Establishment of the Spectral Correction Matrix and Simultaneous Determination of Iron and Zinc with 1-(6-Bromo-2-Benzothiazolylazo)-2-Naphthol as Chromogenic Agent

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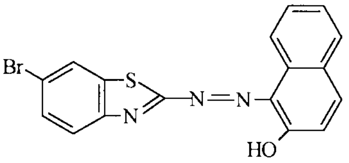
The spectral correction matrix has been established to study the simultaneous complexations between metals and a ligand, and it eliminated the mutual influence among the complexes. The two sensitive reactions between iron (II) and 1-(6-bromo-2-benzothiazolylazo)-2-naphthol (BBTAN) and zinc (II) and BBTAN have been investigated and applied to the simultaneous determination of Zn and Fe at pH 8.5 in the presence of triton x-100. This method gave the simple determination of the characteristic constants of the complexes. For the analysis of natural water, foods and other samples, the recoveries of Fe and Zn were between 92 and 109% and between 91 and 104%, and their relative standard deviations were less than 5.5 and 11%, respectively.

Keywords : Spectral correction technique, Matrix, Simultaneous determination of Fe and Zn, 1-(6-Bromo-2-benzothiazolylazo)-2-naphthol (BBTAN).

Introduction

Both iron (Fe) and zinc (Zn) are essential elements for animals. They often exist in food, drink and grains. The chromogenic reagents, *e.g.* 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol,[1] dithizone, di-2-pyridyl-methanone-2-(5-nitropyridyl) hydrazone[2] were earlier applied to the determination of iron or zinc by spectrophotometry. Recently, the synthesis of the new ligand, 1-(6-bromo-2-benzothiazolylazo)-2-naphthol (BBTAN), was made and its structure is given below.



The reactions between Fe(II) and BBTAN and Zn(II) and BBTAN were sensitive at pH 8.5 in the presence of the nonionic surfactant, triton x-100. The absorption peak of the Fe-BBTAN complex was located at 540 nm and that of the Zn-BBTAN complex at 580 nm, but that of BBTAN at 460 nm. The spectral correction technique was applied instead of ordinary spectrophotometry because several color compounds existed simultaneously in solution, *e.g.* free BBTAN, Fe-BBTAN and Zn-BBTAN complexes. It is difficult by the conventional method to obtain an accurate measurement of each of the mixed complexes. The spectral correction technique was earlier applied to the investigation of metal complex,[3-6] and it may solve the above problem. This work established the spectral correction matrix for the simultaneous determination of complexes. It eliminated the mutual effect among the complexes and gave the real absorbance of each complex. In addition, the simple determination of the characteristic constants of each complex is made, *e.g.* complex ratio, stepwise real absorptivity and stability constant. The recommended method was more acceptable in principle than the classical methods of the molar ratio,[7] the continuous variation[8] the equilibrium movement,[9] *etc.*

Principle

The following expression is developed for the determination of the real absorbance ($\mathbf{A_c}$) of metal (**M**) complex ($\mathbf{ML_\gamma}$) produced with a ligand (**L**).

$$A_c = \frac{A_\beta}{1-\alpha\beta}, \quad \text{where} \quad A_\beta = \Delta A - \beta\Delta A'$$

Here, the term, A_β was named β-correction absorbance, which was proportional to M concentration. ΔA and $\Delta \boldsymbol{A}'$ are the absorbances of the reacted solution between **M** and **L** at wavelengths λ_2 and λ_1 against the reagent blank, respectively. The coefficients, α and β are named correction factors and they can be measured from only $\mathbf{ML_\gamma}$ solution and **L** solution then computed as follows:

$$\alpha = \frac{\varepsilon_{ML\gamma}^{\lambda 1}}{\varepsilon_{ML\gamma}^{\lambda 2}} \quad \text{and} \quad \beta = \frac{\varepsilon_{L}^{\lambda 2}}{\varepsilon_{L}^{\lambda 1}}$$

The terms $\varepsilon_{\mathbf{ML}\gamma}^{\lambda 1}$, $\varepsilon_{\mathbf{ML}\gamma}^{\lambda 2}$, $\varepsilon_{\mathbf{L}}^{\lambda 1}$ and $\varepsilon_{\mathbf{L}}^{\lambda 2}$ are the molar absorptivities of $\mathbf{ML_\gamma}$ and **L** at wavelengths λ_1 and λ_2, respectively.

Each of the metals will form a color complex with ligand (L) and the fractional absorbance of each color compound (free L and complexes) can be calculated by the following correction equation

$$\sum_{j=1}^{n} a_{ij}x_j = \Delta A_i - \beta_i \Delta A' \ (i = 1, 2, \ldots n) \text{ or}$$

$$\begin{pmatrix} a_{11} & a_{12} & \cdots & a_{1n} \\ a_{21} & a_{22} & \cdots & a_{2n} \\ \cdots & \cdots & \cdots & \cdots \\ a_{n1} & a_{n2} & & a_{mn} \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \\ \cdots \\ x_n \end{pmatrix} = \begin{pmatrix} \Delta A_1 - \beta_1\, \Delta A' \\ \Delta A_2 - \beta_2\, \Delta A' \\ \cdots\cdots\ \cdots\cdots \\ \Delta A_n - \beta_n\, \Delta A' \end{pmatrix}.$$

Here, the symbol $\beta_i = \varepsilon_{ligand}^{\lambda i}/\varepsilon_{ligand}^{\lambda o}$ and $a_{ij}=A_{\beta ij}/x_j$. They are named the correction coefficients. The former is determined from the ligand solution at wavelengths λ_i and λ_0, and the latter indicates the sensitivity of *j*-th metal at *i*-th wavelength λ_i (x_j-microgram of *j*-th metal).

The molar ratio (γ') of the effective **L** to **M** may be calculated by:

$$\gamma' = \eta \times \frac{C_L}{C_M} \quad \text{where} \quad \eta = \frac{\alpha\Delta A - \Delta A'}{(1-\alpha\beta)A_o'}.$$

Here, the term η indicates the reacted percentage of **L**. The factors, $\mathbf{C_M}$ and $\mathbf{C_L}$ are the concentrations (mol/l) of **M** and **L** in the beginning. $\mathbf{A'_o}$ is the absorbance of the blank reagent measured at wavelength λ_1. If γ' reaches the maximum and remains constant, we think that $\gamma = \gamma'$, where γ is a natural number and it is named the stoichiometric ratio of the complex produced. In addition, the following expression was established for calculation of the stepwise stability constant ($\mathbf{K_n}$) of complex $\mathbf{ML_\gamma}$.

$$K_n = \frac{\gamma'+1-n}{(n-\gamma')(C_L-\gamma' C_M)}$$

The cumulative constant (**K**) of complex $\mathbf{ML_\gamma}$ can be calculated by

$$\mathbf{K = K_1 \times K_2 \times \ldots \times K_n \ldots \times K_\gamma}.$$

In addition, from such a **M-L** reaction the stepwise absorptivity (real $\boldsymbol{\varepsilon}_{\mathbf{MLn}}^{\lambda 2}$ not apparent $\boldsymbol{\varepsilon}_{\mathbf{a}}^{\lambda 2}$, $n = 1, 2, \ldots, \gamma$) of complex $\mathbf{ML_\gamma}$ may be obtained by:

$$\varepsilon_{ML_n}^{\lambda_2} = \frac{A_c}{\delta C_M(\gamma' + 1 - n)} - \frac{n-\gamma'}{\gamma' + 1 - n}\varepsilon_{ML_{n-1}}^{\lambda_2},$$

where the δ is the cell thickness (cm) and the other variables have the same designations as above. In fact, the beta-correction method was one of the dual-wavelength spectrophotometric methods, but it was different from the others[10-12] in principle and in operation.

Experimental Section

Apparatus and Reagents. Absorption spectra were recorded with a Shimadzu UV-VIS 265 spectrophotometer (Shimadzu, JAPAN) with 10- mm glass cells, and the pH was measured with a Model PHS-2C acidimeter (Xiaoshan, China). The content of Fe and Zn in samples was determined with a WFX-1F2 atomic absorption spectrophotometer (Beijing Sec. Opt. Instrument Works, China).

The standard Fe(II) and Zn(II) stock solutions (both 1.000 g/L) were prepared with 1.000 g of high-purity metal dissolved in 10 mL of 1 mol/L hydrochloric acid and then diluted to 1000 mL with deionized water.

Standard Fe(II) and Zn(II) work solutions (both 10.0 mg/L) were prepared daily.

The BBTAN solution (1.00 mmol/L) was prepared by dissolving 1-(6-bromo-2-benzothiazolylazo)-2-naphthol (BBTAN, purified and provided by Changke Reagents Institute, Shanghai) in dimethylformamide (DMF, A. R., Shanghai Chemicals), which was then diluted to 1000 mL with deionized water. It was stored in a dark bottle.

The buffer solution (pH 8.5) was prepared with sodium borate and hydrochloric acid for adjusting the pH of the reactions.

The hydroxylamine chloride (A. R., Beijing Chemicals) solution (1%) was prepared with deionized water, and it was used to change Fe(III) into Fe(II).

The masking reagent solution was prepared by mixing 100 mL of 2% ethylenediamine (A. R., Shanghai chemicals) and 100 mL of 10% sulfourea (A. R., Beijing Chemicals).

The triton x-100 (Shanghai Organic) solution (2%) was prepared with deionized water so as to increase the reaction sensitivity.

Experimental Procedures. To a 25-mL volumetric flask we added 2 mL of pH 8.5 buffer solution, 1 mL of triton x-100 solution, 1 mL of 1% hydroxylamine chloride, 2 mL of the masking reagent solution, and 2.00 mL of BBTAN solution. Finally, we added a known volume of a sample containing less than 30.0 μg of Fe and 20.0 μg of Zn. The solution was diluted to volume and mixed well. After 10 min, we measured absorbances at 460, 540 and 580 nm against a reagent blank.

For analysis of a solid sample, *e.g.* grain and food, the sample must be ground and then dipped in 1 mol/L hydrochloric acid or ashed at 800 °C and then dissolved.

Results and Discussion

Absorption Spectra. Figure 1 shows the absorption spectra of *BBTAN*, Fe-BBTAN and Zn-BBTAN solutions in the presence of various surfactants, e.g. triton x-100, sodium dodecyl benzene sulfonate (SDBS) and celtriphenylphosphonium bromide (CTMAB). From curves 2-x and 3-x, it is clear SDBS can inhibit the reaction. However, triton x-100 gives the highest peak and lowest valley both more than double those observed in absence of surfactant. Three wavelengths, such that the positive and negative absorbances approach the maximum, 460, 540 and 580 nm, were selected and they just corresponded with the peak absorption of BBTAN, the Fe-BBTAN complex and the Zn-BBTAN complex, respectively. From curve 1, the correction coefficient was calculated to be $\beta = 0.447$ at 540 nm and $\beta = 0.200$ at 580 nm. From curves 4 and 5, the correction coefficient was calculated to be $\alpha_{540}^{\mathrm{ZnL}} = 0.437$, $\alpha_{580}^{\mathrm{ZnL}} = 0.394$, $\alpha_{540}^{\mathrm{FeL}} = 0.638$ and $\alpha_{580}^{\mathrm{FeL}} = 1.04$. The following expressions were used: $\mathbf{A_c = 1.09A_\beta = 1.09(\Delta A - 0.200\Delta A')}$ (Zn-BBTAN complex at 580 nm) and $\mathbf{A_c = 1.40A_\beta = 1.40(\Delta A - 0.447\Delta A')}$ (Fe-BBTAN complex at 540 nm).

Effect of the Addition of BBTAN Solution. Figure 2 shows the effect of *BBTAN* on the measured absorbance of

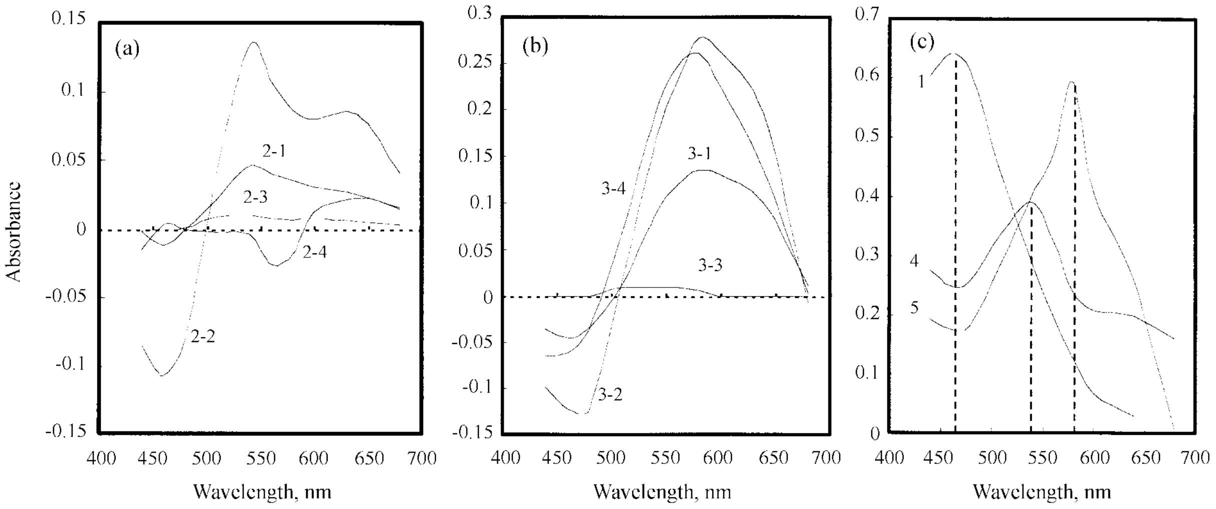


Figure 1. Absorption spectra of *BBTAN*, Fe-BBTAN and Zn-BBTAN complex solutions at pH 8.5 and in the presence of triton x-100: 1- *BBTAN* (1.00 μmol) in presence of triton x-100, 2-x- Fe (20 μg)-BBTAN solution, 3-x- Zn(10 μg)-BBTAN solution, 4- Fe (10 μmol)-BBTAN (1.00 μmol) complex solution, 5- Zn (10 μmol)-BBTAN (1.00 μmol) complex solution. 1, 4 and 5 against water refereence and the others against blank reference (no containing Fe or Zn). Curves 2-1 and 3-1 are in the absence of surfactant, 2-2 and 3-2- in the presence of triton x-100, 2-3 and 3-3- in the presence of SDBS and 2-4 and 3-4- in the presence of CTMAB.

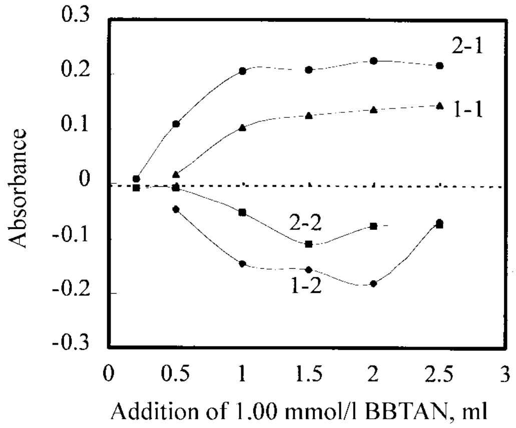


Figure 2. Effect of the addition of *BBTAN* solution on absorbances of Fe(20 μg) -BBTAN and Zn(10 μg)-BBTAN solutions at pH 8.5 and in the presence of triton x-100: x-1 ΔA against reagent blank, x-2 ΔA' against reagent blank, 1-x Fe-BBTAN solution at 540 and 460 nm and 2-x Zn-BBTAN solution at 580 and 460 nm.

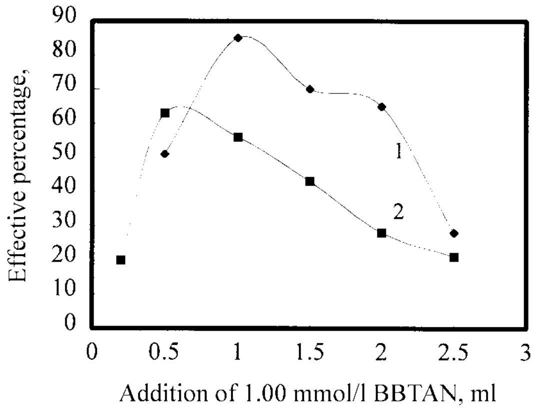


Figure 3. Effect of the addition of *BBTAN* solution on η: 1- Fe(20 μg)-BBTAN solution and 2- Zn(10 μg)-BBTAN solution.

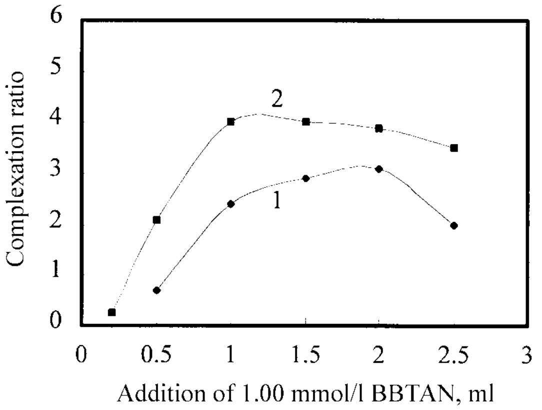


Figure 4. Effect of of the addition of *BBTAN* solution on γ': 1-Fe(20 μg)-BBTAN solution and 2- Zn(10 μg)-BBTAN solution.

the solutions. From curves x-1, it was difficult to calculate accurately the complex ratio of Fe or Zn to *BBTAN* by the molar ratio method due to the unclear inflexion point. The Fe-BBTAN and the Zn-BBTAN solutions approached the maximal absorbances when the addition of 1.00 mmol/L BBTAN was more than 1.5 mL. Therefore, 2.00 mL of the BBTAN solution was added in the simultaneous determination of Fe and Zn. Both η and γ' of each solution was calculated and their curves are shown in Figure 3 and 4, respectively. From curves in Figure 4, γ' reaches maximum 4 (Zn-BBTAN reaction) and 3 (Fe-BBTAN reaction). Therefore, the two complexes Fe(*BBTAN*)$_3$ and Zn(*BBTAN*)$_4$ were formed here. From curves in Figure 3, the effective fraction of BBTAN was 65% in Fe-BBTAN solution and 28% in Zn-BBTAN solution when 2.00 mL of the BBTAN solution was added. Therefore, 35% and 72% of BBTAN were free in solution. Undoubtedly, such an excess of BBTAN affects the

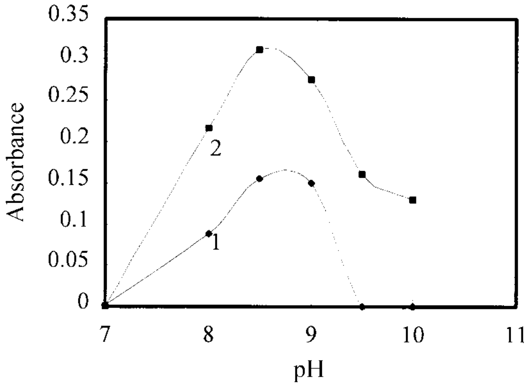


Figure 5. Effect of pH: 1- ΔA of Fe-BBTAN solution at 540 nm and 2- ΔA of Zn-BBTAN solution at 580 nm, both against reagent blank.

absorbance measurement of the complex.

Effect of pH and Time. The effect on the absorbances of the solutions of varying the pH of solution is shown in Figure 5. The absorption of the Fe-BBTAN and the Zn-BBTAN solutions both approach maximum at pH 8.5-9. In the present study, pH 8.5 was used. Figure 6 shows the effect of time on the absorbances of the solutions. We observed that the two reactions are complete in 5 min at room temperature. This shows that BBTAN demonstrates strong coordination with Fe(II) and Zn(II), where N, O and S elements all may provide lone-pair electron for Fe^{2+} and Zn^{2+}.

Determination of Stepwise Absorptivity and Stability Constant. The following solutions were prepared for determination of the stepwise real absorptivity and the stability constant of the Fe-BBTAN and the Zn-BBTAN complexes: 20.0 μg of Fe(II) with 0.50, 0.75, 1.00 μmol of BBTAN and 20.0 μg of Zn(II) with 0.30, 0.50, 0.80, 1.20 μmol of BBTAN. Three replicate determinations of each solution were made. The stepwise real absorptivity and stability constant of the Fe-BBTAN and the Zn-BBTAN complexes were calculated and the results are listed in Table 1. The cumulative stability constant of complexes $Fe(BBTAN)_3$ and $Zn(BBTAN)_4$ is 3.95×10^9 and 7.91×10^{22}, respectively, in ionic strength 0.025 M and at temperature 10 °C. From Table 1, we observe that the stability constant of the *n*-th step complex is always less than that of the *n-1*-th step complex. On the contrary, the absorptivity of the *n*-th step complex is always more than that of the *n-1*-th step complex. These findings are in accordance with the dynamical law of chemical reactions and the light-absorption law of color complex.

Table 1. Determination of stepwise stability constant and real absorptivity $(l\ mol^{-1}.cm^{-1})$ of complex $Zn(BBTAN)_4$ and Fe $(BBTAN)_3$ in ionic strength 0.025 M and at temperature 10 °C

n-th	$Zn(BBTAN)_4$		$Fe(BBTAN)_3$	
	K_n	ε_r at 580 nm	K_n	ε_r at 540 nm
1st	1.86×10^6	1.16×10^4	2.48×10^3	5.42×10^3
2nd	4.19×10^5	2.16×10^4	1.56×10^3	1.30×10^4
3rd	3.61×10^5	3.70×10^5	1.02×10^3	3.03×10^4
4th	2.81×10^5	5.65×10^5		

Calibration Graph. A series of standard Fe (0-40.0 μg) or Zn (0-20.0 μg) solutions was prepared and the absorbance of each was measured. The beta-correction absorbance A_β was calculated and the standard curves are shown in Figure 7. The following equations were expressed: $A_{\beta1\text{-}Fe} = \mathbf{0.0093x_{Fe}}$ at 540 nm, $A_{\beta2\text{-}Fe} = \mathbf{0.0074x_{Fe}}$ at 580 nm for Fe-BBTAN solution and $A_{\beta1\text{-}Zn} = \mathbf{0.0200x_{Zn}}$ at 540 nm, $A_{\beta2\text{-}Zn} = \mathbf{0.0358x_{Zn}}$ at 580 nm for Zn-BBTAN solution. Therefore, $a_{11} = 0.0093$, $a_{21} = 0.0074$, $a_{12} = 0.0200$ and $a_{22} = 0.0358$. From curves 1-x and 2-x, the beta-correction absorbance of the independent Fe-BBTAN and Zn-BBTAN solution is linear between 0 and 30.0 μg Fe and between 0-20 μg Zn. Consequently, the following matrix was established for the simultaneous determination of Fe (0-30.0 μg) and Zn (0-20.0 μg):

$$\begin{pmatrix} 0.0093 & 0.0200 \\ 0.0074 & 0.0358 \end{pmatrix} \begin{pmatrix} x_{Fe} \\ x_{Zn} \end{pmatrix} = \begin{pmatrix} \Delta A_1 - 0.447\Delta A' \\ \Delta A_1 - 0.447\Delta A' \end{pmatrix}$$

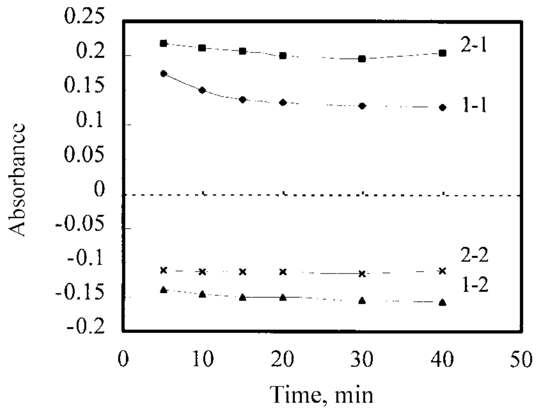


Figure 6. Effect of the reaction time: 1-1 ΔA of Fe(20 μg)-BBTAN solution at 540 nm and 2-1 ΔA of Zn(10 μg)-BBTAN solution at 580 nm, 1-2 ΔA′ of Fe(20 μg)-BBTAN solution at 460 nm and 2-2 ΔA′ of Zn(10 μg)-BBTAN solution at 460 nm, all against reagent blank.

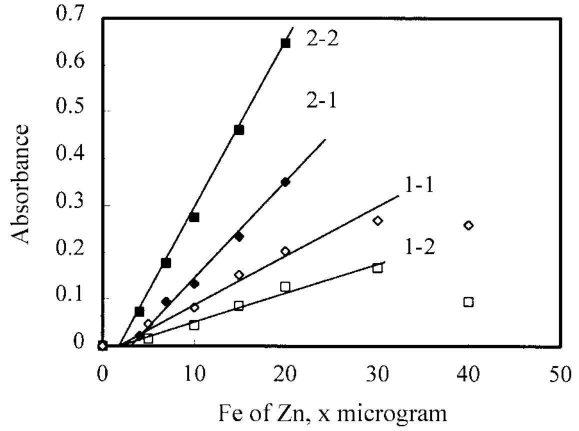


Figure 7. Standard curves for the determination of Fe(II) and Zn(II) at pH 8.5 and in the presence of triton x-100: 1-1- A_β of Fe-BBTAN at 540 nm, 1-2- A_β of Fe-BBTAN at 580 nm, 2-1- A_β of Zn-BBTAN at 540 nm, 2-2- A_β of Zn-BBTAN at 580 nm.

Table 2. The simultaneous determination of Fe and Zn in three samples by the spectral correction matrix (*: mean of two determinations by AAS)

Sample	Added: Fe + Zn	Found: Fe + Zn	Recovery, %: Fe + Zn
Water, mg/l	0 + 0	0.368 + 0.092	
		0.355 + 0.115	
		0.362 + 0.108	
		aver. 0.362 + 0.105	
		RSD: 1.8% + 11%	
		0.349 + 0.099 (by AAS)*	
	0.200 + 0.100	0.561 + 0.196	99.5 + 91.0
		0.569 + 0.199	103 + 93.0
Hair, mg/g	0 + 0	0.084 + 0.030	
		0.076 + 0.032	
		0.078 + 0.031	
		aver. 0.079 + 0.031	
		RSD: 5.3% + 5.1%	
		0.083 + 0.028 (by AAS)*	
	0.100+ 0.050	0.188 + 0.083	109 + 104
		0.171 + 0.077	92.0 + 92.0
Tobaco, mg/g	0 + 0	0.180 + 0.107	
		0.174 + 0.118	
		0.170 + 0.108	
		0.159 + 0.121	
		0.159 + 0.093	
		aver. 0.168 + 0.109	
		RSD: 5.5% + 10%	
		0.149 + 0.111 (by AAS)*	
	0.100 + 0.100	0.274 + 0.203	106 + 94.0
		0.263 + 0.211	94.0+ 102

Precision and Detection Limit. Six replicate determinations of both standard solutions were made: one containing 5.00 μg of Fe and 3.00 μg of Zn and the other containing 15.0 μg of Fe and 10.0 μg of Zn. The relative standard deviations (RSDs) were 6.4, 5.3% and 5.5, 3.4%, respectively. However, the RSDs from ordinary spectrophotometry were all more than 16.4%. Therefore, the precision from the spectral correction technique was better than that from ordinary spectrophotometry.

The formula $\mathbf{L_{min} = kS_b/S}$ was used to calculate the detection limit of Fe and Zn**.** Twenty replicate determinations of the reagent blank (none containing Fe and Zn) showed the detection limit: 1.2 μg of Fe and 0.3 μg of Zn in 25-mL of solution.

Effect of Foreign Ions. Once the masking reagent was added and the recommended procedure was carried out, none of the following ions affected the simultaneous determination of 5 μg of Fe and 5 μg of Zn (<10% error): 1 mg of Cl^-, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, NO_3^-, NH_4^+, K(I), Na(I), Ca(II), Mg(II); 100 μg of Al(III), Sn(II), Pb(II), V(V), Sb(III), Be(II), Mo(VI), Cr(III), I^-, F^-, PO_4^{3-} and 10 μg of Cu(II), Co(II), Ni(II), Cd(II), Mn(II), Hg(II). The heavy metals, such as Hg(II), Cd(II), Ni(II), Cu(II), are hardly present in natural water, drink and food. Therefore, they will not affect the direct determination of Fe and Zn.

Sample Analyzed. As a test of the method, both Fe and Zn in water, hair and tobacco were determined simultaneously. The results are listed in Table 2. We observed that the simultaneous determination of Fe and Zn gives satisfactory recovery: Fe 92.0-109% and Zn 91.0-104% and acceptable precision (RSD): Fe 5.5% and Zn 11%. In order to confirm the detected results obtained by the recommended method, we determined Fe and Zn in the above samples by AAS. The results are listed in Table 2, too. The data obtained by the two methods are identical.

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References

1. Castillo, G. M.; Thibert, R. J.; Seudeal, N. D. *Microchem. J.* **1988**, *37*, 99.
2. Kanetake, T.; Otomo, M. *Anal. Sci.* **1988**, *4*, 411.
3. Gao, H. W.; Zhang, P. F. *Analyst* **1994**, *119*, 2109.
4. Gao, H. W. *Talanta* **1995**, *42*, 891.
5. Gao, H. W.; Shi, H. L. *Zhur. Anal. Khim.* **1997**, *52*, 1152.
6. Gao, H. W.; Zhang, P. F. *Zavod. Lab*. **1998**, *65*, 4.
7. Tikhonov, V. N. *Zhur. Anal. Khim.* **1975**, *30*, 1501.
8. Likussar, W. *Anal. Chem.* **1973**, *45*, 1926.
9. Laearev, A. I. *Zavod. Lab*. **1975**, *41*, 534.
10. Watanabe, H.; Ohmori, H. *Talanta* **1979**, *26*, 959.
11. Valencia, M. C.; Boudra, S.; Bosque-Sendra, M. *Analyst* **1993**, *118*, 1333.
12. Gao, H. W. *Zhur. Anal. Khim.* **1999**, *54*, 941.