# Light-Absorption Ratio Variation Approach to Sensitive and Selective Determination of Iron with Trimethoxyphenylflurone, Cetylpyridinium, and Thioglycollic Acid

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The complexation between trimethoxyphenylflurone (TMPF) and Fe is highly sensitive at pH 11.80 in the presences of cetylpyridinium chloride (CPC) and thioglycolic acid (TGA), where TGA reduced TMPF into a reduced ligand (RTMPF) and Fe(III) into Fe(II). The complexations of RTMPF with CPC and Fe have been characterized by the break point approach and the spectral correction technique. The binuclear complex, $Fe_2(RTMPF)_{10}(CPC)_{20}$ was formed via coordination bond and ion-pair attraction. The Fe-TMPF-CPC complexation is selective in the presence of ethylenediamine tetraacetic acid (EDTA) and Al(III) so it was applied to the spectrophotometric determination of total Fe(II+III) by the light-absorption ratio variation approach (LARVA). Results indicated that $\Delta A_r$ of the Fe-RTMPF solution is linear at 568 and 641.5 nm at the range between 0 and 100 ng/mL Fe. The limit of detection ($3\sigma$) of Fe is only 2 ng/mL. This method was applied to analysis of several samples such as natural waters, cigarette ash, and urine with satisfactory results.

**Keywords:** Light-absorption ratio variation approach; Spectrophotometry; Trimethoxyphenylflurone; Binuclear complex; Determination of iron; Thioglycolic acid.

## INTRODUCTION

Iron is one of the most abundant elements in nature, widely presenting in a variety of rock and soil minerals. Iron can exist as inorganic species[1] of Fe(III) or Fe(II), be organical complexes,[2] exist as colloids[3] of oxides, oxyhydroxides, or mixed with organic material, and be suspended as both biotic and abiotic particles.[4] Iron is important in the biosphere, serving as an active center of a wide range of proteins such as oxidases, reductases, and dehydrases.[5] Iron is the most abundant transition metal present in higher mammals with 3-4 g of the element present in the normal human body. Oxygen transport proteins contain 70% iron; 0.7% is present in other intracellular protein and enzymes. The rest ~ 29% is stored. It plays an essential role in phytosynthesis.[6-7] Microbial processes result in the reduction of Fe(III).[8] Siderophores and some humic and fulvic acid are major ligands for iron(III) in surface and ground water.[9] The observed concentrations of the total dissolved iron in natural water systems vary from 0.2 nmol/l in mid-ocean surface water[10] up to 400 μmol/l in polluted urban cloud.[11] It is well known that iron is a necessary additive in foods and medicines, *e.g.* wine,[12] drinks, milk powder, health products, and multi-vitamins. Human activities have resulted in a series of environmental problems, *e.g.* water acidification, waste discharge, dissolution and digestion of solid substances by acidic rain, soil extract and surface runoff and earth-surface infiltration, so that a large amount of Fe has been released into natural water. Iron has been studied with many techniques[13] such as MS, ICP-AES, stripping voltammetry, flame AAS, flow injection analysis, spectrophotometry, chromatography,[14] colorimetry, and chemillumescence. The MS, GF-AAS and ICP-AES equipment are more expensive. Spectrophotometry has advantages such as low cost, simple operation, easy spread, and wide applications. Up to now, it is still being studied extensively, particularly in developing countries.

It is well known that spectrophotometry has some obvious shortcomings such as in aspects of on-line and real-time analysis, automaticity, miniaturization, and multi-components detection. Nevertheless, more and more ways are

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still being developed to improve sensitivity and selectivity such as H-point standard addition method,[15] synthesis of novel chromophores, and coupling flow injection analysis.[16] It is still very important to establish simple, sensitive, and selective ways for the determination of dissolved Fe. The light-absorption ratio variation approach (LARVA) was established as a novel sensitive method.[17] The appearance of high light-absorption chromophores and low noise spectrophotometry supply LARVA with excellent hardware bases. It can improve significantly the analytical sensitivity. A phenylfluorone derivative, trimethoxyphenylflurone (TMPF), was synthesized and applied to the sensitive determination of Ge.[18] In the present study, a novel method is proposed for the determination of dissolved Fe. The complexation between Fe(III) and TMPF at pH 11.80 is highly sensitive in the presence of cetylpyridinium chloride (CPC) and thioglycolic acid (TGA) and highly selective in the presence of ethylenediamine tetraacetic acid disodium (EDTA) and great amounts of Al(III). The LARVA has been applied to spectrophotometric determination of dissolved Fe in various samples such as natural water, plants, medicine, and body liquids. The application is at the linear range from 0 to 100 ng/mL Fe and the detection limit only 2 ng/mL. In addition, the complexations of the reduced TMPF with CPC and Fe were characterized by the break point approach[19] and the spectral correction technique.[20]

## PRINCIPLE AND CALCULATION

### Spectral Correction Technique[20]

A metal (M) - ligand (L) complexation is often used in analysis of trace M. The reaction equilibrium is expressed as follows:

| | $NL$ | + | $M$ | $\rightleftharpoons$ | $ML_N$ |
|---|---|---|---|---|---|
| Initial state Corresponding to | $C_{Lo}$ $A^L{}_{\lambda 1}$ *and* $A^L{}_{\lambda 2}$ | | $C_{Mo}$ | | $0$ |
| Equilibrium Corresponding to | $C_L$ $(1-\eta)A^L{}_{\lambda 1}$ *and* $(1-\eta)A^L{}_{\lambda 2}$ | | $\sim 0$ | | $C_{Mo}$ $A_c$ |

where both $C_{L0}$ and $C_{M0}$ are the initial molarities of L and M, and $\eta$ indicates the effective fraction of L. The symbol $A_c$ indicates the real absorbance of the ML complex at wavelength $\lambda_2$. Both $A^L{}_{\lambda 1}$ and $A^L{}_{\lambda 2}$ are the absorbances of L solution measured at wavelengths: $\lambda_1$ and $\lambda_2$ against water reference. $N$ refers to the coordination number of L with M.

In fact, a great deal of L is added in order to complex M completely. The excess of L thus occupies a high color fraction in the reaction solution. However, the reaction sensitivity is usually positively correlated to the high light-absorption of L. Recently, a large number of chromophores with big conjugate planes have been synthesized increasingly and applied to the detection of trace M. However, a negative appearance was found to restrict the practical application because the excess of L often influences the measurement of light-absorption of the ML complex. Thus, analytical error increases. Without doubt, this problem must be solved. The spectral correction technique has a specific advancement because it may eliminate the effect of the light-absorption of excess of L in the ML reaction solution. Absorbance of each color component including the reactant and product may be measured and calculated. Thus, not only the light-absorption of ML complex is obtained, but also the complex is characterized clearly. The principal equations are given below:

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

where

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

and

$$\alpha = \frac{A^{ML}{}_{\lambda 1}}{A^{ML}{}_{\lambda 2}} \tag{3}$$

and

$$\gamma = \eta \times \frac{C_{L0}}{C_{M0}} \tag{4}$$

where

$$\eta = \frac{A_c - A_{\lambda 2}}{A^L{}_{\lambda 2}} + 1 \tag{5}$$

Both $\beta$ and $\alpha$ are the correction constants, $\gamma$ the complexation number of L on M. $A_{\lambda 1}$ and $A_{\lambda 2}$, $A^{ML}{}_{\lambda 1}$, and $A^{ML}{}_{\lambda 2}$ are the absorbances of the M-L reaction solution and a ML complex solution without free L, respectively, measured at $\lambda_1$ and $\lambda_2$ against a water reference. From Equation 4, $\gamma$ increases up to a maximal coordination constant $N$ with increase in the molar ratio of L to M. In this study, it was applied to identify the composition of Fe-TMPF complex.

### LARVA[17]

The main equations of the LARVA are described as follows:

$$\Delta A_r^{-1} = p' C_{M0}^{-1} + q' \quad (6)$$

or

$$\Delta A_r = pC'_{M0} + q \qquad (\text{only } C'_{M0} << C_{M0}) \quad (7)$$

where

$$\Delta A_r = A_r - A_{r0} = \frac{A_{\lambda 2}}{A_{\lambda 1}} - \frac{A^L{}_{\lambda 2}}{A^L{}_{\lambda 1}} \quad (8)$$

The symbols $A_{\lambda 1}$, $A_{\lambda 2}$, $A^L{}_{\lambda 1}$ and $A^L{}_{\lambda 2}$ have the same meanings as the equations above. $\Delta A_r$ indicates the absorbance ratio variation of the reaction solution. $C'_{M0}$ is the initial concentration of M but is much lower than $C_{M0}$. All $p'$, $q'$, $p$ and $q$ are constants when both $\lambda_1$ and $\lambda_2$ and the reaction conditions are selected. Such two theoretical equations can be directly used in the quantitative detection of trace M. From the equation above, the sensitivity factor $p$ is the inverse ratio to $C_{L0}$. Therefore, the less L is added, the higher the analytical sensitivity will go. However, too low L will cause a raising of the measurement error because of the instrument's noise.

The LARVA is different from two earlier absorbance ratio ways: the first utilizes the variation of ratio of an absorbance to two additives absorbance with pH[21] to determine impurity of a medicine, and the other utilizes the absorbance ratio at two wavelengths to examine purity of an organic compound *e.g.* protein,[22] or to identify a molecular structure.

## EXPERIMENTAL

### Apparatus and Reagents

The absorption spectra of the TMPF and its complex solutions were recorded with a Perkin-Elmer Model Lambda-25 spectrometer. The spectrometer was computer controlled using a UV WinLab software (Version 2.85.04). A Model KQ318T supersonic wave cleaner (Kunshan Analytical Instruments, China) was used for rapid dissolution of TMPF and EDTA in solvent. The pH of solution was measured with a Model pHS-25 acidity meter (Shanghai Precise Sci. Instrum., China). A Model BCD-196 refrigerator freezer (Meiling Production of Anhui Province, China) was used to store the dilute Fe(II) and TMPF solutions.

1000 mg/l iron standard solution (National Certified, GSB 07-1264-2000) was purchased from the Institute for Reference Materials of SEPA of China. Both 1.00 and 10.0 μg/mL Fe solutions were prepared by diluting the above solution. 0.250 mmol/l TMPF was prepared by dissolving 51.7 mg of purified trimethoxyphenylflurone (provided by Changke Reagents Institute of Shanghai) in 250 mL of ethyl alcohol absolute (AR, Zhenxing Chemicals of Shanghai), and then it was diluted to 500 mL with deionized water. It was used as the chromophore to react with Fe. The ammonia buffer solutions, pH 9.43, 10.0, 10.48, 10.98, 11.53, 11.80 and 12.23 were prepared with ammonia and ammonium chloride, and they were used to adjust the solution acidity. 2.0 mmol/l CPC was prepared by dissolving cetylpyridinium chloride (purchased from Shanghai Chemical Reagents Co.) in deionized water, and it was used to complex TMPF. 2.0% and 1.00 mmol/l TGA was prepared as a reductant by mixing thioglycolic acid (purchased from Shanghai Chemical Reagents Co.) in deionized water. It was used to reduce Fe(III) into Fe(II) and TMPF into a reduced ligand. 0.1 mol/l EDTA was prepared by dissolving ethylenediamine tetraacetic acid disodium (purchased from Shanghai Chemical Reagents Co.) in deionized waterand it was used to mask most metals. In addition, 100 mg/l Al(III) was prepared by diluting 1000μg/mL Al(III) standard solution (National Standard, No. GSB G 62006-90, purchased from the Department of Research and Development of Standard Samples, Shanghai Institute of Materials) and it was used to react with the excess of TMPF in the TMPF-Fe solution.

### General Procedures

*Characterization of Fe-TMPF complexation:* Into a series of 10-mL calibrated flasks, 1 mL of pH 11.80 buffer solution, 0.5 mL of 2 mmol/l CPC, 0.5 mL of 5% TGA and 0.500 μg of Fe were added. 0.250 mmol/l TMPF was added from 0.100 to 0.800 mL and they were diluted to 10 mL and mixed well. After 10 min, the absorbances were measured at 521.5 and 641.5 nm against the reagent blank without Fe. The symbols, β, $A_c$, η and γwere calculated by the equations above.

*Determination of Fe:* Less than 5.00 mL of a sample solution was added into a 10-mL flask. 0.5 mL of 2 mmol/l CPC, 1 mL of pH 11.80 buffer solution, 1 mL of 0.1 mol/l EDTA, 0.50 mL of 5% TGA and 0.400 mL of 0.250 mmol/l TMPF were added. It was diluted to 10 mL and mixed well. After 10 min, 50μl of 100 mg/l Al(III) were added and mixed well immediately. After 10 min, the absorbances ($A_{568nm}$ and $A_{641.5nm}$) were measured at 568 ($\lambda_1$) and 641.5 nm ($\lambda_2$) against water. Simultaneously, a reagent blank without Fe was prepared and then measured at $A^0{}_{568nm}$ and $A^0{}_{641.5nm}$. Thus, $\Delta A_r$ is calculated by the relation:

$$\Delta A_r = \frac{A_{568nm}}{A_{641.5nm}} - \frac{A^0{}_{568nm}}{A^0{}_{641.5nm}} \quad (9)$$

From Equa tion (6) or (7), $C_{Fe}$ in the sam ple was cal cu lated.

## RE SULTS AND DIS CUS SION

### De pend ence of pH

The ab sorp tion spec tra of the Fe-TMPF so lu tions in var i ous pH me di ums are shown in Fig. 1. From curves 1-7 in A, the peaks are lo cated at about 540 nm and the val leys at about 520 nm. From change of the in ter val be tween the peak and val ley shown in B, the TMPF-Fe complexation is more sen si tive at pH be tween 10 and 12. From ex per i ments, we observed that the complexation goes in sen si tive in the pres ence of EDTA at pH less than 10. It is at trib uted to the fact that EDTA co or di nates Fe strongly. If pH is more than 11, the coor di na tion abil ity of TMPF will go much stron ger to re act with Fe than that of EDTA. The rea son is that the dehydrogenation of TMPF will hap pen to form neg a tive bicovalent ions. Thus, it is fa vor able for complexation with CPC. In this work, pH 11.80 am mo nia buffer so lu tion was spec i fied and added. The ab sorp tion peak of such a so lu tion is lo cated at 641.5 nm and the val ley at 521.5 nm, and two such wave-

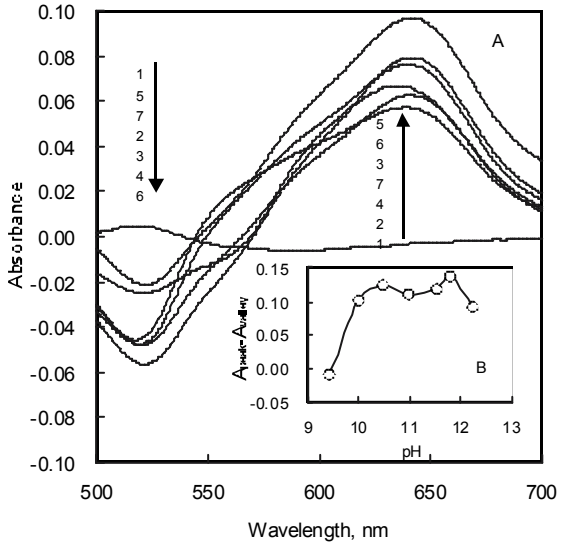


Fig. 1. Ef fect of pH on ab sorp tion spec tra of the Fe-TMPF so lu tions, which con tained 0.010 mmol/l TMPF, 0.050 mg/l Fe(III), 0.20 mmol/l CPC and 0.25% TGA. All against the re agent blank with out Fe. From Curve A-1 to A-7: pH at 9.43, 10.00, 10.48, 10.98, 11.53, 11.80 and 12.23. B: vari a tion of the in ter val be tween peak and valley with pH.

lengths were se lected in char ac ter iza tion of the Fe-TMPF complexation.

### Reaction of CPC and TGA with TMPF

From spec tra A-1, A-2 and A-3 shown in Fig. 2, both CPC and TGA can re act with TMPF at pH 11.80. From curve A-1, the TMPF-$H_2O$ com plex peak is at 507 nm. From curve A-2, the CPC-TMPF com plex peak is at 577 nm. The spec tral red shift (SRS) of the CPC-TMPF com plex is 77 nm so the ion-pair complexation is strong. Also, the light-absorption of spec tra A-2 be comes greater than that of spec trum A-1. TGA is one of the strong reductants. From curve A-3, the peak absorp tion of the TGA-TMPF so lu tion is lo cated at 513 nm with a higher absorbance than that of curve A-1. There fore, TMPF was re duced into the re duced TMPF (RTMPF) as shown in Fig. 3. RTMPF is one of the quadridentate lig ands. In ba sic me dium, its two sides will form quad ri va lent an ions which can all com plex metal ions (see Fig. 3). It is fa vor able for the use of LARVA be cause it has a stron ger light-absorption than TMPF. From curve A-4, the main peak at 577 nm and the shoul der peak at 515 nm in di cate the complexation of CPC with RTMPF. Curve A-5 pres ents spec trum of only the Fe-RTMPF com plex with out free RTMPF in the pres ence of CPC be cause Fe is over RTMPF molarity. The ab sorp tion peak of the com plex is lo cated at 628 nm, and the cor rec tion con stant $\alpha$ of the Fe-TMPF com plex can be cal cu lated for char ac ter iza tion of the Fe-RTMPF complexation. By compar ing curve A-4 with A-5, the SRS of the Fe-RTMPF complex is 51 nm. From spec tra B-1, B-2 and B-4, we ob served that the Fe(III)-TMPF complexation is in sen si tive in the absence of both CPC and TGA. From curve B-5, the Fe(III)-TMPF re ac tion be comes sen si tive when both CPC and TGA ex ist. Fe(II) has a much stron ger co or di na tion to RTMPF than Fe(III) by com par i son of spec tra B-2 and B-4. In ad di tion, TGA re duces Fe(III) into Fe(II) com pletely by com par i son of spec tra B-4 and B-5.

From the de scrip tion above, the CPC-RTMPF complexa tion be longs to the non-covalent in ter ac tion, *e.g.* ion-pair at trac tion and the bind ing ra tio may be de ter mined by break point ap proach.[19] From curve A in Fig. 4, the absorbance ra tio $A_{507nm}/A_{577nm}$ of the CPC-TMPF so lu tion at pH 11.80 de creases with in crease of CPC molarity. The break point is lo cated at 2.0. The com plex can be ex pressed as $RTMPF(CPC)_2$.

### Characterization of Fe-TMPF complexation by spectral correction technique

From curve A in Fig. 5, the cor rec tion con stant, $\beta$ of

RTMPF decreases with increase of RTMPF molarity at pH 11.80 in the presence of CPC. It indicates that the self-aggregation of RTMPF will happen to form a dimer or polymer in such a medium. From curve B, the effective fraction η of RTMPF increases rapidly and then decreases rapidly when RTMPF is more than 0.010 mmol/l in the presence of 0.050 mg/l Fe(III). This is attributed to the effect of the complexation equilibrium between Fe and RTMPF. At the peak, η of 0.010 mmol/l RTMPF is only 41.6%. Therefore, 58.4% RTMPF has not reacted with Fe(III). Without doubt, so high an excess of RTMPF free in the Fe-RTMPF solution will influence the measurement of light-absorption of the Fe-RTMPF complex. Thus, ordinary spectrometry is limited for characterization of the Fe-RTMPF complex and accurate determination of Fe trace. From curve C, γ of TMPF to coordinate Fe(III) increases with increase of RTMPF molarity and then approaches a maximal constant at 5.0. Therefore, the formation of $Fe(RTMPF)_5$ was confirmed at pH 11.80.

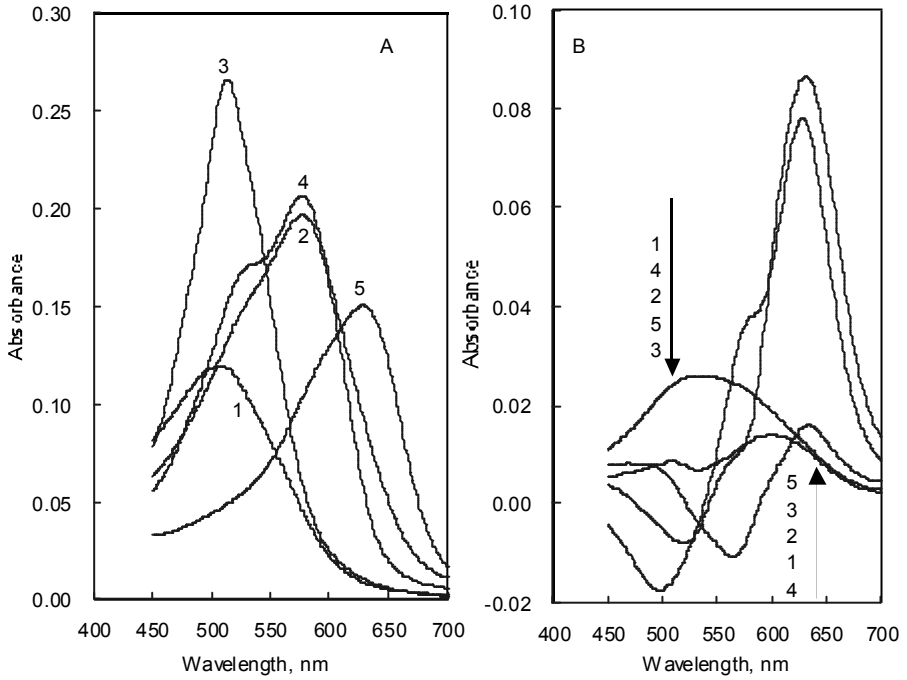


Fig. 2. Absorption spectra of TMPF in the presence of the assistants and Fe(III) at pH 11.80: A-1 - 0.010 mmol/l TMPF; A-2 - 0.010 mmol/l TMPF and 0.10 mmol/l CPC; A-3 - 0.010 mmol/l TMPF in 0.25% TGA medium; A-4 - 0.010mmol/l TMPF and 0.10 mmol/l CPC in 0.25% TGA medium; A-5 - 0.0050 mmol/l TMPF, 0.10 mmol/l CPC and 0.50 mg/l Fe(II); B-1 - 0.010 mmol/l TMPF and 0.050 mg/l Fe(III) in the absence of CPC and TGA; B-2 - 0.050 mg/l Fe(III), 0.010 mmol/l TMPF and 0.10 mmol/l CPC in the absence of TGA; B-3 - 0.010 mmol/l TMPF, 0.050 mg/l Fe(II) and 0.10 mmol/l CPC; B-4 - 0.010 mmol/l TMPF and 0.050 mg/l Fe(III) in 0.25% TGA medium and B-5 same as B-4 but in the presence of 0.10 mmol/l CPC. From A-1 to A-5 against water reference and the others against the corresponding blank.

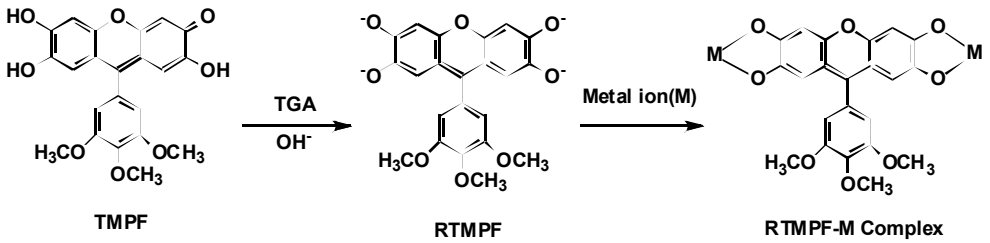


Fig. 3. TMPF and its structural change in the presence of TGA and complexation between metal ion (M) and RTMPF.

From the complexation num bers of CPC and Fe with RTMPF, the binuclear com plex $Fe_2(RTMPF)_{10}(CPC)_{20}$ was formed by co or di na tion bond and ion-pair at trac tion.

**Ef fect of Re ac tion Time and Ad di tion of Al(III)**

From the variation of the absorption spectra of the Fe-TMPF so lu tion at pH 11.80 in the pres ence of CPC, TGA and EDTA, the re ac tion is com plete af ter 10 min. How ever, from the ex per i men tal phe nom e non, the light-absorption of the re agent blank is un sta ble and vari able with time. It is pos-si ble for RTMPF to be ox i dized by dis solved ox y gen in the ba sic me dium. Thus, it will af fect se ri ously the fol low ing ap-pli ca tion of LARVA. If enough Al(III) was added in the so lu-tion to com pletely co or di nate the ex ces sive RTMPF in the Fe-RTMPF so lu tion, such an ef fect can be elim i nated and the se lec tiv ity of the method will im prove ob vi ously for the de-ter mi na tion of Fe trace. The ab sorp tion spec trum of such a so lu tion is shown in Fig. 6(A). By com par ing this spec trum with spec trum 6 in Fig. 1, the peak at 641.5 nm has no change, but the val ley shifts from 521.5 to 568 nm. This is at trib uted to the com plete complexation of RTMPF free in the Fe- RTMPF re ac tion with Al. In the fol low ing ex per i ments, both the wave-lengths 641.5 and 568 nm are used in the de ter mi na tion of Fe trace. From curve B-1, $A_{r0}$ of the RTMPF-CPC so lu tion al-ways in creases with time. This con firms the ex per i men tal phe nom e non ob served above. Af ter 10 min, the ad di tion of Al(III) plays an ob vi ous role in the stablization of $A_{r0}$ from curve B-2 but also $A_{r0}$ is much less than that in curve B-1. This is very im por tant in ap pli ca tion of the LARVA. There-fore, Al(III) must be added while the Fe-RTMPF re ac tion is at 10 min and then both $A_r$ and $A_{r0}$ were mea sured af ter 10 min.

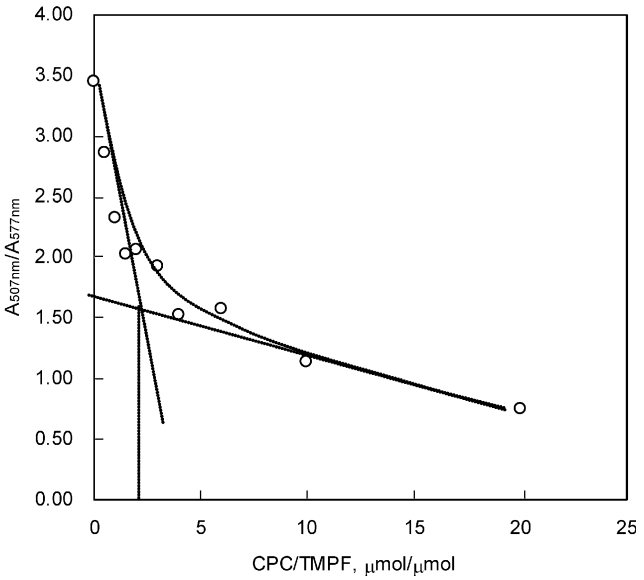


Fig. 4. Ap pli ca tion of break point ap proach to de ter mi-na tion of the bind ing ra tio of CPC to RTMPF at pH 11.80.

**Variation of $\Delta A_r$ and Op tion of RTMPF Molarity**

Change of $\Delta A_r$ of the Fe-RTMPF so lu tions is shown in Fig. 7 with a con stant mo lar ra tio of Fe to TMPF at 0.0716:1

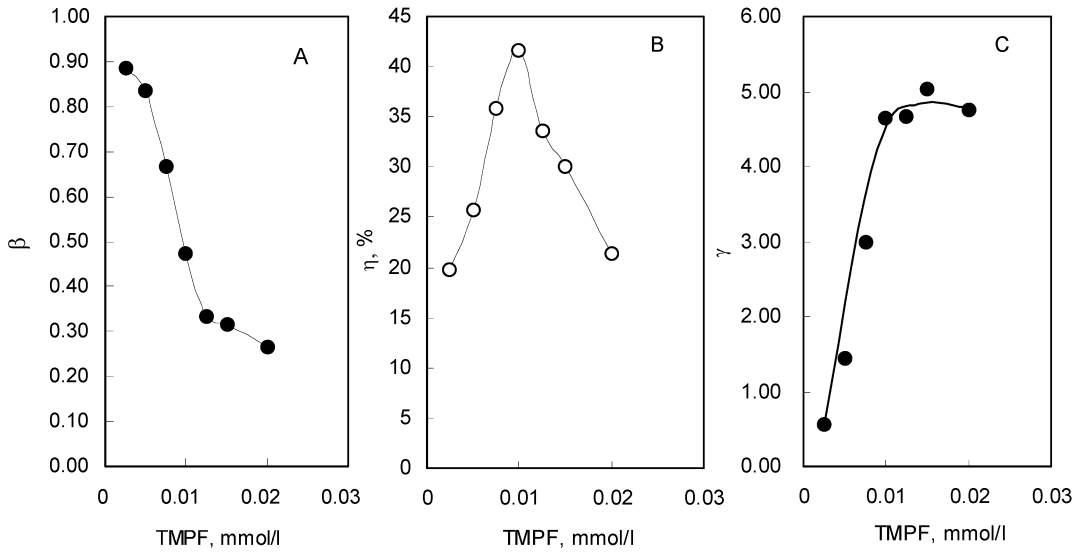


Fig. 5. Vari a tion of β (A), η (B) and γ (C) with TMPF molarity at pH 11.80. The so lu tions con tained 0.050 μg/mL Fe(III), 0.25% TGA, 0.10 mmol/l CPC and TMPF from 0.0025 to 0.020 μmol/mL.

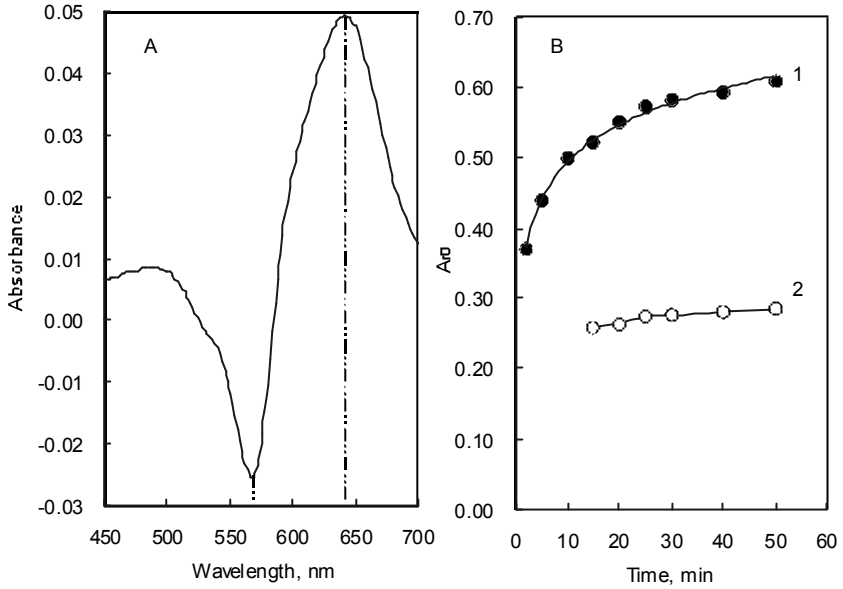


Fig. 6. A. Absorption spectrum of the Fe-TMPF complexation at pH 11.80. The solution contained 0.010 mmol/l TMPF, 0.050 mg/l Fe(III), 0.10 mmol/l CPC, 0.25% TGA and 0.01 mol/l EDTA. After 10 min, 0.50 mg/l Al(III) was added into the solution and it was measured against a blank. B: Variation of $A_{r0}$ with the reaction time at pH 11.80. B-1 - the solution contained 0.010 mmol/l TMPF, 0.10 mmol/l CPC, 0.25% TGA and 0.01 mol/l EDTA and B-2 - same as B-1 but 0.50 mg/l Al(III) was added after the reaction was at 10 min. They were measured at 641.5 and 568 nm against a water reference.

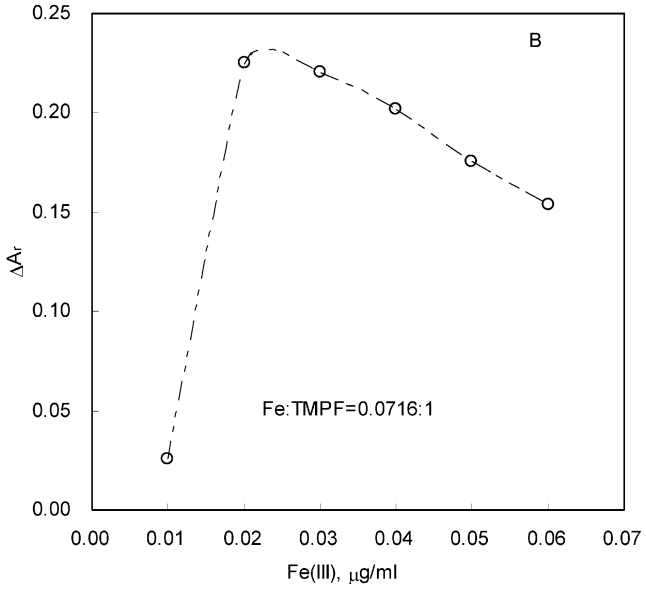


Fig. 7. Variation of $\Delta A_r$ with Fe(III) concentration at pH 11.80: the solutions contained 0.010 mol/l EDTA, 0.10 mmol/l CPC, TMPF from 0.00250 to 0.0150 μmol/mL and Fe(III) from 0.010 to 0.060 μg/mL. The molar ratio of Fe(III) to TMPF always remained constant at 0.0716:1 μmol/μmol and 0.50 mg/l Al(III) was added at 10 min. The solutions were measured at 641.5 and 568 nm, respectively, against a water reference.

μmol/μmol in the presence of EDTA, CPC, TGA and Al(III). $\Delta A_r$ reaches a peak at 0.020 mg/l Fe(III) and then decreases. Similarly, the less TMPF molarity is, the lower the detectable Fe will go. Of course, the fraction of the instrumental noise will increase seriously if the light-absorption is too low. In the following calibration series, three additional volumes, 0.200, 0.300 and 0.400 mL of 0.250 mmol/l TMPF were tried so as to find an optimal addition.

**Calibration Graphs and Limit of Detection**

Three series of standard Fe(III) between 0 and 0.050, 0 and 0.070 and 0 and 0.100 μg/mL were prepared and 0.200, 0.300 and 0.400 mL of 0.250 mmol/l TMPF were added, respectively. The reactions were carried out according to the recommended procedures. The absorbances of each solution were measured at 568 and 641.5 nm and then $\Delta A_r$ was calculated by Equation (9). The linear scope of Fe and the regression equations are given in Table 1. The limit of detection of Fe, defined as the blank values plus 3 times the standard deviation of 10 replicated blanks, was calculated and is given in Table 1, too. Among them, Line 3 is the best because of the good blank precision to result in the lowest limit of detection (LOD) at only 2 ng/mL Fe. Therefore, 0.400 mL of 0.250 mmol/l TMPF is added in analysis of samples. The recom-

Table 1. Regression equations and limit of detection of Fe

| Line | Fe(III), μg/10 mL | TMPF, mM | *p* | $\Delta A_r$ vs. $C_{Fe}$ | R[a] | σ[b] | LOD[c], ng/mL |
|---|---|---|---|---|---|---|---|
| 1 | 0-0.40 | 0.0050 | 1.205 | $\Delta A_r = 1.205C_{Fe} + 0.0391$ | 0.9904 | 0.0238 | 6 |
| 2 | 0-0.70 | 0.0075 | 0.9095 | $\Delta A_r = 0.9095C_{Fe} + 0.0190$ | 0.9962 | 0.0129 | 4 |
| 3 | 0-1.00 | 0.0100 | 0.6428 | $\Delta A_r = 0.6428C_{Fe} + 0.0004$ | 0.9991 | 0.0046 | 2 |

[a] Linear correlation coefficient. [b] Standard deviation of 10 repetitive blanks. [c] Limit of detection of Fe(III) was calculated by LOD = 3σ/*p*.

mended method is one of the most sensitive detections of Fe at present, but also it is simple in operation. It is suitable for natural water, body liquids, food, medicine, materials, and biological and many other samples.

**Effect of Foreign Ions**

The coordination position of RTMPF ligand is $-O^-$ but that of EDTA ligand is –N. The former binds more strongly to Fe(II) than the latter. Therefore, the addition of EDTA will not replace RTMPF in the RTMPF-Fe complex. On the contrary, EDTA can coordinate most heavy metals, so it was used to mask foreign metal ions. In addition, a great deal of Al(III) was added to complex the excessive RTMPF in the Fe-RTMPF solution because of optical instability of RTMPF in basic medium. Thus, the mixed reaction will change to be more favorable to spectrophotometry. Fourteen foreign metal ions were added in the Fe(III)-RTMPF complexation and their effect errors are shown in Table 2. We observed that most metals will not influence the direct determination of dissolved Fe($Fe^{II}$ + $Fe^{III}$) in samples. Therefore, this method is highly selective.

Table 2. Effect of foreign ions on $\Delta A_r$ of the solutions containing 0.50 μg of Fe(III) and error showing

| No. | Ion | Added, μg/10 mL | Error[a] % |
|---|---|---|---|
| 1 | Fe(III) | 0.50 | |
| 2 | Ca(II)[b] | 50.0 | 7.1 |
| 3 | Mg(II) | 20.0 | 4.7 |
| 4 | Co(II) | 5.00 | 2.4 |
| 5 | As(III) | 1.00 | 9.2 |
| 6 | Zn(II) | 5.00 | -3.1 |
| 7 | Cr(III) | 1.00 | 0.2 |
| 8 | Pb(II) | 2.00 | -4.0 |
| 9 | Cd(II) | 1.00 | 0.9 |
| 10 | Cu(II) | 2.00 | -0.8 |
| 11 | Al(III) | 5.00 | 2.1 |
| 12 | V(V) | 2.00 | -7.7 |
| 13 | Ni(II) | 1.00 | -5.1 |
| 14 | Mn(II) | 2.00 | -6.0 |
| 15 | Ge(IV) | 1.00 | -8.9 |

[a] Error = $(\Delta A_r^{No.x} - \Delta A_r^{No.1})/\Delta A_r^{No.1} \times 100$ (x is from 2 to15). [b] Added 0.500 μg of Fe(III) into all the solutions from No. 2 to15.

**Preparation and Analysis of Sample Solution**

Water and liquid samples can be analyzed directly without deep pretreatment. Necessary filtration or elimination of the background colour is often possible. Solid samples, *e.g.* soil, plants, and food, must be dissolved in strong acidic medium and the whole Fe extracted from the sample. The cleaning solution was neutralized to pH of about 4 with 2 mol/l NaOH and then analyzed. Here, nine samples: five natural waters, a tap water sample, a cigarette ash sample and a human urine sample were prepared into the solutions, and total iron was determined according to the recommended procedures. The results are listed in Table 3. The recovery rates of Fe are between 88.0 and 111%. It was seen that results obtained by the recommended method are in good agreement with those obtained by an ISO standard method using 1,10-phenanthroline.[23] The method is simple, inexpensive, accurate, and reproducible and so is suitable for the monitoring of various samples.

## CONCLUSIONS

As a result, it can be concluded that the proposed method enhances sensitivity and improves the detection limit in terms of Fe(III). Also, in the proposed method, none of the metal ions have been found to interfere with the direct determination of Fe(III). Two significant advantages are identified: (i) more sensitive direct spectrophotometric detection of Fe can be performed; and (ii) the presence of EDTA and addition of Al(III) improve greatly the detection selectivity. The performance of the method described here allows the determination of iron species. The LARVA as a novel sepctrophotometric way makes the detection sensitivity over 10 times as high as the ordinary method. Micro-volume of a sample, *e.g.* 0.100 mL of biological or food sample may be

Table 3. Determination of Fe in samples

| Sample from | Fe added, μg/l | Fe found, μg/l | Recovery % |
|---|---|---|---|
| West Lake | 0 | 9.8 ± 2.5[a] | |
| | 10.0 | 18.6-20.9[b] | 88.0 - 111[c] |
| Taihu Lake | 0 | 12.7 ± 1.6[a] | |
| | 10.0 | 22.1-23.8[b] | 94.0 - 111[c] |
| Underground water | 0 | 36.7 ± 4.1[a] | |
| | 40.0 | 73.2-78.9[b] | 91.2 - 106[c] |
| Offshore water | 0 | 69.2 ± 2.8[a] | |
| | 0 | 67.1[d] | |
| Yangtze River | 0 | 37.9 ± 2.3[a] | |
| | 30.0 | 65.2-70.5[b] | 91.0 - 108.7[c] |
| Tap water | 0 | 143.2 ± 3.7[a] | |
| | 0 | 151[d] | |
| Urine | 0 | 164 ± 17[a] | |
| | 100.0 | 259.1-272.4[b] | 95.1 - 108.4[c] |
| Smoking ash (mg/g) | 0 | 1.26 ± 0.03[a] | |
| | 0 | 1.19[d] | |

[a] Average of four determinations. [b] Average of three determinations. [c] *e.g.* 88% = (18.6 - 9.8)/10.0 × 100%. [d] Average of two determinations with 1,10-phenanthroline by spectrophotometry.

analyzed accurately, too. Moreover, the method is very simple in operation. For characterization of a complexation, both the break point approach and the spectral correction technique are more suitable than the other classical methods *e.g.* continuous variations and equilibrium movements because of strong light-absorption of the excessive chromophores in the solution.

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## REFERENCES

1. John son, K. S.; Coale, K. H.; Elrod, V. A.; Tindale, N. W. *Mar. Chem.* **1994**, *46*, 319.
2. Rue, E. L.; Bruland, K. W. *Mar. Chem.* **1995**, *50*, 117.
3. Buffle, J.; Altmann, R. S.; Filella, M.; Tessier, A. *Geochim. Cosmochim. Acta* **1990**, *54*, 1535.
4. Weeks, D. A.; Bruland, K. W. *Anal. Chim. Acta* **2002**, *453*, 21.
5. Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*; Wiley: New York, 1988.
6. Martin, J. H.; Gordon, R. M.; Fitzwater, S. E. *Nature* **1990**, *345*, 156.
7. Magda, A. A. *Microchem. J.* **2003**, *75*, 199.
8. Dichristian, T. G.; Anold, R. G.; Lidstrom, M. E.; Hoffman, M. R. *Water Sci. Tech.* **1988**, *20*, 69.
9. Fang, J. N.; Yu, B. S.; Chen, Y. L.; Song, S. R.; Lo, H. J.; Lin, I. C.; Liu, C. M.; Liu, Y. J. *J. Chin. Chem. Soc.* **2003**, *50*, 465.
10. Martin, J. H.; Gordon, R. M.; Broenkow, S. E.; Fitzwater, W. W. *Deep-Sea Res. Part A* **1989**, *36*, 649.
11. Munger, J. W.; Waldman, J. M.; Jacob, D. J.; Hoffman, M. R. *J. Geophys. Res.* **1983**, *88*, 5109.
12. Riganakos, K. A.; Veltsistas, P. G. *Food Chem.* **2003**, *82*, 637.
13. Pehkonen, S. *Analyst* **1995**, *120*, 2655.
14. Yang, Y. L.; Miao, M. M.; Lin, Q.; Yang, G. Y. *J. Chin. Chem. Soc.* **2004**, *51*, 19.
15. Liu, Y. M.; Yu, R. Q. *Analyst* **1987**, *112*, 1135.
16. Abdollahi, H.; Zolgharnein, J.; Azimi, G. H.; Jafarifar, D. *Talanta* **2003**, *59*, 1141.
17. Gao, H. W.; Xia, S. Q.; Wang, H. Y.; Zhao, J. F. *Water Res.* **2004**, *38*, 1642.

18. Li, Z. J.; Pan, J. M.; Tian, J. *Anal. Chim. Acta* **2001**, *445*, 153.
19. Gao, H. W.; Hu, Z. J.; Zhao, J. F. *Chem. Phys. Lett.* **2003**, *376*, 251.
20. Gao, H. W.; Zhao, J. F. *J. Chin. Chem. Soc.* **2003**, *50*, 329.
21. Tamura, F. Z.; Furuyama, I. S. *Bunseki Kagaku* **1969**, *18*, 446.
22. Murphy, J. B.; Kies, M. W. *Biochem. Biophys. Acta* **1960**, *45*, 382.
23. ISO, Water quality -- Determination of iron -- Spectrometric method using 1,10-phenanthroline, ISO 6332 (1988).