Separation and Kinetic Study of Chromium(III) Chloro Complexes by Capillary Electrophoresis

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Key Words

Capillary electrophoresis
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Kinetics

Summary

Three Cr(III) species (dichlorotetraaquachromium (III), $[CrCl_2(H_2O)_4]^+$; monochloropentaaquachromium(III), $[CrCl(H_2O)_5]^{2+}$; and hexaaquachromium(III), $[Cr(H_2O)_6]^{3+}$) have been separated and determined by capillary electrophoresis. The first two complexes could be detected in direct mode in phosphate buffer, but because the absorption of complex $[Cr(H_2O)_6]^{3+}$ is poor in the UV range, indirect UV detection had to be used. For indirect detection 5 mM imidazole was added to the buffer solution. The formation and decomposition of the different Cr(III) complexes were monitored in time after the preparation of solutions of $CrCl_3.6H_2O$. The slowest process was the decomposition of $[CrCl(H_2O)_5]^{2+}$; 300 h after preparation of a solution of $CrCl_3.6H_2O$ of pH 1 the solution contained only $[Cr(H_2O)_6]^{3+}$. The effects of pH and the content of some matrix ions on the rates of conversion of the complexes were studied. The kinetic characteristics of this complex system could be investigated adequately by means of capillary electrophoresis.

Introduction

There is increasing interest and demand for metal speciation in biological and environmental samples, because the most important factor affecting the toxicity of metals is the nature of their bonding, or the form in which they are complexed, rather than their concentration. One of the most often mentioned examples of metal speciation is the determination of chromium(III) and chromium(VI), because of their highly different toxicity [1]. These species are readily separated by a variety of techniques, because their chemical properties are very different. A greater challenge is to extend the speciation analysis to different chromium(III) species as chloro complexes, i.e. to determine ‚free' and complexed chromium(III).

Capillary electrophoresis (CE) seems to be a technique of great promise for the separation of complexed metal ions. In these applications kinetic considerations are particularly important [2]. The kinetics of exchange between the different complexes in solution must be sufficiently slow, compared with the time required for the separation, that individual peaks are obtained for each species. The mechanisms of separation in CE are different from those in liquid chromatography (LC). Also, in comparison with HPLC, CE method development is usually simpler, smaller volumes of sample are required, and resolving power is almost always better.

Cr(III) readily forms complexes with species with a free electron pair [3]. The different complexes formed from $CrCl_3.6H_2O$ in water are well-known. The reason for the co-existence of these species is the slow rate of ligand exchange in the coordination sphere of the central metal ion. On dissolution of solid $CrCl_3.6H_2O$ in water, first the dichlorotetraaquachromium(III) complex, then the monochloropentaaquachromium(III) complex, and finally the hexaaquachromium(III) complex are formed [4].

The use of CE is expected to be ideal for the separation of these chromium(III) species, because of the large differences between their charge-to-size ratios. Despite this, only one paper on the CE separation of chromium(III) chloro complexes has been published. Glod et al. [5] determined the dichloro- and monochlorochromium(III) complexes by CE with camphorsulfonic acid as running buffer. Because of the direct detection used only these two complexes could be detected. Half-lives of 2.5 and 700 h were estimated for the dichlorotetra-

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aquachromium(III) and monochloropentaaquachromium(III) complexes, respectively [5].

The aim of our work was to investigate the use of CE for kinetic and solution-dynamic studies of chromium chloro complexes and to show the complexity and difficulty of speciation analysis in fresh solutions prepared from solid chromium chloride.

Experimental

Instrumentation

Capillary electrophoresis was performed with HP 3D instrumentation (Hewlett-Packard, Waldbronn, Germany). For all measurements 100 mbar pressure was used to inject samples. Separations were performed in polyimide-coated, extended light-path, bare fused-silica capillary tubing (Hewlett-Packard), 65 cm × 50 μm i.d., effective length 56 cm. The applied voltage was +25 kV. Detection was by on-column photometric measurement at 200 nm. The electropherograms were recorded and processed by ChemStation software, version 1.02 (Hewlett-Packard).

Chemicals

Reagents were analytical grade from various distributors. The chromium sample solutions were prepared by dissolving solid $CrCl_3.6H_2O$ (Reanal, Hungary) in degassed doubly distilled water, and in different acids and phosphate buffers. Sometimes the prepared sample was analyzed as soon as possible, although at least 20–25 min (approx.) was necessary for suitable sample preparation, sampling, and to start the electrophoretic measurements. The pure hexaaquachromium(III) solution was prepared daily by reducing CrO_3 with hydrogen peroxide in presence of excess perchloric acid [6]. Before CE analysis all the samples and buffers were filtered through a 0.45-μm membrane filter.

Separation Procedure

Direct and indirect UV detection was used for detection of the different chromium complexes. For both the running buffer was 20 mM phosphoric acid (pH 2.2), but for indirect detection 5 mM imidazole was added to the buffer as chromophoric reagent [7]. Between measurements, the capillary was rinsed with running buffer for 10 min.

Results and Discussion

Separation and Detection of Chromium(III) Chloro Complexes

The most important factors in selection of an appropriate buffer were that it must not react with, or form a strong complex with, Cr(III). Also, because the Cr(III) complexes can be converted into hydrolytic polymers at high pH [8], the buffer pH should be low. The pH also has a strong effect on the solution dynamics of the Cr(III) chloro complexes (see below). Furthermore, requirements for the separating conditions are in contrast with those usually used for metal speciation by CE. Neither complexing nor micellar electrolytes can be used, otherwise the stability of the complexed metal species during electrophoresis, and hence the reliability of speciation results, cannot be guaranteed. The most important rule is that the number of electrolyte constituents should be kept to a minimum. Because of these requirements 20 mM phosphoric acid, pH 2.2, was used for direct detection and 5 mM imidazole protonated with phosphoric acid for indirect UV detection. By use of these buffers the formation of hydrolytic polymers or the conversion of the different Cr(III) chloro complexes could be considered to be negligible during the electrophoretic separation. The peak symmetry of the signals of the chromium species was acceptable. Changing the amount of sample injected, the applied voltage, or the concentration of the buffer did not result in considerably improved electropherograms. The pH of the sample solutions had a large effect on the transformation of the different chromium(III) species; this effect is discussed later.

Although the different chromium chloro complexes could be separated without difficulty, because of the large differences between their electrophoretic mobilities, sensitive detection of all the species during a single run was problematic, because of their different UV-absorption characteristics. By use of direct UV detection (at 200 nm) two species, the dichloro and monochloro complexes, could be monitored; the hexaaquachromium complex could, however, be detected sensitively only by indirect UV detection. The electropherograms obtained by direct and indirect UV detection of chromium chloro complexes and of the hexaaqua complex, respectively, are shown in Figure 1. Also apparent from this figure is the transformation of the different complexes with time.

The detection limits assessed at 200 nm with direct detection and calculated as three times the average baseline noise, were 0.2 mM for hexaaquachromium and 0.005 mM for the dichloro- and the monochloro complexes. By use of indirect detection the detection limit for the hexaaquachromium complex could be improved to 0.004, but no better results were achieved for the dichloro- and the monochloro complexes. The CE measurements for calculation of detection limits were conducted 5.00 h after dissolving $CrCl_3.6H_2O$ in 0.1 M nitric acid. A calibration graph was constructed for the hexaaquachromium complex only, because only this complex is stable in time and available as a standard. Standards of the hexaaquachromium complex were prepared according to Ref. [6]. The calibration curves for this system are shown in Figure 2. Linearity of peak area response was observed over the range 0.02–4 mM. The relative standard deviations of the peak areas of all

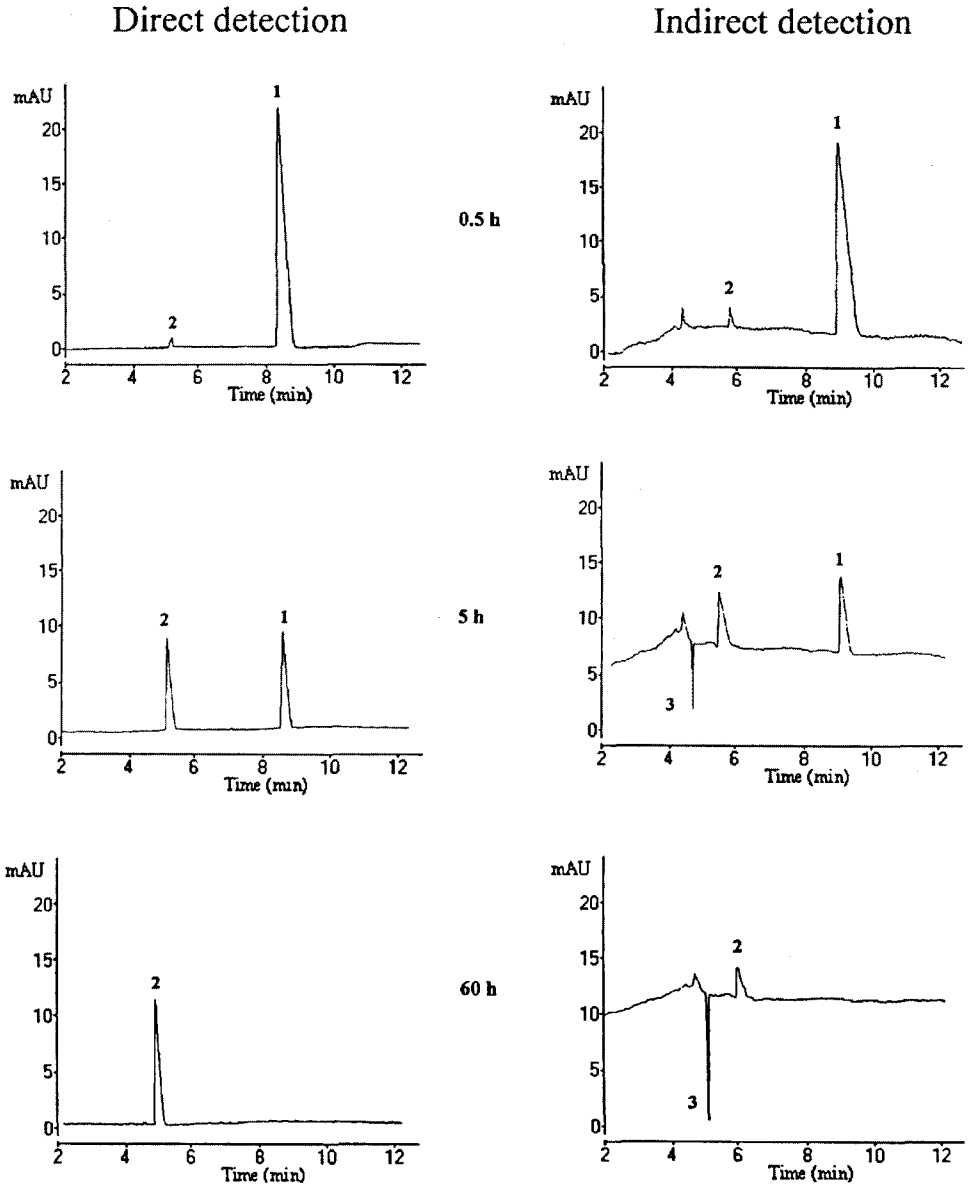

Figure 1

Electropherograms of chromium species in solution obtained by dissolving chromium(III) chloride in 0.1 M nitric acid. Direct and indirect UV detection were conducted 0.5, 5, and 60 h after the preparation of the solution. **1** = $[CrCl_2(H_2O)_4]^+$, **2** = $[CrCl(H_2O)_5]^{2+}$, **3** = $[Cr(H_2O)_6]^{3+}$. Sample, 2 mM $CrCl_3$; λ, 200 nm; the buffer for direct detection was 20 mM phosphoric acid (pH 2.2); the buffer for indirect detection was 20 mM phosphoric acid containing 5 mM imidazole, pH 2.4.

three chromium complexes (seven consecutive runs) were < 2.5 %. The CE measurements for the *RSD* % calculations were obtained 5.00 h after dissolving identical amounts of $CrCl_3.6H_2O$ in identical volumes of 0.1 M nitric acid.

Identification of the hexaaquachromium complex was performed by standard addition and the amount of hexaaquachromium was determined by means of a calibration curve. Because of the lack of standards of the other two species, they were identified by consideration of their charge-to-size ratios and the migration times of the complexes, and by use of literature data. No other unidentified peaks were obtained with direct detection, and only a system peak (constant peak area) was obtained with indirect detection. Because the total amount of the dichloro and monochloro complexes was the difference between the amount of the dissolved chromium chloride and the amount of the hexaaquachromium complex, the amount of a particular chloro complex could be calculated at all times on the basis of the ratio of the peak heights of the complexes. In our work the use of the peak height ratio was more advantageous than use of peak area ratio because of the slower migration of the dichlorochromium complex through the detection cell. The spectra of the three complex ions were recorded on-column by the CE instrument (Figure 1). The chloro complexes have strong absorbance in the UV range whereas the hexaaqua complex does not absorb in the

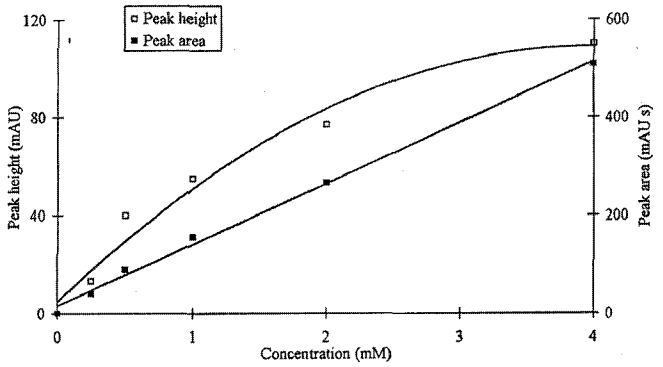

Figure 2

Calibration curves for the hexaaquachromium complex. Indirect detection; conditions as for Fig. 1; correlation coefficients for calibration curve of peak area: $R^2 = 0.996$.

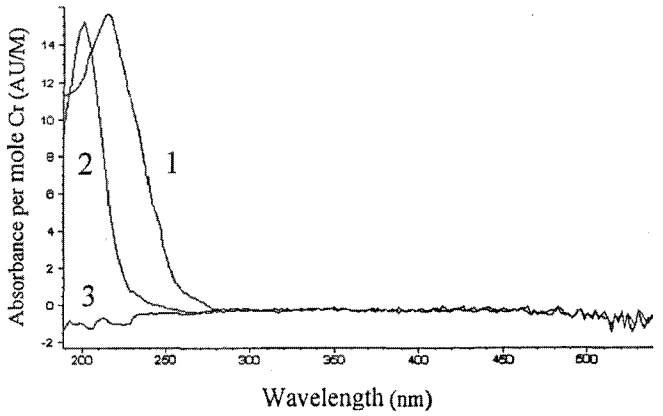

Figure 3

Absorption spectra of chromium(III) complex ions recorded by on-column detection by the CE instrument. **1** = $[CrCl_2(H_2O)_4]^+$, **2** = $[CrCl(H_2O)_5]^{2+}$, **3** = $[Cr(H_2O)_6]^{3+}$.

whole UV-visible region. A small shift of the spectrum of the dichloro complex can be observed toward the higher wavelengths. Figure 3 also shows that the molar absorptivity of the monochloro complex at 200 nm is larger by 20 % than that of the dichloro complex. These different molar absorptivities had to be taken into consideration when calculating the concentrations of the species.

Kinetic Study of Chromium(III) Chloro Complexes

Solid $CrCl_3.6H_2O$ contains discrete $[CrCl_2(H_2O)_4]^+$, Cl^-, and H_2O species [9]. When solid $CrCl_3.6H_2O$ is dissolved in water different species are formed. Some minutes after dissolution of the compound almost all of the chromium exists as the dichlorotetraaquachromium(III) complex. The transformation of dichlorotetraaquachromium(III) to monochloropentaaquachromium(III) and later to hexaaquachromium(III) is well-known [5]. The pH-dependence of these transformations is clearly apparent from the graphs shown in Figure 4. To produce these graphs CE measurements of the chromium species were conducted after dissolution of identical amounts of solid $CrCl_3.6H_2O$ in solutions of pH 1 (0.1 M nitric acid), pH 2.2 and pH 4.5 (20 mM phosphate buffer). Indirect UV detection was used for the hexaaqua complex, direct UV detection for the chloro complexes. Increasing the pH of the solution substantially increased the rate of transformation of the dichloro complex. Above pH 4.5 the dichloro complex could be detected only until 2 h after dissolution (Figure 4a). Initially the amount of the monochloro complex decreases quickly; after 300 min conversion occurs more slowly (Figure 4b). With lower pH the formation of the hexaaqua complex becomes increasingly slow (Figure 4c).

In practice chromium(III) salts are usually dissolved in dilute strong acids. Figure 5 shows the change in the concentrations of the three different chromium(III) species when the $CrCl_3.6H_2O$ was dissolved in 0.1 M HCl (pH 1).

Approximately 2 weeks after dissolving $CrCl_3.6H_2O$ in 0.1 M HCl only one chromium species, the hexaaquachromium complex, can be detected in the solution. For solvents of higher pH the chloro complexes exist for less time in solution. Above pH 3 different hydrolytic polymers of chromium(III) are formed [6, 10] and the chance of wall adsorption or precipitation of the chromium complexes increases. Before trace analysis all metal standard solutions (and thus the solutions prepared from Cr(III) chloride) should usually be acidified. The results obtained show that the solutions prepared from $CrCl_3.6H_2O$ do not contain only free Cr(III) ions, i.e. hexaaquachromium(III) complexes. This is true only 300 h after preparation of the solution. The simplest way of obtaining solutions containing pure, free Cr(III) is to use other chromium compounds (e.g. chromium(III) nitrate or CrO_3) to prepare the solution.

The literature gives half-life data for the transformation of chromium chloro complexes without indicating the pH of the chromium solution used [5]. Our work has shown that transformations of Cr(III) chloro complexes are highly dependent on solution pH, and that the reactions do not seem to behave as characteristic first-order processes. On the other hand, the two transformation processes happen simultaneously, therefore correct half-life data cannot be determined, but it is obvious from the results obtained that the rate of transformation of monochloropentaaquachromium(III) to hexaaquachromium(III) is much slower than the rate of transformation of dichlorotetraaquachromium(III) to monochloropentaaquachromium(III).

The effect of the matrix on the rate of transformation was also studied. The same amounts of $CrCl_3.6H_2O$ were dissolved in different 0.1 M acids (nitric, hydrochloric, sulfuric, perchloric, and phosphoric acids) and the Cr(III) species present were determined after different times. The electropherograms obtained and the concentrations of the Cr(III) species did not differ ap-

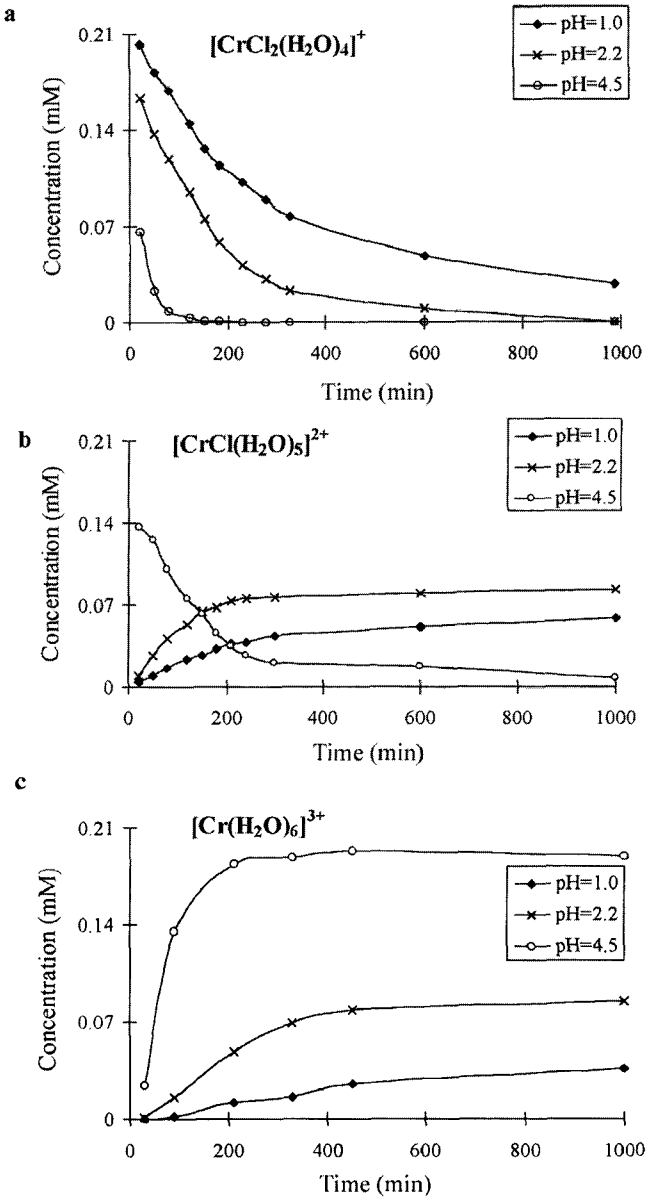

Figure 4

Changes in the concentrations of $[CrCl_2(H_2O)_4]^+$, $[CrCl(H_2O)_5]^{2+}$, and $[Cr(H_2O)_6]^{3+}$ in solutions of different pH obtained by dissolution of chromium(III) chloride. Conditions as for Figure 1.

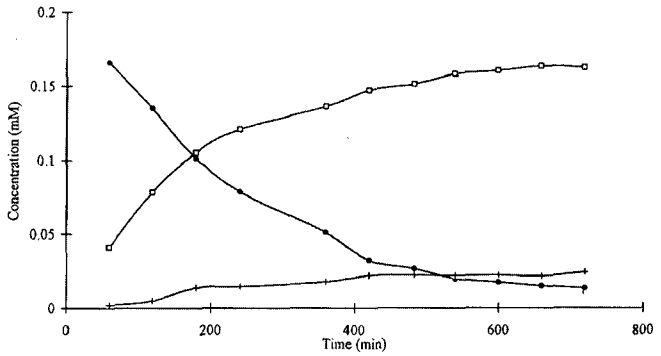

Figure 5

Changes in concentrations of the three different chromium(III) species when $CrCl_3.6H_2O$ was dissolved in 0.1 M HCl. ● = $[CrCl_2(H_2O)_4]^+$, □ = $[CrCl(H_2O)_5]^{2+}$, + = $[Cr(H_2O)_6]^{3+}$. Conditions as for Figure 1.

preciably, indicating the absence of significant interaction and complex formation between Cr(III) and the corresponding anions under these conditions.

Conclusions

This work indicates that CE can be regarded as a powerful new tool for kinetic investigation of metal complexes if the exchange kinetics of the species are sufficiently slow. The kinetic study of chromium(III) chloro complexes showed that the solution obtained by dissolving Cr(III) chloride cannot be regarded as a pure, one-component system but as a mixture of three Cr(III) species the concentrations of which change with time, depending on the pH of the solution. For these reasons most environmental and biological samples containing Cr(III) should be regarded as a mixture of different Cr(III) species with relatively long lifetimes. The solutions after two weeks will not contain any chloro complexes. In the near future further work will be performed to study the separation and kinetics of the chromium complexes occurring in the systems Cr(III)-NO_3^- and Cr(III)-SCN^-.

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