Cu(II)-3-(5-Chlor-2-hydroxy-3-sulfophenylazo)-6-(2,4,6-tribromophenylazo)-4,5-dihydroxynaphthalene-2,7-disulfonic Acid-Co(II) Binuclear Complexation and Its Application to the Selective Determination of Coblat at ng/ml Level

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The chromophore, 3-(5-chlor-2-hydroxy-3-sulfophenylazo)-6-(2,4,6-tribromophenylazo)-4,5-dihydroxynaphthalene-2,7-disulfonic acid (CSTDD) was used to complex Cu(II) and Co(II) in aqueous solution at pH 9.43. A binuclear complex of Cu-CSTDD-Co was formed and showed a high selectivity for the determination of Co(II). The spectral correction technique was applied to characterize the complexes. The results showed the formation of complexes of Cu(CSTDD), Co(CSTDD)$_3$ and Cu$_2$(CSTDD)$_2$Co. The quantitative analysis of Co(II) at ng/ml level was carried out by the light-absorption ratio variation approach (LARVA). The results showed that the technique is satisfactory to determine Co(II) at trace level in water samples with a detection limit of 2.3 ng/ml.

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Introduction

Many analytical techniques, *e.g.* atomic absorption spectrophotometry, ICP-EMS, electrochemical methods, spectrophotofluorimetry, and chromomatography, have been developed to monitor heavy metals at trace level. The light-absorption spectrometry is one of such techniques that has been extensively applied for trace metal analysis particularly in developing countries, due to low costs of instruments and their maintenance. In addition, such spectrometry has also been used in many studies *e.g.* organic structural characterization, molecular interaction, molecular biology and toxicology because of its easy operation.

For analysis of heavy metals at trace level in water samples, many chromophores with high sensitivity and selectivity have been synthesized[1,2] and new approaches and calculations, *e.g.* H-point standard-additions,[3] chemometrics[4,5] have been developed. The complexation of metal-chromophore is the most common reaction in detecting trace heavy metals. Although formations of binuclear complexes have been reported recently in the literature,[6,7] only a few are found to form metal-metal binuclear complexes,[8,9] particularly in trace metal analysis.

Chromophore 3-(5-chlor-2-hydroxy-3-sulfophenylazo)-6-(2,4,6-tribromophenylazo)-4,5-dihydroxynaphthalene-2,7-disulfonic acid (CSTDD) was synthesized in 1989,[10] and soon was used to determine trace scandium in steel samples.[11] In addition, CSTDD is one of the asymmetric chromotropic-acid azo derivatives; and it has a high light absorptivity coefficient with ε of 1.09×10^4 l mol^{-1} cm^{-1} at 577 nm in aqueous medium at pH 9.43. Due to interactions with many transition metals *e.g.* Fe(II, III), Ni(II), Cu(II), Zn(II), Cd(II), Mn(II), V(V) and Co(II), CSTDD has a poor selectivity in reacting with transition metals. However, when a new chromophore, *e.g.* Cu(CSTDD) formed by reaction of CSTDD with Cu(II), is used to react with Co(II), the sensitivity of determination of Co(II) increases due to the presence of excess of Cu(II) in the ion aqueous solution.

In the present work, we fully studied the binuclear complexation of CSTDD with Cu(II) and its application in determination of Co(II) in water samples. The complexes of Cu-CSTDD, Co-CSTDD and Cu-CSTDD-Co were characterized by the spectral correction technique.[12,13] The results showed the formation of complexes of Cu(CSTDD), Co(CSTDD)$_3$ and Cu$_2$(CSTDD)$_2$Co at pH 9.43 in aqueous medium. The molar absorbance coefficients of the complexes were obtained by calculation as follows: $\varepsilon^{613\text{nm}}_{\text{Cu(CSTDD)}} = 1.45 \times 10^4$, $\varepsilon^{649\text{nm}}_{\text{Co(CSTDD)3}} = 2.98 \times 10^4$, $\varepsilon^{684\text{nm}}_{\text{Cu2(CSTDD)2Co}} = 2.65 \times 10^4$ and $\varepsilon^{634\text{nm}}_{\text{Cu2(CSTDD)2Co}} = 2.64 \times 10^4$ l mol^{-1} cm^{-1}. Also, we found that CSTDD can react simultaneously with Cu(II) and Co(II) at pH 9.43 to form a metal-metal binuclear complex, resulting in an increase in selectivity in determination of Co(II) in water samples. In order to improve the sensitivity in determination of trace Co(II) bound to binuclear complex of Cu(CSTDD), a new technique named light-absorption ratio variation approach (LARVA)[14] was applied. Such binding as has been confirmed by experiments greatly increases sensitivity and high selectivity in determination of Co(II) in water samples.

Theories

Description of spectral correction technique[12,13]

Complexation of a metal (M) and a ligand (L) often occurs during analysis of the metals by light-absorption spectrometry.

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The reaction equilibrium is expressed in the following equation:

$$\begin{array}{llcll} & N\mathbf{L} & + & \mathbf{M} \rightleftharpoons & \mathbf{ML}_N \\ \text{Initial state} & C_{L0} & & C_{M0} & 0 \\ \text{Corresponding to} & A^L_{\lambda1} \text{ and } A^L_{\lambda2} & & & \\ & & & & \\ \text{Equilibrium} & C_L & & \sim 0 & C_{M0} \\ \text{Corresponding to} & (1-\eta)A^L_{\lambda1} \text{ and } (1-\eta)A^L_{\lambda2} & & & A_c \end{array}$$

where C_{L0} and C_{M0} are the initial molarities of L and M; η is the effective fraction of L; A_c is the real absorbance of ML complex at wavelength λ_2; $A^L_{\lambda1}$ and $A^L_{\lambda2}$ are the absorbances of L solution measured at wavelengths of λ_1 and λ_2 using water reference, N is the coordination number between L and M.

In order to complex M completely, an extra amount of L is generally added, resulting in a high fraction of L in solution. On the other hand, the reaction sensitivity is direct ratio to the fraction of L, usually with a high light-absorption. Recently, a great numbers of chromophores with big conjugate planes have been synthesized and applied to detect trace metals in different aqueous samples. However, due to influences of redundant L in measurements of light-absorption of ML complex, the technique has a great inevitable analytical error and thus is restricted in practical applications. This problem must be solved before the technique can be practically used to determine trace metals in aqueous samples. The spectral correction technique has shown a specific advancement because it may eliminate the effect of the light-absorption caused by excess L in the ML reaction solution. Absorbances of color components including the reactants and products may be either quantitatively or qualitatively measured. Thus, not only the absorbances of all components in solution need to be clearly distinguished but also the complex of ML needs to be characterized. The equations for the calculation of constants involved in the reactions are given as follows:

$$A_c = \frac{A_{\lambda2} - \beta A_{\lambda1}}{1 - \alpha\beta} \tag{1}$$

where

$$\beta = \frac{A^L_{\lambda2}}{A^L_{\lambda1}} \tag{2}$$

and

$$\alpha = \frac{A^{ML}_{\lambda1}}{A^{ML}_{\lambda2}} \tag{3}$$

and

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

where

$$\eta = \frac{A_c - A_{\lambda2}}{A^L_{\lambda2}} \tag{5}$$

where β and α are the correction constants; γ is the complexation number between L and M, which increases with an increase of molar ratio of L to M and has been applied to identify the composition of Cu-CSTDD and Co-CSTDD complexes and the Cu-CSTDD-Co binuclear complex in this study; and $A_{\lambda1}$ and $A_{\lambda2}$, $A^{ML}_{\lambda1}$ and $A^{ML}_{\lambda2}$ are the absorbances of reaction of M with L and of ML complex formed in solution without free L measured at λ_1 and λ_2 respectively using deionized water as a reference.

LARVA[14]

The equations of LARVA are given as follows:

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

or

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

where

$$\Delta A_r = A_r - A_{r0} = \frac{A_{\lambda2}}{A_{\lambda1}} - \frac{A^L_{\lambda2}}{A^L_{\lambda1}} \tag{8}$$

where $A_{\lambda1}$, $A_{\lambda2}$, $A^L_{\lambda1}$ and $A^L_{\lambda2}$ are the same as described in Eqs. (1) - (5) above; ΔA_r is the difference of absorbance ratio; C'_{M0} is the initial concentration of M but is much lower than C_{M0}. All p', q', p and q are constants when λ_1, λ_2 and reaction conditions are selected. Thus Eqs. (6) and (7) can be used to determine trace metals quantitatively. As given in Eq. (6) above, p' is the factor of sensitivity and is the inverse reverse ratio of C_{L0}. Therefore, the less that L is added, the higher the analytical sensitivity that is obtained. However, too low in a value of L can cause a great error of measurement because of the noise of instrument background. Proper concentrations of L light-absorption are suggested with their absorbances between 0.01 and 0.1. LARVA is different from the previous similar methods.[15]

Experimental

Apparatus and reagents

The absorption spectra of CSTDD and its metal complexes were recorded on Perkin-Elmer Model Lambda-25 spectrometer. The spectrometer was computer controlled using a UV WinLab software (Version 2.85.04). A supersonic cleaner of Model KQ318T (Kunshan Analytical Instruments, China) was used to dissolve CSTDD completely in deionized water. The pH of the solution was measured on a Model pHS-25 meter (Shanghai Precise Sci. Instruments, China). A water-circulated thermostatic oven of Model TS-030 (Shanghai Yiheng Sci. Technol. Co. China) was used for the binuclear complexation of Cu-CSTDD-CO. The diluted solutions of Cu(II), Co(II) and CSTDD were kept in a refrigerator freezer of Model BCD-196 (Meiling Productions of Anhui Province, China).

Standard solutions of 1000 mg/l Cu(II) (GSB07-1257-2000) and 1000 mg/l Co(II) (GSB07-1255-2000) were purchased from Beijing Kanglin Sci. Technol. Co., where the solutions were made by the Institute for Reference Materials of SEPA, Beijing, China. Solutions of 10.00, 1.00 mg/l Co(II) and 10.00 mg/l Cu(II) were prepared by diluting the standard solutions and were kept at a temperature < 5˚C before using. CSTDD was purchased from Shanghai Changke Reagents Institute (content 50%) and a solution of 0.1435 mmol/l CSTDD was prepared by dissolving 0.0644 g of CSTDD completely in 100 ml of acetone. The solution then was diluted to 250 ml with deionized water. The solution was kept at a temperature < 5˚C

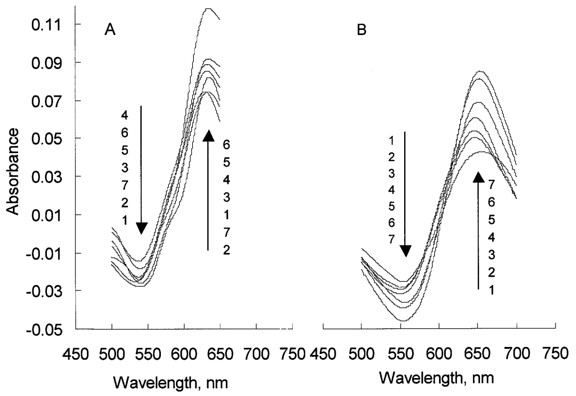

Fig. 1 Effect of pH on absorption spectra of (A) Cu-CSTDD complex in solution containing 0.01435 mmol/l CSTDD and 1.00 mg/l Cu(II), (B) Co-CSTDD complex in solution containing 1.00 mg/l Co(II), with corresponding pH labeled from 1 to 7: 8.13, 8.65, 9.00, 9.24, 9.43, 10.00 and 10.60, respectively.

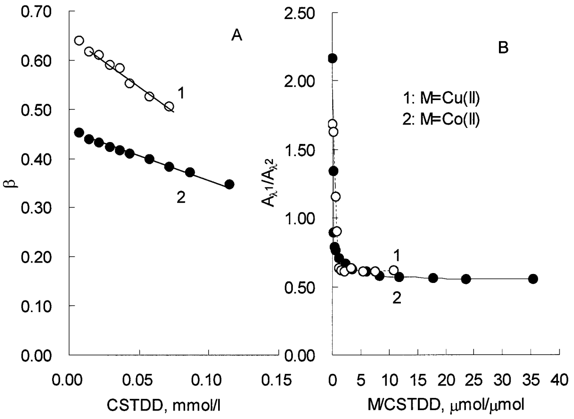

Fig. 2 (A) Variation of β with CSTDD molarity, (B) variation of the absorbance ratio of (1) Cu-CSTDD with the molar ratio of Cu (M = Cu(II)) to CSTDD at pH 9.43, (2) Co-CSTDD with the molar ratio of Co (M = Co(II)) to CSTDD at pH 9.43.

before using.

The Cu-CSTDD chromophore of 0.07175 mmol/l was prepared by mixing 50.0 ml of 0.1435 mmol/l CSTDD, 2.50 mg of Cu(II) and 10 ml of pH 9.43 buffer solution thoroughly. The solution was then diluted into 100 ml with deionized water and kept in dark at a temperature < 5°C before using.

Ammonia buffer solutions from pH 8.13 to 10.60 were prepared and were used to adjust the pH of the solution in order to optimize the complexation conditions.

General procedures

Determination of composition of complexes. One ml of pH 9.43 buffer was added into the solutions of 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00 and 6.00 ml of 0.1435 mmol/l CSTDD in the 10-ml calibrated flasks. The solutions in the flasks were diluted to 10 ml with deionized water and mixed thoroughly. Optical measurements of the solutions were performed with 2 ml optic cells at room temperature (20°C) by using deionized water as a reference. Solutions of 10.0 μl of 200 mg/l Cu(II) and 10.0 μl of 100 mg/l Co(II) were added into two separate sets of the solutions described above. After mixing for 5 min, they were measured on the spectrophotometer in the same way as described above at wavelengths of 634.5 and 540 nm for Cu-CSTDD solutions and at 651 and 556.5 nm for Co-CSTDD solutions. A_c, η and γ were obtained by calculation according to Eqs. (1), (5) and (4) respectively.

Similarly, 1 ml of pH 9.43 buffer was added into the solutions of 0.50, 1.00, 2.00, 3.00, 4.00, 5.00, 7.00 and 9.00 ml of 0.07175 mmol/l Cu(CSTDD) complex in eight of 10 ml flasks. The solutions were diluted to 10 ml with deionized water and were mixed for 2 min. A 5.00 ml portion of the solution was transferred into pre-cleaned and dried flasks and then 20.0 μl of 100 mg/l Co(II) solution was added. After mixing well, the solutions were settled in a water bath at a constant temperature of 60°C for 60 min before they were cooled on the laboratory desktop until room temperature. The absorbances of the Cu-CSTDD-Co dinuclear complex and a reference without Co(II) were measured in the same way described above at wavelengths of 684 and 558.5 nm. A_c, η and γ were obtained by calculation according to Eqs. (1), (5) and (4), respectively.

Determination of Co(II) in water samples. About 5.00 ml of a water sample was added into a 10-ml flask containing 1 ml of pH 9.43 buffer solution and 0.500 ml of 0.07175 mmol/l Cu-CSTDD. After diluting to 10 ml and mixing well, the sample was kept in the water bath at 60°C for 30 min before it was cooled until room temperature. The absorbances ($A_{684\text{nm}}$ and $A_{558.5\text{nm}}$) were measured at 684 and 558.5 nm by using deionized water as a reference. A reagent blank was prepared and measured in the same procedure as the water sample described above. Thus, ΔA_r is obtained by calculation according to the relation given in the following equation:

$$\Delta A_r = \frac{A_{684\text{nm}}}{A_{558.5\text{nml}}} - \frac{A^L{}_{684\text{nm}}}{A^L{}_{558.5\text{nm}}} \tag{9}$$

Thus, concentrations of Co(II), C_{Co}, in water samples were obtained by calculation according to Eq. (6) or (7).

Results and Discussion

pH dependence

The spectra of CSTDD, Cu-CSTDD and Co-CSTDD solutions at different pH valuesare shown in Fig. 1. It is evident, as shown in Fig. 1 (A), that the formation of Cu-CSTDD is sensitive to pH changes in basic solution since the spectra have a great increase in intensity from pH 10.0 to 10.60. However, the formation of Cu-CSTDD is less sensitive at pH values between 8.13 and 10.0. A similar phenomenon is observed in the formation of Co-CSTDD complex as shown in Fig. 1 (B). Therefore, the buffer solution of pH 9.43 was selected in the present work. The wavelengths at 634.5 nm and 540 nm are used to characterize Cu-CSTDD complex, since the absorption peak and the valley position as shown in Fig. 1 (A) are located at two wavelengths in the spectrum at pH 9.43. Similarly, wavelengths of 651 and 556.6 nm are also used to characterize the Co-CSTDD complex.

Determination of spectral correction constants (α and β)

Figure 2(A) shows the results of absorbance ratio of the solutions with different CSTDD molarity measured at 540, 556.5, 634.5 and 651 nm, respectively, using deionized water as a reference, where β decreases with an increase of CSTDD molarity, suggesting that self-aggregation of CSTDD molecules has occurred at pH 9.43. Figure 2(B) shows the absorbance ratios of the complexation solutions containing different molarity values of Cu(II) and Co(II) but a constant molarity of

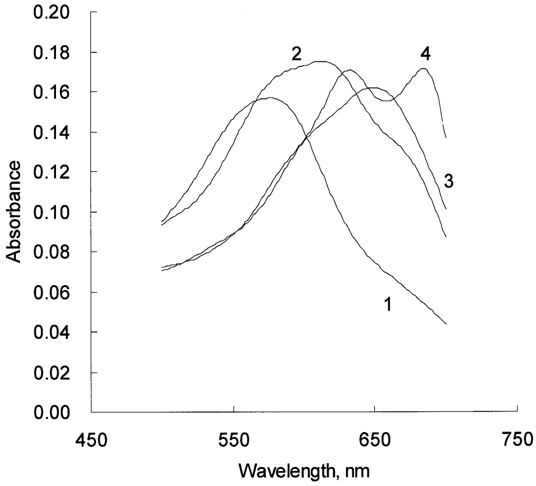

Fig. 3 Absorption spectra, recorded at room temperature, of CSTDD, Cu-CSTDD complex, Co-CSTDD complex and Cu-CSTDD-Co binuclear complex in corresponding solutions containing: (1) 0.01435 mmol/l CSTDD; (2) 0.01435 mmol/l Cu(CSTDD); (3) 3.00 mg/l Co(II) and 0.01435 mmol/l CSTDD, and (4) 0.01435 mmol/l Cu(CSTDD) and 3.00 mg/l Co(II) at pH 9.43.

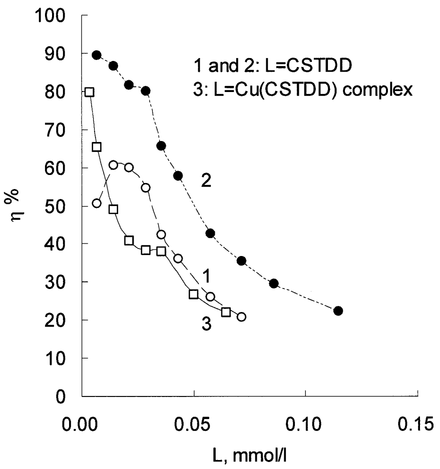

Fig. 4 Variation of η with CSTDD and Cu(CSTDD) complex molarity in the corresponding solutions containing: (1) 1.00 μg/ml Cu(II), L = CSTDD; (2) 0.500 μg/ml Co(II), L = CSTDD and (3) 0.400 μg/ml Co(II), L = Cu(CSTDD) complex.

CSTDD. Curve 1 in Fig. 2 (B) shows that $A_{540\text{nm}}/A_{634.5\text{nm}}$ decreases rapidly with an increase of Cu(II) when Cu(II) molarity is smaller than the CSTDD molarity. However, the ratio stabilizes at a constant number of 0.610 when the molarity of Cu(II) is 2 times greater than the CSTDD molarity, indicating that all CSTDD has been converted into Cu(CSTDD) complex when the molar ratio of Cu(II) to CSTDD is over 2. Therefore, the number of α (0.610) is obtained for the Cu-CSTDD complex. Similarly, the number of α (0.553) can be estimated from curve 2 in Fig. 2(B).

Composition of complexes

Figure 3 shows the spectra of CSTDD, Cu-CSTDD, Co-CSTDD and Cu-CSTDD-Co. Spectra 4 and 3 in Fig. 3 show differences in absorption pattern, suggesting the formation of a new Cu-CSTDD-Co binuclear complex and no replacement of Cu(II) in Cu-CSTDD by Co(II) when enough Co(II) is added to Cu-CSTDD solution. In addition, a red shift of 36 nm from CSTDD at 577 nm to Cu-CSTDD complex at 613 nm shown in the spectra 1 to 3 is observed. Also, a bigger red shift of 72 nm from CSTDD at 577 nm to Co-CSTDD complex at 649 nm is evident, suggesting that Co(II) may have a stronger influence on the big conjugate π electron atmosphere of CSTDD than Cu(II) due to the disturbance caused by complexation. This may also imply that Cu and Co are coordinated into different complexing positions. The peak at 634 nm in spectrum 4 is derived from the spectral red shift from the Cu-CSTDD complex to Co(II) complexation, while the right one is because of the red shift from the Co-CSTDD complex to Cu(II) complexation, suggesting formation of Cu-CSTDD-Co binuclear complex. In order to determine A_c, η and γ in the binuclear solution, we selected the wavelengths of 684 and 558. 5 nm because of the maximal interval between spectra 2 and 4. Thus, an α value of 0.549, the spectral correction constant of binuclear complex is obtained by calculation from spectrum 4 and β of 0.687, the Cu(CSTDD) complex, is obtained from spectrum 2.

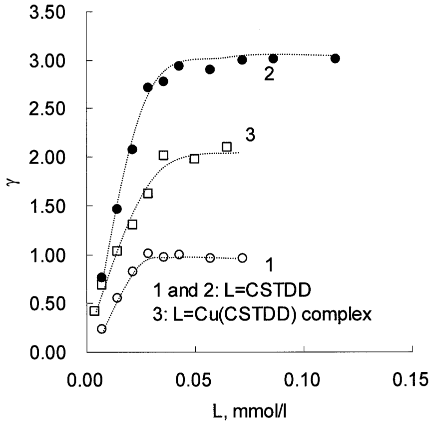

Fig. 5 Variation of γ with CSTDD and Cu(CSTDD) complex molarity in the corresponding solutions: (1) 1.00 μg/ml Cu(II), L = CSTDD; (2) 1.00 μg/ml Co, L = CSTDD and (3) 0.400 μg/ml Co(II), L = Cu(CSTDD) complex.

The absorbances for a series solutions with various CSTDD molarities but a constant molarity of Cu(II) were measured at 540 and 634.5 nm using the corresponding reagent without Cu(II) as a blank. All A_c, η and γ in the Cu-CSTDD solutions obtained by calculation according to Eqs. (5) and (4) are shown in Figs. 4 and 5 respectively. As shown in curve 1 in Fig. 4, η increases from about 50% to 60% and then decreases with an increase of CSTDD; η drops 20% when CSTDD is greater than 0.05 mmol/l, indicating that over 70% CSTDD does not react with Cu(II). Curve 2 in Fig. 4 shows that η decreases from 90% with an increase of CSTDD and drops to less than 50% when the molarity of CSTDD is greater than 0.05 mmol/l, indicating that over half of the CSTDD does not react with Co(II). Similarly, curve 3 shows that η decreases from 80% with an increase of the Cu(CSTDD) complex chromophore molarity and is less than 30% when the molarity of CSTDD is greater than 0.05 mmol/l, indicating that over 70% of Cu(CSTDD) complex has not reacted with Co(II). It is clear that excessive chromophores of [CSTDD and Cu(CSTDD)] influence the measurement of absorbance because both the reactant and the product [CSTDD and Cu(CSTDD)] have an additive light-absorption. It is difficult for conventional spectrophotometry to measure the light-absorption fraction of an individual chromophore in the solution. In order to solve the problem, the

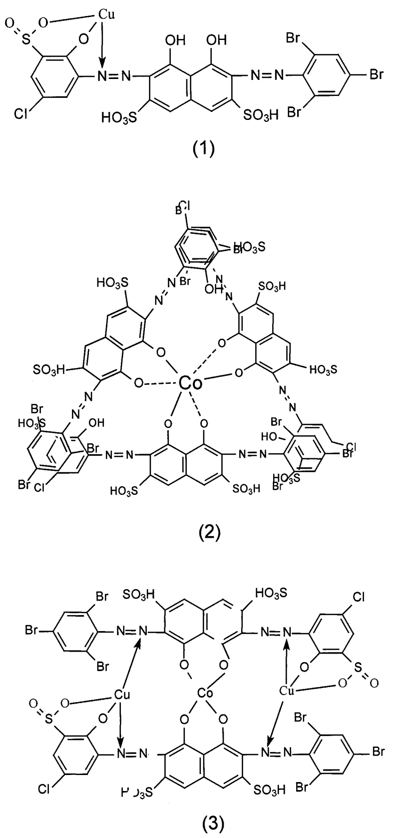

Fig. 6 The possible structure s of complexes: (1) Cu(CSTDD), (2) $Co(CSTDD)_3$, and (3) $Cu_2(CSTDD)_2Co$.

spectral correction technique was thus applied in the present paper.

Figure 5 shows that all γ increase with an increase of molarity of CSTDD and Cu(CSTDD) complex and approach to a maximum of 1 in Cu-CSTDD solution, 2 in the Cu(CSTDD)-Co solution, and 3 in the Co-CSTDD solution when molarities of chromophores are greater than 0.035 mmol/l. Figure 5 shows that the maximum coordination ratio (N) of CSTDD to Cu(II) is 1:1, that N of Cu(CSTDD) to Co(II) is 2:1, and that N of CSTDD to Co(II) is 3:1. It is clear that two complexes of Cu(CSTDD) and $Co(CSTDD)_3$ are formed at pH 9.43 and so is the binuclear complex, where the complexing reaction can be expressed as below:

$$Co^{2+} + 2[Cu(CSTDD)]^- = [Cu_2(CSTDD)_2Co]$$

The possible coordination structures of the three complexes are sketched in Fig. 6. As shown in sketch 1, Cu(II) is bound to oxygen and nitrogen ligands at the right side of CSTDD and so the perturbation of the conjugate π electron atmosphere due to complexation is little. Thus, the spectral red shift caused by formation of Cu-CSTDD complex is about 36 nm from CSTDD at 557 nm to Cu-CSTDD at 613 nm, slightly greater than that of CSTDD. From sketch 2, we can see that that three CSTDDs are overlapped binding of Co(II) to oxygen ligands in CSTDD, giving the hydrogen and hydrophobic bonds between CSTDD molecules. The perturbation due to conjugate plane is so intensified that the spectral red shift of the Co-CSTDD complex is increased to over 70 nm with a high ε value, as described above. Also it is clearly shown in sketch 3 that Co(II) coordinates two Cu(CSTDD) only because of a sterric effect,

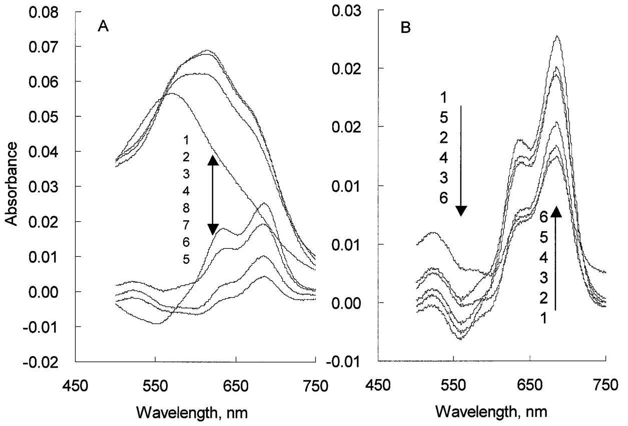

Fig. 7 (A) Effect of temperature on the absorption spectra of 0.007175 mmol/l Cu(CSTDD) complex using water reference (curves 1 – 4) and its 0.400 mg/l Co(II) solution against using reagent reference without Co(II) (curves 5 – 8). Curves 1 – 4 and 5 – 8 correspond to the temperature: 20, 40, 60 and 100°C, respectively. (B) Effect of the reaction time on the absorption spectra of Co-Cu-CSTDD in solution containing 0.007175 mmol/l Cu(CSTDD) complex and 0.400 mg/l Co(II) at 60°C using reagent reference without Co(II). Curves 1 – 6 corresponds to the reaction time: 5, 10, 20, 30, 40 and 60 min, respectively.

which causes a high ε value due to its wide conjugate electron plane.

In addition, the apparent molar absorptivity coefficients (ε) of the complexes were obtained by calculation according to the relation given in the following equation:

$$\varepsilon = A_c/(\delta C_M) \tag{10}$$

where δ is the optical path in 1 cm. The results are given as below: $\varepsilon^{613nm}_{Cu(CSTDD)} = 1.45 \times 10^4$, $\varepsilon^{649nm}_{Co(CSTDD)3} = 2.98 \times 10^4$, $\varepsilon\varepsilon^{684nm}_{Cu2(CSTDD)2Co} = 2.65 \times 10^4$ and $\varepsilon^{634nm}_{Cu2(CSTDD)2Co} = 2.64 \times 10^4$ l mol^{-1} cm^{-1} in aqueous medium at pH 9.43. The technique used in the present paper is sensitive and has been applied to determine selectively trace amounts of Co(II) in water samples due to formation of binuclear complexes with their molar absorptivity coefficients.

Effects of temperature and reaction time on binuclear complexation

The absorption spectra of the binuclear complex recorded at different temperatures (A) and the different reaction time (B) are shown in Fig. 7. In comparison, it is evident that the intensity of the spectra (from 1 to 3) decreases with an increase of temperature and that the pattern of spectrum 4 changes when the temperature rises over 60°C, suggesting that Cu(CSTDD) complex become less stable with an increase of temperature. On the other hand, the intensities of light-absorption spectra from 5 to 8 decreases with a decrease in temperature, suggesting that the formation of binuclear complex is slow at low temperature. A constant temperature of 60°C was used in order to ensure completion of the binuclear complexation. From the spectra in Fig. 7(B) one can observe that the interval between peak at 684 nm and valley at 558.5 nm increases with an increase in the reaction time period from 30 to 40 min. In addition, it was found that the binuclear complexation needed at least 5 h to complete at room temperature but needed only 1 h at 60°C. Therefore, two wavelengths: at 684 and 558.5 nm were

Table 1 Regression equations and detection limit of Co(II)

Series	0.07175 mmol l^{-1} Cu(CSTDD)/ml	Linear scope, µg Co(II)/10 ml	Calibration graph	R^a	σ^b	DL^c/ng ml^{-1}
1	0.500	0 - 0.30	$\Delta A_r = 0.416C_{Co} - 0.0033$	0.9983	0.0032	2.3
2	1.00	0 - 2.00	$\Delta A_r = 0.1950C_{Co} + 0.0145$	0.9968	0.0062	9.5
3	1.50	0 - 2.00	$\Delta A_r = 0.1532C_{Co} + 0.0093$	0.9952	0.0074	14.5

a. Linear correlation coefficient. b. Standard deviation of 8 repetitive reagent blanks. c. Detection limit of Co(II) was calculated by $DL = 3 \times \sigma \times p$.

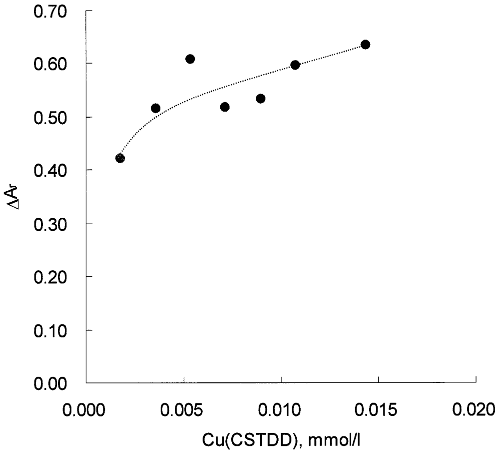

Fig. 8 Effect of molarity of Cu(CSTDD) on ΔA_r of Cu(CSTDD)-Co(II) in the solution where Co(II) was added at a constant ratio at pH 9.43.

Table 2 Effect of foreign ions on ΔA_r of the solutions containing 1.00 µg of Co(II)

No.	Foreign ion	Added, µg/10 ml	$Error^a$, %
1	Co(II)	1.00	
2	$Zn(II)^b$	5.00	1.5
3	Cr(III)	5.00	–6.0
4	Pb(II)	5.00	1.76
5	Ni(II)	5.00	3.86
6	Cd(II)	2.00	0.68
7	Mn(II)	5.00	3.65
8	Al(III)	5.00	0.73
9	Fe(II)	5.00	–13
10	Fe(III)	2.50	–9.6
11	Mg(II)	5.00	3.0
12	Ca(II)	20.0	2.5
13	Mo(VI)	2.00	2.8
14	As(III)	1.00	6.4
15	V(V)	2.00	10.6
16	Sn(II)	5.00	1.5
17	SDS^c	5.00	5.0
18	BSA^d	10.0	6.6

a. Error = $(\Delta A_r^{No.\ x} - \Delta A_r^{No.\ 1})/\Delta A_r^{No.\ 1} \times 100$ (x is from 2 to18). b. Added 1.00 µg of Co(II) into all the solutions from No. 2 to18. c. Sodium dodecyl sulfate. d. Bovine serum albumin.

used in the absorbance measurement. The binuclear complexation should be stopped in 30 min at 60°C before measurement of absorbance is performed at room temperature.

Effect of Cu-CSTDD complex on ΔA_r

Figure 8 shows variations in differences of ΔA_r in the solutions with the constant ratio of Co(II) (2.50 µg) to Cu(CSTDD) (0.07175 µmol). ΔA_r increases when the solution becomes concentrated. Thus, the less of the Cu(CSTDD) complex solution that is added, the lower the amounts of Co(II) that is detected. Therefore, LARVA makes the determination of Co(II) with a high sensitivity when a small amount of chromophore is added. In the successive calibration experiments, three volumes of 0.500, 1.00 and 1.50 ml of 0.07175 mmol/l Cu(CSTDD) complex were added to react with Co(II) in order to optimize the addition of Cu(CSTDD).

Calibration graph and detection limit

Three standard Co(II) solutions with Co(II) concentrations from 0 and 1.00, 0 and 4.00 and 0 and 6.00 µg were prepared. 0.500, 1.00 and 1.50 ml of 0.07175 mmol/l Cu(CSTDD) complex were added into the solutions respectively. The binuclear reaction was performed at 60°C for 30 min according to the procedure described above. After the solution was cooled to room temperature, the absorbances of the solution were measured at 684 and 558.5 nm respectively using deionized water as a reference. ΔA_r of each solution and the detection limit of Co(II) were obtained by calculations according to Eq. (9) and are summarized in Table 1. It is found that they are linear at the concentration range given in Table 1. In comparison, series 1 has the least σ and the lowest detection limit of Co(II) with only 2.3 ng/ml. In order to determine ultra-trace metals, 0.500 ml of 0.07175 mmol/l Cu(CSTDD) complex was added into water samples.

Effect of foreign ions

Eighteen metals were added into the Co-Cu(CSTDD) complexation solution and their effects on ΔA_r are summarized in Table 2. Most of the metals give satisfactory results (error below 10%); the exceptions are Fe(II), Fe(III) and V(V). There is no evidence that the metals listed in Table 2 react with the Cu(CSTDD) complex. Because over 2 mg/l Cu^{2+} remains in the reaction flask, many metals *e.g.* Pb(II), Cd(II), Mn(II), Ni(III), Zn(II), Fe(II) do not affect the formation of binuclear complex of Co(II). In addition, a citrate was recommended to mask Fe(II, III) by addition into the reaction solution. Therefore, determination of Co(II) due to formation of the binuclear complex of Co-Cu(CSTDD) is highly selective at pH 9.43.

Analysis of water samples

Co(II) concentrations in three water samples from river, wastewater and municipal sewage were determined. The recovery of Co(II) added into the samples was estimated. The results are summarized and listed in Table 3. The concentrations of Co(II) in the samples are comparable to those reported in the literature (Ref.) and the recovery of Co(II) is found to be between 99.8 and 120% with the RSD less than 4.8%.

Table 3 Determination of Co(II) in water samples

Sample from	Co(II) added/ μg l^{-1}	Co(II) found/ μg l^{-1}	RSD, %	Rec., %
River	0	< 2.3[a]		
	5.00	6.02[b]		120[c]
Wastewater	0	54.3 ± 2.6[a]	4.8	
	25.0	79.5[b]		101[c]
Sewage	0	< 2.3[a]		
	5.00	4.99[b]		99.8[c]

a. Average of four replicated determinations. b. Average of two replicated determinations. c. For example, 101% = (79.5 – 54.3)/25.0 × 100

Conclusions

Like many other heavy metals, cobalt can be spectrophotometrically detected upon reacting with many organic compounds, *e.g.* with PAN,[16,17] 5-(6-methoxy-2-benzothiazoleazo)-8-aminoquiinoline,[2] pyrolidine,[18] 2-(2-quinolinylazo)-5-diethylaminophenol,[19] and 2-(2-benzothiazolyazo)-*p*-cresol,[20] which show a high sensitivity. However, complexation of metal-metal binuclear with a new chromophore is useful in determining trace metals with a high selectivity. Using LARVA and formation of binuclear complex play important roles in analyzing Co(II) at trace level in the water samples tested. In addition, the spectral correction technique is much more useful in characterizing complex composition and in determining the ε value of a metal complex with a chromophore than other conventional methods.

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