



ELSEVIER

Journal of Chromatography A, 740 (1996) 151–157

JOURNAL OF  
CHROMATOGRAPHY A

Short communication

## Direct determination of iron(II), iron(III) and total iron as UV-absorbing complexes by capillary electrophoresis

S. Schäffer<sup>a</sup>, P. Gareil<sup>a,\*</sup>, C. Dezael<sup>b</sup>, D. Richard<sup>b</sup>

<sup>a</sup>Laboratoire d'Electrochimie et de Chimie Analytique (URA CNRS 216), Ecole Nationale Supérieure de Chimie de Paris, 75231 Paris Cedex 05, France

<sup>b</sup>Institut Français du Pétrole, B.P. 311, 92506 Rueil-Malmaison Cedex, France

Received 11 October 1995; revised 31 January 1996; accepted 31 January 1996

### Abstract

Capillary electrophoresis (CE) was investigated for the separation of iron(II) and iron(III) after selective complexation of these species by *o*-phenanthroline and EDTA added in excess to the sample solution. The cationic and anionic natures of the ferrous and ferric complexes, respectively, allow their easy electrophoretic analysis in a single run in the presence of an electro-osmotic flow. It was ascertained that the addition of the mixed complexing solution does not alter the iron(II)/iron(III) ratio of the sample and no significant evolution of this ratio was observed over a period of ten days. Using direct UV absorbance detection, the developed method allows the simultaneous determination of iron(II) and iron(III) in less than 3 min with R.S.D.<1% for migration times and <3.5% for corrected peak areas. Excellent detection linearity was also obtained. When only EDTA is added as complexing agent, CE allows the rapid direct determination of total iron content, which provides a means for checking total iron recovery. Both methods were successfully applied to monitor a wet chemistry process converting iron(II) into iron(III).

**Keywords:** Complex formation; Phenanthroline; Iron; Metal ions; Ethylenediaminetetraacetic acid

### 1. Introduction

Capillary electrophoresis (CE) is being increasingly applied in the determination of alkaline [1–3], alkaline-earth [1–3] and transition metal ions [3–8], primarily because of its great flexibility and easy implementation. Although the absolute mobility values of most of the metal ions in their free form do not differ enough from one another to envision electrophoretic separations of practical interest, the use of complexing agents, either for complexation before injection or as electrolyte additives for in situ

reaction, offers a powerful means of manipulating separation selectivity. In this trend,  $\alpha$ -hydroxyisobutyrate [2,5,6], EDTA [3,7,8] or cyclohexanediaminetetraacetate [7,8] were recently proposed as complexing agents for the separation of various metal ions including either iron(II) or iron(III). For this latter case detection modes were currently direct [4,7,8] or indirect [1,2,5,6] UV absorbance, but indirect laser induced fluorescence was also experienced lately [3].

In addition, like ion chromatography, CE inherently has the capability of resolving speciation problems that could be of paramount importance for a thorough understanding of chemical, biotechnologi-

\*Corresponding author.

cal, biological or environmental processes. Till now, little has been shown in this direction in CE, except for the separation of hexacyanoferrate(II) and (III) applied to monitor an electroplating solution [9], the separation of free and complexed forms of aluminum(III) [10] and, very recently, the separation of four forms of arsenic [11]. Nevertheless the simultaneous determination of iron(II) and iron(III) employing a less drastic ligand than cyanide is still an analytical challenge in CE.

In a previous work [12], we already applied CE for the determination of iron in samples originating from a desulfuration process of industrial exhaust gases. The washing solutions of the gases contained iron(II) and iron(III) and the complexing agent nitrilotriacetate (NTA). The developed method used the stronger complexing agent EDTA to quantitatively displace ferrous and ferric NTA complexes. However, under these conditions, injection of iron(II)–iron(III) mixtures always resulted in a sharp single peak. We report now on the simultaneous use of *o*-phenanthroline and EDTA to complex iron(II) and iron(III) selectively and to achieve the separation of iron under its two oxidation states. The quantitative aspects of the method were evaluated with a series of samples collected over the duration of an oxidation reaction of iron(II) into iron(III) involved in the desulfuration process.

## 2. Experimental

### 2.1. Apparatus

CE was performed with a Hewlett-Packard HP<sup>3D</sup>CE system (Hewlett-Packard, Waldbronn, Germany) with a built-in UV diode-array detector and DOS windows-type, data-analysis software. All separations were carried out in fused-silica capillaries of 30 cm effective length (38.5 cm total length) and 50  $\mu\text{m}$  I.D. The window for on-column detection was created by burning off a small section (0.5 cm) of the polyimide coating. Samples were introduced hydrodynamically by applying a pressure of 50 mbar for 2 or 5 s. The corresponding volumes were 4.5 nl or 11.3 nl, respectively. The separation voltage was set at 30 kV. The temperature of the capillary was kept

at 25°C using the thermoregulation equipment of the instrument. Detection was performed at 245, 254 or 266 nm depending on the ion of interest.

The colorimetric determinations of iron(II) were carried out on a UV-160A spectrophotometer (Shimadzu Europa, Duisburg, Germany). The studied complexes were detected at 505 nm.

### 2.2. Reagents and solutions

All solutions, electrolytes and standards were prepared from 18 M $\Omega$  water generated by a Milli-Q laboratory water purification system (Millipore, Milford, MA, USA).

$\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ,  $\text{FeSO}_4$ ,  $(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$  and  $\text{Na}_2\text{B}_4\text{O}_7$  dihydrate were obtained from Prolabo (Paris, France). The complexing agents ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) and *ortho*-phenantroline monohydrate (*o*-phen) were purchased from Sigma (St. Louis, MO, USA).

Standard solutions containing iron(II) and iron(III) at a concentration of 1 mM were prepared by dissolving the solid iron salts in a solution of EDTA and *o*-phen, 10 mM each. This complexing solution was made up by mixing equal volumes of a 20 mM aqueous EDTA solution and a 20 mM ethanolic *o*-phen solution.

The samples used for method testing were collected over the duration of an oxidation step of iron(II) into iron(III). They were first filtered through a 0.45- $\mu\text{m}$  membrane. The samples from a first series, intended for iron(II) and iron(III) determinations, were diluted 1:50 in the 10 mM EDTA, 10 mM *o*-phen complexing solution. The samples from a second series, intended for total iron determination, were each obtained at the same time as those of the first series and diluted 1:50 in 10 mM EDTA.

The carrier electrolytes for iron(II) and iron(III) analysis and for the direct total iron analysis were prepared from a 25 mM sodium tetraborate solution, pH 9.2. For quantitative analysis of iron(II) and iron(III) the carrier electrolyte also contained 0.2 mM EDTA and 0.2 mM *o*-phen to prevent dissociation of the formed complexes. For quantitation of total iron the borate buffer was only supplemented with 0.2 mM EDTA.

### 2.3. Oxidation of iron(II) into iron(III)

The working solution, initially containing 68 mM total iron, predominantly in ferrous form, and 93 mM NTA was reoxidized according to a procedure previously described [13]. 860 ml of this solution, kept at pH 7 by a regulator, was allowed to circulate in a tube (flow-rate: 33 l/h), producing a falling film on its inside wall on which iron(II) was rapidly converted to iron(III) by air introduced at the bottom of the tube (flow-rate: 20 l/h).

## 3. Results and discussions

### 3.1. Complexation of iron(II) and iron(III)

The development of a CE separation of ferrous and ferric ions cannot be contemplated without the use of a strong complexing agent of iron(III), intended to extend its range of solubility towards neutral and possibly slightly alkaline aqueous media. Furthermore, the redox potential of the Fe(III)/Fe(II) system must also be maintained at a high level, in order to preserve the stability of samples with respect to oxido-reductive phenomena. This in turn requires consideration of a strong complexing agent of iron(II).

In addition, as electrophoretic separations are based on differences in mobility, the separation will only be possible if the complexed forms of iron(II) and iron(III) differ in charge or in size. The complexing agents selected for this study were *o*-phen and EDTA. *o*-phen and EDTA can both complex iron(II) and iron(III) but, as can be inferred from Table 1, *o*-phen and EDTA are expected to be the preferred complexing agents of iron(II) and iron(III), respectively. In effect, using a simulation software

for chemical equilibria in solution [17], it was ascertained that, in a solution containing a ligand to metal ion ratio of 5, iron(II) remains quantitatively under the form  $\text{Fe}(\textit{o}\text{-phen})_3^{2+}$  below pH 9.5, whereas between pH 4 and 11 iron(III) is quantitatively complexed by EDTA under the form of either  $\text{FeY}^-$  or  $\text{Fe}(\text{OH})\text{Y}^{2-}$ . Moreover, the addition of *o*-phen to a solution of ferric and ferrous ions will also increase the redox potential of Fe(II)/Fe(III), making the oxidation of iron(II) into iron(III) more difficult. The cationic and anionic natures of the ferrous and ferric complexes allow their easy electrophoretic separation in a single run in the presence of an electro-osmotic flow. It is to be noted that this approach of the separation will remain consistent with sample solutions initially containing other ligands of iron(II) and iron(III) in so far as *o*-phen and EDTA will be strong enough to quantitatively displace the initial complexes. This point was checked for the case of NTA and HEDTA (results not shown).

### 3.2. Separation of a standard solution

Several carrier electrolytes were studied to find out the best conditions for the separation and detection of complexes  $\text{Fe}(\textit{o}\text{-phen})_3^{2+}$  and, according to pH,  $\text{FeY}^{2-}$  or  $\text{Fe}(\text{OH})\text{Y}^{2-}$ . Satisfying conditions for a standard mixture, containing iron(II), iron(III) and complexing agents *o*-phen and EDTA were obtained with a 100 mM borate buffer, pH 9.2 (Fig. 1). This solution, prepared from aqueous and ethanolic stock solutions of the complexing agents, also contains 50% (v/v) ethanol. At this pH, free *o*-phen carries no charge and migrates along with the neutral species. Iron(III) is almost entirely under the form  $\text{Fe}(\text{OH})\text{Y}^{2-}$ . The peaks corresponding to iron(II) and iron(III) are detected on both sides of the signal due to electro-osmosis, which clearly illustrates this unique capability of electroosmotic flow CE to analyze anions and cations in a single run. Also shown in Fig. 1 are the UV absorbance spectra of the iron(II) and iron(III) complexes collected directly during the migration. Direct UV absorbance can be carried out to detect the *o*-phen and EDTA complexes if a highly UV transparent buffer such as borate is used. The more favourable detection wavelengths are 266 nm for iron(II) and 245 nm for iron(III).

Table 1  
Complex formation constants of ferrous and ferric ions with *o*-phenanthroline (*o*-phen) and EDTA ( $\text{Y}^{4-}$ ) (from Refs. [14–16])

|                  | <i>o</i> -Phenanthroline  | EDTA   |                                   |
|------------------|---|--|-----------------------------------|
| $\text{Fe}^{2+}$ | $\text{Fe}(\textit{o}\text{-phen})_3^{2+}$<br>$\log \beta_3 = 21.2$ | $\text{FeY}^{2-}$<br>$\log K = 14.3$                   |                                   |
| $\text{Fe}^{3+}$ | $\text{Fe}(\textit{o}\text{-phen})_3^{3+}$<br>$\log \beta_3 = 14.4$ | $\text{Fe}(\text{OH})\text{Y}^{2-}$<br>$\log K = 31.6$ | $\text{FeY}^-$<br>$\log K = 25.2$ |

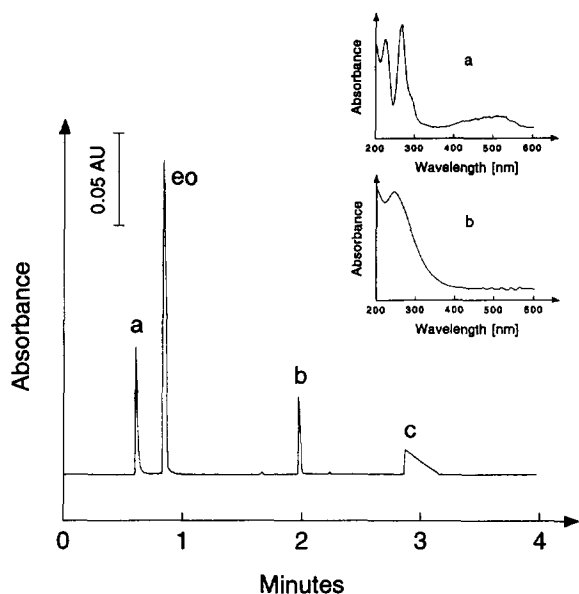


Fig. 1. Electropherogram of a standard solution of iron(II) and iron(III), 1 mM each and *o*-phenanthroline and EDTA, 10 mM each. Electrolyte: 100 mM borate, pH 9.2. Direct UV detection at 200 nm. Hydrodynamic injection for 2 s. Peak identification: a=Fe(II)-*o*-phenanthroline complex; eo=free *o*-phenanthroline superimposed on electro-osmosis signal; b=Fe(III)-EDTA complex; c=free EDTA. Insert a: UV absorbance spectrum of peak a. Insert b: UV absorbance spectrum of peak b.

In order to envisage quantitative applications, it was first checked that, on injecting standard solutions of either iron(II) or iron(III) into the mixed complexing solution, no trace of the conjugated species was detected. The stability of the standard mixture solution was next studied by injecting it repeatedly over a period of 10 days. No significant evolution was observed for the time corrected peak areas of the two studied complexes. The R.S.D.s for this whole series were 7.6 and 8.1% for the iron(II) and iron(III) complexes, respectively. In addition, small amounts of *o*-phen (as an ethanolic solution) and EDTA were added to the carrier electrolyte to avoid any subsequent dissociation of the complexes during their migration inside the capillary. The linear correlation coefficient calculated from the calibration curve obtained with four different concentrations (0.2–1 mM) of iron(II), detected at 266 nm was 0.998. The repeatability ( $n=4$ ) was less than 5.8% R.S.D. for the corrected peak areas and less than 0.9% R.S.D. for the migration times. The calibration curve ob-

tained with four different concentrations of iron(III) (0.2–1 mM) in the same standard solution using the detection wavelength of 245 nm also showed good linearity with a correlation coefficient of 0.999. The results for iron(III) also showed a good repeatability as indicated by the R.S.D.s ( $n=4$ ) of migration times (<1%) and corrected peak areas (<4.5%).

The detection limits, defined as three times the signal-to-noise ratio at the optimum wavelengths were determined to be 0.3 ppm ( $5 \cdot 10^{-6}$  M) for iron(II) and 0.6 ppm ( $10^{-5}$  M) for iron(III) for a calculated injection volume of 22.6 nl.

### 3.3. Quantitation of iron(II) and iron(III) during an oxidation reaction

The method was applied for the determination of iron(II) and iron(III) during the reoxidation reaction of iron(II) into iron(III) involved in a desulfuration process [12]. Before the development of the CE method, a classical colorimetric technique was commonly used to monitor iron(II) concentration and detect the completion of the reaction, which normally occurred after ca. 80 min. The working solution was sampled every 10 min in order to redetermine iron(II), iron(III) and total iron concentrations by CE. The addition, just after sampling, of *o*-phen and EDTA resulted in the formation of stable iron(II) and iron(III) complexes which prevented further alteration of the iron(II)/iron(III) ratio. The calibration curves obtained with five different concentrations (0.2–1 mM) of iron(II) and iron(III) complexed by *o*-phen and EDTA showed excellent linearities with correlation coefficients of 0.999 for the Fe(II)-*o*-phen complex and of 0.9999 for the Fe(III)-EDTA complex. A common wavelength for both species (254 nm) was chosen for this particular application to demonstrate that very current detection conditions can also be employed. Each calibration point was obtained from four consecutive injections. The R.S.D.s of migration times calculated from these data were  $\leq 1\%$  for iron(II) and  $\leq 0.6\%$  for iron(III) and those of corrected peak areas were  $\leq 3.6\%$  for iron(II) and  $\leq 3.7\%$  for iron(III).

The results for the determinations of iron(II) and iron(III) in the samples collected during the oxidation reaction are presented as histograms in Fig. 2. The origin on the time axis in Fig. 2 does not

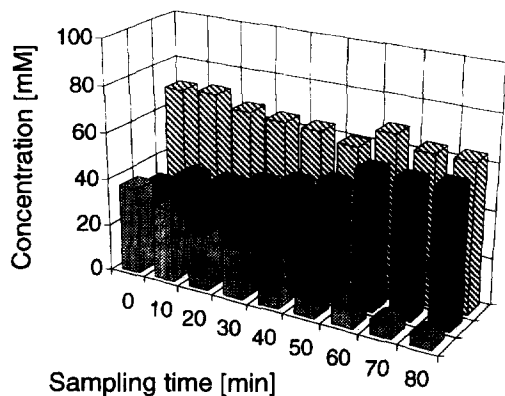


Fig. 2. Variation of concentration of iron(II), iron(III) and total iron determined in the duration of an oxidation process of iron(II) into iron(III). Clear bars in the front: iron(II); dark bars in the middle: iron(III); striped bars in the background: total iron (calculated as the sum of iron(II) and iron(III) contents). Operating conditions: see Section 2.

correspond to the actual start of the oxidation reaction but to the establishment of a steady state regime for the circulating solution a few minutes later. As expected, the concentration of total iron, calculated by addition of iron(II) and iron(III) concentrations, remains stable over the whole experiment.

Special attention was paid to the recovery of total iron with respect to the concentrations of iron(II) and iron(III) found separately. The concentration of total iron was determined directly from a second series of solutions sampled during the oxidation reaction at the same time as the first series. For this purpose, a single complexing agent, EDTA, was added to the samples and was also present in the electrolyte. Fig. 3 shows the electropherogram of a solution sampled at the beginning of the oxidation reaction exhibiting a single peak, identified with an iron(III)–EDTA complex. This behaviour may be accounted for by the fact that the stability constant of the ferric complex is much greater than that of the ferrous one. Consequently, iron(II) can be easily oxidized into iron(III), either chemically by ambient oxygen before injection or most likely electrochemically just after injection on the anodic side. As a result, these conditions were used to monitor the total iron concentration directly. As can be seen from Fig. 4, the results for the total iron determination were well

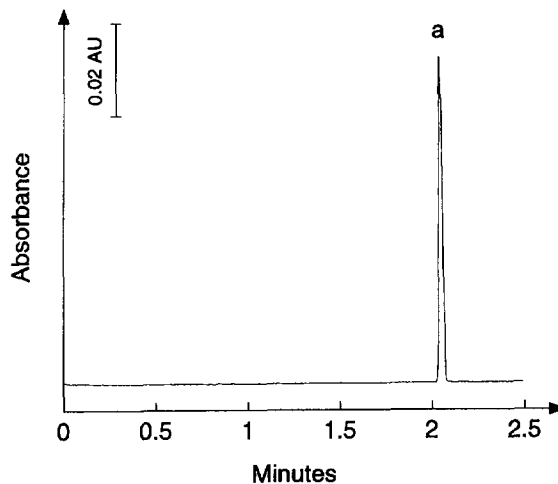


Fig. 3. Electropherogram of a solution sampled at the beginning of an oxidation process of iron(II) into iron(III). Electrolyte: 100 mM borate, 0.2 mM EDTA, pH 9.2. Direct UV detection at 254 nm. Hydrodynamic injection for 5 s. Peak identification: a=Fe(III)–EDTA complex.

correlated to those obtained indirectly by addition of iron(II) and iron(III) concentrations, determined as described above.

#### 3.4. Comparison of electrophoretic and colorimetric determination of iron(II)

In order to gain a further insight into the reliability of the electrophoretic method, the results for iron(II) determinations obtained by CE were compared with those obtained by a classical colorimetry technique.

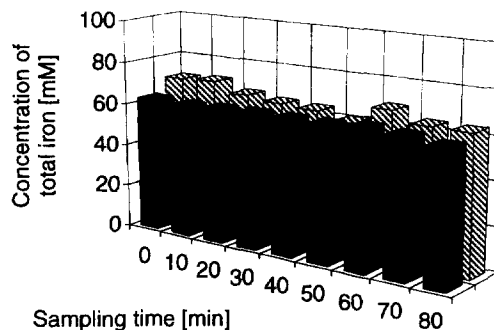


Fig. 4. Comparison of the results for total iron determinations (dark bars) with the sum of the iron(II) and iron(III) concentrations, determined separately (striped bars, also represented in Fig. 2). Operating conditions: see Section 2.

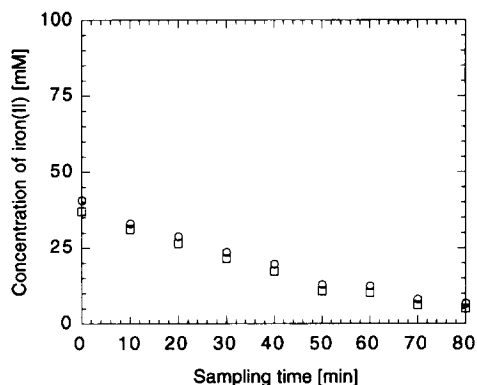


Fig. 5. Comparison of the results for iron(II) determination obtained by CE and colorimetry. See text (Section 2) for experimental conditions and comments. (□): Results obtained by CE. (○): Results obtained by colorimetry.

The use of *o*-phen as complexing agent of iron(II) is well known for this purpose [18]. The measurements were done on the same samples as those previously used for CE analysis (first series, supplemented with *o*-phen and EDTA). The iron(II)–*o*-phen complex was detected at 505 nm. It can be noted that the presence of iron(III) and EDTA in these solutions may result in slight interferences for this method. In spite of this, it can be asserted that the values derived from the two methods are in good agreement, as shown in Fig. 5, which corroborates the use of CE as a rapid and effective technique for ion speciation.

#### 4. Conclusion

CE in the presence of an electro-osmotic flow offers a unique possibility for rapid, reproducible and simple determination of iron(II) and iron(III). The complexing agents *o*-phen and EDTA, involved in the developed method react selectively with iron(II) and iron(III) to form positively and negatively charged species, respectively. They also allow direct UV detection of these species with satisfactory detection limits. The method applied successfully to the quantitation of iron(II) and iron(III) in the duration of an oxidation reaction. The results for iron(II) determinations compare well with those obtained by classical colorimetry, but CE allows determination of both iron(II) and iron(III) without

any risk of interference by preparing only one range of standard solutions. A special stress was paid to the recovery of total iron by comparing total iron contents calculated by addition of iron(II) and iron(III) concentrations to a direct determination obtained by applying slightly modified conditions (use of EDTA as single complexing agent). The CE method is also well suited for determination of iron(II) and iron(III) in solutions initially containing less stable complexing agents than EDTA and *o*-phen. Finally this work highlights that the flexibility of CE can potentially open new methodologies in speciation analysis, which deserve to be exploited further.

#### Acknowledgments

The authors would like to acknowledge the Hewlett-Packard Company (France) and especially D. Frayssinhes for the loan of a HP<sup>3D</sup>CE system and helpful technical discussions.

#### References

- [1] Y. Shi and J.S. Fritz, *J. Chromatogr.*, 640 (1993) 473.
- [2] A. Weston, P.R. Brown, A.L. Heckenberg, P. Jandik and W.R. Jones, *J. Chromatogr.*, 602 (1992) 249.
- [3] P.L. Desbène, C.J. Morin, A.M. Desbène Monvernay and R.S. Groult, *J. Chromatogr. A*, 689 (1995) 135.
- [4] A.R. Timerbaev, W. Buchberger, O.P. Semenova and G.K. Bonn, *J. Chromatogr.*, 630 (1993) 379.
- [5] M. Chen and R.M. Cassidy, *J. Chromatogr.*, 640 (1993) 425.
- [6] Waters Millipore, *Capillary Ion Analysis Methods*, Application sheet.
- [7] S. Motomizu, M. Oshima, S. Matsuda, Y. Obata and H. Tanaka, *Anal. Sci.*, 8 (1992) 619.
- [8] S. Motomizu, S.-I. Nishimura, Y. Obata and H. Tanaka, *Anal. Sci.*, 7, Suppl. (1991) 253.
- [9] M. Aguilar, X. Huang and R.N. Zare, *J. Chromatogr.*, 480 (1989) 427.
- [10] N. Wu, W.J. Horvath, P. Sun and C.W. Huie, *J. Chromatogr.*, 635 (1993) 307.
- [11] L. Lin, J. Wang and J. Caruso, *J. Chromatogr. Sci.*, 33 (1995) 177.
- [12] S. Schäffer, P. Gareil, L. Carpot and C. Dezael, *J. Chromatogr. A*, 717 (1995) 351.
- [13] A. Laurent, C. Prost and J.-C. Charpentier, *J. Chim. Phys.*, 72 (1975) 236.
- [14] L.G. Sillen, A.E. Martell, *Stability Constants of Metal-Ion Complexes*, Chemical Society, London, 1964.

- [15] A.E. Martell, *Stability Constants of Metal-Ion Complexes, Organic Ligands*, Chemical Society, London, 1964.
- [16] D.D. Perrin, *Dissociation Constants of Organic Bases in Aqueous Solution*, London, 1965.
- [17] R. Rosset, D. Bauer and J. Desbarres, *Chimie Analytique des Solutions et Informatique*, Masson, Paris, 1991.
- [18] G. Charlot, *Dosages Colorimétriques des Eléments Minéraux, Principes et Méthodes*, Masson, Paris, 1961.