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Original Paper

Establishment of heterodinuclear replacement complexation and its application to direct determination of iron in natural water with dibromo-*o*-nitrophenylfluorone

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Abstract. The chromophore dibromo-*o*-nitrophenylfluorone (DBNPF) was used to complex Fe(III) and Cu(II) at pH 5.88. Fe(III) can competitively replace Cu(II) from its dinuclear complex Cu(DBNPF)Cu, and forms the Cu(DBNPF)Fe heterodinuclear complex. The Fe-DBNPF and Cu-DBNPF complexes were also characterized by the spectral correction technique. The heterodinuclear replacement complexation (HRC) is proposed and first used for the quantitative detection of iron in trace level with high sensitivity and good selectivity by the light-absorption ratio variation approach. The limit of detection of Fe is 1.0 $\mu g\,L^{-1}$. The method has been successfully applied to the direct determination of Fe(II, III) dissolved and bound to suspended substances in natural water.

Key words: Determination of Fe; dibromo-*o*-nitrophenylfluorone; heterodinuclear replacement complexation; light-absorption ratio variation approach.

Iron is a ubiquitous but essential metal on the Earth. As a vital element in life, iron is indispensable for almost all living things, participating in a wide variety of metabolic processes, including oxygen transport, electron transport, and DNA synthesis [1]. For human beings, the gastrointestinal absorption of certain trace elements is strongly influenced by iron status [2]. The disorders of iron metabolism are among the most common diseases, encompassing a broad spectrum of diseases ranging from anemia to iron overload and, possibly, to neurodegenerative conditions [3]. In aquatic environments, iron is one of the most reactive metal ions. In general, iron concentration in natural water ranges 0.5–14 $nmol\,L^{-1}$ [4–6]. It usually exists as two valence states: Fe(II) and Fe(III). Fe(II) has a very short half-life in natural waters as ambient pH and this most of the dissolved Fe in toxic waters is in the form of Fe(III). These valence states are involved in the formation of soluble inorganic and organic complexes, colloids and particulate phases [7], which may absorb other trace metals [8]. Hence, it could be expected that iron controls the bioavailability, toxicity and mobility of other trace metals in bodies [9].

Today, with increasing concern about the environmental impact of metals on the water bodies and human health, a need for the development of sensitive and selective analytical techniques to predict the fate and mobility of iron occurred. Various well-established methods for the quantitative determination of iron have been proposed, such as AAS, ICP-OES, ICP-MS, ion chromatography, spectrofluorimetry, capillary electrophoresis, chemiluminescence, and stripping

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voltammetry. Among the most widely applied methods are those based on UV-vis spectrophotometry because they are remarkable for their versatility, simple operation, and low instrumentation cost.

For analysis of heavy metal via spectrophotometry, the complexation of metal-chromophore is the most ordinary and effective method. Although syntheses of numerous dinuclear complex have been reported recently [10, 11], few of them are applied in trace analysis. The chromophore, dibromo-*o*-nitrophenylfluorone (DBNPF) is a colorant with a high molar absorptivity, which was used earlier for the determination of Ti [12]. In the present work, DBNPF is used to complex with Cu(II) to form a dinuclear complex Cu(DBNPF)Cu in the presence of plenty of Cu(II). However, Fe(III) can replace one Cu of this complex to form a heterodinuclear complex Cu(DBNPF)Fe. The proposed heterodinuclear replacement complexation (HRC) for the determination of iron is highly sensitive with good selectivity. Fe(II, III) dissolved in water and binding to suspended substances (SS) have been determined and analyzed, which respectively collected from Yangtze River, Huangpu River and Taihu Lake of China.

Principles and calculations

Heterodinuclear replacement complexation (HRC)

The metal (M)–chromophore (L) complexation is usually applied to analyze metal at trace level. In consideration of the poor selectivity of L itself, the heterodinuclear replacement complexation (HRC) is established to enhance the selectivity of M–L reaction. A multi-dentate L can often coordinate two or more M ions (*e.g.* M^1) when excess M^1 is added into L. Thus, a dinuclear complex $\mathrm{M}^1\mathrm{LM}^1$ may be formed under such circumstances. After the addition of M^2, another metal possessing higher complexation stability with L, one M^1 in the dinuclear complex would be replaced by M^2 to form a heterodinuclear complex $\mathrm{M}^1\mathrm{LM}^2$ because of the different connection stability of the coordination groups. The process of HRC can be simply expressed as follows:

$$\mathrm{M}^1\ (\text{excess}) + \mathrm{L} \rightarrow \mathrm{M}^1\mathrm{LM}^1$$

$$\mathrm{M}^2 + \mathrm{M}^1\mathrm{LM}^1 \rightarrow \mathrm{M}^1\mathrm{LM}^2 + \mathrm{M}^1$$

The HRC is the first time to be proposed and applied to the detection of M^2 where the $\mathrm{M}^1\mathrm{LM}^1$ complex is regarded as a new chromophore. Since M^1 is present in a high enough amount, it will prevent the interference from other co-existing ions in the solution. Therefore, the HRC is able to improve the selectivity remarkably.

Light-absorption ratio variation approach (LARVA)

LARVA is a newly proposed method and has been successfully used to determine various kinds of metals with high sensitivity [13–15]. The primary principle of the LARVA can be described by following relations:

$$\Delta A_r^{-1} = p' C_{\mathrm{M0}}^{-1} + q' \tag{1}$$

or

$$\Delta A_r = pC'_{\mathrm{M0}} + q \quad (\text{only when } C'_{\mathrm{M0}} \ll C_{\mathrm{M0}}) \tag{2}$$

where

$$\Delta A_r = A_r - A_{r0} = \frac{A_{\lambda 2}}{A_{\lambda 1}} - \frac{A^{\mathrm{L}}_{\lambda 2}}{A^{\mathrm{L}}_{\lambda 1}} \tag{3}$$

where $A^{\mathrm{L}}_{\lambda 1}$ and $A^{\mathrm{L}}_{\lambda 2}$, $A_{\lambda 1}$ and $A_{\lambda 2}$ are the absorbances of the $\mathrm{M}^1\mathrm{LM}^1$ solution and the $\mathrm{M}^2 - \mathrm{M}^1\mathrm{LM}^1$ reaction solution, measured at wavelengths λ_1 and λ_2 against water reference. Both C_{M0} and C'_{M0} are the initial concentrations of M^2. The symbols p', q', p and q are constants and they can be regressed and calculated from calibration plots ΔA_r^{-1} *vs* C_{M0}^{-1} and ΔA_r *vs* C'_{M0}, respectively. By measuring ΔA_r of an $\mathrm{M}^2 - \mathrm{M}^1\mathrm{LM}^1$ reaction solution, Eq. (1) or (2) can be used to calculate the amounts of M^2 in a sample.

Spectral correction technique

The spectral correction technique can effectively eliminate the interference from excess L existing in the reaction solution, and therefore is applied to the accurate characterization of metal complexes. Its principle can be described by following Eq. [16]:

$$A_c = \frac{A_{\lambda 2} - \beta A_{\lambda 1}}{1 - \alpha\beta} \tag{4}$$

where

$$\alpha = \frac{A^{\mathrm{ML}}_{\lambda 1}}{A^{\mathrm{ML}}_{\lambda 2}} \tag{5}$$

and

$$\beta = \frac{A^{\mathrm{L}}_{\lambda 2}}{\mathrm{A}^{\mathrm{L}}_{\lambda 1}} \tag{6}$$

and

$$\gamma = \eta \times \frac{C_{L0}}{C_{M0}} \tag{7}$$

where

$$\eta = \frac{A_c - A_{\lambda 2}}{A_{\lambda 2}^{L}} + 1 \tag{8}$$

Both $A_{\lambda 1}^{ML}$ and $A_{\lambda 2}^{ML}$ are the absorbances of the ML complex only solution, measured at wavelengths λ_1 and λ_2 against water reference. C_{L0} is the initial molar concentration. A_c is the real absorbance of ML complex at λ_2. Both α and β are the correction constants. η is the effective fraction of L and γ the complexation number of L with M. According to the above equations, α, β, A_c, η, γ can be calculated to characterize the Fe-DBNPF and Cu-DBNPF complexes.

Experimental

Apparatus

The absorption spectra of the DBNPF and its metal complexes were recorded on a Model Lambda-25 spectrometer (Perkin-Elmer Instruments, USA) with UV WinLab software (Version 2.85.04). A Model pHS-25 acidity meter (Shanghai Rex Precise Science Instruments, Shanghai, China) was used for pH measurement. A Model SK-3300H ultrasonic cleaner (Shanghai Kedao Ultrasonic Cleaning Instruments, Shanghai, China) was used to dissolve the SS samples in acidic medium. A Model RO DI Water Ultra Purification System (Hi-tech Instruments, Shanghai, China) was used to produce the deionized water.

Reagents

Standard stock solution of 100 mg L^{-1} 120 Fe(II) was prepared by dissolving 0.7020 g of ammonium iron(II) (A. R., $NH_4)_2Fe(SO_4)$ $2.6H_2O$, Shanghai Chemical Reagents, China) in 50 mL of concentrated sulfuric acid. The solution was then diluted with deionized water to 1000 mL.

Solution of 1.00 mg L^{-1} Fe(II) was prepared by diluting the standard stock solution. Two standard stock solutions, respectively containing 1000 mg L^{-1} Cu(II) (GSB07-1257-2000) and 1000 mg L^{-1} Fe(III) (GSB07-1264-2000) were purchased from the Institute for Reference Materials of SEPA, Beijing, China. Solutions of 200 mg L^{-1} Cu(II), 200 and 1.00 mg L^{-1} Fe(III) were prepared by diluting the above stock solutions. DBNPF was purchased from Shanghai Changke Reagents Institute. A 0.500 mmol L^{-1} DBNPF was prepared by dissolving 65.4 mg of DBNPF completely in 250 mL anhydrous ethyl alcohol. A 0.0500 mmol L^{-1} DBNPF was prepared by dissolving 25 mL of 0.500 mmol L^{-1} DBNPF in 225 mL anhydrous ethyl alcohol. A 0.0500 mmol L^{-1} Cu(II)-DBNPF complex solution was prepared by mixing 25 mL of 0.500 mmol L^{-1} DBNPF with 40 mL of 200 mg L^{-1} Cu(II) and 25 mL of pH 5.88 acetate buffer solution and then diluting to 250 mL with anhydrous ethyl alcohol. All above solutions containing DBNPF were stored at less than 5 °C. A series of buffer solutions from pH 4.37 to 7.11 were prepared with the recrystallized sodium acetate (A. R., Shanghai Chemical Reagents, China) and acetic acid (A. R., Shanghai Chemical Reagents, China). Their accurate pHs were measured with a pH meter (Shanghai Rex Co-Perfect Instruments).

Procedures

Characterization of Fe-DBNPF and Cu-DBNPF complexes

The characterization of DBNPF complex is mainly composed of three steps. First of all, into a series of 10 mL calibrated flasks were added different amounts of Fe(III) from 0 to 50.0 µg. Afterwards, 1 mL of pH 5.88 buffer solution and 1.00 mL of 0.0500 mmol L^{-1} DBNPF were added, respectively. The solutions were diluted to 10 mL with demonized water and mixed well. The absorbances ($A_{533\,nm}$ and $A_{639\,nm}$) of these solutions were measured at 533 and 639 nm against deionized water and the ratio $A_{533\,nm}/A_{639\,nm}$ of each solution was calculated. α is calculated from Eq. (5) and the spectrum of complex. Secondly, into a series of 10 mL calibrated flasks were added 1.00 mL of pH 5.88 buffer solution and 0.0500 mmol L^{-1} DBNPF from 0.0500 to 5.00 mL. The solutions were then diluted to 10 mL with deionized water and mixed thoroughly. The absorbances ($A^0_{533\,nm}$ and $A^0_{639\,nm}$) of these solutions were measured at 533 and 639 nm against water and β was calculated from Eq. (6). Then, 1.00 µg of Fe(III) was added into the solutions above. After mixing, the absorbances ($A_{533\,nm}$ and $A_{639\,nm}$) of these solutions were measured at 533 and 639 nm against deionized water. A_c, η, and γ were calculated according to Eqs. (4), (8) and (7). Thus, the Fe(III)-DBNPF complex was characterized. According to the similar procedures above, the Cu(II)-DBNPF solutions were prepared and measured at 530 and 568 nm. Thus, the Cu(II)-DBNPF complex was characterized, too.

Pretreatment of water samples

Water samples were collected in clean bottles and each of them was split into three portions. (1) 100 mL of water was filtered and the filtrate was collected for the direct determination of dissolved iron. (2) Concentrated hydrochloric acid was dropped into another 100 mL of water to adjust pH at about 1.0 and then it was kept in an ultrasonic cleaner for 30 min. After standing the acidified sample overnight to ensure inorganic iron mainly as Fe oxides binding in SS to be dissolved, it was filtered and the filtrate was neutralized with 10.0 mol L^{-1} sodium hydroxide. (3) Another 100 mL of sample is pretreated according to (2) but it was boiled until only 10 mL to ensure all of inorganic and organic iron binding in SS including the organic complexes to be composed into iron ions. After filtration, the filtrate was neutralized with 10.0 mol L^{-1} sodium hydroxide and diluted to 100 mL with deionized water. These three solutions from a sample were colored and then measured according to the following procedures.

Determination of iron in natural water

A known volume of pretreated sample solution was added into a 10 mL calibrated flask. A 1.00 mL of pH 5.88 buffer solution and 0.500 mL of 0.0500 mmol L^{-1} Cu(II)-DBNPF complex solution were added. After diluting to 10 mL with deionized water and mixing thoroughly, the flask was kept in a constant temperature water bath at 40 °C for 40 min. Then, it was cooled down to room temperature. The absorbances ($A_{660\,nm}$ and $A_{560\,nm}$) of the solution were measured at 660 and 560 nm against water reference. Meanwhile, a corresponding reagent blank without Fe(II, III) was prepared and its absorbances ($A^0_{660\,nm}$ and $A^0_{560\,nm}$) were measured. Thus, ΔA_{r1},

ΔA_{r2} and ΔA_{r3} of the sample solutions pretreated according to procedures (1)–(3) were calculated by the equation:

$$\Delta A_r = \frac{A_{660\,\mathrm{nm}}}{A_{560\,\mathrm{nm}}} - \frac{A^0_{660\,\mathrm{nm}}}{A^0_{560\,\mathrm{nm}}} \qquad (9)$$

From plots ΔA_{r1} vs C_{Fe} the Fe dissolved in water and adsorbed on suspended substances (SS) can be obtained, respectively from ΔA_{r1} and $\Delta A_{r3} - \Delta A_{r1}$. The inorganic Fe absorbed on SS was calculated from $\Delta A_{r2} - \Delta A_{r1}$ and the organic Fe complexing with SS from $\Delta A_{r3} - \Delta A_{r2}$.

Results and discussion

Dependence of pH

To optimize the pH of complexation, the effect of pH on the absorption spectra of the Fe(III)-DBNPF and Cu(II)-DBNPF solutions were analyzed by varying the pH of corresponding solutions, as shown in Fig. 1. From Fig. 1(A), curve 4 shows a maximum peak-valley interval among all 7 curves. It indicates that the complexation between Fe(III) and DBNPF is the most sensitive at pH 5.88. As shown in Fig. 1(B), the sensitivity of the complexation between Cu(II) and DBNPF is always sensitive at pH between 5.88 and 7.11. Thus, pH 5.88 buffer solution was chosen for subsequent experiment. From curve 4 in Fig. 1(A), the peak is located at 639 nm and the valley at 533 nm. From curve 4 in Fig. 1(B), the peak is located at 568 nm and the valley at 530 nm. Such four wavelengths were used respectively for the characterization of the Fe(III)-DBNPF and Cu(II)-DBNPF complexes.

Composition of Fe-DBNPF and Cu-DBNPF complexes

From curve 2 in Fig. 2, the ratio, $A_{530\,\mathrm{nm}}/A_{568\,\mathrm{nm}}$ of the Cu(II)-DBNPF solution decreases rapidly when the molarity of Cu(II) is less than that of DBNPF. The ratio value approaches a minimum at 0.653 when Cu(II)/DBNPF ratio is at 0.6:1, where DBNPF was reacted completely with Cu(II). Therefore, $\alpha = 0.653$ and it will be used in characterization of the Cu-DBNPF complex. However, the ratio increases up to over 1 with increase of Cu(II). Such a phenomenon indicated that DBNPF was binding more Cu(II) to form a dinuclear complex. In fact, DBNPF has the left and right multi-dentate groups and the Cu(DBNPF)Cu binuclear complex will be formed in the presence of plenty of Cu(II). Similarly from curve 1, the ratio $A_{533\,\mathrm{nm}}/A_{639\,\mathrm{nm}}$ of the Fe(III)-DBNPF complex decreases down to a minimum at 0.778 with increase of Fe(III). Thus, α of the Fe-DBNPF complex is 0.778 and it will be used in characterization of the Fe-DBNPF complex. Then, it increase slightly up to over 1 when the molarity of Fe(III) is more than that of DBNPF. Thus, the Fe(DBNPF)Fe binuclear complex will be formed. In addition, β was obtained to be 0.0275 for $A_{639\,\mathrm{nm}}/A_{533\,\mathrm{nm}}$ from Eq. (6).

After obtaining α and β, two series solutions containing various concentrations of DBNPF with a constant molarity of metal ions were measured. A_c, η and γ were calculated from Eqs. (4), (7) and (8) and changes of both η and γ are shown in Fig. 3. From curve 1, η of DBNPF drops down to less than 40% when DBNPF is more than 0.0125 mmol L^{-1}. Thus, over 60% DBNPF has not reacted with Fe(III). Curve 2 indicates that over 80% DBNPF has not reacted with Cu(II). So excess DBNPF will affect seriously the accurate measurement of light-absorption of the complex product. So, an ordinary spectrophotometry becomes helpless. Curves 3 and 4 show the variations of γ of the Fe(III)-DBNPF and Cu(II)-DBNPF com-

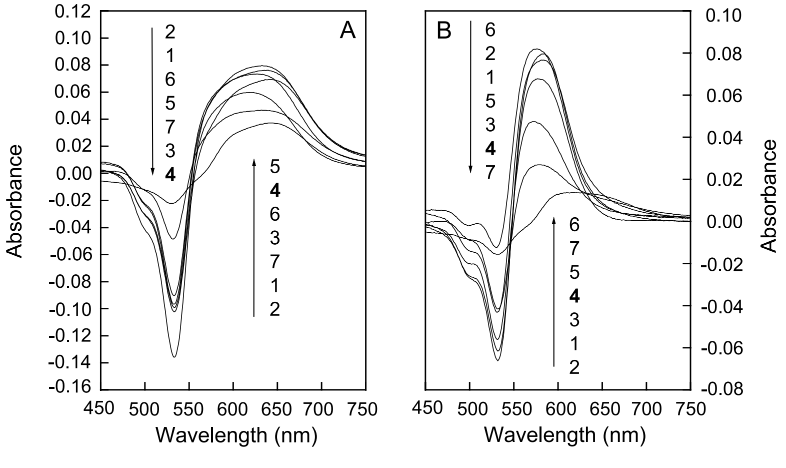

Fig. 1. Effect of pH on the absorption spectra of Fe(II)-DBNPF and Cu(II)-DBNPF complex: (A) solutions that contained 0.00500 mmol L^{-1} DBNPF and 0.100 µg mL^{-1} Fe(II) against a reference without Fe(II); (B) solutions that contained 0.00500 mmol L^{-1} DBNPF and 0.100 µg mL^{-1} Cu(II) against a reference without Cu(II). *From curve 1 to 7*: pH 4.37, 4.88, 5.43, 5.88, 6.22, 6.67, and 7.11

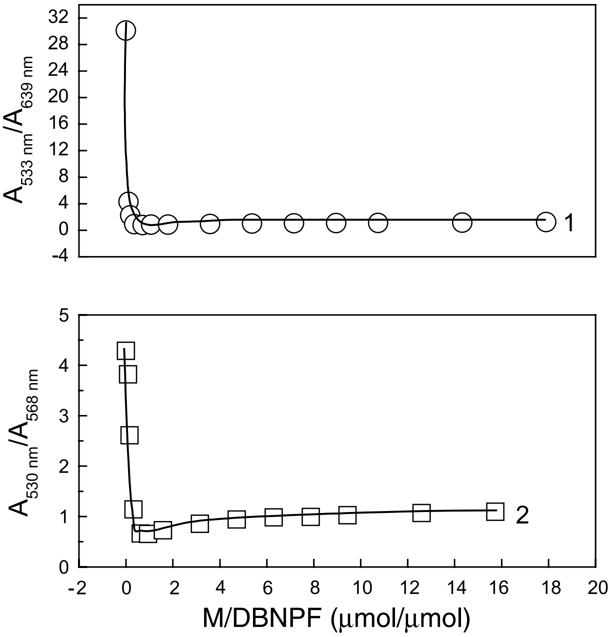

Fig. 2. Variation of the absorbance ratio with molarity of metal ions (*M*) in corresponding solutions containing constant molarity of DBNPF at pH 5.88: (*1*) $A_{533\,\mathrm{nm}}/A_{639\,\mathrm{nm}}$ of the solution containing $0.00500\,\mathrm{mmol\,L^{-1}}$ DBNPF and Fe(III) from 0 to $5.00\,\mu\mathrm{g\,mL^{-1}}$; (*2*) the variation of $A_{530\,\mathrm{nm}}/A_{568\,\mathrm{nm}}$ of the solution containing $0.00500\,\mathrm{mmol\,L^{-1}}$ DBNPF and Cu(II) from 0 to $5.00\,\mu\mathrm{g\,mL^{-1}}$

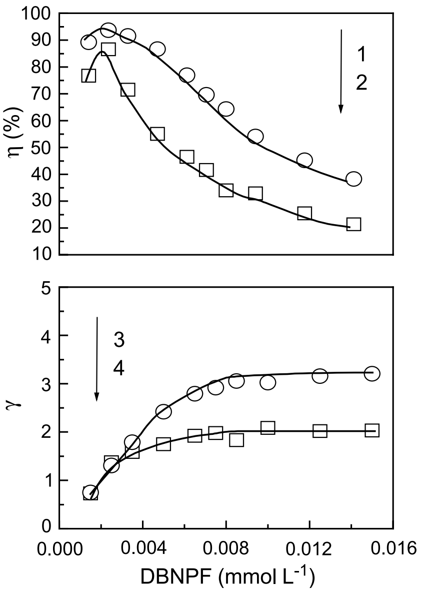

Fig. 3. Variation of η and γ with DBNPF concentration at pH 5.88: (*1 and 2*) η of the Fe(III)-DBNPF solution containing $0.100\,\mu\mathrm{g\,mL^{-1}}$ Fe(III) and the Cu(II)-DBNPF solution containing $0.100\,\mu\mathrm{g\,mL^{-1}}$ Cu(II), respectively; (*3 and 4*) γ of the solutions above

plexes, respectively. Both γ increase with the increase of DBNPF and then approach to a maximum at 3 in the Fe(III)-DBNPF solution and 2 in the Cu(II)-DBNPF solution. Therefore, the final complexes $\mathrm{Fe(DBNPF)_3}$ and $\mathrm{Cu(DBNPF)_2}$ were formed at pH 5.88 in the presence of excess DBNPF.

HRC

The influence of other nine types of metal ions on the absorption spectrum of the Cu(II)-DBNPF solution were investigated in presence of plenty of Cu(II). As shown in Fig. 4(A), only Fe(II) and Fe(III) caused obvious changes of the absorption spectrum of the Cu(II)-DBNPF complex. The other metals, such as Ca(II), Mg(II), Al(III) and heavy metals Zn(II), Ni(II), Mn(II) and Pb(II) hardly affect the absorption spectrum of the Cu(II)-DBNPF complex. Therefore, only Fe(II, III) could replace Cu of the Cu-DBNPF complex. This is different from the earlier work [16] on the simultaneous determination of Fe and Zn where only DBNPF not Cu-DBNPF complex is as the indicator of Fe and Zn. By comparing curves 3 and 4 with curve 2 in Fig. 4(B), the absorption spectrum of the Cu-DBNPF complex generates a red shift of 11 nm from 543 nm of peak of the complex to 554 nm and its shape also changes. However, Fe(II) would not be expected to replace Cu(II) according to Irving-Williams rules, Fe(II) could be oxidized into Fe(III) [17, 18] the presences of both DBNPF and dissolved oxygen as potential oxidants. Thus, only Fe(III) can replace Cu(II) in the Cu-DBNPF complex. Curves 3 and 4 in Fig. 4(B) are coincident with neither curve 2 nor curves 5 and 6. Therefore, Fe(III) could replace only one Cu(II) of the Cu(DBNPF)Cu complex to form a heterodinuclear complex. In fact, the binding of the left group of FBNPF with Cu(II) should be stronger because the coordination ability of C=O is weaker that of –OH. Therefore, the right Cu(II) of the Cu(DBNPF)Cu complex could be replaced by Fe(III) to form the Cu(DBNPF)Fe complex. The process of both complexation of Cu with DBNPF and replacement of Cu by Fe(III) is sketched in Fig. 5. In this work, the HRC is the first to be applied in trace analysis and complex Cu(DBNPF)Cu was regarded as a new chromophore to determine trace amounts of Fe(II, III).

Effect of time and temperature on HRC

From variation of ΔA_r of the solutions containing the Cu(II)-DBNPF complex and Fe(II) and Fe(III) with the reaction time at room temperature and 40 °C

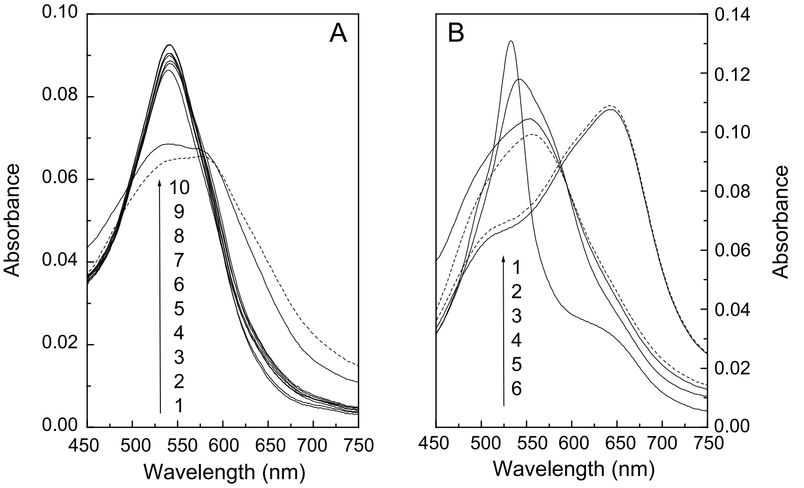

Fig. 4. (A) Effect of metal ions with the same molarity on the spectra of the Cu(II)-DBNPF complex at pH 5.88: (*5*) the solution containing 0.0500 mmol L^{-1} Cu(II), 0.00500 mmol L^{-1} DBNPF; *from 1 to 10 (except for 5)* the solutions containing the same components as 5 but with additional 0.0500 mmol L^{-1} Fe(II), Fe(III), Ni(II), Zn(II), Mg(II), Mn(II), Ca(II), Pb(II) and Al(III), respectively. (B) The absorption spectra variation of DBNPF complexes: (*1*) the solution containing 0.00500 mmol L^{-1} DBNPF complex; (*2*) the solution containing 0.00500 mmol L^{-1} DBNPF complex with additional 0.0500 mmol L^{-1} Cu(II); (*3 and 4*) the solutions containing 0.00500 mmol L^{-1} Cu(II)-DBNPF complex with additional 0.172 mmol L^{-1} Fe(III) and 0.172 mmol L^{-1} Fe(II), respectively; (*5 and 6*) the solutions containing 0.00500 mmol L^{-1} DBNPF complex with additional 0.172 mmol L^{-1} Fe(III) and 0.172 mmol L^{-1} Fe(II), respectively

as shown in Fig. 6. ΔA_r of the two solutions could not reach a final equilibrium within 70 min at room temperature. In addition, ΔA_r of the Cu(II)-DBNPF-Fe(II) solution is only about 50% that of the Cu(II)-DBNPF-Fe(III) solution. Such an obvious difference will bring difficulty for the joint determination of Fe(II, III). On the contrary, both ΔA_r of the Cu(II)-DBNPF-Fe(II) and Cu(II)-DBNPF-Fe(III) solutions remains synchronous and coincident after 40 min at 40 °C. Under such conditions, Fe(II) could be oxidized into Fe(III) completely in the presence of DBNPF. Thus, the joint detection of Fe(II) and Fe(III) becomes easy and simple using the HRC for 40 min at 40 °C.

Effect of Cu-DBNPF complex on ΔA_r

Figure 7 gives the effect of the Cu(II)-DBNPF complex on ΔA_r of the HRC. From curve 1 with a constant ratio of Fe(II) to Cu(II)-DBNPF complex at 10:1 μg μmol^{-1}, ΔA_r remains constant when the Cu(II)-DBNPF complex is more than 0.00150 mmol L^{-1}. From curve 2, the sensitivity factor, $\Delta A_r/C_{\mathrm{Fe(II)}}$ decreases with increase of the Cu(II)-DBNPF complex. Thus, the detection of Fe will become more sensitive when less Cu(II)-DBNPF complex is added. Nevertheless, too low concentration of Cu(II)-DBNPF complex will result in a low absorbance, and the measurement will be affected inevitably by the instrumental noise. Consequently, choosing an optimal addition of the Cu(II)-DBNPF complex solution should consider not only the sensitivity but also the precision of the reagent blanks test.

Calibration graphs and LOD of Fe

Three series of solutions containing standard Fe(II) between 0 and 30.0, 0 and 50.0, and 0 and 100.0 μg L^{-1} and 0.00150, 0.00250, and 0.00500 mmol L^{-1} Cu(II)-DBNPF complex were prepared, respectively. Simultaneously, 10 replicated reagent blanks of each of the Cu(II)-DBNPF complex solutions without Fe were prepared and measured. The regression equations of plots ΔA_r^{-1} vs C_{Fe}^{-1} and the precisions (σ) of the reagent blank tests are given in Table 1. LOD of iron were calculated as shown in Table 1, too. Series 2 is the ideal one because of its satisfactory linearity and the lowest LOD. Hence, it was selected for subsequent analysis of water samples.

Effect of foreign ions

In order to investigate the effect of potential interfering foreign ions on the iron determination, fifteen kinds of the most common foreign ions present in natural water, namely, Ca(II), Mg(II), Zn(II), Al(III), Co(II), Mn(II), Ni(II), Pb(II), As(III), V(V), Mo(VI), Cr(III), Cd(II), sodium dodecyl benzene sulfonate

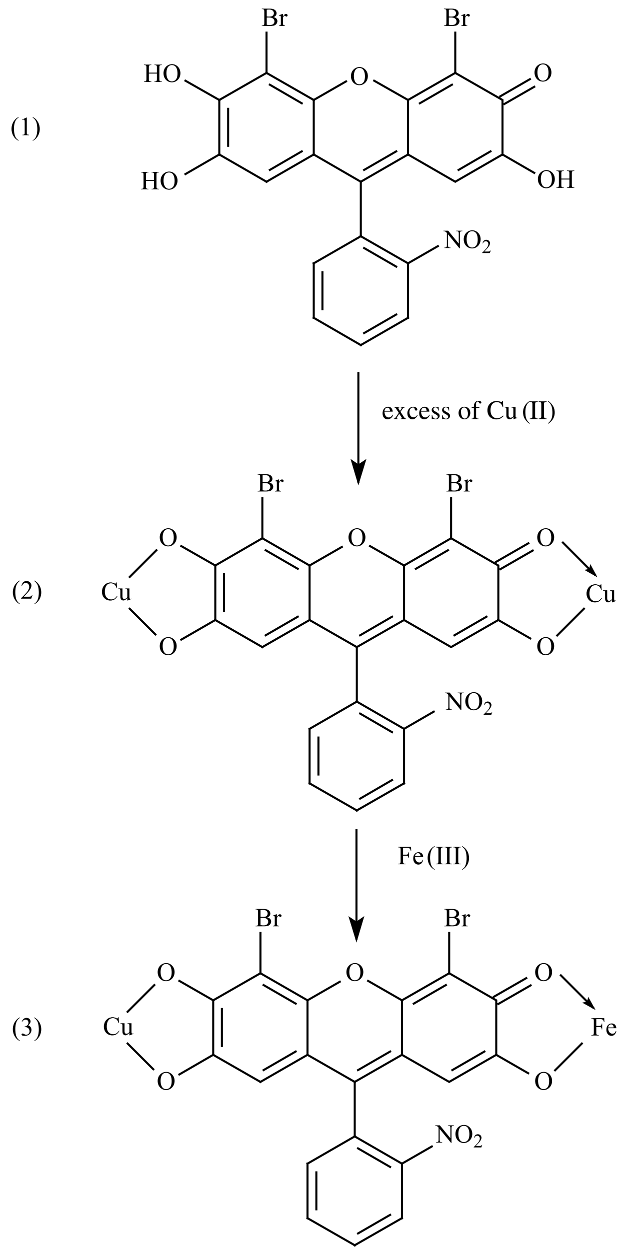

Fig. 5. Structures of DBNPF and its metal complexes: (*1*) DBNPF; (*2*) dinuclear complex Cu(DBNPF)Cu; (*3*) heterodinuclear complex Cu(DBNPF)Fe

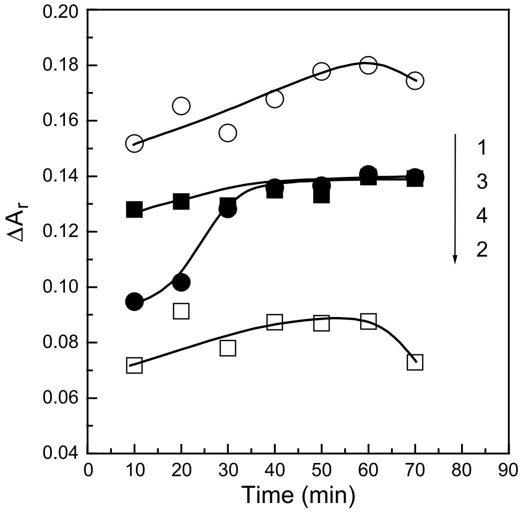

Fig. 6. Effect of time and temperature on ΔA_r of the solutions at pH 5.88. (*1 and 3*) 0.00250 mmol L^{-1} Cu(II)-DBNPF and 0.0200 µg mL^{-1} Fe(III); (*2 and 4*) 0.00250 mmol L^{-1} Cu(II)-DBNPF and 0.0200 µg mL^{-1} Fe(II). (*1 and 2*) At room temperature and (*3 and 4*) at 40 °C

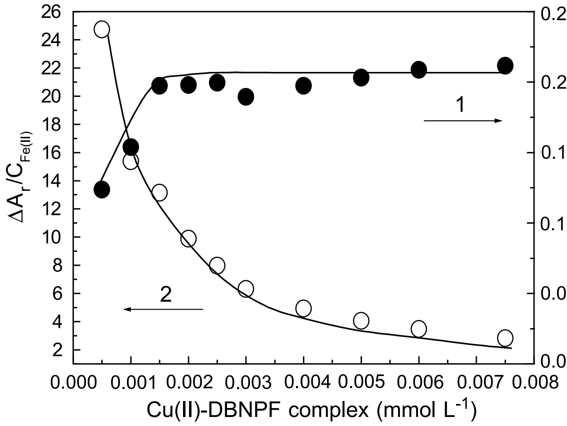

Fig. 7. Effect of the Cu(II)-DBNPF complex on ΔA_r at pH 5.88: (*1*) variation of ΔA_r of the solutions initially containing Cu(II)-DBNPF complex from 0.000500 to 0.00750 mmol L^{-1} and Fe(II) from 0.00500 to 0.0750 mg L^{-1}. (*2*) Variation of the sensitivity factor, $\Delta A_r/C_{\mathrm{Fe(II)}}$ of the solutions above

Table 1. Regression equations and LOD of iron

Series	Cu(II)-DBNPF (mmol L^{-1})	Fe(II), (µg L^{-1})	Calibration graph	R^a	σ^b	LOD^c, (µg L^{-1})
1	0.00150	0–30.0	$\Delta A_r^{-1} = 0.4328 C_{\mathrm{Fe}}{}^{-1} + 2.4020$	0.9959	0.00697	1.1
2	0.00250	0–50.0	$\Delta A_r^{-1} = 0.8215 C_{\mathrm{Fe}}{}^{-1} + 3.7174$	0.9965	0.00444	1.0
3	0.00500	0–100.0	$\Delta A_r^{-1} = 1.5778 C_{\mathrm{Fe}}{}^{-1} + 1.9495$	0.9973	0.00323	1.9

[a] Linear correlation coefficient.

[b] Standard deviation of 10 repetitive reagent blanks.

[c] $\mathrm{LOD} = 3 \times \sigma \times p'$ (p' = line slope of calibration graph).

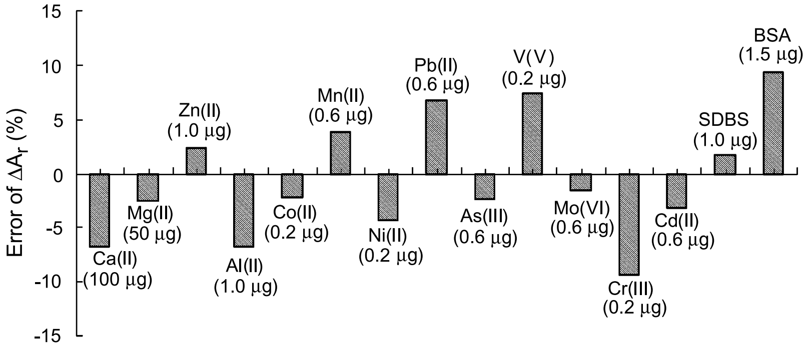

Fig. 8. Effect of foreign ions on ΔA_r in the solutions containing 0.200 μg of Fe(II)

(SDBS) and bovine serum albumin (BSA), were investigated respectively. Each of them was separately added into the solution containing 0.200 μg of Fe and 0.00250 mmol L^{-1} Cu(II)-DBNPF complex, and then ΔA_r of solution was measured according to the recommended procedures. The results are given in Fig. 8. None of the foreign ions and substances caused a serious interference (over 10%) in the determination of Fe even when their concentrations are many times higher than that of Fe, such as Ca(II), Mg(II), Zn(II), Mn(II), SDBS. This is attributed to the fact that the excess Cu(II) existing in the solution plays an important role in eliminating the interference from other ions. In order to investigate the effect of electrolytes, NaCl and KCl and were added into such a Fe(II) solution and 0.5 M of them would not result in over 10% of error. Additionally, some strongly complexing agents such as EDTA, DTPA, salicylic acid and diamninoethane were still added into such a reaction solution and 0.5 μg of them would make over 10% of error. Thus, this method is suitable for the direct analysis of natural waters without adding any mask reagent.

Analysis of natural waters

In the current work, three typical natural water samples were analyzed, which were individually sampled from Yangtze River, Huangpu River and Taihu Lake of China.

From Table 3, the recovery rates of iron are between 98.0 and 108.0% by adding the Fe(II) standard solution. So, the combined HRC-LARVA is accurate and credible for practical analysis. However, iron in natural waters is mainly bounded to SS in the chemical forms of unsolvable oxides or organic complexes. Such iron complexes cannot be determined directly. The acid treatment for a water sample is necessary before analysis of a sample. The concentrated hydrochloric acid was added into the Yangtze River samples to make pH of solutions at 0, 1, 2, and 3, respectively. Iron was determined after mixing for 12–20 h. The experiments indicated that pH 0 and 1 released the same highest amount of iron between 12 and 20 h. The result is consistent with other reports [19, 20] before equilibrating overnight. Different from other solid geological samples [21], SS in natural water is much easier to be dissolved in acidic medium due to so small and loose particles. From the results in Table 2, the dissolved Fe(II, III) in all three samples only occupies no more than 2.0% of the total iron and the majority of iron is bounded to SS. For the sample collected from Yangtze River, inorganic iron existing in SS is more than one time than iron organically complexed in SS. It may be attributed to the fact that SS in Yangtze River contains much unsolvable inorganic iron oxides due to soil erosion of so long upper reaches. For the sample from Huangpu River, iron bounded to SS is the highest among the three samples and iron organically complexing with SS is much higher than that existing in inorganic form. Since Huangpu River passes through the center of Shanghai city, it can be inferred that large amount of urban domestic sewage is discharged into it daily. Undoubtedly, the occupancy of organic matter in total SS would be higher than that of other two water bodies. As a result, the portion of iron organically complexing with SS of Huangpu River is higher. As for Taihu Lake, it is an almost enclosed water body where the content of SS is the lowest, so the total iron detected in Taihu Lake is the least. However, plenty of domestic sewage and industrial wastewater are also discharged into the lake frequently, so the organic iron complexing with SS occupies a higher portion in bounded iron of Taihu Lake than that of Yangtze River.

Table 2. Determination and phase analysis of iron in natural waters

Sample	Fe	Fe added ($\mu g\,L^{-1}$)	Fe found ($\mu g\,L^{-1}$)	Recovery (%)	Occupancy (%)
Yangtze River	dissolved	0	7.6 ± 0.7^a		1.9
		20.0^d	27.2 ± 1.4^a	98.0^c	
	bounded in SS	0	388 ± 1^a		98.1
	inorganic Fe in SS	0	237 ± 1^a		61.1^e
	inorganically complexed in SS	0	152^b		38.9^e
Huangpu River	dissolved	0	5.2 ± 0.2^a		0.5
		20.0^d	26.0 ± 0.8^a	103.8^c	
	bounded in SS	0	1204 ± 19^a		99.5
	inorganic Fe in SS	0	456 ± 29^a		37.9^e
	inorganically complexed in SS	0	748^b		62.1^e
Taihu Lake	dissolved	0	6.7 ± 0.3^a		2.0
		20.0^d	28.3 ± 2.1^a	108.0^c	
	bounded in SS	0	343 ± 8^a		98.0
	inorganic Fe in SS	0	167 ± 2^a		48.7^e
	inorganically complexed in SS	0	176^b		51.3^e

[a] Average of three replicated determinations.
[b] Defined as "Bounded"; minus "Bounded as inorganic oxide"; e.g. $152 = 388 - 237$.
[c] E.g. $98.0\% = (27.2 - 7.6)/20.0 \times 100\%$.
[d] With 10.0 μg of Fe(II) and 10.0 μg of Fe(III) mixed together.
[e] Occupancy in the bounded phase.

Table 3. Analytical performance of various spectrophotometric methods for determination of iron in natural water

Series	Chromophore or method	LOD, $\mu g\,L^{-1}$	Ref.
1	dibromo-*o*-nitrophenylfluorone (DBNPF)	1.0	Current method
2	1, 10-phenanthroline	60	[22]
3	2,4,6-tri(2′-pyridyl)-1,3,5-triazine (TPTZ)	50	[23]
4	2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP)	18	[24]
5	2-carboethoxy-1,3-indandione (CEIDNa)	10	[25]
6	3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT)	1.2	[26]
7	*N*-benzoyl-*N*-phenylhydroxylamine (BPHA)	0.05	[27]
8	*N,N*-dimethyl-*p*-phenylenediamine (DPD)	0.02	[21]
9	DBNPF	12	[16]
10	FerroZine Iron reagent	20	[28]
11	Long pathlength spectroscopy	0.01^a	[29]

[a] The most sensitive spectrophotometric method reported so far.

Conclusions

The establishment of HRC improves remarkably the reaction selectivity and no masking reagent is necessary in the determination of iron. The proposed HRC-LARVA method is simple and easy in operation and it has been directly applied to determine iron in natural waters with good sensitivity, selectivity and recovery. In order to compare this method with the other methods, Table 3 summarizes the analytical performance of various spectrophotometric methods for determination of iron. Some of them are more sensitive for iron determination [21, 27, 29] than the recommended one. Methods [21, 27] often complicated in operation due to catalytic procedures and method [29] needs a spectral liquid core waveguide (LCW). From report [16], DBNPF was early used for determination of Fe and Zn but LOD of Fe is over 10 times that of this method. In this work, not only the recommended method is very sensitive but also the information on iron phases and distribution using the proposed sample pretreatment procedure can be rather useful for the environmental assessment of iron in natural waters.

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