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DETERMINATION OF COPPER AT NG ML^{-1} LEVEL IN SURFACE WATER USING THE ELECTROPHILIC SUBSTITUTION REACTION BETWEEN CU(II) AND 3-(3-SULFOPHENYLAZO)-6-(4-CHLORO-2-PHOSPONOPHEN- YLAZO)-4,5-DIHYDROXYNAPHTHALENE-2,7-DISULFONIC ACID-CA(II) COMPLEX

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The chromophore, 3-(3-sulfophenylazo)-6-(4-chloro-2-phosponophenylazo)-4, 5-dihydroxynaphthalene- 2, 7-disufonic acid (MSCPA) was synthesized to complex Cu(II) and Ca(II) in aqueous solution at pH 6.47. Both the Cu(II)-MSCPA and Ca(II)-MSCPA complexes were characterized by spectral correction technique and the results have shown that complexes $Cu(MSCPA)_3$ and Ca(MSCPA) were formed. An interesting phenomenon was found that Cu(II) substituted Ca(II) of the Ca(II)-MSCPA complex. The competitive substitution complexation (CRC) has been applied to the detection of Cu (II) with good selectivity. The light-absorption ratio variation approach (LARVA), which produces an obvious increase of sensitivity, has been applied to detection of copper in trace level in surface water. The limit of detection of Cu(II) is only 2.0 ng mL^{-1}. The percentage of recovery of Cu(II) in samples and the experience compared with AAS both obtained satisfactory results.

INTRODUCTION

Copper is a kind of trace element that human body and animal need. The lack of a recommended dietary allowance for copper may be hazardous to human health.[1] For example, copper intake in the UK population is rarely above adequate levels, which is a matter of some concern, both in terms of public health and possible clinical consequences. [2] A form of chlorosis found by determination of copper in red blood cells is also due to copper deficiency. [3] Similarly, animals cannot live without copper, and copper as an essential trace element has been added into all kinds of new forages.[4,5] However, the ingestion of excessive copper may be detrimental to the health of human beings.[6] Copper excess has been regarded as leading to potential health problems especially for infants and children worldwide.[7] Plethoric copper has carcinogenic effect and can give rise to a form of liver cirrhosis in childhood.[8] The high consumption of copper inevitably leads to environmental pollution.[9] Widespread copper pipes also bring out some adverse effects on drinking water because some researches suggested that the presence of bacteria could contribute to copper dissolution and increased copper levels in domestic water systems.[10,11] Consequently, it is of great significance to detect trace amounts of copper in water.

Recently, a number of methods detecting trace copper have been proposed. For example, atomic absorption spectrometry (AAS), reversed-phase high performance liquid chromatography and kinetic catalytic method are presented for determination of copper in water.[12-14] Compared with these methods, the ultraviolet-visible spectrophotometry as a kind of conventional method has its own advantages such as simple operation, low-priced instrumentation, and wide owning all over the world. Especially, the development of light absorption ratio variation approach (LARVA) [15] increases the analytical sensitivity and expands the availability of spectrophotometry.

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The chromophore, 3-(3-sulfophenylazo)-6-(4-chloro-2-phosponophenylazo)-4, 5-dihydroxynaphthalene-2, 7-disufonic acid (MSCPA), was synthesized and used in detection of Th and Ce about twenty years ago.[16] Due to its poor selectivity, people conducted few researches with this chromophore later. In the present work, its novel usage was found. Trace amounts of Cu(II) can competitively substitute Ca(II) from the Ca-MSCPA complex at neutral medium. The combination of LARVA and the competitive substitution complexation (CRC) has been used in determination of trace amounts of Cu(II) in natural water with high sensitivity and good selectivity.

PRINCIPLE

1. Spectral Correction Technique[17]

A metal-ligand complexation is often used in the analysis of trace metals. The reaction of Cu/Ca with MSCPA is expressed as follows:

$$N\text{MSCPA} + \quad \text{Cu/Ca} \rightleftharpoons \text{Cu(MSCPA)}_N/\text{Ca(MSCPA)}_N$$

Initial state C_{MSCPA0} C_{Cu0}/C_{Ca0} *0*

Corresponding to $A_{\lambda 1}^{MSCPA}$ and $A_{\lambda 2}^{MSCPA}$

Equilibrium C_{MSCPA} *~0* C_{Cu0}/C_{Ca0}

Corresponding to *(1-η)* $A_{\lambda 1}^{MSCPA}$ and *(1-η)* $A_{\lambda 2}^{MSCPA}$ A_c where C_{MSCPA0} and C_{Cu0}/C_{Ca0} are the initial molarities of MSCPA and Cu/Ca, η is the effective fraction of MSCPA and N is the coordination number of MSCPA with Cu/Ca. The symbol A_c indicates the real absorbance of the Cu-MSCPA /Ca-MSCPA complex at wavelength λ_2. Both $A_{\lambda 1}^{MSCPA}$ and $A_{\lambda 2}^{MSCPA}$ are the absorbances of MSCPA solution measured at wavelengths: λ_1 and λ_2 against water reference. The complex reaction was characterized by the relations as below:

$$A_c = \frac{A_{\lambda 2} - \beta A_{\lambda 1}}{1 - \alpha\beta} \quad \text{.....(1)}$$

where

$$\beta = \frac{A_{\lambda 2}^{MSCPA}}{A_{\lambda 1}^{MSCPA}} \quad \text{..... (2)}$$

and

$$\alpha = \frac{A_{\lambda 1}^{Cu-MSCPA}}{A_{\lambda 2}^{Cu-MSCPA}} \quad \text{or} \quad \alpha = \frac{A_{\lambda 1}^{Ca-MSCPA}}{A_{\lambda 2}^{Ca-MSCPA}} \text{.....(3)}$$

and

$$\gamma = \eta \times \frac{C_{MSCPA0}}{C_{Cu0}} \quad \text{or} \quad \gamma = \eta \times \frac{C_{MSCPA0}}{C_{Ca0}} \quad \text{..... .....(4)}$$

where

$$\eta = \frac{A_c - A_{\lambda 2}}{A_{\lambda 2}^{MSCPA}} + 1 \quad \text{..... (5)}$$

where both β and α are the correction constants, γ is the complexation ratio of MSCPA to Cu/Ca. $A_{\lambda 1}$ and $A_{\lambda 2}$, $A_{\lambda 1}^{Cu-MSCPA}$ / $A_{\lambda 1}^{Ca-MSCPA}$ and $A_{\lambda 2}^{Cu-MSCPA}$ / $A_{\lambda 2}^{Ca-MSCPA}$ are the absorbances of the Cu-MSCPA/Ca-MSCPA solution and Cu-MSCPA /Ca-MSCPA complex solution without free MSCPA, respectively measured at λ_1 and λ_2 against water reference. So A_c, η and γ can be calculated by equations (1)-(5).

2. Development of the Absorbance Ratio Difference Spectrometry

The equations of absorbance ratio difference spectrometry[15] were established for the determination of trace amounts of Cu(II).

$$A_{\lambda 1} = A_{Ca-MSCPA1} + A_{Cu-MSCPA2}$$

and

$$A_{\lambda 2} = A_{Ca-MSCPA2} + A_{Cu-MSCPA2}$$

$$A_r = \frac{A_{\lambda 2}}{A_{\lambda 1}} = \frac{A_{Ca-MSCPA2} + A_{Cu-MSCPA2}}{A_{Ca-MSCPA1} + A_{Cu-MSCPA2}} = \frac{\delta C_{Ca-MSCPA} \varepsilon_{\lambda 2}^{Ca-MSCPA} + \delta C_{Cu0} \varepsilon_{\lambda 2}^{Cu-MSCPA}}{\delta C_{Ca-MSCPA} \varepsilon_{\lambda 1}^{Ca-MSCPA} + \delta C_{Cu0} \varepsilon_{\lambda 1}^{Cu-MSCPA}}$$

$$A_{r0} = \frac{A^0_{\lambda 2}}{A^0_{\lambda 1}} = \frac{\delta C_{Ca-MSCPA0} \varepsilon_{\lambda 2}^{Ca-MSCPA}}{\delta C_{Ca-MSCPA0} \varepsilon_{\lambda 1}^{Ca-MSCPA}}$$

$$\Delta A_r = A_r - A_{r0} = \frac{C_{Ca-MSCPA} \varepsilon_{\lambda 2}^{Ca-MSCPA} + C_{Cu0} \varepsilon_{\lambda 2}^{Cu-MSCPA}}{C_{Ca-MSCPA} \varepsilon_{\lambda 1}^{Ca-MSCPA} + C_{Cu0} \varepsilon_{\lambda 1}^{Cu-MSCPA}} - \frac{\varepsilon_{\lambda 2}^{Ca-MSCPA}}{\varepsilon_{\lambda 1}^{Ca-MSCPA}}$$

$$= \frac{(\varepsilon_{\lambda 1}^{Ca-MSCPA} \varepsilon_{\lambda 2}^{Cu-MSCPA} - \varepsilon_{\lambda 2}^{Ca-MSCPA} \varepsilon_{\lambda 1}^{Cu-MSCPA}) C_{Cu0}}{(C_{Ca-MSCPA0} - nC_{Cu0})(\varepsilon_{\lambda 1}^{Ca-MSCPA})^2 + C_{Cu0} \varepsilon_{\lambda 1}^{Cu-MSCPA} \varepsilon_{\lambda 1}^{Ca-MSCPA}}$$

$$\Delta A_r^{-1} = \frac{C_{Ca-MSCPA0} (\varepsilon_{\lambda 1}^{Ca-MSCPA})^2}{\varepsilon_{\lambda 2}^{Cu-MSCPA} \varepsilon_{\lambda 1}^{Ca-MSCPA} - \varepsilon_{\lambda 1}^{Cu-MSCPA} \varepsilon_{\lambda 2}^{Ca-MSCPA}} \cdot C_{Cu0}^{-1} + \frac{\varepsilon_{\lambda 1}^{Ca-MSCPA} (\varepsilon_{\lambda 1}^{Cu-MSCPA} - n\varepsilon_{\lambda 1}^{Ca-MSCPA})}{\varepsilon_{\lambda 2}^{Cu-MSCPA} \varepsilon_{\lambda 1}^{Ca-MSCPA} - \varepsilon_{\lambda 1}^{Cu-MSCPA} \varepsilon_{\lambda 2}^{Ca-MSCPA}}$$

$$\therefore \Delta A_r^{-1} = pC_{Cu0}^{-1} + q \qquad \text{.....(6)}$$

where $p = \frac{C_{Ca-MSCPA0} (\varepsilon_{\lambda 1}^{Ca-MSCPA})^2}{\varepsilon_{\lambda 2}^{Cu-MSCPA} \varepsilon_{\lambda 1}^{Ca-MSCPA} - \varepsilon_{\lambda 1}^{Cu-MSCPA} \varepsilon_{\lambda 2}^{Ca-MSCPA}}$

and $q = \frac{\varepsilon_{\lambda 1}^{Ca-MSCPA} (\varepsilon_{\lambda 1}^{Cu-MSCPA} - n\varepsilon_{\lambda 1}^{Ca-MSCPA})}{\varepsilon_{\lambda 2}^{Cu-MSCPA} \varepsilon_{\lambda 1}^{Ca-MSCPA} - \varepsilon_{\lambda 1}^{Cu-MSCPA} \varepsilon_{\lambda 2}^{Ca-MSCPA}}$.

The symbols $\varepsilon_{\lambda 1}^{\text{Ca-MSCPA}}$, $\varepsilon_{\lambda 2}^{\text{Ca-MSCPA}}$, $\varepsilon_{\lambda 1}^{\text{Cu-MSCPA}}$ and $\varepsilon_{\lambda 2}^{\text{Cu-MSCPA}}$ are the molar absorptivities of Ca-MSCPA and Cu-MSCPA complex at λ_1 and λ_2. n is the coordination number of MSCPA with Cu/Ca, δ is the optical length equal to the cell thickness and C_{Cu0} is the initial concentration of Cu(II). From Equation (6), Plots ΔA_r^{-1} vs. C_{Cu0}^{-1} is linear. Both p and q are constants when the wavelengths: λ_1 and λ_2 and the reaction conditions, such as the pH, temperature and reaction time, are fixed. Such a theoretical equation (6) can be directly used in the quantitative detection of trace amounts of Cu(II). From the theory of LARVA, the sensitivity factor p is the positive ratio to $C_{Ca\text{-}MSCPA0}$. Therefore, the less the chromophore Ca-MSCPA complex is added and the higher the analytical sensitivity goes and the lower the detection limit of a component goes by LARVA. However, too low chromophore will certainly bring out an obvious error because of the increase of instrument's noise.

EXPERIMENTAL

1. Apparatus

A model Lambda 25 (PerkinElmer Instruments, USA) Spectrometer, which was connected to a computer with UV Winlab software (version 2.85.04) installed, was used to record the adsorption spectra of the reaction solutions A model pHS-25 meter (Shanghai Precise Instruments, Shanghai, PRC) was used to adjust the acidity of the solutions. A model Aanalyst 600 (PerkinElmer Instruments, USA) graphite furnace atomic absorption spectrometer with Winlab 32 software and As-800 automatic injector installed was used to determine the amount of copper in water samples.

2. Reagents and Solutions

Cu(II) and Ca(II) standard solutions containing 200 mg L^{-1} Cu(II) and 200 mg L^{-1} Ca(II) respectively were prepared from their stock solutions: 1000 mg L^{-1} Cu(II) (GSB07-1257-2000) and 1000 mg L^{-1} Ca(II) (GSB07-1263-2000), which were purchased from the Institute for Reference Materials of State Environmental Protection Agency (SEPA), Beijing, PRC. The standard use solutions containing 1.00 and 10.0 mg L^{-1} Cu(II) and 10.0 mg L^{-1} Ca(II) respectively were prepared by diluting the above solutions. All the solutions were stored at less than 5 °C. A standard MSCPA solution containing 0.100 mmol L^{-1} MSCPA was prepared by dissolving 0.0180 g MSCPA in 250 mL of deionized water. It should be stored at less than 5 °C. A Ca-MSCPA complex solution containing 0.050 mmol L^{-1} Ca-MSCPA complex was prepared by mixing 10 mL of 2% calcium chloride (A. R, Silian Chemical Plant of Shanghai) and 50 mL of 0.100 mmol L^{-1} MSCPA and then diluting to 100 mL with deionized water. It was used as a chromogenic reagent in determination of Cu(II). A series of phosphate buffer solutions between pH 5.8 and 8.0 were prepared by mixing 50 mL of 1 mol L^{-1} KH_2PO_4 with a series of various volume of 1.0 mol L^{-1} NaOH and then diluting to 100 mL. Each of the solutions was measured accurately with pH meter, and their pH values were 5.85, 6.47, 7.30 and 8.00, respectively. A series of NaAc-HAc buffer solutions were prepared by mixing NaAc and HAc solutions. Their pH values were 3.60, 4.17 and 5.34, respectively. In addition, nitric acid, surlfuric acid, chlorhydric acid, sodium carbonate, sodium nitrite, lithium hydroxide, metanilic acid, ureophil, chlorophosphonazo I and sodium hydroxide were used in synthesis of MSCPA and pretreatment of water samples. All of them were in A. R. grade and were purchased from Sinopharm

Chemical Reagent Co., Ltd. Hydrogen peroxide solution (30%, A. R) was used to eliminate the interference of reductants and to change Cu(I) into Cu(II).

3. Procedures

3.1. Synthesis and Purification of MSCPA

Both 1.0 g of metanilic acid and 0.50 g of sodium carbonate were dissolved into 5.0 mL of deionized water. The solution was cooled at 0 °C. 0.50 g of sodium nitrite and 8.0 mL of chlorhydric acid were added into the solution. Some ureophil was added slowly at 0 °C until no air bubble was formed. Thus, the solution of diazonium salt was prepared for following use. The second solution was prepared by dissolving 2.0 g of chlorophosphonazo I into 15 mL of 10% lithium hydroxide at 0 °C. Then above diazonium salt solution was added drop by drop in the second solution while maintaining pH at 9-10 by adding lithium hydroxide solution. After 12 h, chlorhydric acid was added into the solution, and the solution was filtrated and washed with 6 mol L^{-1} chlorhydric acid 24 h later. Finally, the solution was dried at 80 °C and about 1.5 g of the product was obtained.

3.2. Characterization of the Composition of the Complexes

Into a series of 10 mL calibrated flasks were added 2.00 μg of Cu(II), 1 mL of pH 6.47 buffer solution, and a known volume of 0.100 mmol L^{-1} MSCPA. The solution were diluted to 10 mL with deionized water and mixed thoroughly. After 10 min, the absorbances of the solution were measured at 519 and 610 nm against water reference.

With the same method, 1.00 μg of Ca(II) was added to substitute Cu(II). The Ca-MSCPA solutions were measured at 533 and 650 nm. Thus, the complex reaction was characterized by the relations (1)-(5).

3.3. Determination of Copper

In a water sample, copper does not necessarily exist in terms of Cu(II). For example, widespread food-associated copper or organically associated copper exists in seawater .[18,19] Similarly, cuprous ion and its complex compound can be found in river and groundwater.[9,20]

So prior to detection，the samples must be pretreated by digesting with nitric acid and surlfuric acid in order to convert various states of copper, *e.g.* associated copper and cuprous ion, to Cu(II). The digestive method is as follows: 100 mL water sample, 1 mL of concentrated surlfuric acid and 5 mL of concentrated nitric acid were mixed into a 250 mL beaker. The solution was boiled and digested on electric hot plate until SO_3 fumes appeared. After the solution was cooled, 80 mL of deionized water was added and boiled for 3 min. Then 1 mol L^{-1} NaOH was added drop by drop until pH approached to about 6. After cooling, the solution was diluted to 100 mL with deionized water.

Into a 10 mL calibrated flask were added less than 5 mL of the solution above, 0.5 mL of pH 6.47 buffer solution, 0.1 mL of hydrogen peroxide solution and 0.300 mL of 0.050 mmol L^{-1} Ca-MSCPA complex. The solution was diluted to 10 mL with deionized water and mixed thoroughly. After 10 min, the absorbances of solution, A_{560nm} and A_{650nm}, were measured at 560 and 650 nm against deionized water. At the same time, the absorbances of the reagent blank, $A_{560nm}^{Ca-MSCPA}$ and $A_{650nm}^{Ca-MSCPA}$, were measured at 560 and 650 nm against deionized water. The absorbance ratio variation (ΔA_r) was calculated by equation (7):

$$\Delta A_r = A_r - A_{r0} = \frac{A_{560nm}}{A_{650nm}} - \frac{A_{560nm}^{Ca-MSCPA}}{A_{650nm}^{Ca-MSCPA}} \quad (7)$$

ΔA_r was obtained from equation (7) and then C_{Cu0} in the sample was calculated from equation (6).

RESULTS AND DISCUSSION

1. pH Dependence of Absorption Spectra

The absorption spectra of the Cu-MSCPA and Ca-MSCPA solutions were sketched in Fig.1 in a series of various buffer solutions from pH 3.60 to 8.00. From the left curves, the peak-valley interval reaches a maximum at pH 6.47, which means that Cu-MSCPA solution was most sensitive at this pH. From the right curves, the Ca-MSCPA solution was very sensitive at pH 6.47, too. So pH 6.47 buffer solution was added. From curves, the absorption peak of Cu-MSCPA solution is located at 610 nm and the valley at 519 nm. In the same way, both 533 and 650 nm were selected for characterization of the Ca-MSCPA complex.

2. Composition of the Complexes

In the reaction solution, excessive MSCPA is added in order to complex Cu /Ca completely. The free MSCPA often occupies a high fraction in the solution and it will influence the measurement of light-absorption of the complex product. Thus, the application of the ordinary spectrophotometry leads to a high error. Fortunately, the spectral correction technique[17] can solve the problem because it may eliminate the effect of the light-absorption of the excessive reactant and absorbance of each color component in the reaction solution may be calculated. By means of using the spectral correction technique, not only the light-absorption of Cu-MSCPA/Ca-MSCPA complex is obtained, but also the complexation can be characterized from equations (1)-(5).

By varying the MSCPA molarity in the reagent blanks, β remained constant in the vicinity of 0.64 for A_{610nm}/A_{519nm} and in the vicinity of 0.40 for A_{650nm}/A_{533nm} with increase of MSCPA molarity. It indicates that self-aggregation of MSCPA molecules did not occur in aqueous solution at pH 6.47. By varying Cu(II) or Ca(II) molarity in solutions with constant MSCPA molarity, the absorbance ration of the complexation solutions

showed in Fig. 2 were found. Fig. 2 displays that the ratio A_{519nm}/A_{610nm} decreases rapidly when Cu(II) is less than MSCPA molarity and the ratio remains almost constant at 0.656 when the molarity of Cu(II) is more than MSCPA. This indicates that all of the MSCPA molecules had coordinated with Cu(II) to form the Cu-MSCPA complex under such condition Therefore, α=0.656 is obtained from the Cu-MSCPA complex solution. The same method was applied to the determination of α for the Ca-MSCPA complex and α=0.455.

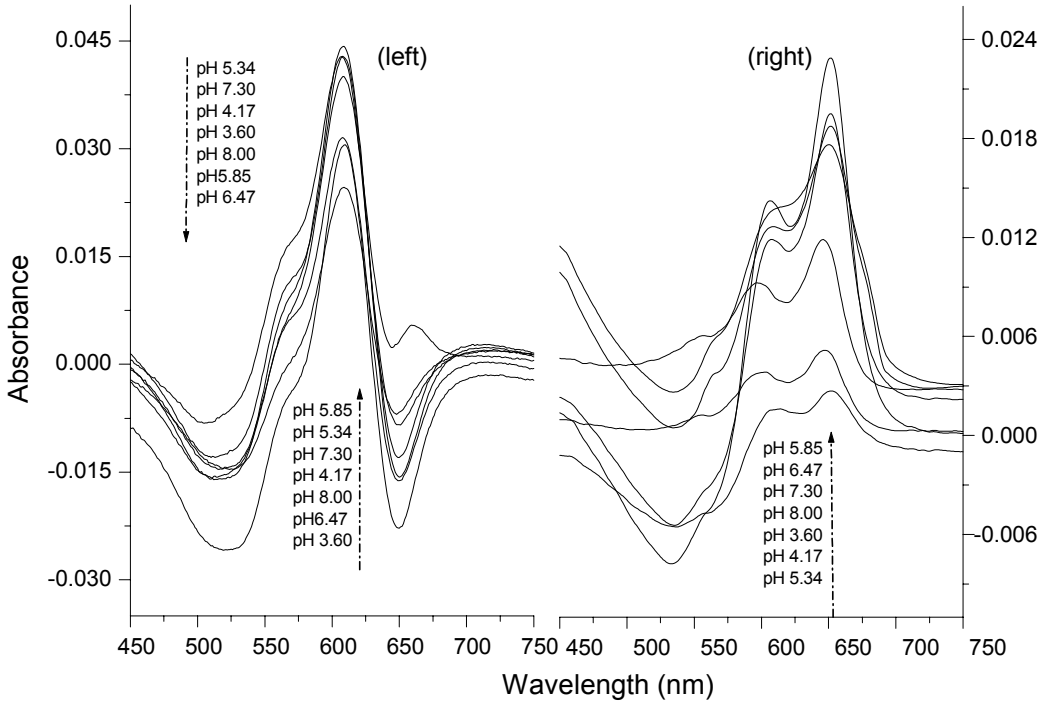


Fig. 1 – Effect of pH on the absorption spectra of Cu-MSCPA (left) and Ca-MSCPA (right) solutions against the reagent blank without Cu(II) or Ca(II), in which 0.500 mL of 0.100 mmol L^{-1} MSCPA, 1 mL of 1 mol L^{-1} different buffer solution from pH 3.60 to 8.00, 5.0 μg of Cu(II) (left) or 10.0 μg of Ca(II) (right) are added to a series of calibrated flask and then diluted to 10 mL.

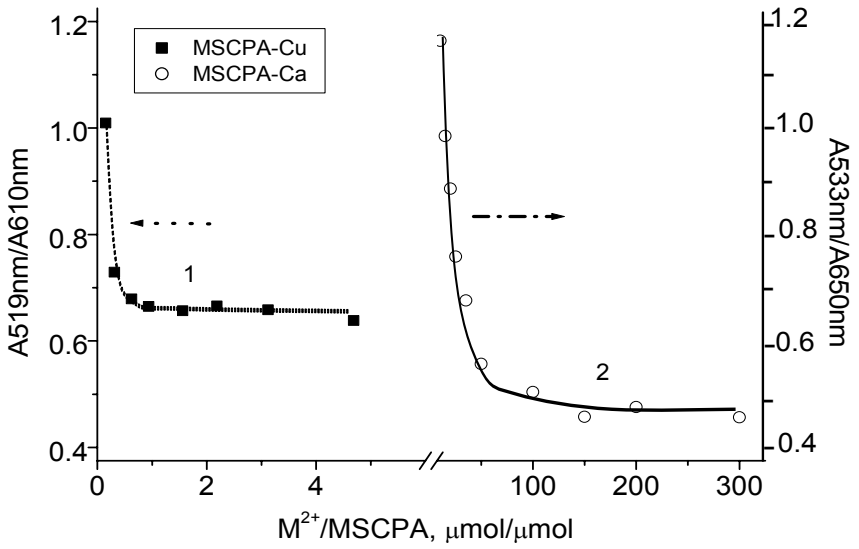


Fig. 2 – Variation of the absorbance ratio of the Cu-MSCPA solution and the Ca-MSCPA solution with increase of the molar ratio of Cu(II) or Ca(II) to MSCPA at pH 6.47. In both 1 and 2, MSCPA remains at 0.005 mmol L^{-1} and the absorbance ratios approach constant minima (α) when the molar ratio of Cu(II) or Ca(II) to MSCPA is over 1.0 and 100.0 respectively.

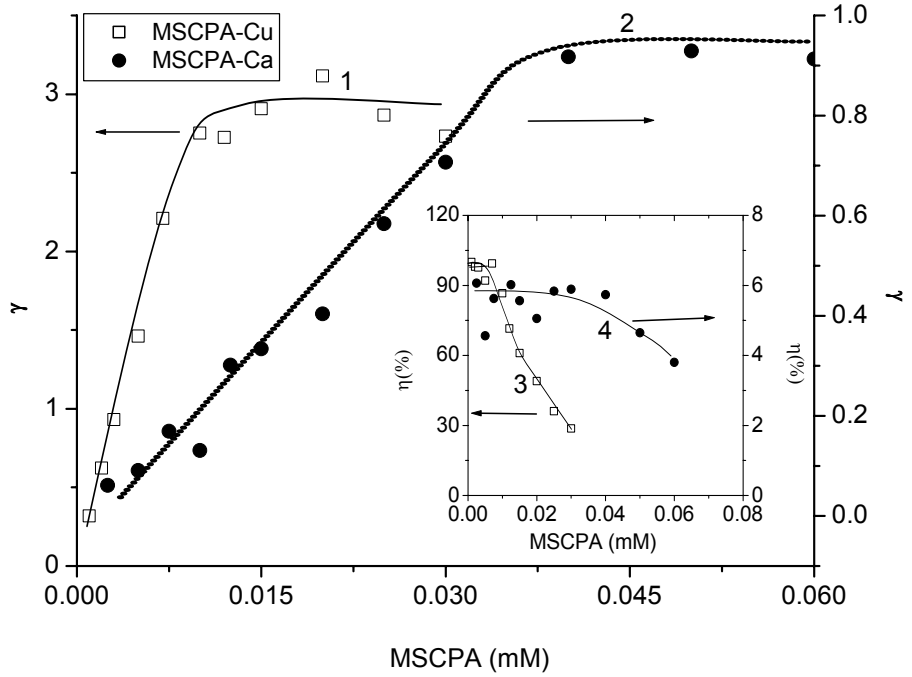


Fig. 3 – Variation of η and γ with MSCPA molarity: Cu-MSCPA solution containing 0.200 μg mL^{-1} of Cu(II), Ca-MSCPA solution containing 0.100 μg mL^{-1} of Ca(II). The decrease of η is correlated with increased excess of MSCPA. γ increases up to a constant maximum at 1.0 for Ca-MSCPA solution and at 3.0 for Cu-MSCPA solution, corresponding to maximum coordination number of MSCPA.

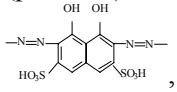
By varying the MSCPA molarity in Cu(II) or Ca(II) solutions, Change of A_c, η and γ values are shown in Fig. 3. From curve 3, η decreases from about 100 to 30% with the increase of the MSCPA molarity. From curve 4, η is less than 6%. It indicates that a high fraction of MSCPA has not reacted with Cu(II) or Ca(II) in such solutions. Without doubt, the excess of MSCPA will influence the measurement of absorbance of the complex product (Cu-MSCPA / Ca-MSCPA). It is impossible for ordinary spectrophotometry to measure the accurate light-adsorption of each color compounds in the reaction solution. However, the spectral correction technique solved the problem.

From curve 1, γ of MSCPA to Cu approaches a constant maximum at 3.0 when MSCPA is more than 0.015 mmol L^{-1}. This confirms that complex $Cu(MSCPA)_3$ is formed at pH 6.47. With the same method, complex Ca(MSCPA) is characterized, too.

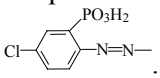
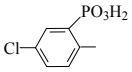
3. Electrophilic Substitution Complexation

Fig. 4 is composed of a series of spectra of MSCPA, Ca-MSCPA and Cu-MSCPA complexes with structures. The color solutions were photographed as shown in Fig. 4, too. Solution (a) contains 0.005 mmol L^{-1} MSCPA plus 0.5 mol L^{-1} buffer solution at pH 6.47, solution (b) is the same as (a) plus 40.0 μg mL^{-1} Ca(II), solution (c) is the same as solution (a) plus 1.00 μg mL^{-1} Cu(II), and solution (c) is also the same as (b) plus 1.00 μg mL^{-1} Cu(II) all against deionized water. Interestingly, spectra (c) can be obtained by adding plenty of Cu(II) to solution (a) or (b). This states that there is only an identical kind of color compound, Cu-MSCPA complex formed in solution (c). Therefore, Cu(II) can substitute Ca(II) of the Ca-MSCPA complex. From the photographs, Cu(II) and Ca(II) can complex MSCPA to form a deeply blue Cu-MSCPA complex and a sky blue Ca-MSCPA complex, respectively. Therefore, from comparison of the spectra and photograph colors, Cu(II) was confirmed to substitute Ca(II) of Ca-MSCPA complex.

From the structure of MSCPA, many groups, such as -OH, -N=N-, HO_3S- and $-PO_3H_2$ may all complex a metal ion. The position where the most stable complex will be formed with a metal by chelation is denoted with a dot cycle in Fig. 4 (a). From the spectrum of MSCPA, the main peak (peak 1) at 550 nm is corresponding to



, with the effect of two electron with-

drawing groups of two ends: chlorobenzenephosphonic acid and benzene sulfonic acid. The weak peak at 650 nm (peak 2) should correspond to  . When plenty of Ca(II) are added in a MSCPA solution, peak 2 enhances greatly and peak 1 has a red shift from 550 to 600 nm by comparison of (b) with (a). This is attributed to the chelation of Ca(II) with $-PO_3H_2$, -N=N- and -OH by the tridentate connection. The chelation of Ca makes the electronic cloud of  more dispersed and induces hyperchromicity and the effect of the chelated Ca on the middle structure causes the peak wavelengths red shift for 50 nm. The possible structure of Ca-MSCPA complex is showed in Fig. 4 (b). Calcium, as a kind of alkali earth metals, is often weak to coordinate a ligand. Thus it can be substituted easily by a transition metal.

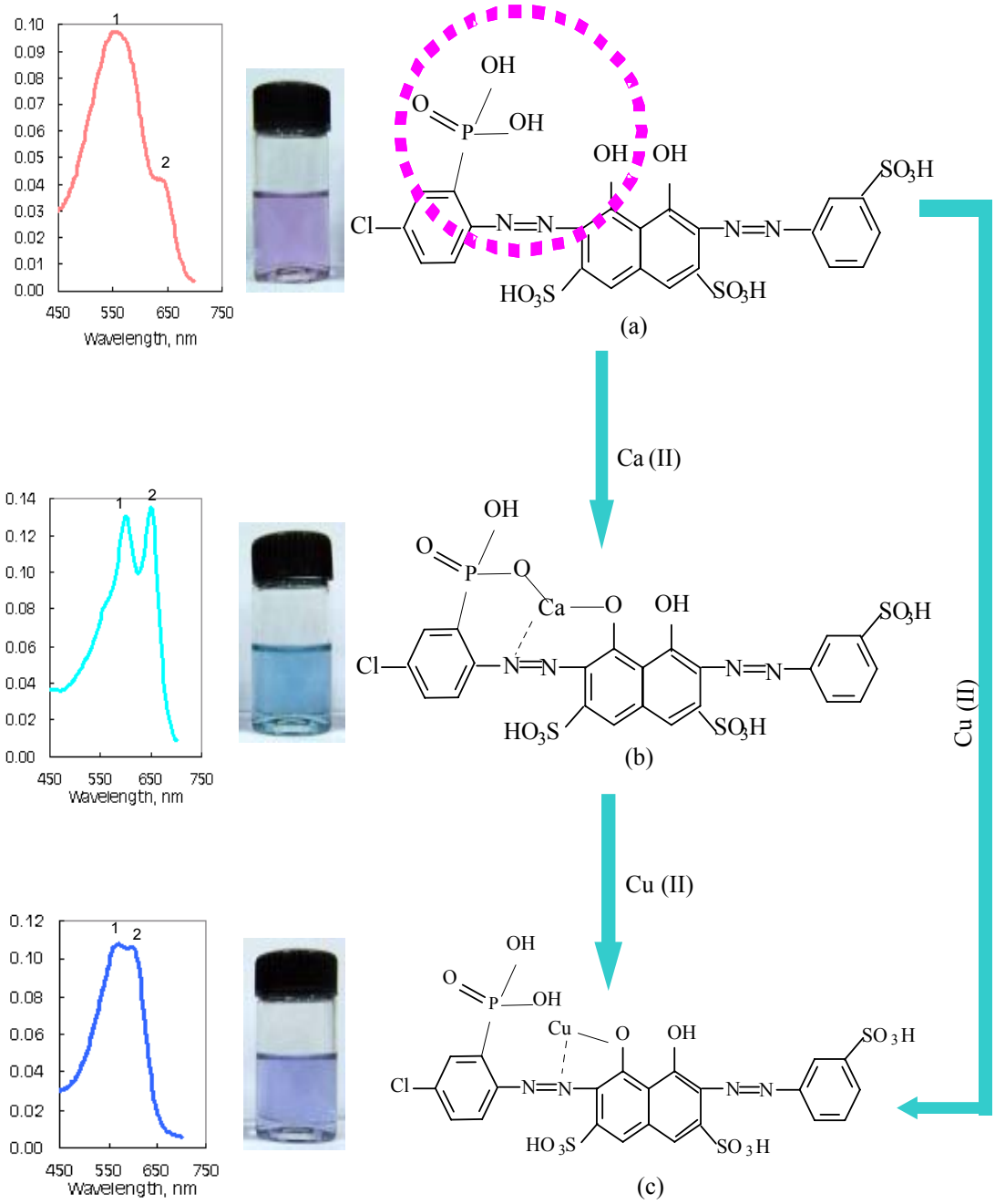
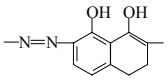


Fig. 4 – The spectrum and pictures of MSCPA and the possible structures of complexes: (a) MSCPA (b) Ca(MSCPA) (c) $Cu(MSCPA)_3$.

By comparison of (c) with (b), peak 1 returns from 600 to 550 nm and peak 2 decreased greatly, when plenty of Cu(II) are added into the Ca-MSCPA Complex solution. This indicates that $-PO_3H_2$ group is released and the coordination region of Cu(II) becomes little. Thus, the possible complex structure of Cu-MSCPA complex is shown in Fig. 4 (c). The peak 2 indicates the notable effect of Cu on the  group. The same result is obtained when plenty of copper are added into MSCPA solution, which indicates Cu(II) can substitute Ca(II) of Ca-MSCPA complex. The obvious reduction of the peak at 650 nm shows that the Ca-MSCPA connection has been destroyed. Compared with spectrum of MSCPA, peak 2 of spectrum of the Cu-MSCPA complex has a blue shift from 650 to 600 nm and intensifies. Therefore, the possible structure of the Cu-MSCPA complex is showed in Fig. 4 (c). The substitution reaction is as below:

$$Cu^{2+} + 3[Ca(MSCPA)]^{3-} \rightleftharpoons 3Ca^{2+} + [Cu(MSCPA)_3]^{13-}$$

It is possible to determine trace amounts of Cu(II) in water by using Ca-MSCPA as a new chromophore. So Fig. 5 shows the adsorption spectrum of this substitution reaction solution against the reagent blank with only the Ca-MSCPA complex. Such a solution was prepared by adding 1 mL of 0.050 mmol L^{-1} Ca-MSCPA complex solution, 1.00 μg of Cu(II) and 1 ml of pH 6.47 buffer solution. From the curve, 560 and 650 nm were selected for the determination of Cu(II) in water. The light-adsorption approached a maximum in 3 min at 20 ~ 25 °C and then remained constant for at least 2 h.

4. Effect of the Ca-MSCPA Complex on ΔA_r

From variation (in Fig. 6) of ΔA_r of the solutions with the constant ratio of Cu(II) to Ca-MSCPA complex at 0.3, ΔA_r change slightly when the Ca-MSCPA complex was more than 2.00 μmol L^{-1}. When the concentration of Ca-MSCPA solution was less than 1.00 μmol L^{-1}, ΔA_r increased rapidly. The primary reason is that the self-aggregation of Ca-MSCPA complex will not occur in an extremely diluted solution. If a ligand is too diluted, the fraction of instrumental noise will increase to influence the accurate measurement of the product. Three series of 0.200, 0.300 and 0.500 mL of the Ca-MSCPA solution were added in order to obtain the most satisfactory calibration graph and to give the lowest limit of detection of Cu.

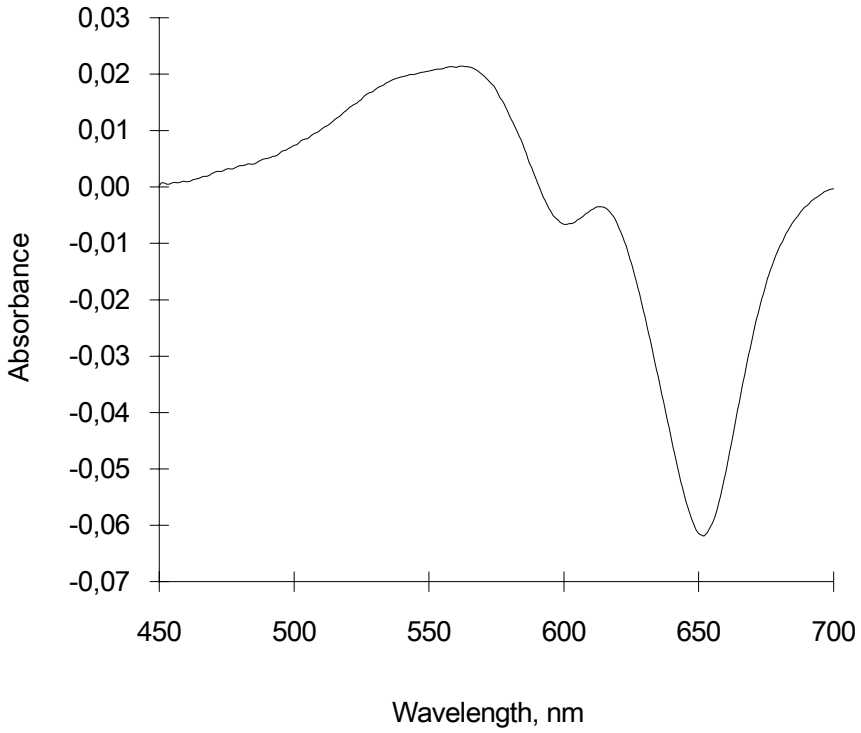


Fig. 5 – Adsorption of spectrum of Cu-MSCPA against the Ca-MSCPA complex reagent blank.

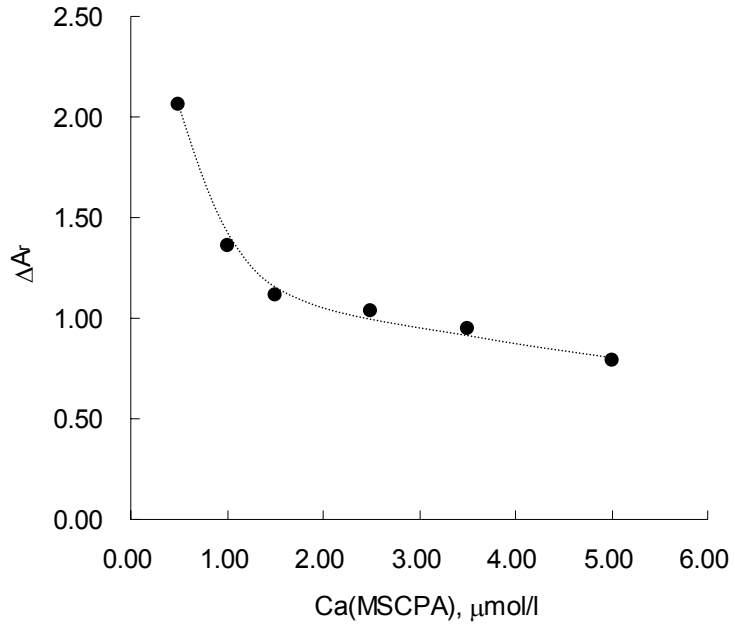


Fig. 6 – Effect of addition of 0.05 mmol L^{-1} Ca-MSCPA complex (from 0.100 mL to 1.00 mL) on ΔA_r of Ca-MSCPA-Cu(II) solution at pH 6.47, where Cu(II) was added according to the constant ratio of Cu(II) to Ca-MSCPA complex at 0.3.

5. Calibration Graph and Limit of Detection

Three series of standard Cu(II) between 0 and 0.300, 0 and 0.500 and 0 and 0.700 μg were prepared, 0.200, 0.300 and 0.500 mL of the Ca-MSCPA solution were added, respectively. According to the recommended procedures, ΔA_r was calculated according to equation (6). Their regression equations, ΔA_r^{-1} vs C_{Cu}^{-1}, are showed in Table 1, all of which are linear. The detection limit (DL) of Cu(II), defined as the reagent blank plus 3 times of the standard deviation, was calculated and showed in Table 1. By comparing the three series of data, the second series has the widest linear scope and the lowest DL at only 2.0 ng mL^{-1}. Therefore, 0.300 mL of the Ca-MSCPA solution was added and the second standard line was used.

Table 1

Regression equations and limit of detection of Cu(II)

Series	0.050 mmol/L Ca(MSCPA), mL	Linear scope, μg Cu(II)	Calibration graph	R [1)]	σ [2)]	DL[3)], ng mL^{-1}
1	0.200	0-0.300 (7) [4)]	$\Delta A_r^{-1}=0.420C_{Cu}^{-1}-1.15$	0.9970	0.020	2.5
2	0.300	0-0.500 (8)	$\Delta A_r^{-1}=0.613C_{Cu}^{-1}-1.13$	0.9994	0.010	2.0
3	0.500	0-0.700 (7)	$\Delta A_r^{-1}=1.20C_{Cu}^{-1}-1.45$	0.9974	0.007	2.5

[1)] Linear correlation coefficient; [2)] Standard deviation of 10 repetitive reagent blanks; [3)] Limit of detection of Cu(II) was calculated by DL=3×σ×*p* (*p*=line slope of plots ΔA_r^{-1} vs C_{Cu}^{-1}); [4)] number of solutions

6. Effect of Foreign Ions

Because plenty of Ca(II) exist in the reaction solution without free MSCPA contained, it is possible that the majority of metal ions will not affect the direct determination of copper. The addition of hydrogen peroxide may eliminate the effect of reductants and also transfer some low valent metals into high valent state, *e.g.* Fe(II), Mn(II) which could interfere the determination of Cu(II). Nineteen foreign ions and sodium dodecyl benzene sulfonate (SDBS) were added into the reaction solution containing 0.300 μg of Cu(II) and their effects were shown in Table 2. As a result, none of them affected the direct determination of Cu(II). Therefore, the recommended method is suitable for analysis of surface water.

Table 2

Error showing of foreign ions on ΔA_r of the solutions containing 0.300 μg of Cu(II) in 10 mL calibrated flask

No.	Foreign ion	Added, μg	Error %
1	Cd(II)	1.00	-7.3
2	Zn(II)	3.00	5.0
3	Mg(II)	100.0	4.0
4	Sn(II)	1.00	-1.7
5	As(III)	1.00	0.4
6	Mo(VI)	1.00	9.3
7	V(V)	3.00	10.0
8	Fe(II)	0.500	3.9
9	Fe(III)	0.500	5.1
10	Pb(II)	0.500	10.0
11	Ti(IV)	2.00	9.0
12	Ni(II)	0.500	3.3
13	Al(III)	0.500	8.0
14	Cr(III)	0.300	6.4
15	Co(II)	1.00	8.7
16	Ge(IV)	0.300	8.4
17	Mn(II)	2.00	-3.9
18	F^-	100.0	-3.7
19	NH_4^+	40	-5.7
20	sodium dodecyl benzene sulfonate	10.0	1.7

Table 3

Determination of copper in surface water

Water sample	Added into flask: Cu, μg	ng mL^{-1} in sample		Recovery, %
		This method	AAS	
East sea of China	0	11.3±1.0 [1)]	11.0	
	1.00	12.23 [2)]		93.4
Yangtze River	0	2.9±0.7 [1)]	2.7	
	1.00	3.90 [2)]		100.2
Huangpu River	0	24.8±0.9 [1)]	26.0	
	1.00	25.72 [2)]		92.3
Taihu Lake	0	21.2±1.2 [1)]	18.0	
	1.00	22.14 [2)]		93.9

1) Average of 5 replicated determinations; 2) Average of 2 replicated determinations

7. Analysis of Water Samples

As a test of the method, total copper in 4 samples (East Sea of China, Yangtze River, Huangpu River and Taihu Lake) were determined. The results are listed in Table 3. The percentage of recovery of Cu(II) was between 92.3 and 100.2%. In addition, all the samples were analyzed by atomic absorption spectrometry (AAS). By comparison, the two methods have similar determination results. Therefore, the LARVA method is accurate and credible for the determination of trace amounts of copper.

CONCLUSIONS

The LARVA as a kind of new analytical method can detect heavy metals in water with a high sensitivity. The CRC can notably increase the selectivity. Therefore, the combination of CRC and LARVA will be suitable for the direct determination of natural water. In fact, lots of present colorimetric reactions with deep-color chromogenic reagents can be improved obviously via LARVA in terms of the analytical sensitivity. For example, it can also be applied to analyze micro-volume samples, *e.g.* body liquid and medicinal samples. Compared with traditional methods, the spectral correction technique still plays an important role in the characterization of chemical reactions, *e.g.* coordination chemistry, macromolecular assembly, oxidation-reaction and so on.

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REFERENCES

1. L. M. Klevay, *J. Am. Coll. Nutr.*, **1998,** *17*, 322-326.
2. L. Gambling and H. J. McArdle, *P. Nutr. Soc*., **2004**, *63*, 553-562.
3. P. L. Fox , *Biometals*, **2003,** *16*, 9-40.
4. W. C. Gardner, K. Broersma, J. D. Popp, Z. Mir, O. S. Mir and W. T. Buckley, *Can. J. Anim. Sci.*, **2003**, *83*, 479-485.
5. T. N. Barry, S. O. Hoskin and P. R. Wilson, *New Zeal Vet. J.,* **2002**, *50*, 244-251.
6. B. P. Zietz, J. D. Vergara and H. Dunkelberg, *Environm. Res*., **2003**, *92*, 129-138.
7. M. Araya, B. Koletzko and R. Uauy, *J. Pediatr. Gastr. Nutr*., **2003**, *37*, 422-429.
8. L. Fewtrell, D. Kay, F. Jones, A. Baker and A. Mowat, *Public Health*., **1996,** *110,* 175-177.
9. A. Klumpp, K. Bauer, C. Franz-Gerstein and M. Menezes, *Environ. Int.,* **2002**, *28*, 165-171.
10. A. M. Dietrich, D. Glindemann, F. Pizarro, V. Gidi, M. Olivares, M. Araya, A. Camper, S. Duncan, S. Dwyer, A. J. Whelton, T. Younos, S. Subramanian, G. A. Burlingame, D. Khiari and M. Edwards, *Water. Sci. Technol.,* **2004**, *49*, 55-62.
11. D. Davidson, B. Beheshti and M. W. Mittelman, *Biofouling.,* **1996**, *9*, 279.
12. Z. H. Shi , H. Y. Zhang and C. G. Fu, *J. Liq. Chromatogr. Relat. Technol.,* **2000**, *23,* 209-222.
13. A. P. Fernandes, M. Moraes and J. A. G. Neto, *Atom. Spectrosc.,* **2003**, *24,* 179-184.
14. A. Rustoiu-Csavdari, D. Mihai and I. Baldea, *Anal. Bioanal. Chem.,* **2005**, *381*, 1373-1380.
15. H. W. Gao, S. Q. Xia, H. Y. Wang and J. F. Zhao, *Water Res.,* **2004,** *38,* 1642-1650.
16. J. M. Pan and M. L. Zhou, *Huaxueshiji,* **1984**, *6,* 43-47.
17. H. M. Gao, *Talanta*, **2000,** *52,* 817-823.
18. M. C. P. Absil, M. Berntssen and L. J. A. Gerringa, *Aquat. Toxicol.,* **1996**, *34,* 13-29.
19. L. Brugmann, R. Hallberg, C. Larsson and A. Loffler, *Ambio*, **1997**, *26,* 107-112.
20. A. E. Edet, B. J. Merkel and O. E. Offiong, *Environ. Geol.,* **2004**, *45*, 1025-1035.