

Ultrasound-assisted Replacement Reaction for the Simultaneous Speciation Analysis of Fe(II)-EDTA and Fe(III)-EDTA in Absorbing Liquid of Denitrification System with LC-ICP-OES

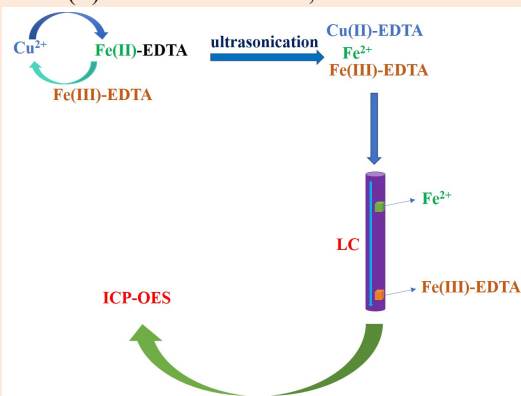
Liwei Liu, Yong Cheng,* Jinhong Yuan, and Yang Su

State Key Laboratory of Vanadium and Titanium Resources Comprehensive Utilization, Pangang Group Research Institute Co., Ltd., Panzhihua 617000, Sichuan, P. R. China

Received: August 30, 2020; Revised: September 12, 2020; Accepted: September 12, 2020; Available online: September 16, 2020.

DOI: 10.46770/AS.2020.06.004

ABSTRACT: A procedure was developed for the fast simultaneous speciation analysis of Fe(II)-EDTA and Fe(III)-EDTA in the absorbing liquid of a denitrification system with LC-ICP-OES based on ultrasound-assisted replacement reaction. Under ultrasonication, Fe^{2+} was replaced from Fe(II)-EDTA by Cu^{2+} at pH = 1, subsequently Fe^{2+} and Fe(III)-EDTA were separated by LC before introduction into the ICP-OES for measurement. The effects of several key factors, such as pH, concentration of Cu^{2+} , and ultrasonication time on the efficiency of the replacement reaction were investigated. Under the optimized conditions, the limits of detection of $1.2 \times 10^{-5} \text{ mol L}^{-1}$ for Fe(III)-EDTA and $1.6 \times 10^{-5} \text{ mol L}^{-1}$ for Fe(II)-EDTA were obtained, with a relative standard deviation ($n=11$, at $1.79 \times 10^{-4} \text{ mol L}^{-1}$ for both) of 4.7% for Fe(II)-EDTA and 2.1% for Fe(III)-EDTA. These results successfully fulfill the requirement of speciation analysis of Fe(II)-EDTA and Fe(III)-EDTA (typical concentration: $0.025 \sim 0.1 \text{ mol L}^{-1}$) in the absorbing liquid of the denitrification system. Common interferences from Na^+ , K^+ , SO_3^{2-} , SO_4^{2-} , Ca^{2+} , Mg^{2+} were also investigated. Routine spike recovery tests with randomly selected samples showed the satisfactory results of 84.0% ~ 93.0% and 92.0 ~ 101.3% for Fe(II)-EDTA and Fe(III)-EDTA, respectively. The proposed method is simple yet sensitive for screening of Fe(II)-EDTA and Fe(III)-EDTA in the absorbing liquid samples of a denitrification system, it is also a potential analytical method for other sample matrices.



INTRODUCTION

Nitrogen oxide (NO_x) emission from combustion of coal and fuel oils has a significantly negative impact on the ecosystem, air quality and human health, leading to the depletion of the ozone layer, global warming, acidification of soil and water bodies.¹⁻⁴ Additionally, it is one of the principal causes of smog formation. Among all of the NO_x pollution species above, nitric oxide (NO) accounts for 90 ~ 95% overall.^{5,6} Therefore, removal of NO from flue gas is most important in air pollution control. As a cost-effective and environmentally sustainable technology for NO removal, use of Fe(II)-EDTA solution has been most widely adopted as the absorbing liquid to combine with NO providing fast

absorption rate and high absorption capacity. Thus, this technology has always been among hot research topics.⁷⁻¹¹

However, Fe(II)-EDTA will be gradually oxidized to Fe(III)-EDTA, which has no ability to absorb NO. For recycling, Fe(III)-EDTA will be reduced to Fe(II)-EDTA for re-use and at the same time, NO will be released as harmless N_2 .^{12,13} Therefore, to evaluate the performance of an absorbing liquid in a denitrification system and to decide when Fe(III)-EDTA should be reduced, the determination of Fe(II)-EDTA and Fe(III)-EDTA are of great importance. To the best of our knowledge, little investigation was focused on the determination of Fe(II)-EDTA. The traditional indirect assay of Fe(III)-EDTA was based on the dissociation of

Fe(III)-EDTA into Fe³⁺ in strong acid condition, subsequently reduced to Fe²⁺ and detected by UV/Visible spectrophotometry with 1,10-phenanthroline colorimetric method.^{10,14,15} But this method involved too many pre-treatments and calculations, which were time-consuming and laborious. Many other methods were also developed for the determination of Fe(III)-EDTA, such as liquid chromatography,¹⁶⁻¹⁸ capillary isotachopheresis and capillary zone electrophoresis with UV detection,¹⁹⁻²⁰ graphite furnace atomic absorption spectrometry,²¹ and photoluminescent carbon dot.²²

As we know, the stability constant of Fe(II)-EDTA ($\lg K_{\text{Fe(II)-EDTA}} = 14.32$) is much lower than that of Fe(III)-EDTA ($\lg K_{\text{Fe(III)-EDTA}} = 25.1$), so when another metal ion (M^{n+}) is chelated by EDTA with the stability constant in between ($\lg K_{\text{Fe(II)-EDTA}} < \lg K_{M-EDTA} < \lg K_{\text{Fe(III)-EDTA}}$), Fe²⁺ will be replaced by M^{n+} from Fe(II)-EDTA. Given the big molecular structure difference between Fe²⁺ and Fe(III)-EDTA, they could be separated by LC and subsequently become subject to elemental analysis technology, which is routinely adopted for iron determination.²³⁻²⁸

Therefore, based on the replacement reaction theory, separation technology, and elemental analysis for iron, in this study a new method is proposed for the simultaneous speciation analysis of Fe(II)-EDTA and Fe(III)-EDTA in the absorbing liquid of a denitrification system by LC-ICP-OES.

EXPERIMENTAL

Instrumentation. Fe²⁺, Fe³⁺, Fe(II)-EDTA and Fe(III)-EDTA were separated by liquid chromatography (LC) (Flexar LC, PerkinElmer, Inc., USA), equipped with a LC pump (Flexar Binary LC pump, part No. N2910401, PerkinElmer, Inc., USA), a special column (Dionex IonPac™ CG5A RFIC™ 4×50 mm Guard, Thermo Scientific, USA) and an eluent (Dionex MetPac PDCA Eluent Concentrate P/N 046088, Lot Number: 190417) designed for Fe³⁺/Fe²⁺ separation. The emission intensity measurements of iron were performed using an Avio 200 inductively coupled plasma optical emission spectrometer (ICP-OES) (PerkinElmer, Inc., USA). The spectrometer was equipped with a concentric nebulizer, a rotary spray chamber, and can be easily connected to LC. An ultrasonic cleaner bath (SK7200LHC, Shanghai Kedao Ultrasonic Machine Co., Ltd.) was employed to accelerate the replacement reaction rate. The working parameters for LC and ICP-OES are listed in Tables 1 and 2, respectively.

Reagents and standard solutions. All reagents used in this work are at least of analytical grade. Ultra-pure quality water (electrical resistivity: 18.25 MΩ cm⁻¹) was obtained with a water purification system (RiOs™ 100, Millipore Corporation, USA). The water did not contain any detectable concentration of iron under optimal working conditions by ICP-OES. All plastic and glassware were cleaned by soaking in dilute HNO₃ (1%, V/V) and rinsed

Table 1. LC Working Parameters

Step	Hold time (min)	Flow rate (mL min ⁻¹)	Percentage of eluent (%)
Equilibrium	0.2	1.8	100
1	3.0	1.8	100
2	4.0	2.2	100

Table 2. ICP-OES Working Parameters

Parameter	Setting
Plasma flow (mL min ⁻¹)	12,000
Auxiliary flow (mL min ⁻¹)	200
Nebulizer flow (mL min ⁻¹)	600
Plasma power (W)	1150
Analytical line (nm)	239.562
Integration time (sec)	0.1

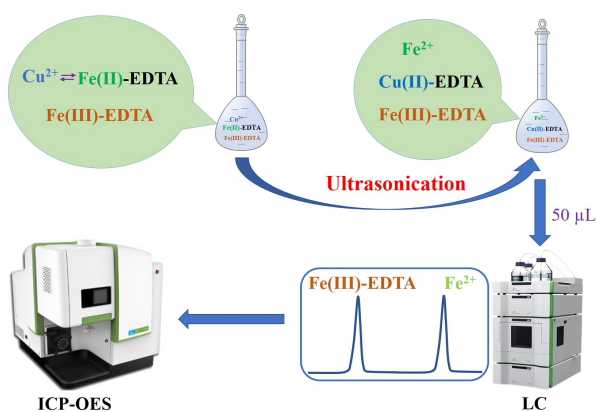


Fig. 1 Schematic diagram of the analysis procedure.

sequentially with ultra-pure water prior to use. 1000 mg L⁻¹ Fe²⁺ and Fe³⁺ standard stock solutions were obtained by dissolving 0.4978 g FeSO₄·7H₂O (Chengdu Kelong Reagent Co., Ltd.) and 0.4840 g FeCl₃·6H₂O (Chengdu Kelong Reagent Co., Ltd.) in 100 mL ultrapure water containing 1% H₂SO₄, respectively. 2000 mg L⁻¹ Cu²⁺ was obtained by dissolving 0.7858 g CuSO₄·5H₂O (Chongqing Reagent Factory) in 100 mL ultra-pure water. Hydroxylamine hydrochloride and EDTA were purchased from Chengdu Kelong Reagent Co., Ltd. The Fe(II)EDTA and Fe(III)EDTA complex stock solution was prepared by introducing Fe²⁺, Fe³⁺ and EDTA with equal concentration to degassed deionized water, preferably under anaerobic conditions. The working solutions were prepared fresh daily by serial dilution of the standard stock solution. All solutions were stored in the refrigerator at 4°C.

Procedure. As illustrated in Fig. 1, first, 1 mL analyte (primarily Fe(II)-EDTA and Fe(III)-EDTA), 0.55 mL concentrated sulfuric acid and a certain amount of Cu²⁺ were pipetted into a 100 mL volumetric flask, and made up to the mark with ultra-pure water. The amount of Cu²⁺ was decided by the sum of concentration of Fe(II)-EDTA and EDTA in the analyte. In this research, Cu²⁺ at the

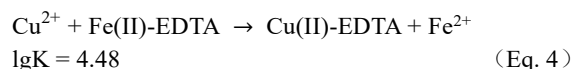
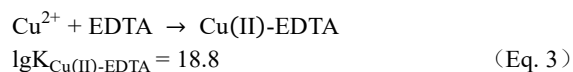
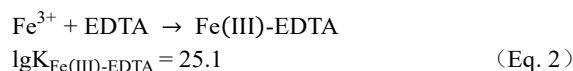
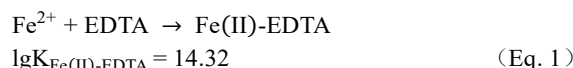
concentration of 5 times the sum of concentration of the Fe(II)-EDTA and EDTA was added to make sure that Fe²⁺ could be completely replaced by Cu²⁺ from Fe(II)-EDTA. Second, to accelerate the replacement reaction rate, the above 100 mL mixture was placed into an ultrasonic cleaner bath under ultrasonic irradiation for 15 min with the working frequency of 53 KHz. Third, 50 μL analyte solution was injected into the LC for the separation of Fe²⁺ and Fe(III)-EDTA, and then introduced into the ICP-OES for sequential measurement of the integrated intensity signals of peak area at the analytical line of 239.562 nm.

RESULTS AND DISCUSSION

Replacement reaction

As we know, EDTA is a most widely adopted chelating agent because it can form a very stable chelate compound with the majority of metal ions. However, the stability constant²⁹ varies largely when EDTA chelates with different metal ions. As shown in the following four equations, Fe²⁺, Fe³⁺ and Cu²⁺ can form a stable chelate compound with EDTA; however, the stability constant of Fe(III)-EDTA (Equation 2) is about 10¹¹ times that of Fe(II)-EDTA (Equation 1), and the stability constant of Cu(II)-EDTA (Equation 3) is in between. This tremendous gap between the stability constants leads to the feasibility that Fe²⁺ can be replaced by Cu²⁺ from Fe(II)-EDTA, while Fe³⁺ is unable to be replaced by Cu²⁺ from Fe(III)-EDTA. It is very easy to calculate that the chemical reaction rate constant of this replacement reaction (Equation 4) is 10^{4.48} ($\lg K_{\text{Cu(II)-Fe(II)-EDTA}} = \lg K_{\text{Cu(II)-EDTA}} - \lg K_{\text{Fe(II)-EDTA}}$), which is theoretically possible for a chemical reaction to proceed in the right direction in chemical thermodynamics, regardless of chemical kinetics. Because the stability constant of Cu(II)-EDTA is in between and Cu²⁺ is very

cost-effective, easily available, and less toxic than other metal ions, Cu²⁺ was selected in this study as the replacement reagent.



Separation of Fe(II)-EDTA and Fe(III)-EDTA by LC

A special column (Dionex IonPac™ CG5A RFIC™ 4×50 mm Guard) and eluent (Dionex MetPac PDCA Eluent Concentrate P/N 046088) designed for Fe³⁺/Fe²⁺ separation was adopted for this work. As shown in Fig. 2 (a), Fe³⁺ and Fe²⁺ can be effortlessly separated and detected by ICP-OES. Nevertheless, in Fig. 2(b), Fe(II)-EDTA and Fe(III)-EDTA had the same short retention time (50 seconds), suggesting Fe(II)-EDTA and Fe(III)-EDTA cannot be retained and separated in this LC condition. This might be due to the significant difference of the molecular structure and electrical charge between Fe³⁺/Fe²⁺ and Fe(II)-EDTA/Fe(III)-EDTA. When Cu²⁺ was added to replace Fe²⁺ from Fe(II)-EDTA, the chromatogram of Fe(II)-EDTA disappeared and an obvious peak appeared in the retention time of Fe²⁺ (about 300 seconds), indicating Cu²⁺ was successful in replacing Fe²⁺ from Fe(II)-EDTA (see Fig. 2(c)). In Fig. 2(d), a mixture of Fe(III)-EDTA, Fe(II)-EDTA, and Cu²⁺ was injected into the LC, and a peak appeared in the retention time of 50 seconds in comparison to Fig. 2(c), indicating that Cu²⁺ cannot replace Fe³⁺ from Fe(III)-EDTA. So Fe(II)-EDTA and Fe(III)-EDTA can be discriminated by LC based on the chemical replacement reaction with Cu²⁺.

Fig. 2 Chromatogram: (a) Fe³⁺ + Fe²⁺, (b) Fe(II)-EDTA or Fe(III)-EDTA, (c) Fe(II)-EDTA + Cu²⁺, (d) Fe(III)-EDTA + Fe(II)-EDTA + Cu²⁺.

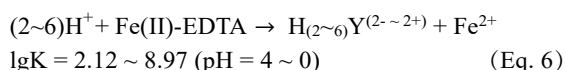
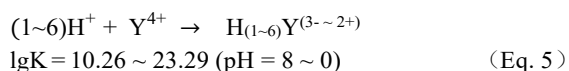
Table 3. Stability Constant of EDTA at Different pH

Compound	Stability constant	pH (approximately)
H ₁ Y ³⁻	lgK _H ¹ = 10.28	8
H ₂ Y ²⁻	lgK _H ² = 6.16	4
H ₃ Y ⁻	lgK _H ³ = 2.67	3
H ₄ Y	lgK _H ⁴ = 2.0	2
H ₅ Y ⁺	lgK _H ⁵ = 1.6	1
H ₆ Y ²⁺	lgK _H ⁶ = 0.6	0

Fig. 3 pH on the effect of replacement reaction.

pH on the effect of replacement reaction

EDTA, namely ethylene diamine tetra-acetic acid (H₄Y), is a weak tetrahydric acid. It will decompose into H⁺ and Y⁴⁻ in alkaline condition, while in acid condition, Y⁴⁻ will combine 1~6 H⁺ to form H_(1~6)Y^(3~-2+) (see Equation 5) based on the concentration of H⁺(pH). The higher the concentration of H⁺, the more H⁺ combines with Y⁴⁻. The stability constants³⁰ of H_(1~6)Y^(3~-2+) are listed in Table 3.



In other words, in acid condition, H⁺ can be captured by EDTA, so H⁺ is also likely to replace Fe²⁺ from Fe(II)-EDTA (see Equation 6). Theoretically, when the pH = 4, EDTA can combine with 2 H⁺ to form H₂Y²⁻ with a stability constant of lgK_{pH4} = 16.44 (lgK_{pH4} = lgK_H¹ + lgK_H²), and the chemical reaction rate constant of this replacement reaction (Equation 6) is 10^{2.12} (lgK_{pH4-Fe(II)-EDTA} = lgK_{pH4} - lgK_{Fe(II)-EDTA}). When the pH = 0, EDTA can combine 6 H⁺ to form H₆Y²⁺ with a stability constant of lgK_{pH0} = 23.29 (lgK_{pH0} = lgK_H¹ + lgK_H² + lgK_H³ + lgK_H⁴ + lgK_H⁵ + lgK_H⁶), and the chemical reaction rate constant of this replacement reaction (Equation 6) is 10^{8.97} (lgK_{pH0-Fe(II)-EDTA} = lgK_{pH0} - lgK_{Fe(II)-EDTA}), which is much higher than that of Equation 4, proving in theory that H⁺ might also be able to replace Fe²⁺ from Fe(II)-EDTA.

Generally, metal ions keep stable in acid condition, but H⁺ can be captured by EDTA in acidic conditions, so the participation of

H⁺ in the replacement reaction by Cu²⁺ must be taken into account. In order to investigate the contribution of H⁺ to replace Fe²⁺ from Fe(II)-EDTA, a comparison experiment was conducted in different pH with/without Cu²⁺. As illustrated in Fig. 3, without adding Cu²⁺, it was impossible for H⁺ to replace Fe²⁺ from 1.79 × 10⁻⁴ mol L⁻¹ Fe(II)-EDTA when the pH = 4, which might be because the replacement reaction rate constant is not high enough (lgK_{pH4-Fe(II)-EDTA} = 2.12). When the pH ≤ 2, H⁺ began to replace Fe²⁺, but with very low efficiency. At pH = 1, the efficiency peaked at 30.1% which was much lower than expected, even though the replacement reaction rate constant is very high (lgK_{pH1-Fe(II)-EDTA} ≈ 8). This phenomenon probably needs to be explained by the chemical kinetics theory, which is beyond the scope of the discussion in this work. When the pH ≤ 0.5, a flattened tailing peak was observed, and the width of the peak at least doubled, indicating that the separation condition of LC was interrupted by H⁺. The reason is that, because too much H⁺ would exceed the buffer tolerance of the eluent (buffer solution, pH = 4), leading to a significant pH change in the eluent, and subsequently result in an inferior separation in LC. Therefore, the replacement efficiency of H⁺ decreased.

However, in the presence of Cu²⁺, the case was quite different. The efficiency of the replacement rate was greatly enhanced. Viewing from the molecular structure, this was probably due to an empty orbital (3d) provided by Cu²⁺, which could accommodate surplus unpaired electrons from N atoms in EDTA, forming a very stable chelate compound. Since at pH > 5, Cu²⁺ and Fe²⁺ would hydrolyze to form precipitations of Cu(OH)₂ and Fe(OH)₂, the maximum pH of the investigation was set at pH = 5. As can be seen in Fig. 3, in the presence of 8.95 × 10⁻⁴ mol L⁻¹ Cu²⁺ (Fe(II)-EDTA concentration: 1.79 × 10⁻⁴ mol L⁻¹), the replacement efficiency was about 30% at pH = 5, much higher than without Cu²⁺. With the increase of H⁺, the replacement efficiency kept an upward trend and reached maximum at pH of 1. When the pH < 1, too much H⁺ will change the pH of the eluent (buffer solution), depressing the separating efficiency, thus an inferior replacement efficiency was observed.

Therefore, from the comparison investigation with/without Cu²⁺, it was easy to reach the conclusion that both H⁺ and Cu²⁺ contributed to the replacement of Fe²⁺ from Fe(II)-EDTA, and a pH of 1 was selected as the optimal acidity for the replacement reaction in this work.

Concentration of Cu²⁺ on the effect of replacement reaction.

Under the fixed pH at 1, the dosage of Cu²⁺ on the effect of replacement efficiency was investigated. 1.79 × 10⁻⁴ mol L⁻¹ Fe(II)-EDTA was prepared by mixing 1.79 × 10⁻⁴ mol L⁻¹ Fe²⁺ (10 mg L⁻¹) and 1.79 × 10⁻⁴ mol L⁻¹ EDTA, then kept standing for 20 min. To make sure the reaction was complete, the mixture was detected by LC-ICP-OES, and the results showed that no Fe²⁺ was present, but Fe(II)-EDTA was found, suggesting that all the Fe²⁺ was completely chelated by EDTA.

Fig. 4 Dosage of Cu^{2+} on the effect of replacement reaction.

Fig. 5 Ultrasonication time on the effect of replacement reaction.

As demonstrated in Fig. 3, without adding Cu^{2+} , H^+ can replace Fe^{2+} from Fe(II)-EDTA with a maximum efficiency of about 38%. However, when $\text{Cu}^{2+}/\text{Fe}^{2+}$ (mol/mol) was increased to 0.5, which was lower than the theoretical stoichiometric ratio 1:1 (Equation 4), the replacement efficiency soared dramatically to about 93% (see Fig. 4), which is probably due to the contribution of H^+ in the replacement reaction. When $\text{Cu}^{2+}/\text{Fe}^{2+}$ (mol/mol) reached the theoretical stoichiometric ratio of 1:1, the replacement efficiency attained about 98%, indicating that almost all of the Fe^{2+} was replaced from Fe(II)-EDTA. A further increase of the dosage of Cu^{2+} just kept the replacement efficiency stable at close to 100%.

Considering the practical application, excess dosage of EDTA is likely to be adopted and in this work, the ratio (mol/mol) of 5:1 for $\text{Cu}^{2+}/\text{Fe}^{2+}$ was applied. The researcher can modify the ratio of $\text{Cu}^{2+}/\text{Fe}^{2+}$ in real sample analysis based on the composition of the sample of interest.

Time of ultrasonication on the effect of replacement reaction.

As shown in Fig. 5, without ultrasonication, Fe^{2+} was very slow to be replaced from Fe(II)-EDTA by Cu^{2+} . It took almost 300 min to totally finish the replacement reaction. Obviously, it is too time-consuming for real analysis. This might be explained by the following reasons: First, in Equation 4, the reaction constant of this replacement reaction is $10^{4.48}$, in fact not high enough for a reaction proceeding fast (usually above 10^5). Second, the reactant concentrations of Cu^{2+} and Fe(II)-EDTA were 8.95×10^{-4} mol L^{-1}

and 1.79×10^{-4} mol L^{-1} , respectively, which were too low for a chemical reaction to go fast. Ultrasound was widely employed in the chemical pretreatment.^{31,32} Under ultrasonication, molecule collision probability enhanced greatly together with the increase of the reaction temperature, leading to a much faster reaction. Further discussion of the mechanism under ultrasonication was excluded in this work. So, in this study, in order to shorten the analysis time, a mixture of 8.95×10^{-4} mol L^{-1} Cu^{2+} and 1.79×10^{-4} mol L^{-1} Fe(II)-EDTA ($\text{Cu}^{2+}/\text{Fe}^{2+} = 5$) was placed into an ultrasonic cleaner bath, which was filled with an appropriate amount of water. It is very clear from Fig. 5 that under ultrasonic irradiation, the efficiency of the replacement reaction increased sharply and almost linearly with the increase in ultrasonication time. After 10 min ultrasonication, the efficiency of the replacement reaction reached a plateau at about 99% and kept stable even when the ultrasonication time was further prolonged, indicating that Fe^{2+} was totally replaced by Cu^{2+} . Thus, 15 min of ultrasonic irradiation was selected for this work.

Effect of concomitants.

To evaluate the analytical applicability of the proposed method, the effect of some concomitants was investigated. In the absorbing liquid of the denitrification system, the concomitants involved in the preparation of Fe(II)-EDTA were $\text{Na}_2\text{SO}_3/\text{K}_2\text{SO}_3$, which were used to stop Fe(II)-EDTA from oxidizing to Fe(III)-EDTA. Thus, Na^+ , K^+ , SO_3^{2-} , SO_4^{2-} were studied to establish whether they interfered in the determination of Fe(II)-EDTA/Fe(III)-EDTA. The results showed that 1.79×10^{-2} mol L^{-1} Na^+ , K^+ , SO_3^{2-} , and SO_4^{2-} did not interfere at the fixed concentration of 1.79×10^{-4} Fe(II)-EDTA. For most other metal(M) ions, except Ca^{2+} and Mg^{2+} , they could form more stable chelate compounds with EDTA than that with Fe^{2+} ($\lg K_{\text{M-EDTA}} > \lg K_{\text{Fe(II)-EDTA}}$), influencing the stability of Fe(II)-EDTA. In the preparation of Fe(II)-EDTA, these metal ions should be removed first or chelated by excessive EDTA. Therefore, for other metal ions, only Ca^{2+} and Mg^{2+} were selected for the interference study, and the results showed that 1.79×10^{-2} mol L^{-1} Ca^{2+} and Mg^{2+} did not interfere at the fixed concentration of 1.79×10^{-4} mol L^{-1} Fe(II)-EDTA.

Analytical figures of merit and sample analysis.

Under the optimal experimental conditions, the analytical performance of this proposed method for the speciation of Fe(II)-EDTA and Fe(III)-EDTA in the absorbing liquid of the denitrification system was investigated (Table 4). The limit of detection (LOD) was defined as three times the standard deviation of 11 measurements of a blank divided by the slope of the calibration curve. After the replacement reaction, an LOD of 1.2×10^{-5} mol L^{-1} for Fe(III)-EDTA and an LOD of 1.6×10^{-5} mol L^{-1} for Fe(II)-EDTA were obtained, which can successfully fulfill the requirement of the speciation of Fe(II)-EDTA (typical concentration: 0.025–0.1 mol L^{-1}) and Fe(III)-EDTA in the absorbing liquid of the denitrification system.

Table 4. Analytical Characteristics of the Proposed Method

Details	Fe(II)-EDTA	Fe(III)-EDTA
Sample volume (μL)	50	
Calibration equation (X in mmol L^{-1})	$Y=1.6\times 10^6 X-1926$	$Y=2.1\times 10^6 X-1525$
Correlation coefficient R	0.9991	0.9995
Limit of detection (mmol L^{-1})	1.7×10^{-2}	1.2×10^{-2}
Precision at $0.179 \text{ mmol L}^{-1}$ (RSD, n = 11)	4.7%	2.1%

Note: Samples in this table were prepared by mixing Fe^{2+} , Fe^{3+} and EDTA, not real samples.

Table 5. Recovery Test of Proposed Method

Sample	Spiked (mol L^{-1})	Found (mol L^{-1}) ^c	Recovery (%)
^a A	0.025	0.021 ± 0.002	84.0
^a B	0.050	0.046 ± 0.004	92.0
^a C	0.075	0.069 ± 0.005	92.0
^a D	0.100	0.093 ± 0.006	93.0
^b A	0.025	0.023 ± 0.002	92.0
^b B	0.050	0.048 ± 0.003	96.0
^b C	0.075	0.076 ± 0.004	101.3
^b D	0.100	0.098 ± 0.004	98.0

^a Sample spiked with Fe(II)-EDTA, ^b Sample spiked with Fe(III)-EDTA.

^c Average \pm one standard deviation of three trials.

Since there are no commercially available certified reference materials for evaluation of the accuracy of the proposed method, four absorbing liquid samples of the denitrification system were collected from the Environmental Technology Research Center of Pangang Group Research Institute Co., Ltd., for the recovery test. As shown in Table 5, the recoveries for Fe(II)-EDTA and Fe(III)-EDTA were in the range of 84.0%–93.0% and 92.0–101.3%, respectively, indicating that this method is applicable to speciation analysis of Fe(II)-EDTA and Fe(III)-EDTA in real samples from a denitrification system.

CONCLUSIONS

To enable successful simultaneous speciation of Fe(II)-EDTA and Fe(III)-EDTA in absorbing liquid samples of a denitrification system, the hyphenated technique of LC-ICP-OES based on ultrasound-assisted replacement reaction is proposed which enjoys several distinct advantages. First, Fe(II)-EDTA and Fe(III)-EDTA can be simultaneously analyzed. Second, the operation of this method is relatively simple and green, only Cu^{2+} and H_2SO_4 are involved in pre-treatment. Third, the volume of sampling is as small as $50 \mu\text{L}$ and finally, it is a potential method for speciation of Fe(II)-EDTA and Fe(III)-EDTA for other samples.

AUTHOR INFORMATION

Corresponding Author

*Y. Cheng

Email address: chengyongpg@163.com

Notes

The authors declare no competing financial interest.

REFERENCES

- Q. Guo, Y. He, T. Sun, Y. Wang, and J. Jia, *J. Hazard. Mater.*, 2014, **276**, 3713-76. <https://doi.org/10.1016/j.jhazmat.2014.05.058>
- Y. Shi, Y. F. Xia, B. H. Lu, N. Liu, L. Zhang, S. J. Li, and W. Li, *J. Zhejiang Univ-Sci. A*, 2014, **15**, 454-464. <https://doi.org/10.1631/jzus.A1300379>
- Y. M. Jin, M. C. Veiga, Mand and C. Kennes, *J. Chem. Technol. Biotechnol.*, 2005, **80**, 483-494. <https://doi.org/10.1002/jctb.1260>
- W. P. Flanagan, W. A. Apel, J. M. Barnes and B. D. Lee, *Fuel*, 2002, **81**, 1953-1961. [https://doi.org/10.1016/S0016-2361\(02\)00130-8](https://doi.org/10.1016/S0016-2361(02)00130-8)
- X. L. Long, Z. L. Xin, M. B. Chen, W. Li, W. D. Xiao, and W. K. Yuan, *Sep. Purif. Technol.*, 2008, **58**, 328-334. <https://doi.org/10.1016/j.seppur.2007.05.004>
- H. F. Jin, D. E. O. Santiago, J. Park, and K. Lee, *Biotechnol. Bioprocess Eng.*, 2008, **13**, 48-52. <https://doi.org/10.1007/s12257-007-0164-z>
- Y. F. Xia, J. K. Zhao, M. F. Li, S. H. Zhang, S. J. Li, and W. Li, *Environ. Sci. Technol.*, 2016, **50**, 3846-3851. <https://doi.org/10.1021/acs.est.5b05861>
- W. Li, Y. F. Xia, J. K. Zhao, N. Liu, S. J. Li, and S. H. Zhang, *Environ. Sci. Technol.*, 2015, **49**, 4530-4535. <https://doi.org/10.1021/es5058488>
- J. Chen, L. Wang, J. Zheng, and J. M. Chen, *Bioprocess Biosyst. Eng.*, 2015, **38**, 1373-1380. <https://doi.org/10.1007/s00449-015-1378-7>
- W. Li, C. Z. Wu and Y. Shi, *Chem. Technol. Biotechnol.*, 2006, **81**, 306-311. <https://doi.org/10.1002/jctb.1394>
- B. Yan, J. H. Yang, M. Guo, G. D. Chen, Z. Li, and S. C. Ma, *J. Ind. Eng. Chem.*, 2014, **20**, 2528-2534. <https://doi.org/10.1016/j.jiec.2013.10.036>
- X. Y. Dong, Y. Zhang, J. T. Zhou, M. X. Chen, X. J. Wang, and Z. Shi, *Bioresour. Technol.*, 2013, **138**, 339-344. <https://doi.org/10.1016/j.biortech.2013.03.181>
- X. Y. Dong, Y. Zhang, J. T. Zhou, H. Y. Li, X. J. Wang and M. X. Chen, *J. Chem. Technol. Biotechnol.*, 2014, **89**, 111-116. <https://doi.org/10.1002/jctb.4112>
- W. Li, C. Z. Wu, S. H. Zhang, K. Shao, and Y. Shi, *Environ. Sci. Technol.*, 2007, **41**, 639-644. <https://doi.org/10.1021/es061757e>
- N. Li, Y. Zhang, Y. M. Li, M. X. Chen, X. Y. Dong and J. T. Zhou, *J. Chem. Technol. Biotechnol.*, 2013, **88**, 311-316. <https://doi.org/10.1002/jctb.3833>

16. China National Standard, Determination of sodium iron(III) ethylenediaminetetraacetate (NaFeEDTA), trihydrate in iron fortified soy sauce, GB/T 21234-2007.
 17. B. Nowack, F. G. Kari, S. U. Hilger, and L. Sigg, *Anal. Chem.*, 1996, **68**, 561-566. <https://doi.org/10.1021/ac9507505>
 18. M. Sillanpaa, R. Kokkonen, and M. L. Sihvonen, *Anal. Chim. Acta*, 1995, **303**, 187-192. [https://doi.org/10.1016/0003-2670\(94\)00535-T](https://doi.org/10.1016/0003-2670(94)00535-T)
 19. P. Blatny, F. Kvasnlek, and E. Kenndler, *J. Chromatogr. A*, 1997, **757**, 297-302. [https://doi.org/10.1016/S0021-9673\(96\)00668-1](https://doi.org/10.1016/S0021-9673(96)00668-1)
 20. B. Baraj, M. Martinez, A. Sastre, and M. Aguilar, *J. Chromatogr. A*, 1995, **695**, 103-111. [https://doi.org/10.1016/0021-9673\(94\)01078-S](https://doi.org/10.1016/0021-9673(94)01078-S)
 21. R. Manjusha, R. Shekhar, and S. J. Kumar, *Food Chem.*, 2019, **294**, 384-389. <https://doi.org/10.1016/j.foodchem.2019.04.104>
 22. N. Wang, H. J. Chai, X. L. Dong, Q. Zhou, and L. H. Zhu, *Food Chem.*, 2018, **258**, 51-58. <https://doi.org/10.1016/j.foodchem.2018.03.050>
 23. S. K. Pradhan, B. Ambade, and P. K. Tarafdera, *At. Spectrosc.*, 2019, **40**, 145-151. <https://doi.org/10.46770/AS.2019.04.006>
 24. R. Clough, C. F. Harrington, S. J. Hill, Y. Madrid and J. F. Tyson, *J. Anal. At. Spectrom.*, 2018, **33**, 1103-1149. <https://doi.org/10.1039/c8ja90025f>
 25. B. Wang, T. P. Liu, J. X. Liu, X. F. Mao, X. Na, G. Y. Chen, Z. H. Lv, and Y. Z. Qian, *At. Spectrosc.*, 2020, **41**, 119-126. <https://doi.org/10.46770/AS.2020.03.004>
 26. V. Padmasubashini, V. V. Hanuman, S. B. Singh, S. Hamilton, and M. B. Verma, *At. Spectrosc.*, 2019, **40**, 179-187. <https://doi.org/10.46770/AS.2019.05.005>
 27. C. F. Harrington, S. Elahi, S. A. Merson, and P. Ponnampalavanar, *Anal. Chem.*, 2001, **73**, 4422-4427. <https://doi.org/10.1021/ac010279h>
 28. G. AlChoubassi, J. Aszyk, P. Pisarek, K. Bierla, L. Ouerdane, J. Szpunar, and R. Lobinski, *Trac-Trend Anal. Chem.*, 2018, **104**, 77-86. <https://doi.org/10.1016/j.trac.2017.11.006>
 29. Wuhan University, Analytical Chemistry, the Fifth Edition, Section 1, Higher Education Press, 2006, Beijing, China, p. 393.
 30. Wuhan University, Analytical Chemistry, the Fifth Edition, Section 1, Higher Education Press, 2006, Beijing, China, p. 169.
 31. G. S. Zhou, Y. C. Yuan, Y. Yin, Y. P. Tang, R. J. Xu, Y. Liu, P. D. Chen, L. Yin, and J. A. Duan, *Anal. Chim. Acta*, 2020, **1107**, 74-84. <https://doi.org/10.1016/j.microc.2020.105018>
 32. M. Krawczyk and E. Stanisz, *Talanta*, 2016, **161**, 384-394. <https://doi.org/10.1016/j.talanta.2016.08.071>
-