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Determination of iron(II) in natural waters by capillary zone electrophoresis using on-capillary complexation with 2,4,6-tri(2′-pyridyl)-1,3,5-triazine

Johan Dahlén*, Stefan Karlsson

Department of Water and Environmental Studies, Linköping University, SE-58183 Linköping, Sweden

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Abstract

A new capillary zone electrophoretic method, based on on-capillary complexation with 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ), was developed for the determination of Fe(II) in natural waters. A carrier electrolyte consisting of 50 m*M* ammonium acetate at pH 5.0 with 0.2 m*M* TPTZ and 20% (v/v) ethanol allowed for proper determinations at 254 nm. Both hydrostatic and electrokinetic injection were evaluated and linear calibration functions were in both cases obtained over at least two orders of magnitude. The repeatability of the peak area was better than 3% and 5% for the hydrostatic and electrokinetic injection and the corresponding limits of detection (two×noise) were 0.050 and 0.010 mg/l, respectively. The method is easy to use and requires a minimum of sample pretreatment. Analysis of real samples was compared to an established colorimetric method. Good agreement ($p=0.05$) was obtained and elimination of interferences due to the electrophoretic separation was indicated. Preservation of Fe(II) with H_2SO_4 was evaluated by additions of the acid to two surface waters.

Keywords: Water analysis; Complexation; On-capillary derivatization; Environmental analysis; Iron; Tripyridyltriazine; Metal cations

1. Introduction

In the environment, total concentration of iron as well as its speciation are of vital importance in chemical and microbial redox processes [1]. Determination of Fe(II) is often made by colorimetric analysis. Two common standardized colorimetric methods employ complexation with 1,10-phenantroline (phen) [2] and 2,4,6-tri(2′-pyridyl)-1,3,5-triazine (TPTZ) [3], respectively. The use of TPTZ for colorimetric determinations of iron goes back to the works by Collins and co-workers in the late 1950s [4]. TPTZ forms a violet 2:1 complex with Fe(II), which is stable in the pH range 3.4–5.8 [4]. This chromogen has a local absorption maximum at 593 nm. A minimum of interferences are affecting the analysis at this wavelength, which allows for successful colorimetric quantifications of Fe(II). Reported stability constants (log β_2) for this complex are in the range 10.2–12.4, somewhat depending on the applied method and the experimental conditions [5–9]. The use of phen is also possible, but then the recommended wavelength is 510 nm. These two

*Corresponding author. Tel.: +46-13-282-951; fax: +46-13-133-630.

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methods are accurate, but rather tedious and time-consuming, why attempts have been made to automatize the determination of Fe(II) by the use of high-performance liquid chromatography (HPLC) [10], and more recently by capillary electrophoresis (CE).

CE has experienced an enormous growth since the advent of commercial instruments in the late 1980s. While metal ion analysis with CE only to a minor extent has contributed to this expansion, the number of publications within this field is steadily increasing. Normally, CE in its simplest form, i.e. capillary zone electrophoresis (CZE), is employed for metal ion analysis. Since the differences between the hydrated radii of metal ions are small [11], their separation in CZE are usually achieved by the introduction of an auxiliary complexing agent that alters their electrophoretic mobility. There are two different strategies in introducing the complexing agent into the analytical system. First, it can be used as a carrier electrolyte additive, which leads to formation of complexes in the capillary (on-capillary complexation). In this case, the choice is generally on reagents that form rather weak complexes with the metal ions, for example α-hydroxybutyric acid (HIBA) [12–14], lactic acid [12,14] and citric acid [13]. Separation relies on differences in equilibria, i.e. to what extent the metal ions are complexed. To obtain a satisfactory limit of detection (LOD), a UV-absorbing co-ion is normally added to the carrier electrolyte, which allows for indirect UV-detection. The other approach is referred to as pre-capillary complexation and involves addition of the complexing agent to the sample before it is injected. Ligands that form stable complexes with UV absorbing properties are suitable, and detection is normally performed by direct UV detection. Examples of reagents that have proved to be useful are polyaminocarboxylic acids like ethylenediaminetetraacetic acid (EDTA) [15,16], cyclohexane-1,2-diaminetetraacetic acid (CDTA) [17–19] and diethylenetriaminepentaacetic acid (DTPA) [20], cyanide [21–23] 4-(2-pyridylazo)resorcinol (PAR) [24,25]. A more elaborate discussion on metal ion analysis with CE can be found in recent reviews [11,26,27].

There are several studies on CZE analysis of Fe(II) or Fe(III), along with other metal ions that are based on pre-capillary or on-capillary complexation [11,26]. The simultaneous determination of the two oxidation states of iron has been made as pre-capillary formed cyano complexes [21–23] and as EDTA complexes in a mixed salicylate–EDTA buffer [28]. The use of PAR [25] and CDTA [19] have also been successful in differentiating Fe(II) and Fe(III). Schäffer et al. [29] successfully employed CZE for the simultaneous determination of Fe(II) and Fe(III). This was accomplished by adding phen and EDTA to the samples, resulting in a selective formation of cationic Fe(II)–phen3 and anionic Fe(III)–EDTA complexes. Similarly, Fe(II) and Fe(III) have been determined as the corresponding phen and CDTA complexes [30,31]. The use of phen as a single complexing agent has been reported for the total analysis of iron after reduction to Fe(II) [32–34] and for the determination of Fe(II) [34].

In colorimetry, the use of TPTZ is well established for the determination of Fe(II) as well as for total iron analysis after reduction of Fe(III). While the colorimetric reagent phen has been employed as a complexing agent for the determination of ferrous iron with CZE, there are to our knowledge no such reports on the use of TPTZ.

This study presents a CZE method for the analysis of Fe(II) in natural waters that is based on on-capillary complexation with TPTZ.

2. Experimental

2.1. Instrumentation

The CZE analysis was made with a Quanta 4000 (Waters). The internal diameter of the capillary (J&W Scientific) was 50 μm and the total and effective lengths were 38 and 30 cm, respectively. The carrier electrolyte consisted of a 50 m*M* ammonium acetate buffer with 0.2 m*M* TPTZ and 20% (v/v) ethanol, adjusted to pH 5 with a 5% (v/v) acetic acid solution. The ethanol was used in order to maintain the TPTZ in solution. Filtration of the carrier electrolyte through a thoroughly rinsed 0.20 μm cellulose acetate membrane was employed to protect the capillary from particulates. The separation voltage was 15 kV (cathodic detection side) giving a current through the capillary of approximately 25 μA. Samples were introduced by hydro-

static injection (10 cm for 30 s) or electrokinetically (5 kV for 45 s). Detection was made at 254 or 546 nm.

A special sequence was employed to recondition the capillary at the beginning of each day. The procedure included Milli-Q water (5 min), 0.1 *M* HCl (5 min) and Milli-Q water (5 min). The capillary was then allowed to equilibrate with the ammonium acetate buffer by purging for another 30 min. Prior to each injection, the capillary was purged with carrier electrolyte for three min or with 0.1 *M* HCl (3 min) followed by carrier electrolyte (6 min). The electropherograms were recorded and the peaks were integrated by use of the software Millenium 2.0 (Waters). Corrected peak area (i.e. peak area/migration time) was used in order to compensate for peak area variations that are attributed to differences among the migration times.

All samples and standard solutions were preserved by the addition of 100 μl concentrated H_2SO_4/100 ml, unless other proportions are stated. Sample colloids were removed by filtration through 0.40 μm polycarbonate membranes (diameter=47 mm; Millipore).

Absorbance measurements were made with a DU-64 Spectrophotometer (Beckman) equipped with a 10 mm path length quartz cell. An Elan 6000 (Perkin-Elmer Sciex) was employed for inductively coupled plasma mass spectrometry (ICP–MS) analysis.

2.2. Reagents

All solutions were prepared with Milli-Q water (Millipore). Ammonium acetate (analytical-reagent grade), TPTZ (analytical-reagent grade), iron(II) sulfate heptahydrate (analytical-reagent grade), iron(III) nitrate nonahydrate (analytical-reagent grade), acetic acid (100%, analytical-reagent grade), hydrochloric acid (37%, analytical-reagent grade) and sulfuric acid (95–97%, analytical-reagent grade) were all purchased from Merck. Ethanol (95%) was supplied by Kemetyl.

2.3. The Fe(II)–$TPTZ_2$ complex

A maximum of two TPTZ molecules coordinate to Fe(II) in the colored, positively charged complex (Fig. 1). Each TPTZ molecule acts as a tridentate ligand with two pyridyl nitrogen and one ring nitrogen attached to the Fe(II) [5]. It is assumed that the two TPTZ molecules are perpendicular to each other [5].

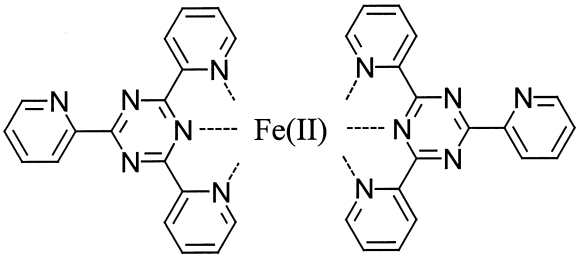

Fig. 1. The positively charged Fe(II)-$TPTZ_2$ complex.

2.4. Linearity, limits of detection and repeatability

2.4.1. Hydrostatic injection

The suitability of hydrostatic injection was tested with respect to linearity and linear range by use of least squares regression analysis. The choice of detection wavelengths was restricted to the optical filters that are provided by the instrument supplier. Detection at 546 and 254 nm were chosen since they were as close as possible to the wavelength recommended by the colorimetric Swedish reference method (593 nm) and to the absorption maximum of the Fe(II)–TPTZ complex (289 nm) that we obtained by spectrophotometric measurements. The LOD (two×noise) was determined and the repeatability was calculated from nine consecutive injections of Fe(II) solutions of 0.5 and 10 mg/l. Finally, the presence of Fe(III) in the samples was evaluated in order to assure that no reduction occurs in the capillary and that possible TPTZ–Fe(III) complexes do not interfere in the electropherograms.

2.4.2. Electrokinetic injection

The electrokinetic injection mode was tested for linearity (least squares), linear range, repeatability and LOD. It is well known that a high sample conductivity reduces the amount of introduced sample when employing this injection mode [35,36], and as a consequence the LOD is also negatively affected. Since the samples were preserved with H_2SO_4 in order to prevent oxidation of Fe(II), it is obvious that this treatment is crucial when minimizing the sample

conductivity. The results of the preservation study (Section 2.5) gave guidance in reducing the concentration of acid.

2.5. Preservation and stability

All samples and standard solutions in this study were preserved with concentrated H_2SO_4 in order to minimize oxidation of ferrous iron. An experiment was made to elucidate what proportion of concentrated H_2SO_4 that is required to preserve a typical surface water. Water from the dystrophic Lake Bjän and the eutrophic River Stångån (Östergötland, South Sweden) were filtered through 0.40 μm polycarbonate membranes (diameter=47 mm; Millipore) to remove particles that might have a negative influence on the performance of the CE instrument. Each of the two filtrates was divided into four sub samples, which were transferred to acid washed 100 ml polyethene bottles. These sub samples were then preserved with 0, 25, 50 and 100 μl concentrated H_2SO_4 per 100 ml and were spiked with Fe(II) to an additional concentration of 0.500 mg/l. The sub samples from Lake Bjän and River Stångån were then analyzed immediately as well as after 25, 46, 72, 148 and 330 h (two weeks). All samples were analyzed in triplicate using hydrostatic injection and detection at 254 nm. The preservative efficiency of the various additions of H_2SO_4 was evaluated as a function of time.

2.6. Comparison with the colorimetric swedish reference method

2.6.1. Swedish reference method

This colorimetric method is normally used for determinations of total iron and includes reduction of Fe(III) to Fe(II) by the addition of hydroxylammonium chloride followed by complexation with TPTZ and photometric measurement at 593 nm. By omitting the reduction step it can also be employed for the analysis of Fe(II). In this study a slightly modified version of the Swedish reference method for analysis of Fe(II) was employed as follows:

(1) To samples and calibration standards were added 100 μl concentrated H_2SO_4 per 100 ml in order to prevent oxidation of Fe(II).

(2) TPTZ of 0.2 ml 2.4 m*M* (with a few drops of concentrated HCl) followed by 0.2 ml 3 *M* sodium acetate were added to 10 ml sample or calibration solution.

(3) The absorbance at 593 nm was measured after 10±1 min.

2.6.2. Statistical evaluation

The results of the colorimetric reference method were compared with CZE employing the hydrostatic injection mode. The same calibration solutions were used for both methods to avoid sources of error that are dependent on the preparation of standards.

Five real samples and two defined solutions were analyzed in six replicates with both methods. The precision of the two methods was compared by the employment of an two sided *F*-test [37]. The mean values, was compared by use of a two-sided *t*-test according to Miller and Miller [37] in order to estimate the accuracy of the CZE application.

3. Results and discussion

3.1. On-capillary complexation

Initially, both on-capillary and pre-capillary complexation with TPTZ were tested. The pre-capillary alternative did, however, give distorted peaks and poor repeatability of the peak area. On-capillary complexation was more successful and was therefore chosen for further investigation (cf. Fig. 8). In addition, on-capillary complexation was less labour extensive, since a minimum of sample treatment prior to injection was needed.

3.2. Hydrostatic injection

Both tested detection wavelengths gave linear calibration curves over two orders of magnitude. The peak shape of concentrations exceeding 25 mg/l was slightly distorted. Improved performance can, however, be obtained by reducing the injection time, or by diluting the samples. Standard solutions with the concentrations 0.1; 0.5; 1.0; 5.0; 10; 25; 50 and 100 mg/l were used for the calibration.

Correlation coefficients (r^2) and calibration equations in the ranges 0–5, 0–25 and 0–100 mg/l are shown in Table 1.

Table 1
Correlation coefficients (r^2) and calibration equations for the hydrostatic injection mode. Detection was made at 254 or 546 nm. Other conditions as in Fig. 3

Wavelength (nm)	Concentration range (ppm)	Correlation coefficient (r^2)	Calibration equation
254	0–5	0.9996	$y=3867.7x + 136.6$
254	0–25	0.9997	$y=4088.8x - 258.6$
254	0–100	0.9999	$y=4190.4x - 1041.3$
546	0–5	0.9996	$y=1749.9x - 59.3$
546	0–25	0.9998	$y=1852.4x - 214.0$
546	0–100	0.9998	$y=1924.6x - 720.5$

According to the Swedish colorimetric reference method [3] detection should be performed at 593 nm. The reason for this choice is the local absorption maximum of the Fe(II)–$TPTZ_2$ complex and the minimum of interferences from other possible sample components at this wavelength [4,38]. The absorptivity of the complex is, however, higher at 254 nm (maximum at 289 nm) than at 593 or 546 (Fig. 2), why a better sensitivity is obtained at the former wavelength. The absorptivity at this wavelength (254 nm) is, however, not specific for the Fe(II)–$TPTZ_2$ complex, since it is dominated by the aromatic structure of the TPTZ molecules (Fig. 2). In addition, there are other sample constituents, including uncomplexed TPTZ that would interfere. Consequently, colorimetric analysis at 254 nm is not suitable. The separation that occurs in the capillary in CZE analysis provides a mean to eliminate such interferences, which allows for proper quantifications at 254 nm. The LOD (two×noise) at 254 and 546 nm was determined to 0.050 and 0.125 mg/l, respectively. Since both wavelengths gave linear calibration functions the one with the lowest LOD was preferred, i.e. 254 nm. Accordingly, all further studies were carried out at this wavelength.

The repeatability of the peak area ($n=9$), expressed as the relative standard deviation (RSD), was 2.5 and 0.4% for Fe(II) solutions of 0.5 and 10.0

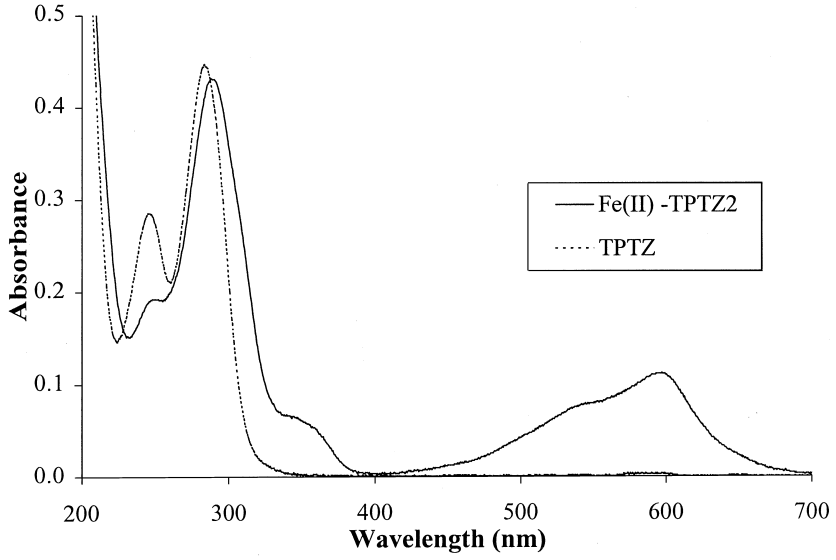

Fig. 2. UV spectra of the Fe(II)–$TPTZ_2$ complex and TPTZ. The concentrations of Fe(II) and TPTZ were 7.2 μ*M* and 14.4 μ*M*, respectively.

mg/l, respectively. The variance was acceptable in both cases, though the relative standard deviation was higher for the lower concentration.

3.3. Electrokinetic injection

Calibration was made in the range 0–5 mg/l (0.05; 0.1; 0.25; 0.5; 1.0 and 5.0 mg/l). An addition of 25 μl of concentrated H_2SO_4 per 100 ml solution was used for preservation since this was the lowest volume, of those tested, that quantitatively preserved ferrous ions in a typical Swedish surface water (Section 3.4). The calibration functions were linear (r^2=0.9998) and the LOD (two×noise) was 0.010 mg/l.

The concentration of H_2SO_4 that is used for preservation has a significant influence on the LOD, as can be seen in Fig. 3. This negative effect is due to the increased sample conductivity that follows the addition of acid, which decreases the field strength over the sample plug during the injection and thereby also the amount of injected sample. By lowering the volume of added acid the LOD would be further reduced. It is, however, questionable if less than 25 μl prevents oxidation of Fe(II). The LOD might also be reduced by increasing the injection time, which on the other hand would result in broader peaks.

The repeatability of the peak area, based on nine consecutive injections of 0.05 and 1.0 mg/l solutions yielded RSDs of 4.9 and 1.8%, respectively. While being acceptable, these results was not as good as the outcome of the hydrostatic alternative. The relative standard deviation was higher for the lower concentration as was also the case for the hydrostatic injection. Based on these results the hydrostatic injection mode is recommended for samples where the Fe(II) content is sufficiently high.

3.4. Preservation

There were only small differences in preservation efficiency among the various volumes of added H_2SO_4 that were tested (Figs. 4 and 5). The Fe(II) content in River Stångån (Fig. 4) seems to be efficiently preserved by 25, 50 as well as 100 μl H_2SO_4 for at least 150 h (6 days). Unpreserved river water resulted, not surprisingly, in an instantaneous oxidation of Fe(II). For storage exceeding 150 h, there was a slight decrease of the Fe(II) concentration for all additions of H_2SO_4.

The behavior of the Lake Bjän sample is some-

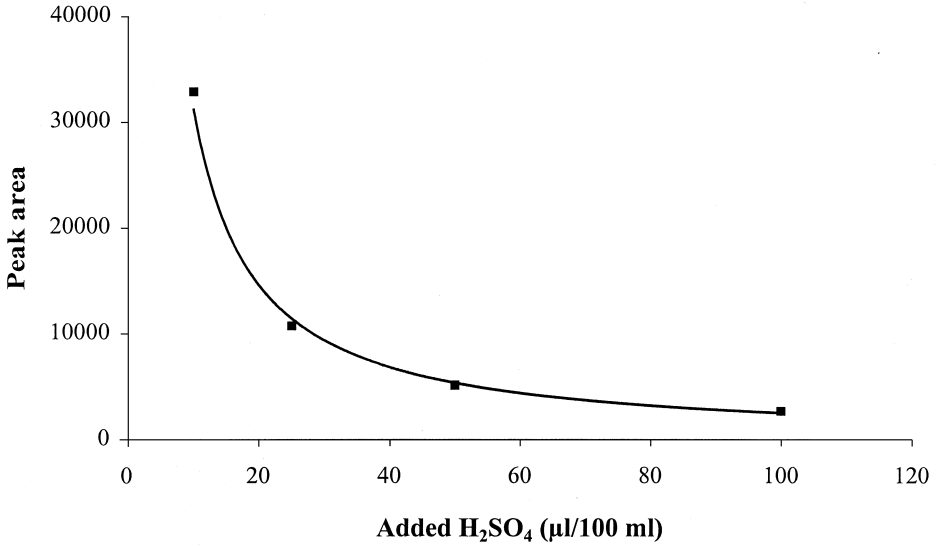

Fig. 3. The peak area of a 0.250 mg/l Fe(II) solution versus the volume of added concentrated H_2SO_4. Carrier electrolyte: 50 m*M* ammonium acetate at pH 5 with 0.2 m*M* TPTZ and 20% (v/v) ethanol. Voltage: 15 kV. Injection: electrokinetically at 5 kV for 45 s. Detection: direct UV at 254 nm.

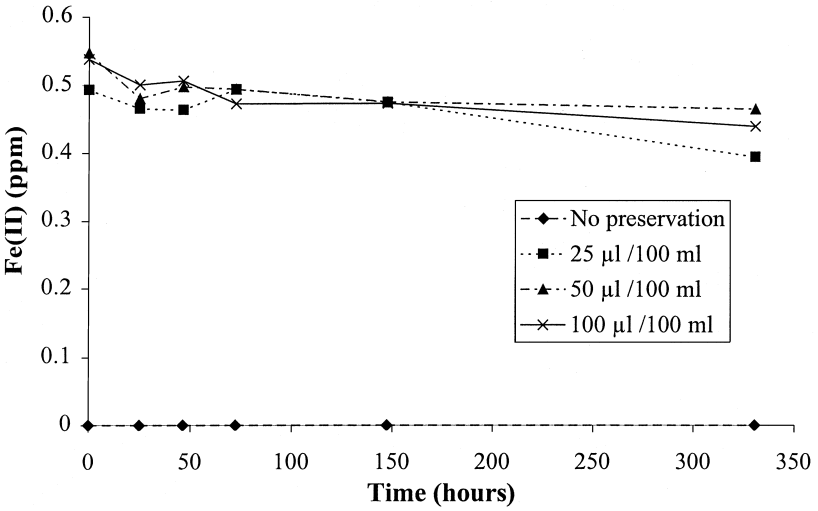

Fig. 4. Concentration of Fe(II) versus time for River Stångån water preserved with various additions of concentrated H_2SO_4. Injection: hydrostatically at 10 cm for 30 s. Other analytical conditions as in Fig. 3.

what different (Fig. 5), probably because of its high content of fulvic acids (some 25 mg/l; [39]). Here the Fe(II) concentration remained constant for only 50 h (2 days) for all additions of H_2SO_4. After 50 h there was a significant increase of the Fe(II) concentration. Possibly, a slow release of Fe(II) that initially was sorbed to humics and dispersed solids in the lake water can account for this behavior. As expected, the concentration of aqueous Fe(II) in the unpreserved Lake Bjän water decreased, and after 50 h the concentration was below detection. A probable explanation is removal by oxidation and sorption.

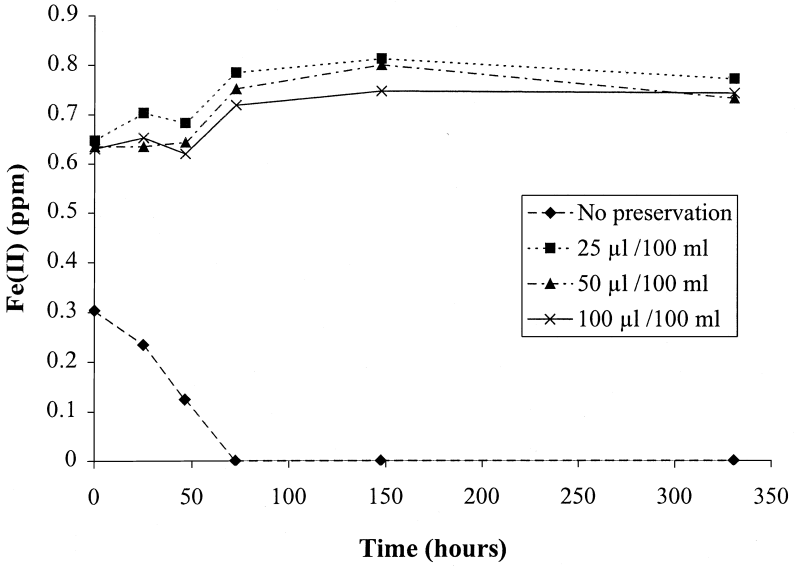

Fig. 5. Concentration of Fe(II) versus time for Lake Bjän water preserved with various additions of concentrated H_2SO_4. Injection: hydrostatically at 10 cm for 30 s. Other analytical conditions as in Fig. 3.

According to the results it seems like 25 μl of concentrated H_2SO_4 per 100 ml sample is sufficient to preserve a typical Swedish surface water with respect to Fe(II).

For the electrokinetic injection mode this information is important, since the LOD is decreasing with the volume of added acid (i.e. the conductivity of the sample). It is, however, recommended that pH is measured after addition of H_2SO_4. As a rule of thumb it should be lower than pH 2, since the oxidation rate increases rapidly above this pH [1]. When the hydrostatic injection mode is chosen, one might just as well use 100 μl, since LOD will not be affected in this case.

3.5. Impact of the Fe(III) concentration on the Fe(II) peak area

To ascertain that no reduction of Fe(III) occurs in the sample after it has been introduced on the capillary, a series of Fe(II) solutions with various concentrations of Fe(III) was injected. Four 1 mg/l Fe(II) solutions with 0, 1, 5 and 10 mg/l Fe(III) were analyzed in triplicate by the hydrostatic injection mode. The peak area did not significantly differ between the four solutions (Fig. 6), which indicates that reduction of Fe(III) does not take place. Furthermore, it can be concluded that there are no interfering Fe(III)–TPTZ complexes.

3.6. Stability of migration times

One well known problem in CE is to establish reproducible migration times. A stable electroosmotic flow (EOF) is here the most important factor and requires control of the pH of the carrier electrolyte and of the capillary temperature. Sorption to the capillary surface is another process that might occur in samples with high contents of iron, where precipitation of ferric hydrous oxides reduces the EOF.

Nine consecutive runs of a solution containing 1 mg/l Fe(II) and 5 mg/l Fe(III) were made, and the migration times were found to be positively correlated with the injection number (Fig. 7). This situation is undesirable, since peak area increases with migration time due to a prolonged time at the optical window. Even though corrected peak area can be used to compensate for this kind of bias, as was made throughout this study, the situation is not fully satisfactory. Commonly, a NaOH solution is employed to recondition the capillary. This treatment will, however, not remove freshly precipitated ferric

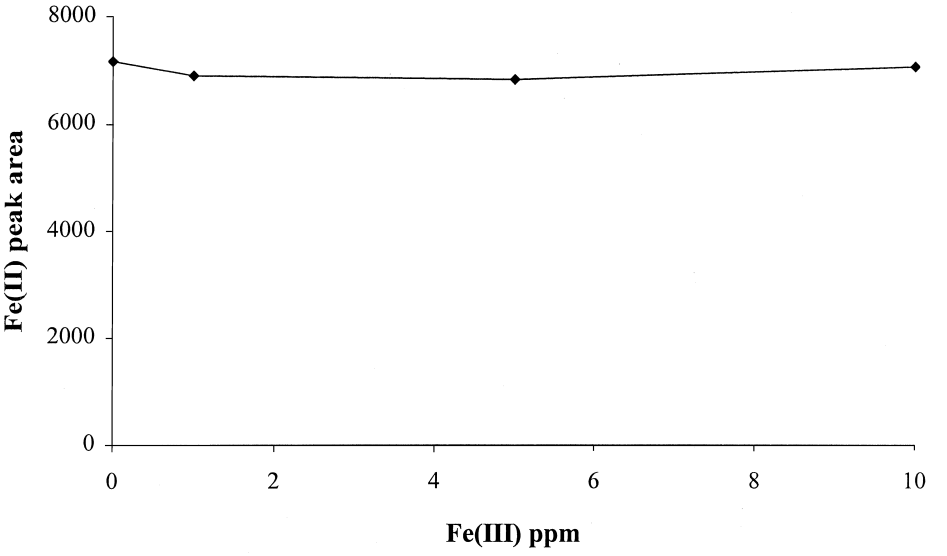

Fig. 6. The peak area of a 1 mg/l Fe(II) solution versus the concentration of Fe(III). Injection: hydrostatically at 10 cm for 30 s. Other analytical conditions as in Fig. 3.

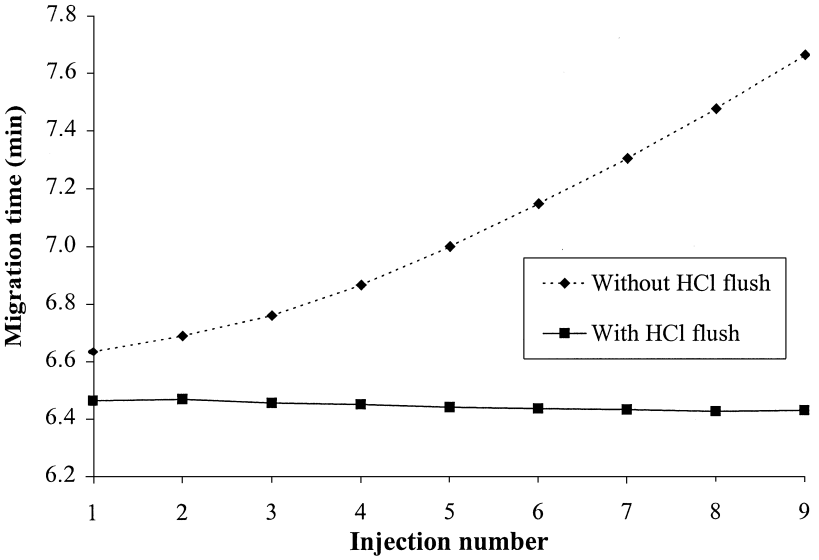

Fig. 7. Effect of flushing the capillary with 0.1 *M* HCl prior to injection. Without HCl flush: rinsing with carrier electrolyte (3 min). With HCl flush: rinsing with 0.1 *M* HCl (3 min) followed by carrier electrolyte (6 min). Injection: hydrostatically at 10 cm for 30 s. Other analytical conditions as in Fig. 3.

hydrous oxides, and therefore rinsing with 0.1 *M* HCl was tested. By purging with this solution for 3 min followed by 6 min with carrier electrolyte prior to each injection the capillary was effectively reconditioned. This procedure led to significantly improved repeatability of the migration times of the Fe(II) complex as is shown in Fig. 7. As a consequence, rinsing with 0.1 *M* HCl is recommended for samples with high contents of Fe(III).

3.7. Comparision with the colorimetric swedish reference method

Four real samples and two defined solutions (approximately 0.5 and 1.0 mg/l) were analyzed in six replicates by the hydrostatic injection mode. All solutions were preserved with 100 μl H_2SO_4 per 100 ml sample. The result of the comparison is summarized in Table 2.

Table 2
Summary of the method comparison. Mean±SD are the mean value and the corresponding standard deviation (n=6). Dilution factors for sample dilutions are listed. Hydrostatic injection was employed for sample introduction in the CZE analysis.

Sample	Dilution factor	Mean±SD (mg/l)	
		Colorimetry	CZE
0.5 mg/l solution	1	0.509±0.004	0.511±0.014
1.0 mg/l solution	1	0.941±0.005	0.948±0.014
River Stångån + 0.5 mg/l	1	0.562±0.006	0.553±0.016
Lake Bjän + 0.5 mg/l[a]	1	0.798±0.002 (0.860±0.002)	0.798±0.013
Mine waste drainage (Bersbo)[b]	10	0.510±0.003 (0.320±0.010)	0.510±0.020 (0.570±0.020)
Groundwater (Kristineberg)	1000	0.799±0.003	0.797±0.011

[a] Colorimetry: Corrected for absorption of HSs. Uncorrected value within parenthesis.
[b] Colorimetry: 1.4 ml 2.4 m*M* TPTZ solution was added, 0.2 ml TPTZ within parenthesis. CZE: Concentration of Fe(II) corrected for Co(II) and Ni(II). Uncorrected concentration within parenthesis.

A visual inspection of the standard deviations in Table 2 indicates that the precision of the CZE method is slightly poorer than that of the colorimetric method. A two sided F-test ($p=0.05$) confirmed this suspicion.

When it comes to the comparison of the mean values, i.e. the accuracy, the interpretation is not that straightforward. The two methods gave the same mean values ($p=0.05$) for the 0.5 mg/l solution, the 1.0 mg/l solution, River Stångån and the Kristineberg groundwater. The level of agreement was verified by a two sided t-test ($p=0.05$) for comparisons of means [37]. When employing the colorimetric method for determination of the Fe(II) concentration in the dystrophic Lake Bjän water, the effects of the high contents of humic substances (HSs) had to be considered. Since HSs have a small, but not negligible, absorption at 593 nm there is an obvious risk for overestimation of the Fe(II) content. When the background absorption of the Lake Bjän matrix was subtracted from the absorption of the corresponding colorimetric measurement, the obtained mean value agreed ($p=0.05$) with the mean of the CZE method. Without such a correction there was a significant ($p=0.05$) difference between the two methods, probably due to an overestimation by the colorimetric method. This indicates that the presence of HSs introduces a serious bias. Since separation of negatively charged HSs and the positively charged Fe(II)–$TPTZ_2$ complex occurs in the capillary, these interferences are eliminated in the CZE method.

According to the *t*-test, the two methods gave different mean values for the mine waste drainage water ($p=0.05$). It was obvious that the matrix of this sample interfered with the colorimetric determinations. When using 0.2 ml 2.4 m*M* TPTZ solution, as was prescribed by the employed method, there was an obvious underestimation of Fe(II). Improved results were obtained when 1.4 ml of the TPTZ solution was added, but there still was a significant difference between the methods. We suggest that other metal ions present in this sample compete in forming complexes with TPTZ, thereby leading to a situation where Fe(II) is not quantitatively complexed. In the CZE method there is an excess of TPTZ, since the metal ions, including Fe(II), migrate through the carrier electrolyte, which continuously provide uncomplexed TPTZ. Therefore the TPTZ is not limiting, why all Fe(II) will be complexed. In addition, separation occurs in the capillary, as is demonstrated in the electropherogram of the mine waste drainage sample (Fig. 8). Spiking was employed for a tentative identification of Cu(II), Zn(II), Mn(II) and Fe(II).

It should, however, be mentioned that the TPTZ complexes of Co(II) and Ni(II) are not separated from that of Fe(II) at the employed CZE conditions (Fig. 8). ICP–MS analysis of the mine waste drainage water sample determined the concentrations of Co(II) and Ni(II), which were 0.054 and 0.006 mg/l, respectively. Since the response of Co(II)–TPTZ, Ni(II)–TPTZ and Fe(II)–TPTZ are about the same (not shown), the sum of the two former (0.060 mg/l) can be subtracted from the mean value obtained by the CZE method. After this correction the mean values of the two methods were the same ($p=0.05$).

Detection at 546 (593) nm would reduce the interferences of the TPTZ complexes of Co(II) and Ni(II) due to their lower absorptivity at this wavelength. Since the absorptivity of the Fe(II)–TPTZ complex would also be reduced, the usefulness of 546 nm is limited to samples with higher concentrations.

Even though samples where the concentrations of Co(II) and Ni(II) are high enough to substantially interfere with the Fe(II) determinations are rare, an improvement of the CZE method would be desirable. Attempts to optimize the separation of Fe(II), Ni(II) and Co(II) have therefore been initiated.

4. Conclusions

A new CZE application for the determination of Fe(II) in natural waters is presented. On-capillary complexation with TPTZ and direct detection at 254 nm was employed. The method is easy and straightforward, since the only sample pretreatment that is needed is filtration and preservation with concentrated H_2SO_4. The LODs (two×noise) were determined to be 0.050 and 0.010 mg/l for the hydrostatic and electrokinetic injection modes, respectively.

The accuracy compares well with a colorimetric method that is based on the same complexing agent,

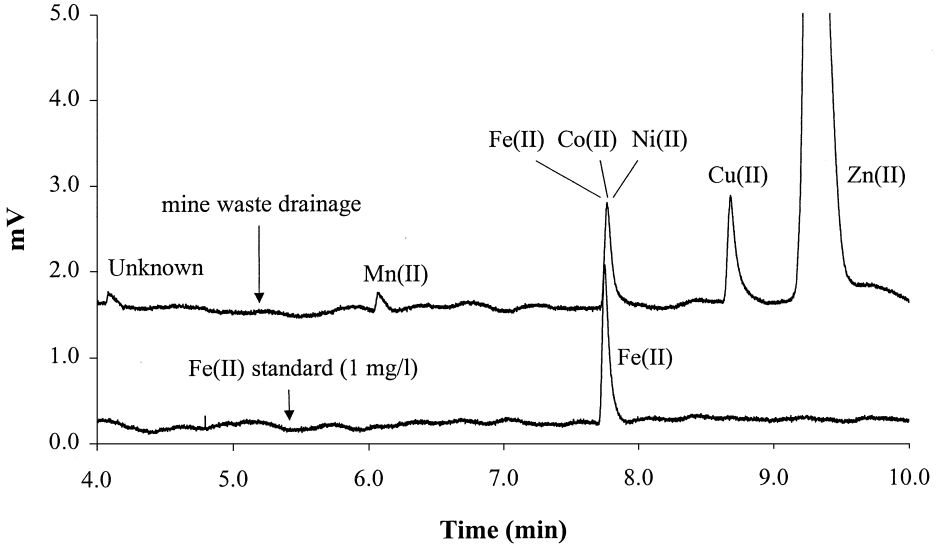

Fig. 8. Electropherograms of the mine waste drainage water and a Fe(II) standard (1 mg/l). Injection: hydrostatically at 10 cm for 30 s. Other analytical conditions as in Fig. 3.

while the precision is a bit poorer. Further, it is indicated that interferences caused by UV absorbing humic substances, that might cause a bias in the colorimetric alternative, are eliminated in the electrophoretic separation. Most metal ions that interfere in the colorimetric determination are effectively separated in the capillary, except for Ni(II) and Co(II) that co-migrate with Fe(II). These ions are, however, generally present at low levels in natural waters and will not significantly affect the analysis.

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