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Trace determination of iron in water at the μg/l level by on-line coupling of capillary isotachophoresis and capillary zone electrophoresis with UV detection of the EDTA–Fe(III) complex

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Abstract

The determination of iron in water at the trace level by on-line coupled capillary isotachophoresis and capillary zone electrophoresis (CITP–CZE) with a commercial column coupling device is described. Iron is determined as the negatively charged complex with EDTA which is highly UV-absorbing and thus enables photometric detection at 254 nm. The analyses are performed using 10 mmol/l HCl+20 mmol/l L-histidine+0.1% hydroxypropylmethyl cellulose (pH 6.0), 5 mmol/l MES, and 25 mmol/l MES+10 mmol/l bis-tris-propane (pH 6.6) which served as leading, terminating and background electrolyte, respectively. Samples are acidified with HNO_3, diluted and EDTA added (to a final concentration of 10^{-4} mol/l) prior to CITP–CZE analysis. The detection limit of Fe(III) is 10 μg/l, and is given by the chemical noise due to the impurities of the chemicals used enriched by the ITP preconcentration step. The precision of the CE measurement, expressed by the relative standard deviation, is about 3% (at the 400 μg/l level); the recovery is between 80 and 115% depending on the iron concentration level (40–400 μg/l).

Keywords: Water analysis; Column switching; Iron; EDTA–iron(III) complex

1. Introduction

Iron is commonly present in surface and drinking water. It stems from dissolution processes of iron-stones in nature and partially from the corrosion processes in pipes [1]. Industrial waste water is also a significant source of iron.

The concentration of iron in water can vary over several orders of magnitude from tens of μg/l (sea water) to tens of mg/l (some ground and mineral waters). The iron concentration in most surface and common ground water is not very important from the hygienic point of view; its content influences, however, the organoleptic properties of drinking water – mainly the color, taste and turbidity. Iron concentration is therefore a parameter for the quality of drinking water and the World Health Organization (W.H.O.) recommends 0.3 mg/l [2] as upper concentration limit of iron in drinking water.

There are also concentration limits for waste waters, but these limits differ for various industrial

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branches (e.g., in the Czech Republic the upper limits are set between 5 and 10 mg/l [3]).

The iron content in water can cause technical faults rather than sanitary problems, as an iron concentration of just 0.5 mg/l can lead to turbidity of water. In addition, even small amounts of Fe(II) can cause iron bacteria growth leading to the clogging of pipes and a bad smell of the water.

Several methods are used for the determination of iron in water. HPLC methods [4–9] are based on the separation of various complexes of iron and other cations, offering the possibility of multielement analysis. A disadvantage of these methods is the necessity of sample pretreatment such as SPE or solvent extraction. Another group of methods consists of various spectrophotometric procedures [10–12]. Some of them are combined with flow injection analysis [13]. Atomic absorption spectrometry [14,15] and inductively coupled plasma spectrometry [16] have been also applied for the determination of iron in water. Spectroscopic and electrochemical determinations have been demonstrated for the analysis of ultra traces of iron and other cations [17].

Iron has been separated by capillary isotachophoresis as an EDTA complex for the simultaneous determination of EDTA–Fe(III) and free EDTA [18] not in water, but in wash liquids used for the desulfurization of gases. However, as the iron contents were rather high in this case, a single capillary instrument could be used to determine iron content. The EDTA–Fe(III) complex has been also used for the determination of EDTA in mayonnaise. The EDTA–Fe(III) complex was determined by CITP in single capillary arrangement with a rather complex sample pretreatment [19] or by combination of CITP and CZE in a column-coupling device [20].

Such on-line coupling of isotachophoresis and zone electrophoresis in the capillary format offers the advantages of both methods and makes this combination very suitable for trace analysis. The low concentration sensitivity of capillary zone electrophoresis is overcome by the high sample load of isotachophoresis [21–23]. The resolution power of CZE enables us, on the other hand, to resolve initially isotachophoretic zones of UV-active compounds without the use of spacers. However, iron cannot be detected with sufficient sensitivity by UV absorbance, the normally-used detection method in CZE. Therefore in the present work the high sample load of isotachophoresis as a preconcentration method is combined with capillary zone electrophoresis for the determination of trace levels of iron in water based on the stable, charged, UV active EDTA–Fe(III) complex.

2. Experimental

2.1. Chemicals

The following chemicals were used: hydrochloric acid, (A.C.S. reagent grade, volumetric standard, 0.1 mol l^{-1} solution), hydroxypropylmethylcelullose (HPMC, 4000) all from Aldrich (Steinheim, Germany); morpholinoethanesulphonic acid (MES, 99.5% purity), L-histidine (His, 99% purity), 1,3-bis[tris(hydroxymethyl)methylamino)propane (bis-tris-propane, BTP, 99% purity) all from Sigma (Deisenhofen, Germany); ethylenediaminetetraacetic acid (EDTA, p.a.), disodium salt, nitric acid (p.a., 65% content), hydrogen peroxide (p.a., 29–32% content) all from Lachema (Brno, Czech Republic); iron standard for atomic absorption spectrometry (1000 mg/l), E. Merck (Darmstadt, Germany).

Deionised water was used for the preparation of the buffers.

The solution of HPMC (1% in water) was purified by shaking with a mixed-bed ion-exchanger.

2.2. Apparatus

The column-coupling instrument (ItaChrom EA 101, E. Merck) was equipped with two serially coupled capillaries made from a fluorinated ethylene–propylene copolymer (pre-separation capillary 160×0.8 mm I.D., analytical capillary 90×0.3 mm I.D.). Detection was carried out with a contact conductivity detector at the pre-separation capillary and with a UV absorbance detector (operated at 254 nm) in the analytical capillary, respectively. Samples were injected via a sample valve of 25 μl fixed volume.

2.3. Conditions of analysis

The pre-separation capillary was filled with lead-

ing electrolyte (10 mmol/l HCl+20 mmol/l L-His+ 0.1% HPMC, pH 6.0); MES (5 mmol/l) served as the terminating electrolyte. The analytical capillary was operated with a background electrolyte consisting of 25 mmol/l MES+10 mmol/l BTP, pH 6.6. The driving current was set constant at 250 μA in the ITP step (running only in the pre-separation capillary) and was 120 μA in the CZE step (running through both capillaries).

2.4. Calibration

An external standard calibration method was used. Six calibration points were measured in the calibration range between 0 and 500 μg/l Fe(III). All calibration solutions contained 10^{-4} mol/l of EDTA.

2.5. Sample preparation

The acidified sample (1 ml of 65% w/v HNO_3/l for drinking and table water, 4 ml for mineral and river water) was placed into an ultrasonic bath for 5 min and filtered if necessary. The mixture was diluted (2–50 fold) and EDTA was added to the final concentration of 10^{-4} mol/l prior to analysis.

3. Results and discussion

3.1. Qualitative analysis

EDTA is a strong complexing agent to Fe(III). The logarithm of the stability constant of this complex is 25.1 (20°C) [24]. EDTA forms even stronger complexes with Zr(IV), Hf(IV), Mn(III), In(III), Tl(III), Sb(III) and Bi(III). However, in contrast to the latter complexes the EDTA–Fe(III) complex strongly absorbs UV light at 254 nm (λ_{max}=256 nm, ε=9290 M^{-1} cm^{-1} [25]). As this complex is charged, capillary electrophoresis can be adequate to separate it from interfering material and could enable its quantification by selective use of UV absorbance detection.

An electropherogram from an iron standard solution is shown in Fig. 1, where the EDTA–Fe(III) zone appearing as very tiny shoulder in the ITP conductivity trace (Fig. 1a) is cut electrically onto the analytical capillary and run by CZE. The resulting peak recorded on the UV trace after CZE separation is depicted in Fig. 1b.

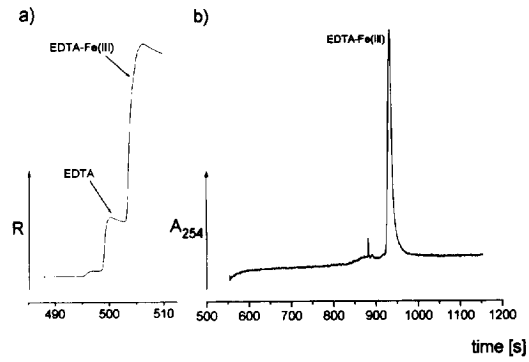

Fig. 1. Electropherograms of the EDTA–Fe(III) complex from a standard iron solution; 400 μg/l of Fe(III)+10^{-4} mol/l EDTA were injected; (a) conductivity record of the CITP step; (b) UV trace from the CZE step.

In practically all published papers dealing with the combination of CITP–CZE in column-coupling systems, an additive is introduced to the CZE background electrolytes in order to reduce electroosmosis [22,23,26]. Since we observed no significant improvement in efficiency with an additive in the background buffer the addition of e.g., modified cellulose, as recommended in the literature, was omitted.

Electropherograms of two real samples (tap and river water) are shown in Fig. 2. It can be seen that no peak other than that of the Fe-complex is recorded. This result is typical for all samples investigated; it points to the conclusion that interferences from other sample components are not to be expected.

3.2. Quantitation

3.2.1. Calibration

The necessity for control of the purity of the entire measuring system is evident from the electropherogram of a blank run, shown in Fig. 3. Despite the fact that the quality of all chemicals used was of the highest obtainable grade a peak of the Fe-complex is visible on the low μg/l range. In our case, the blank analysis of the reagents resulted in a concentration of 13 μg Fe/l. Although the chemicals used may be specified with a lower Fe concentration, it must be

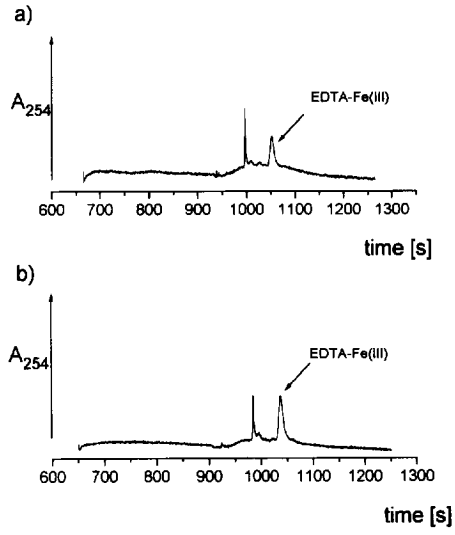

Fig. 2. Electropherograms of two typical real samples. (a) River water (10-fold diluted sample+10^{-4} mol/l EDTA), (b) tap water (2.5-fold diluted sample+10^{-4} mol/l EDTA). In both cases the sample was acidified by addition of 1 ml of 65% HNO_3 to 250 ml of river or 1000 ml of tap water, respectively. UV records are shown.

realized that Fe is stacked and therefore enriched in the ITP step as the complex with EDTA. The presence of Fe in the chemicals at that low trace level represents a kind of chemical noise and thus limits the analysis of Fe at lower concentrations in the sample. Chemicals with higher purity (lower iron content, respectively) would enable still lower detection limits.

It is noted at this point that the cleanness of the whole separation unit is of key importance, also.

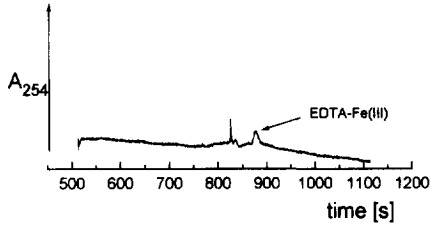

Fig. 3. Electropherogram of a blank run (injection of 10^{-4} mol/l EDTA); iron stems from impurities of the chemicals, which are enriched by the ITP stacking process. The peak corresponds to 13 μg/l Fe(III). UV trace is shown.

After each day the separation unit was washed with 0.1 mol/l hydrochloric acid. If a significantly higher iron content was found in the blank run, a careful cleaning of all parts of the separation unit was done in an ultrasonic bath.

For quantitation of the iron in samples the area of the peaks in the zone electropherograms was used. The calibration line measured between 0 and 500 μg/l obeys the equation $Y=22.13X+281.39$, where Y is the peak area and X is the concentration of iron in μg/l. Good linearity is observed in this region: the linear correlation coefficient R is 0.99967.

The timing of the current switching between the columns is a further important aspect of analysis with column-coupling instruments. Theoretically the current has to be switched when the ions of interest reach the bifurcation block of the instrument. In practice, switching should be carried out earlier to ensure that the transfer of the ions of interest is quantitative.

If, on the other hand, the current switching is performed too early, the amount of leading zone transferred to the analytical capillary can lead to the migration of isotachophoretically stacked compounds of interest through the UV detector in the capillary. The detection in the CZE step in the CITP–CZE should proceed after the zone has been destacked. This means that the so-called destacking time must be shorter than the migration time of the compound. As the destacking time is directly proportional to the amount of leading zone transferred to the analytical capillary [21] the time of current switching is a rather important parameter in CITP–CZE.

During this investigation it was found that the peak of the analyte was deformed in the electropherogram if the time interval for the current switching was set between 3 and 15 s before the zone of the complex reaches the bifurcation block. This deformation disappeared, however, if the current was switched at a time interval of about 20 s. One explanation of this phenomenon could be that two or more sample components migrate stacked together with the zone of the complex and longer destacking time is necessary for their resolution. Peak deformation occurred, however, also in the absence of such components, namely with the standard solution of the EDTA–Fe(III) complex. Therefore we assume that the deformation is probably

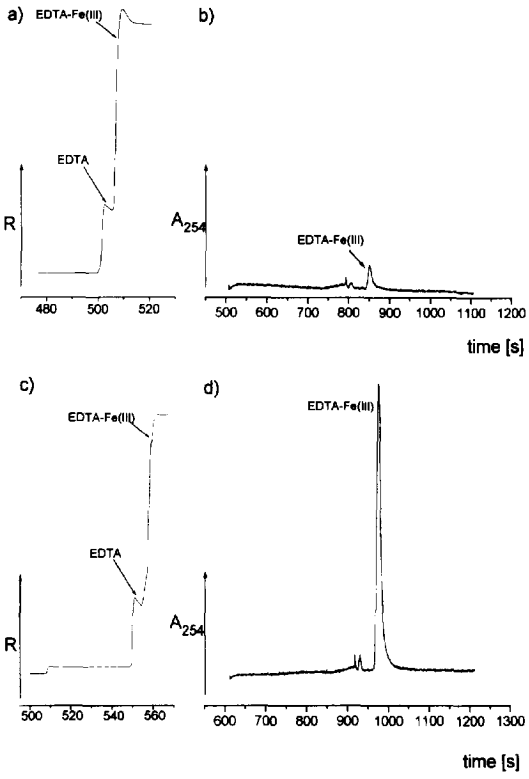

Fig. 4. Effect of acidification of the sample on the iron analysis of a mineral water with high Fe(III) content; conductivity and UV records of 25-fold diluted sample+10^{-4} mol/l EDTA; (a), (b) without acidification; (c), (d) after addition of 1 ml HNO_3 (65%) to 250 ml of mineral water.

caused by the geometry of the bifurcation block. It follows that in practice it seems necessary that there is some leeway in the transfer process of the EDTA–Fe(III). A leeway of 20 s leads to an actual distance of the isotachophoretic EDTA–Fe(III) zone between 6 and 8 mm from the bifurcation point, which is surprisingly large.

3.2.2. Determination of iron in real samples

During the evaluation of the method for quantitative analysis of real samples it was found that significantly lower iron contents are obtained if samples were analysed without addition of nitric acid (see Fig. 4). In these cases also iron added was not recovered quantitatively. The reason for this effect may lie in the low solubility of iron(III) hydroxide, which has a solubility product of about 10^{-38}, and precipitates even at moderate acidic pH. $Fe(OH)_3$ may still remain in the sample as a colloid, an effect already observed in another context concerning CE [27]. The analytical conditions are, however, poorly defined in this case. The addition of nitric acid to the sample leads to a well-defined strongly acidic medium. Additionally, in combination with an ultrasonic treatment, the sample is degassed and the carbon dioxide, which is naturally present in mineral water or is occasionally added to table water, is released. Higher amounts of carbonate salts in mineral waters require the application of a greater amount of nitric acid (see Section 2.5).

When evaluating the present analytical method, hydrogen peroxide (together with nitric acid) was applied to ensure that iron is quantitatively oxidized to Fe(III). Since the addition of peroxide had no observed effect on the results of the analysis, this step was also omitted.

A series of different water samples, including drinking and surface water, were analysed with the method presented here. Some typical results of these determinations are shown in Table 1. The recovery at iron levels between 80 and 11 000 μg/l varied from

Table 1
Results of the quantitative analysis of real water samples

Sample	Mean value of iron concentration (μg/l)[a]	Span (μg/l)[b]
Tap water	85	19
Table water	150	38
Table water	11	3
River water	100	31
Mineral water	10 730	550

[a] Mean values from three independent measurements.
[b] Span is the difference between the highest and lowest value of the measurement.

80 to 115%. The precision of the determination, expressed by the relative standard deviation of ten independent CE measurements (injected iron concentration was about 400 μg/l) was 3%. The reproducibility of the entire determination of Fe for real samples, expressed by the range (the span between the highest and the lowest measuring value of a particular sample) obviously depends on the concentration level of Fe and lies between 6 and 40% (see Table 1).

4. Conclusion

The determination of iron by on-line combination of CITP (as sample preconcentration step) and CZE based on the charged UV active complex with EDTA enables trace analysis in water (i) with appropriate yield; (ii) with acceptable precision and accuracy; (iii) within acceptable time of analysis due to the minimal sample pretreatment and the reasonable CE run times; (iv) at low cost of analysis.

It can be concluded that, besides the longterm problem of the acceptance of CITP in the field of separation methods, the analytical method evaluated is favourable for the trace determination of Fe(III) in water samples of different origin.

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