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On-column chelation of metal ions in capillary zone electrophoresis

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Abstract

A method for the chelation of metal ions is described in order to apply direct UV detection for the determination of these species in capillary zone electrophoresis. The chelating reaction was carried out on-column by mixing the zones of the reactants during the electrophoretic migration. The reaction, the stability of the products and the separation of the products from the chelating agent were investigated by the chelation of metal ions with EDTA. In comparison with a common indirect UV detection method the limit of detection was decreased by a factor of up to 10.

1. Introduction

The main features of the most common method for the determination of inorganic ions in capillary zone electrophoresis (CZE) include the use of indirect UV detection, additives such as weak complexing agents for supporting the separation and an electroosmotic flow modifier if necessary. This method is called inorganic capillary electrophoresis (ICE) or capillary ion electrophoresis (CIE). It is a powerful technique for the determination of both inorganic cations and anions, as shown by many applications published in recent years [1–9]. It is also possible to determine a large number of transition metals simultaneously with alkali and alkaline earth metal ions using ICE [7–9].

However, this method has also some limitations, since the electrolyte system must fulfil requirements for both an effective separation and for indirect detection. The indirect detection is based on a charge displacement of the detectable background electrolyte caused by the analyte ions. Therefore, the change in the concentration of all ionic species such as additives in the electrolyte or matrix ions in the sample is sensed by the detector and may result in system peaks or disturbance of the baseline. During the separation of metal ions the effective positive charge may also be reduced by the equilibrium reaction between these ions and the anionic complexing agents, which decreases the charge displacement and results in an increase in the limits of detection. For this reason it is useful to develop methods for direct detection, especially for the determination of transition metal ions.

One possibility of applying the direct detection of metal ions in CZE is the formation of stable metal chelates, which can be measured using UV–Vis detection. There are different ways to perform the chelation. The precolumn reaction was used for the chelation of metal ions, while the products were separated by micellar electrokinetic capillary chromatography (MECC)

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[10]. The precolumn reaction is also possible in cases where the products can be separated with zone electrophoresis [11–17]. The separation of the metal chelates by zone electrophoresis is often difficult, however, especially for equally charged metal ions that form chelates with the same stoichiometry. In this case there is only a small difference in the ratio of charge to radius, which results in nearly identical electrophoretic mobilities of the products. Another possibility is chelation after the separation of the metal ions by the use of a postcolumn reactor [18–24]. This method requires modification of the CE equipment and a significant difference in the absorptivities of the reagent and the product, because both appear in the detector at the same time.

This paper describes the development of an on-column chelation method with the example of the chelation of metal ions with EDTA. The chelating reaction, which occurs inside the capillary, is carried out by mixing the analyte zone and the reagent zone during the electrophoretic migration. The possibility of performing a chemical reaction during the contact time of zones in CZE is based on the technique of electrophoretically mediated microanalysis (EMMA), which has already been demonstrated by the enzymatic oxidation of ethanol [25].

2. Experimental

The CZE system was laboratory built and allows the injection of the sample and the electrolyte at both ends of the capillary. The equipment, which includes a UV detector (Dionex, Sunnyvale, CA, USA), has been described previously [26]. All solutions, electrolytes and standards were prepared using water purified with a Milli-Q system (Millipore, Eschborn, Germany). All other reagents were of analytical-reagent grade from Merck (Darmstadt, Germany). Stock solutions (10 mmol/l) of each cation were used to prepare the sample solutions. The fused-silica capillary (75 μm I.D.) was rinsed for 5 min with 0.1 mol/l sodium hydroxide solution, water and electrolyte at the beginning of each day and for 2 min with electrolyte between all electrophoretic separations. The data were processed with an A/D board from ERC (Altegolfsheim, Germany) using the APEX chromatography software (Autochrom, Milford, MA, USA). The absorbance units are converted into microvolts by the A/D board. Therefore, the output of the data is in the voltage mode ($19 \cdot 10^3$ μV = 0.001 absorbance).

3. Results and discussion

Fig. 1 shows the principle of the mechanism of the on-column zone reaction in CZE. The first step is the injection of the sample and the chelating agent. Then an electrophoretic separation of the metal ions with either organic solvents or weak complexing agents such as lactic acid or hydroxyisobutyric acid is carried out (Fig.

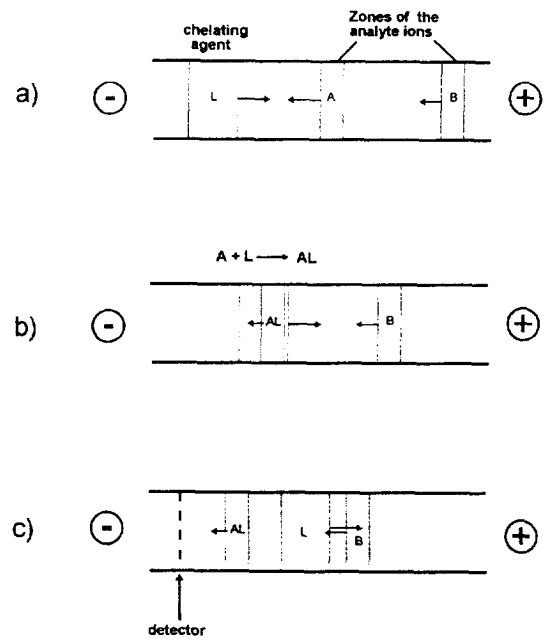

Fig. 1. Principle of on-column chelation. L = chelating agent; A and B = analyte ions. (a) Injection of the sample (A, B) and the reagent (L) and electrophoretic separation of the metal ions. (b) Mixing of the analyte zone and the reagent zone. (c) Separation of the products and the chelating agent followed by detection.

1a). After the separation, the analytes enter the reagent zone and the reaction takes place (Fig. 1b). Since the resulting products have different charges and therefore different electrophoretic mobilities compared with the chelating agent, they migrate out of the reaction zone towards the detector before the next analyte enter this area (Fig. 1c).

Some requirements have to be fulfilled for a successful on-column chelation. A fast reaction must occur during the contact time of the zones of the analyte and the chelating agent. After leaving the reaction zone, the resulting products must be stable enough to reach the detector. There should be significant differences in the electrophoretic mobilities of the metal chelates and the chelating reagent. Further, the products should be detectable.

For the development of the CZE on-column reaction, EDTA was used as a chelating agent. The chelation of metal ions with EDTA has been well investigated and used in chromatographic separations with UV detection [27–31]. EDTA forms stable anionic chelates with many metals and some of these, such as lead–, copper–, cobalt–, nickel–, iron– and bismuth–EDTA chelates, are detectable by UV detection. The optimum experimental conditions for parameters such as the mobility of the reactants and the products, the required length of the capillary for supporting the separation and the required direction and velocity of the electroosmotic flow, can be precalculated.

Fig. 2 shows the principle of the on-column reaction, which was developed especially for the chelation of the metal ions with EDTA. First the capillary is filled with electrolyte solution. The detector is placed near the anodic end of the capillary, where the sample is injected (Fig. 2a). Therefore, both hydrodynamic and electrokinetic injection are possible. The EDTA solution will be introduced at the cathodic end of the capillary. After the high voltage has been applied (Fig. 2b), the separation of the analyte ions starts and the first analyte migrates through the EDTA zone followed by the reaction. Then the EDTA anions and the metal–EDTA chelates, which are also anions but less negatively charged, can be separated by electrophoresis (Fig. 2c).

Fig. 3 shows an electropherogram for the determination of four metal ions carried out with the on-column chelation technique. The electrolyte used was 10 mmol/l sodium acetate solution (pH 4.8) containing 2 mmol/l tartaric acid for supporting the separation of the metal ions. The concentration of the EDTA solution was 1 mmol/l. The electroosmotic flow is reversed by the addition of tetradecyltrimethylammonium bromide (TTAB). This allows the slow-moving metal–EDTA species of the trivalent cations also to reach the detector in a short time, whereas the peak broadening is still acceptable. EDTA, which is present in an excess concentration compared with the concentration of the analyte ions, is detectable at 242 nm and appears as the first peak in the electropherogram. Lead and nickel are separated before the reaction because the EDTA chelates of this species have the same electrophoretic mobility. Iron(III)–EDTA appears as the last peak because the mobility of this product differs most from the mobility of the EDTA anions.

In the following, investigations of some important aspects of on-column chelation in CZE are described, viz., the chelation of the analytes in the reaction zone, the stability of the chelates and the separation of the products from the chelating agent.

Fig. 4 shows the results of the investigations of the chelating process with the example of the reaction between EDTA and lead. The EDTA concentration was changed in a range between 10 μmol/l and 10 mmol/l whereas the lead concentration was constant at 100 μmol/l. Curve A shows the peak area of the EDTA peak at different concentrations. As can be seen from curve B, the area of the lead–EDTA peak is constant for EDTA concentrations between 10 mmol/l and 100 μmol/l. At lower EDTA concentrations, there is not enough chelating agent present and the lead–EDTA peak decreases. These results indicate that there is a quantitative and equimolar reaction during the contact time of the zones.

After the reaction, the metal chelates have to

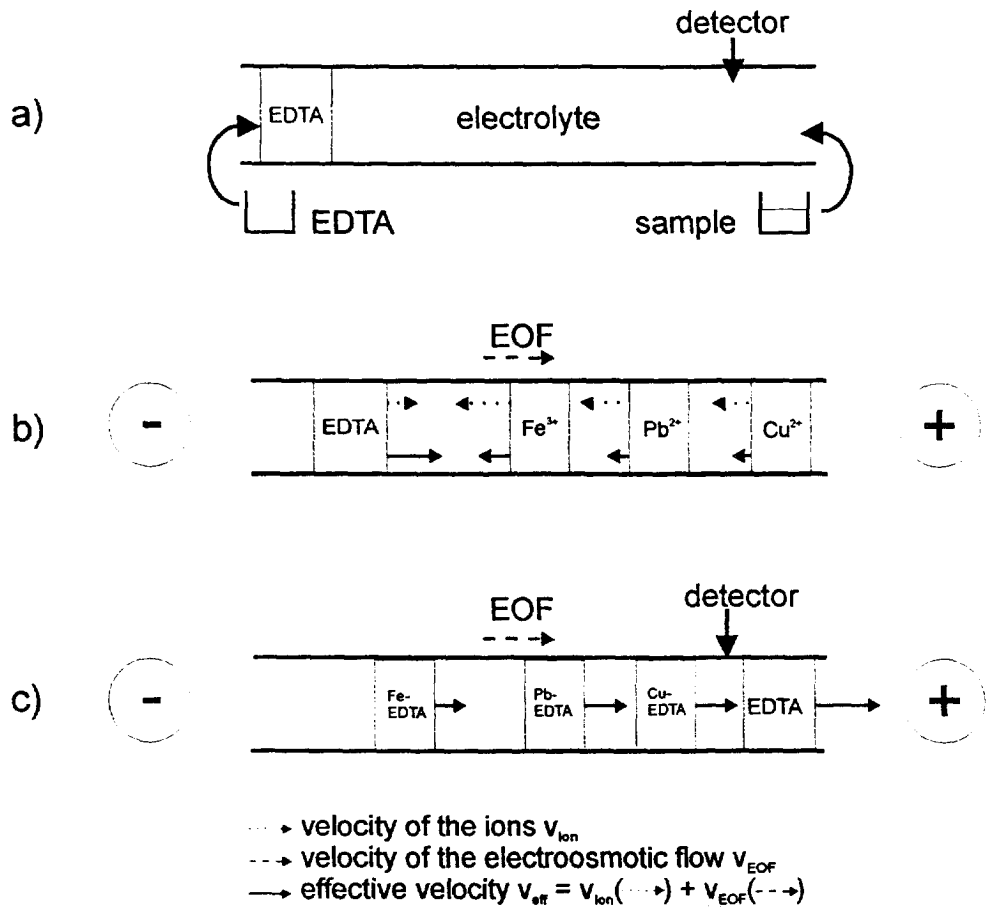

Fig. 2. Mechanism of the on-column reaction with EDTA as chelating agent. (a) Filling of the capillary with electrolyte and chelating agent and sample injection. (b) Electrophoretic separation of the metal ions. (c) Migration of the products towards the detector after the mixing of the zones and the reaction.

be stable in order to migrate through the electrolyte solution towards the detector. For the investigation of the stability of the products in the electrolyte system used, first the analyte ions and second the EDTA solution were introduced by hydrostatic injection at the cathodic end of the capillary. Therefore, the reaction occurs at the inlet side and the chelates are forced to migrate through nearly the whole capillary until they arrive at the detection window. Fig. 5 shows electropherograms of the EDTA chelates of copper, lead and iron. For these ions, separation before the reaction is not necessary, because the differences in the mobilities of these metal chelates are sufficient for an electrophoretic separation. The measurements were carried out by applying different high voltages. Therefore, the metal chelates migrate with different velocities and have different contact times with the electrolyte solution. Nevertheless, no decrease can be observed, in either peak height or peak area. Table 1 gives the values of peak height and peak area. Because of the different velocities of the analyte ions passing the detection window, the values of the peak areas are corrected by the migration time. Under these conditions, the stabilities of the metal chelates are sufficient for on-column zone chelation.

The chelation technique described here requires the separation of the metal chelates and

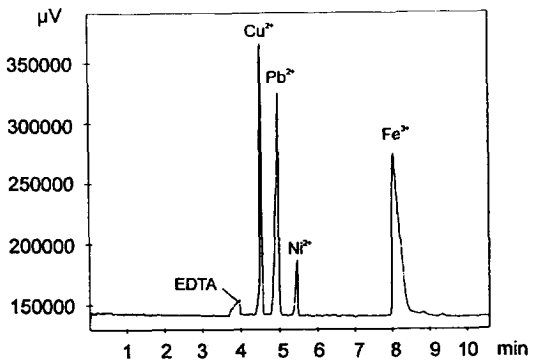

Fig. 3. Electropherogram of four metal ions after on-column chelation. Electrolyte: 10 mmol/l sodium acetate–2 mmol/l tartaric acid–0.2 mmol/l TTAB (pH = 4.8). Injection: (1) hydrostatic, 10 cm, 30 s, 1 mmol/l EDTA at the cathodic capillary inlet; (2) hydrostatic, 10 cm, 30 s, 50 μmol/l Pb, 75 μmol/l Cu, Ni and Fe at the anodic capillary inlet. Detection: UV at 242 nm. Separation voltage: 30 kV.

the chelating agent after the reaction. If EDTA is used as the chelating agent, the most important parameter for a successful separation of the reagents and the products is the pH of the electrolyte system. As some dissociation equilibria for EDTA in the pH range between 2 and 10 have to be considered, EDTA species of different charges exist at different pH values [32].

After the reaction with doubly negatively charged EDTA the chelates of the bivalent

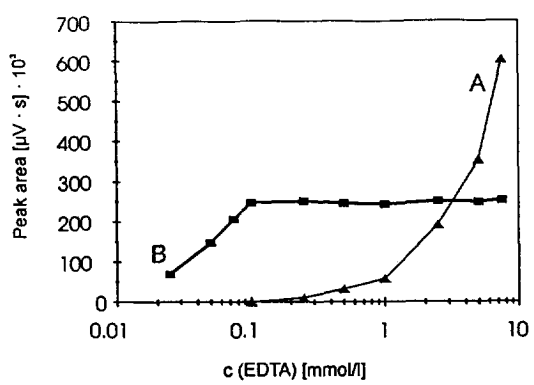

Fig. 4. Investigation of the reaction during the contact time of the zones. ■ = Peak area of 100 μmol/l Pb^{2+}; ▲ = area of the EDTA peak.

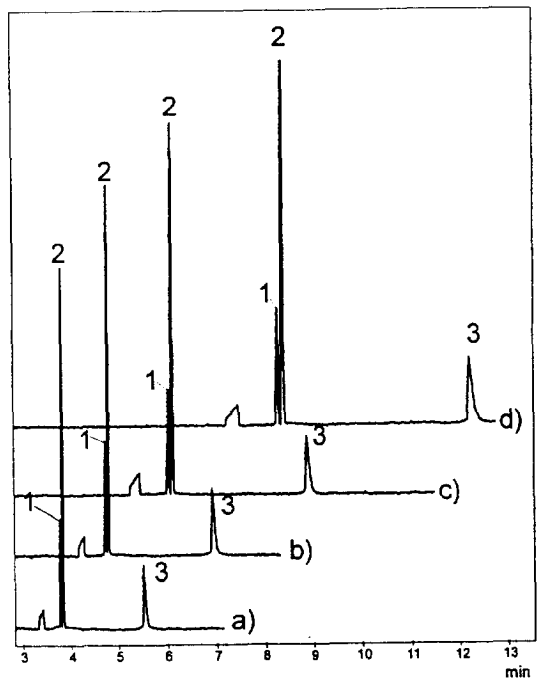

Fig. 5. Investigation of the stability of the metal chelates during the migration in the electrolyte system. 1 = Cu–EDTA; 2 = Pb–EDTA; 3 = Fe–EDTA. Separation voltage: (a) 30; (b) 25; (c) 20; (d) 15 kV.

cations have the same charge as the EDTA anions and separation by zone electrophoresis is not possible. In this case, a higher pH is necessary for the separation. Fig. 6 shows the differences between the electrophoretic mobilities of EDTA and EDTA chelates of bi- and trivalent metal ions depending on the pH value. The mobilities are calculated from experimentally determined migration velocities. It can be seen that for bivalent cations a pH of 6 or higher is

Table 1
Corrected peak areas of metal–EDTA chelates (Fig. 5) under different separation conditions

Separation voltage (kV)	Peak area (μV s)/migration time (s)		
	Cu–EDTA	Pb–EDTA	Fe–EDTA
30	21946	61306	19010
25	20273	60780	19590
20	20695	61454	19596
15	21254	60603	18586

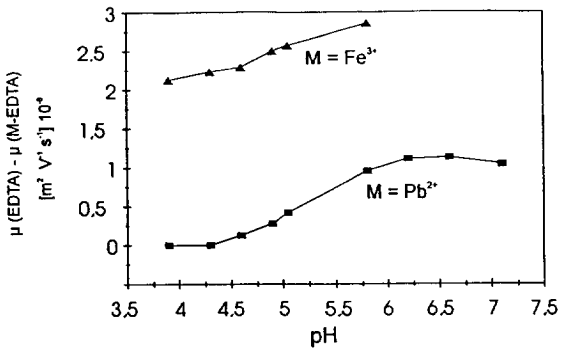

Fig. 6. Differences between the electrophoretic mobilities of EDTA and the metal–EDTA chelates depending on the pH of the electrolyte.

best for the separation. Because of hydroxide formation, some metals cannot be separated under these conditions. In this case the pH of the electrolyte has to be decreased and a less efficient separation has to be accepted.

The metal–EDTA species, which can be measured using UV detection, have absorption maxima at different wavelengths. Therefore, the limits of detection (LODs) of the different analyte ions depend on the wavelength used. In Table 2, the detection limits measured with the on-column chelating technique at 242 nm are compared with indirect detection which was carried out with a 4-aminopyridine electrolyte. The LOD was defined as three times the signal-to-baseline noise ratio. The experimental conditions were the same. A 75 μm I.D. fused-silica capillary with a total length of 70 cm was used. The sample injection was hydrostatic by raising the capillary inlet side 10 cm for 15 s. With direct detection 3–10-fold lower detection limits can be achieved, depending on the position of the absorption maxima of the metal–EDTA ions and of their molar absorptivity at the wavelength used.

Table 2
Detection limits (μmol/l) measured with electrolyte systems for indirect and direct UV detection (signal-to-noise ratio = 3)

Metal	Indirect UV detection[a]	Direct UV detection[b]
Cu	10	1
Pb	10	0.5
Ni	8	3
Co	5	2
Fe	10	2

Injection: hydrostatic (10 cm, 15 s). Capillary: fused silica (70 cm × 75 μm I.D.).
[a] Electrolyte: 5 mmol/l 4-aminopyridine–2 mmol/l tartaric acid (λ = 264 nm).
[b] On-column chelation: 10 mmol/l sodium acetate–2 mmol/l tartaric acid (pH 4.8) (λ = 242 nm).

The calibration with hydrostatic injection is linear up to about three orders of magnitude above the detection limit. The correlation coefficients ranged from 0.991 for nickel to 0.999 for lead. The determination of high concentration levels of the analyte ions is limited by the amount of chelating agent that is available in the reaction zone and by the CZE separation of the metal ions, which must occur before the chelating reaction.

Using EDTA as the chelating agent, alkali metal ions up to a concentration of 50 mmol/l can be present in the sample without interferences, because the alkali metal ions migrate through the EDTA zone without a reaction. Fig. 7 shows the electropherogram for copper, lead, nickel and cobalt in the presence of 20 mmol/l sodium chloride.

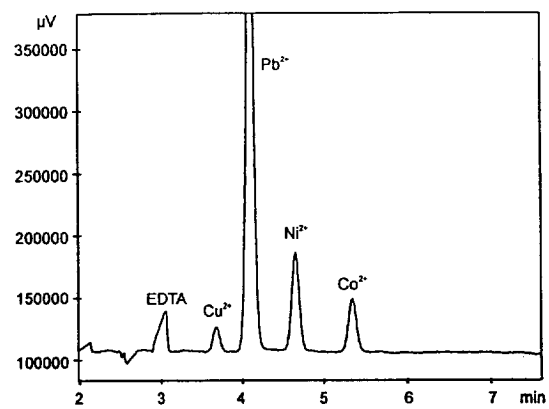

Fig. 7. Electropherogram of metal ions in sodium chloride solution. Sample: 20 μmol/l Cu–100 μmol/l Pb–50 μmol/l Ni–25 μmol/l Co–20 mmol/l NaCl. Electrolyte: 10 mmol/l sodium acetate–2 mmol/l tartaric acid–0.2 mmol/l TTAB (pH 4.8). Injection: hydrostatic (10 cm, 15 s). Separation voltage: 25 kV. Detection: UV at 242 nm.

4. Conclusion

The "on-column" reaction in CZE is an additional method complementing the commonly used pre- or postcolumn chelation of metal ions. It offers the possibility of carrying out chemical reactions of oppositely charged ions by mixing the zones of these species inside the capillary and during an electrophoretic separation. This may be useful, for example, if small sample volumes do not allow a precolumn reaction or if a postcolumn reaction disturbs the detection.

The possibility of mixing zones containing oppositely charged species is an advantage of the electrophoretic separation mechanism compared with a chromatographic separation, where the zones do not migrate in opposite directions.

The method allows the complexation or derivatization of analytes and therefore the direct detection in CZE determinations of UV-inactive species such as inorganic cations. This technique was developed with the example of the chelation of metal ions with EDTA because the equimolar reaction makes it easy to precalculate important parameters of the separation. The method may be improved by choosing other chelating agents to permit the determination of more metal ions. If the resulting products have a higher molar absorptivity than the metal–EDTA chelates, the detection will be more sensitive. This requires a knowledge of the chemistry of the reaction and parameters such as the stability and the charge of the reagents and the products in order to optimize the conditions for the reaction, separation and detection. Possibly it will be necessary to change the instrumental or the chemical conditions such as the sample injection, the position of the detector or the direction of the electroosmotic flow.

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