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Analytical Methods

# Detection of Fe(III)EDTA by using photoluminescent carbon dot with the aid of $F^-$ ion

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ABSTRACT

Iron(III) ethylenediaminetetraacetate (Fe(III)EDTA) is widely used in iron fortification for reducing iron deficiency, and its determination is urgently needed. The present work developed a fluorescent method to straightforwardly determine Fe(III)EDTA by using photoluminescent carbon dots (C-dots) with the aid of $F^-$ ions as the masking agent of free $Fe^{3+}$ ions. In the presence of $F^-$ ions, only Fe(III)EDTA selectively quenched the photoluminescence of C-dots, and both $Fe^{3+}$ and $Fe^{2+}$ as well as other carboxylic acids have no effect on the emission of C-dots. The sensing mechanism was attributed to the ligand-tailored electron transfer process from C-dots to $Fe^{3+}$. Under optimum conditions, this method showed a linear calibration plot over the Fe(III)EDTA range of 1.0–200 μmol $L^{-1}$ and a detection limit of 0.4 μmol $L^{-1}$. The proposed method was successfully applied to determine Fe(III)EDTA in real samples with acceptable recoveries of spikes (95% − 110%) and repeatability (RSD, 4.2% − 9.5%).

## 1. Introduction

Iron deficiency is the common worldwide nutritional deficiency. Food fortification programs are cost effective means for reducing the prevalence of iron deficiency (Hurrell, 1997). A number of iron forms including inorganic iron salts, elemental iron powders and iron chelates are used in the food vehicles such as rice, milk, soy sauce, fish sauce and fruit juices (Huang et al., 2009; Hurrell, Reddy, & Cook, 2000; Thuy et al., 2003; Yang, Siekmann, & Schofield, 2011). Among these iron status, iron(III) ethylenediaminetetraacetate (Fe(III)EDTA) has attracted considerable interest, because of its high stability, good solubility, and superior iron bioavailability (Huang et al., 2009; Hurrell et al., 2000; Thuy et al., 2003; Yang, et al., 2011). Hurrell et al. reported that when used to fortify foods rich in phytic acid and polyphenol, iron bioavailability from NaFeEDTA was 2–3 times higher than that of $FeSO_4$ (Huang et al., 2009). However, high doses of iron also result in some disorders like depression, rapid and shallow respiration, coma, convulsions and cardiac arrest (Kumar et al., 2014; Zhao et al., 2016).

The iron level is a critical issue in governing the functionality of iron fortified food. A variety of analytical methods, such as atomic absorption spectroscopy, spectrophotometric detection using chromogenic reagents, and inductively coupled plasma mass spectrometry have been developed to determine either $Fe^{3+}$, $Fe^{2+}$ or total iron (Amonette and Matya, 2015; Kumar et al., 2014; Zhao et al., 2016). Since different iron species have different iron bio-accessibility, the analysis of iron speciation becomes more important. In general, the determination of iron from Fe(III)EDTA is based on the detection of either $Fe^{3+}$ or $EDTA^{2-}$ after a dissociation (Cai, Cheng, Wu, & Yu, 2012; Kosse, Yeung, Gil, & Miller, 2001). The Fe(III)EDTA is mainly in the chelate form at pH $> 5$, and it is totally disassembled to free $Fe^{3+}$ ions at pH $\leq 0.5$. By using chromogenic reagents like thiocyanate or bathophenanthroline disulfonic acid, the spectrophotometric method measures the amount of "free" $Fe^{3+}$ if present in the sample at pH 5 and also the amount of total $Fe^{3+}$ at pH 0.5 after a dissociation of Fe(III)EDTA (China, 2007; Kosse et al., 2001). The concentration of Fe(III)EDTA was calculated by subtracting the "free" $Fe^{3+}$ at pH 5 from the total $Fe^{3+}$ at pH 0.5. Nevertheless, $Fe^{3+}$ ions would undergo a hydrolysis and/or a precipitation at pH 5, resulting in a great interference in the determination of "free" $Fe^{3+}$ ions. Based on the determination of $EDTA^{2-}$ anions, Cai et al. proposed an ion chromatography (IC) method to detect NaFeEDTA in cookies (Cai et al., 2012). However, it was rather difficult to discriminate Fe(III)EDTA from other food additives like $Na_2EDTA$ and CaEDTA (Krokidis, Megoulas, & Koupparis, 2005). By combining UV detector with high performance liquid chromatography (HPLC), Lucena

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et al. developed an ion-pairing reversed-phase HPLC method to directly determine $Fe^{3+}$-chelates like EDTA in commercial fertilizers (Lucena, Barak, & Hernández-Apaolaza, 1996). The similar HPLC methods have been also proposed to determine free EDTA in feed, pharmaceutical formulations and sea water after adding $Fe^{3+}$ to form Fe(III)EDTA (Chiumiento, D'Aloise, Marchegiani, & Melai, 2015; Kemmei, et al., 2012, 2013). Owing to the nature of the metal chelate equilibrium, this HPLC method requires very special elution conditions (pH, buffer and solvents). Moreover, the use of ion-pair reagents needs a considerable long time to flush out mobile phase, unless, the column lifetime would be shorten. Therefore, the identification of the same valent metal ions but at different states is still a great challenge, and the methods for the direct determination of Fe(III)EDTA are urgently needed.

Recently, fluorimetric methods based on carbon nanomaterials like carbon dots (C-dots), graphene quantum dots (GQDs) and organic nanorods have been developed for imaging live cell and determining metal ions like $Fe^{3+}$, $Cu^{2+}$, $Al^{3+}$, $Pb^{2+}$ and $Hg^{2+}$ (Anand, Sivaramana, Mahesh, & Chellappa, 2015; Feng et al., 2016; Gong et al., 2015; Liang, Zhang, Zhang, & Chen, 2015; Lu et al., 2015; Maniyazagan, et al., 2018; Ranee, Sivaraman, Pushpalatha, & Muthusubramanian, 2018; Vandarkuzhali et al., 2017; Zhang, Cui, Song, Liu, & Hu, 2016; Zhou, Sheng, Han, Zou, & Li, 2012; Zhou, Zhou, Gong, Zhang, & Li, 2015). Because of the strong electron-accepting ability, $Fe^{3+}$ could accept easily the electrons of photo-excited C-dots, and consequently quenched the photoluminescence (PL) of C-dots or GQDs (Feng et al., 2016; Gong et al., 2015; Lu et al., 2015; Zhang et al., 2016; Zhou et al., 2015). This process led to a turn-off photoluminescent sensor for the determination of $Fe^{3+}$ with C-dots. Some researchers found that the oxidation of $Fe^{2+}$ by $H_2O_2$ to $Fe^{3+}$ also induced a photoluminescence quenching of graphene oxide, and constructed a combinational logic gate to discriminate $Fe^{3+}$ and $Fe^{2+}$ (Mei et al., 2012) or to determine $H_2O_2$ (Song et al., 2014). After introducing reducing reagents like ascorbic acid and chelating agents such as dopamine or $F^-$, the quenched luminescence of C-dots by $Fe^{3+}$ could be effectively recovered (Mandal et al., 2016; Qu, Wang, Ren, & Qu, 2013; Tian et al., 2017). Based on this property, the systems of C-dots and $Fe^{3+}$ have been developed to determine ascorbic acid, dopamine, and $F^-$ ions (Mandal et al., 2016; Qu et al., 2013; Tian et al., 2017). However, Huang et al. found that the $Fe^{3+}$-quenched emission of GQDs could not be recovered with the addition of EDTA (Huang et al., 2013). This indicates that Fe(III)EDTA can also quench the emission of C-dots. Since EDTA possesses a much stronger complexing ability ($10^{25.1}$) for $Fe^{3+}$ ions than $F^-$ does ($10^{15.8}$), we anticipated that the addition of $F^-$ ions hardly influenced the quenched emission of C-dots by Fe(III)EDTA. Therefore, by combining photoluminescent C-dots and $F^-$ ions, the present work firstly developed a simple photoluminescent method to determine Fe(III)EDTA. This sensing system has been successfully used for the analysis of Fe(III)EDTA in iron fortified food.

## 2. Experimental section

### 2.1. Chemicals and materials

Phenylboronic acid (97%) was purchased from Energy Chemical (Shanghai, China). NaFeEDTA $3H_2O$ was purchased from aladdin reagents (shanghai) Co., Ltd. Acetic acid (HOAc), sodium acetate (NaOAc), and all the other chemical reagents were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All the chemical reagents were of analytical grade and used without further treatments. The dialysis bag (molecular weight cutoff = 1000) was purchased from Shanghai Green Bird Science & Technology Development Co. (Shanghai, China). Ultrapure water (18.2 MΩ cm) was used throughout.

### 2.2. Preparation and characterizations of C-dots

C-dots were synthesized with phenylboronic acid as carbon source according to the hydrothermal method with some modification (Shen et al., 2014). Briefly, 0.7 g of phenylboronic acid was dissolved in 70 mL of ultrapure water. After adjusting the solution pH to 9 by adding a 1.0 mol $L^{-1}$ NaOH solution, the mixture was purged with nitrogen gas for 1 h to remove dissolved $O_2$. Then, the reaction mixture was transferred to the Teflon-lined autoclave chamber and heat to 160 °C for 12 h. After being cooled down to room temperature, the C-dots containing solution was dialyzed in a dialysis bag for 24 h (molecular weight cutoff = 3500). The purified C-dots were kept at 4 °C for further use. The characterization and results were shown in Section S1, S2 and Fig. S1 of Supplementary Materials.

### 2.3. Determination of Fe(III)EDTA

Unless specified otherwise, the basic reaction conditions for the determination of Fe(III)EDTA were as follows: 0.5 mL of C-dots stock dispersion (200 mg $L^{-1}$), and 0.4 mL HOAc-NaOAc buffer were rapidly mixed, followed by the addition of a given volume of Fe(III)EDTA standard solution and $F^-$ ions. Then, the mixture solution was diluted to 2 mL with water. After 5 min, the emission spectra were recorded under excitation at 290 nm. Both the excitation and emission slit widths were set at 5 nm.

### 2.4. Analysis of real samples

Two types of iron-fortified samples were analyzed including the artificial iron-fortified fish sauce and the iron supplement granule. The nonfortified fish sauce (Feng Qiu, Yi Food Co., Ltd. China) was purchased from a local market, and was used to be fortified with NaFeEDTA as the similar method in the previous literature (Thuy et al., 2003). A concentration of 4.5 mmol $L^{-1}$ Fe as NaFeEDTA in the fish sauce was achieved by mixing 0.1895 g NaFeEDTA·$3H_2O$ with 100 mL fish sauce for 2 h before bottling. Prior to analysis, 4 mL fish sauce was mixed with 4 mL methanol, which helps the denaturation of proteins. The resulting mixture was shaken for 12 h at 140 rpm and 303 K in a shaking water bath. After removing protein aggregates by centrifugation at 14000 rpm for 10 min and filtration with a 0.22 μm membrane filter, the filters are diluted to 10 times with water. Then, 1 mL dilution solution was passed through a hydrophobic solid phase extraction cartridge (Bond-Elut C18 column, 500 mg, Agilent) for a further purification. The organic matrix compounds retained on the cartridge, while most fraction of Fe(III)EDTA species was eluted. The cartridge was then rinsed with 3.5 mL water, because water can elute Fe(III)EDTA, but not elute organic matrixes. When applying 1 mL of 100 μmol $L^{-1}$ Fe(III)EDTA standard solution to the cartridge, it was found that almost all of the Fe(III)EDTA was eluted by 3 mL water. Therefore, 3.5 mL water should be enough to elute Fe(III)EDTA in the fish sauce. Finally, the collected eluate was diluted to 5 mL with water, followed by measurements. If needed, the samples were spiked with Fe(III)EDTA stock solution before pretreatment. The measurement processes of Fe(III)EDTA were the same as those in the section of 2.3. To further reduce the possible matrix effect of fish sauce, the fluorescence intensity of C-dots in the presence of pretreated fish sauce without Fe(III)EDTA was taken as the initial value of $I_0$.

The iron supplement granule was purchased from Shanghai Gold-Banded Cudgel Healthcare Product Co. Ltd, and it contained 1.5 mg $g^{-1}$ Fe from NaFeEDTA. To analyze the iron supplement granule, 0.5 g sample was dissolved in 50 mL water under stirring. If needed, the samples were spiked with Fe(III)EDTA during the dissolving process. The resulting mixture was centrifuged at 14000 rpm for 10 min and filtered through 0.22 μm membrane filters. The collected filtrates were directly measured by the proposed fluorescence method, because the ingredients in the iron supplement granule including sucrose, starch,