effective electrophoretic mobility of  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  versus  $\text{SO}_4^{2-}$  concentration. The buffers consisted of 1–100 mM  $\text{H}_2\text{SO}_4$ , and each buffer was adjusted to pH 7.4 using Tris. Data were otherwise obtained under the same experimental conditions as in Fig. 5.



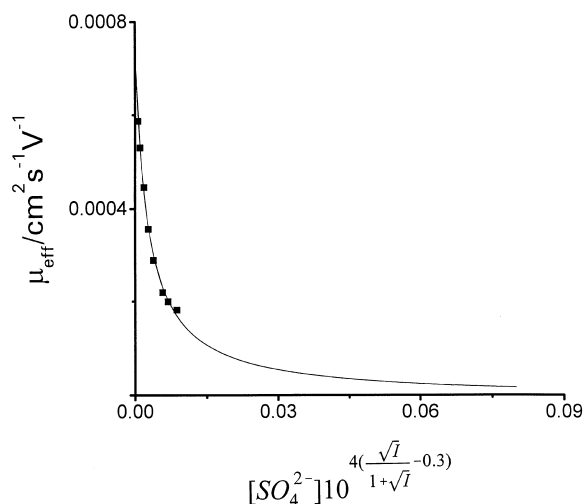


Fig. 7. Effective electrophoretic mobility of  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  versus a corrected  $\text{SO}_4^{2-}$  concentration according to Eq. 9. Data were obtained under the same experimental conditions as in Fig. 6.

electrophoretic mobility will be proportional to the ionic charge of the compound similar to what is shown in Fig. 2 for different charged compounds of similar size. This approximation makes it possible to calculate the association constants not only by solving Eq. 9 using nonlinear fitting, but also by direct application of Eq. 5, since the mobility of the associated compound can be estimated once the mobility of the unassociated compound is known:

$$\mu_{\text{ML}} = \frac{z_{\text{ML}}}{z_{\text{M}}} \cdot \mu_{\text{M}} \quad (11)$$

where  $z_{\text{ML}}$  is the ionic charge of the associated compound ML, and  $z_{\text{M}}$  is the charge of the unassociated coordination compound M. Using Eq. 5 with this approximation means that one association constant can be obtained for every measurement.  $K^0$  can thus be obtained after calculation of  $K$  at different ionic strengths and extrapolation to zero ionic strength, according to Eq. 12 which is a combination of Eqs. 6 and 7:

$$\log K = \log K^0 + 0.5(z_{ij}^2 - z_i^2 - z_j^2) \left( \frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3I \right) \quad (12)$$

Table 1

Constants for the association of either  $\text{ClO}_4^-$  or  $\text{SO}_4^{2-}$  with  $\text{Co}(\text{III})$  coordination compounds of charge +1 or +2<sup>a</sup>

					Literature
$[\text{Co}(\text{en})_2\text{Cl}_2]^+$	$\text{ClO}_4^-$	Direct	$\langle K \rangle$	$7 M^{-1}$	6 <sup>b</sup> [16]
		Davies	$K^\circ$	$9 M^{-1}$	
$[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$	$\text{ClO}_4^-$	Direct	$\langle K \rangle$	$9 M^{-1}$	11 [17]
		Davies	$K^\circ$	$17 M^{-1}$	
	$\text{SO}_4^{2-}$	Direct	$\langle K \rangle$	$136 M^{-1}$	
		Davies	$K^\circ$	$363 M^{-1}$	

<sup>a</sup> The constants are obtained either by direct non linear fitting of data to Eq. 5 or after application of Davies' equation using Eq. 9.

<sup>b</sup> The literature value for the association of  $[\text{Co}(\text{en})_2\text{SO}_4]^+$  with  $\text{ClO}_4^-$  at 25°C.

Figs. 8 and 9 shows  $\log K$  versus the ionic strength for  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}/\text{ClO}_4^-$  and  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}/\text{SO}_4^{2-}$ , respectively. The two plots result in straight lines with slopes in accordance with Eq. 12. The obtained  $K^0$  is also in close agreement with the literature value (Table 2).

The more complicated systems involving  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  separated with  $\text{ClO}_4^-$  and  $[\text{Co}(\text{dien})_2]^{3+}$  separated with either  $\text{ClO}_4^-$  or  $\text{SO}_4^{2-}$  are more difficult to handle partly because of the possibility of forming 1:2 ion pairs and partly because activity correction for 3+ ions becomes very large. However, as can be seen from Table 1 a reliable value is obtained for  $K^0$  for the association

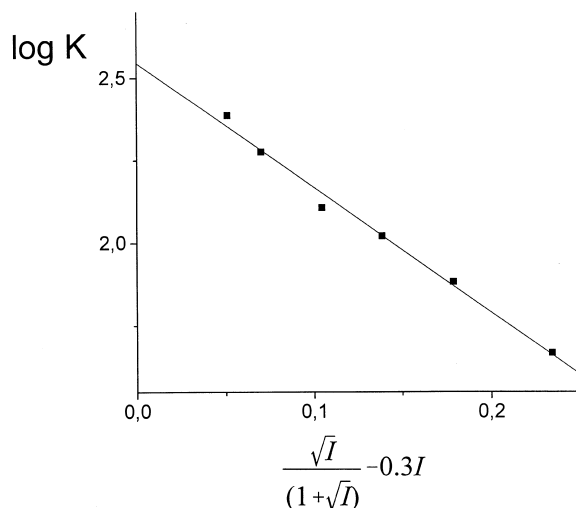


Fig. 8. Log  $K$  versus  $\sqrt{I}/(1+\sqrt{I})-0.3I$  for the association of  $\text{SO}_4^{2-}$  with  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  at  $40^\circ\text{C}$ . The association constants at each ionic strength was obtained using Eq. 5 after estimation of  $\mu_{\text{ML}}=0$ . The data points obtained at the highest and lowest concentration on  $\text{SO}_4^{2-}$  were removed due to the high uncertainties of  $K$  determined at those concentrations (see Eq. 5). After linear regression a slope of  $-3.8$  and a value of  $\log K^0$  of  $2.5$  was found. Data were obtained under the same experimental conditions as in Fig. 6.

of  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  with  $\text{ClO}_4^-$ , indicating that this Co(III) coordination compound does not form 1:2 ion pairs to a significant degree in the investigated concentration range. However, as can be seen from Table 3, all  $+3$  ions investigated, especially with

$\text{SO}_4^{2-}$  as a counterion, showed quite big deviations between the  $K^0$  obtained from Eq. 9 and the expected values. Expected values for the constant for the outersphere association between a  $+3$  charge cobalt(III) coordination compound and a  $-1$  charge

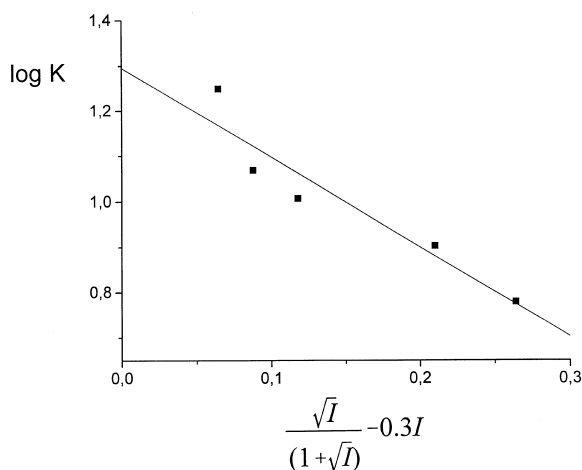


Fig. 9. Log  $K$  versus  $\sqrt{I}/(1+\sqrt{I})-0.3I$  for the association of  $\text{ClO}_4^-$  with  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  at  $40^\circ\text{C}$ . The buffers consisted of  $5\text{--}300\text{ mM}$   $\text{HClO}_4$ , and each buffer was adjusted to pH 7.4 using Tris. The experiments were performed at  $15\text{ kV}$ . Data were otherwise obtained under the same experimental conditions as in Fig. 5. The association constant at each ionic strength was obtained using Eq. 5 after estimation of  $\mu_{\text{ML}}=0.5\ \mu_{\text{M}}$ . After linear regression a slope of  $-2.0$  and a value of  $\log K^0$  of  $1.3$  was found.

Table 2  
 Constants for the association of either  $\text{ClO}_4^-$  or  $\text{SO}_4^{2-}$  with  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$ <sup>a</sup>

$[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$	$K^0/M^{-1}$	$K^0/M^{-1}$ literature values	Slope	Slope (calculated)
$\text{ClO}_4^-$	20	11 [17]	-2	-2
$\text{SO}_4^{2-}$	346	333 [17]	-3.9	-4

<sup>a</sup> The constants are obtained after extrapolation to zero ionic strength using Eq. 12 and Eq. 5 after estimation of  $\mu_M$  from  $\mu_{ML}$  (Eq. 11).

Table 3  
 Constants for the association of either  $\text{ClO}_4^-$  or  $\text{SO}_4^{2-}$  with Co(III) coordination compounds of charge +3<sup>a,b</sup>

	$K^0/M^{-1}$
$[\text{Co}(\text{dien})_2]^{3+}$	
$\text{ClO}_4^-$	7
$\text{SO}_4^{2-}$	404
$[\text{Co}(\text{NH}_3)_6]^{3+}$	
$\text{ClO}_4^-$	38
$\text{SO}_4^{2-}$	383
$[\text{Co}(\text{en})_3]^{3+}$	
$\text{ClO}_4^-$	17

<sup>a</sup> The constants are obtained by nonlinear fitting of data after application of Davies' equation (using Eq. 9).

<sup>b</sup> The literature values for the association of  $[\text{Co}(\text{NH}_3)_6]^{3+}$  with  $\text{ClO}_4^-$  are  $25 M^{-1}$  [18] and for the association with  $\text{SO}_4^{2-}$  it is reported to be  $3200 M^{-1}$  [19] at 25°C.

anion like  $\text{ClO}_4^-$  is approximately  $25 M^{-1}$  [18] and approximately  $3200 M^{-1}$  for a -2 charge anion like  $\text{SO}_4^{2-}$  [19].

The formation of 1:2 ion pairs in the  $[\text{Co}(\text{NH}_3)_6]^{3+}/\text{ClO}_4^-$  system can be deduced from Fig. 10. Log  $K$  in this plot is calculated from Eq. 5 and the approximation given in Eq. 11, and shows that a linear relationship with a negative slope exists at low ionic strengths. This is what is expected from Eq. 12. However, at high ionic strengths it seems that  $K$  is increasing due to binding of a second  $\text{ClO}_4^-$ . To handle systems with ions having charges higher than +2 more elaborated models are obviously needed.

#### 4. Discussion

Capillary electrophoresis has in our hand proven to be a very useful analytical tool for separating inorganic coordination compounds of either same or different charge. Especially the outstanding ability to separate geometric isomers due to different outer

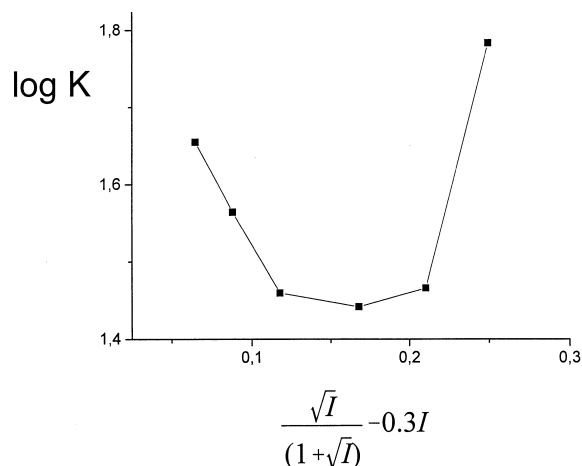


Fig. 10. Log  $K$  versus  $\sqrt{I}/(1+\sqrt{I})-0.3I$  for the association of  $\text{ClO}_4^-$  with  $[\text{Co}(\text{NH}_3)_6]^{3+}$  at 40°C. The association constants at each ionic strength were obtained using Eq. 5 after estimation of  $\mu_{ML}=2/3 \mu_M$ . Data were obtained under the same experimental conditions as in Fig. 9.

sphere complexation is a useful application in inorganic coordination chemistry.

In this study, an ordinary fused-silica capillary has been used for simple analytical separations but this capillary was found unsuitable for the determination of outersphere association constants, since the coordination compounds adsorbed to the silica surface at low ionic strengths. An ionic strength of at least 0.030 was necessary to obtain a peak, and at this ionic strength it is certainly still possible, that an equilibrium exists between free and silica adsorbed coordination compounds. An adsorption to the silica surface will cause an error in the association results, since the influence on the mobility is not constant but will decrease as the ionic strength of the ligand solutions is increasing. To overcome this problem a capillary coated with linear polyacrylic amide has been employed for the association studies. Using this capillary no adsorption was discovered even at ionic strengths as small as 0.003. A further advantage of the coated capillary is that the electroosmotic flow is eliminated.

For the association of  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  with either  $\text{ClO}_4^-$  or  $\text{SO}_4^{2-}$  we have presented two different methods for obtaining  $K^0$ : Nonlinear fit of data to Eq. 9, which include activity correction according to Davies' equation (Eq. 7), and extrapolation to zero ionic strength after obtaining  $K$  at fixed ionic strengths using Eq. 5 and the assumption in Eq. 11. For the association of  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  with  $\text{ClO}_4^-$ , a value of  $17 \text{ M}^{-1}$  was found using activity correction, while  $20 \text{ M}^{-1}$  was found using extrapolation. For the association of  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$  with  $\text{SO}_4^{2-}$  a slightly smaller value of  $K^0$  ( $347 \text{ M}^{-1}$ ) was found by extrapolation than the one obtained using nonlinear fit of the data ( $363 \text{ M}^{-1}$ ). In both cases are the obtained values of  $K^0$  close to the literature values, showing that in the very simple systems that we have studied, it is possible to use either of the two methods.

An important conclusion of this study is, that to understand the observed variation of the electrophoretic mobilities with buffer concentration and to obtain accurate values for the association constants, it is necessary to correct for the different activities of the ions in the solutions inside the capillary. We have found that Davies' equation is a good choice for calculating activity coefficients for such correction.

Using Davies' equation in solutions with ionic strengths up to 0.2 allows for the determination of association constants in close agreement with literature values for inorganic compounds of small charge with a simple 1:1 coordination. It is even possible to do the measurements with more than one coordination compound at a time, as long as the compounds have no association with each other.

Since the obtained association constants are close to values obtained employing other methods it can also be concluded, that the association is only affected to a minor degree by the electric field in the capillary. However, it is certainly possible that this result is only found for very simple systems, and that f.x. protein ligand binding is influenced by the electric field.

Analysis of association between compounds of higher charge than +2 is complicated by association with more than one counter ion. This problem can hopefully be overcome, if the association of the second counter ion does not affect the size of the coordination compound significantly, as we have found in our study to be the case for the association of the first ligand. In that case  $\mu_{\text{ML}2}$  as well as  $\mu_{\text{ML}}$  can be determined once  $\mu_{\text{M}}$  is known. However, we have not studied this possibility thoroughly and the question is still under investigation.

## 5. Symbols

A:	General outersphere counter ion
ACE:	Affinity capillary electrophoresis
CE:	Capillary electrophoresis
daes:	Di(aminoethyl)sulfide
dien:	Diethylentriamine
en:	1,2-ethandiamine
gly:	Glycine
$\gamma_i$ :	Activity coefficients of compound $i$
$I$ :	Ionic strength
$K$ :	Association constant at a given ionic strength
$K^0$ :	Association constant at zero ionic strength
$\langle K \rangle$ :	Average value of association constant in a given concentration range
L:	Innersphere ligand in coordination compound
M:	General coordination compound
$\mu_{\text{app}}$ :	Apparent mobility
$\mu_e$ :	Electrophoretic mobility

$\mu_{\text{eff}}$ : Effective electrophoretic mobility  
 $\mu_{\text{eo}}$ : Electroosmotic mobility  
 $\mu_{\text{M}}$ : Electrophoretic mobility of an unassociated coordination compound M  
 $\mu_{\text{MA}}$ : Electrophoretic mobility of an associated coordination compound MA  
 tn: 1,3-propanediamine  
 Tris: Tris(hydroxymethyl)aminomethan  
 z: Ionic charge

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