Spectroscopic and Electrochemical Studies on the Interaction Mechanism of Tetraploid (imidazole) Copper(II) Terephthalate with DNA

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Summary: The crystal structure of Tetraploid (imidazole) Copper(II) Terephthalate ($Cu(Im)_4^{2+}$) was determined by X-ray crystallography. The interaction between DNA and the $Cu(Im)_4^{2+}$ complex ion was studied by cyclic voltammetry, linear sweep voltammetry and UV/Vis spectroscopy. The results depicted an obvious decrease of the peak current with a positive shift of the peak potential in cyclic voltammogram after the interaction between the two studied species. The diffusion coefficient decreased. Hypochromicity was observed at 259 nm after the interaction of DNA and $Cu(Im)_4^{2+}$. The competitive reaction of $Cu(Im)_4^{2+}$ and ethidium bromide (EB) confirmed that the interaction of DNA with $Cu(Im)_4^{2+}$ complex ion was intercalation.

Introduction

Deoxyribonucleic acid (DNA) is an important genetic material in organism. It is significant for designing medical molecule whose target is DNA to study the interaction between small molecule and DNA. It is generally accepted that there are three major modes in the small molecule binding to DNA: intercalation, simple outside (external) binding, and outside binding with self-stacking along the DNA surface. The latter two are unintercalation [1].

In 1969, it was reported that Cis-Diaminedichloroplatinum had a strong anticarcinogenesis. It has stimulated interest in the studies of the metal complexes anticarcinogenesis since 1969. A lot of metal complexes have been synthesized which include complexes of aurum, platinum, tin, rhodium or ruthenium. In recent years, it has been confirmed that many metal complexes have anticarcinogenesis. Among these compounds, people have paid the greatest attention to the platinic coordination complex [2]. There are many documents [3-6] reported that $Fe[EDTA]^{2-}$, $Cu(phen)_2^{2+}$, RuNi binuclear complex, dicyclopentadienyliron etc. have functionalities of splitting DNA and distinguishing DNA. The interaction between anticarcinogen and DNA was also reported.

According to the documents [7,8], we find that copper and its coordination complexes have pharmacological actions. Therefore, in this paper, we studied the interaction of Tetraploid (imidazole) Copper(II) Terephthalate with DNA by electrochemical methods and ultraviolet-visible spectrophotometry.

Results and Discussion

X-ray Crystal Structure of Title Compound

The X-ray crystal structure of the title compound is composed of discrete monomeric molecule of [Cu(Im)$_4$](teph)] (Im = imidazole, teph = terephthalate). Fig. 1 shows a perspective view of the title compound with atomic numbering scheme. The crystal structure of the complex consists of monomeric $Cu(Im)_4^{2+}$ cations and terephthalate anions linked by electrostatic forces and hydrogen bonds. The coordination model of the copper(II) atom can be

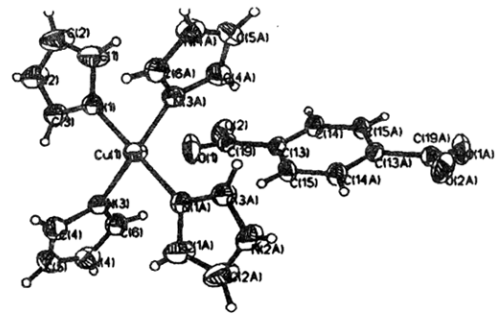

Fig. 1: The X-ray crystal structure of [Cu(Im)$_4$](teph).

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described as CuN_4 chromophore. The copper(II) ion has a square plane geometry. Four imidozole molecules are coordinated through its tertiary nitrogen atoms to each copper(II) ion and one terephthalate is out of coordination sphere, in general, balances the charge. Selected bond lengths and angles and hydrogen bond distances are presented in Tables-1 and 2.

Table-1:Selected bond distances (nm) and bond angles (°) of title compound

Cu(1)-N(3)	0.20027(1)	N(2)-C(3)	0.1324(3)
Cu(1)-N(1)	0.20427(1)	N(2)-C(2)	0.1334(4)
N(1)-C(3)	0.1304(3)	N(3)-C(4)	0.1307(3)
N(1)-C(1)	0.1360(3)	N(3)-C(6)	0.1367(3)
N(4)-C(4)	0.1330(3)	N(4)-C(5)	0.1350(4)
O(1)-C(7)	0.1244(3)	O(2)-C(7)	0.1255(3)
N(1)-Cu(1)-N(3A)$1	89.72(7)	C(2)-C(1)-N(1)	109.8(2)
N(1)-Cu(1)-N(1A)$1	180.00(6)	C(3)-N(2)-C(2)	107.5(2)
N(3)-Cu(1)-N(1)	90.28(7)	C(6)-N(3)-Cu(1)	128.66(15)
C(4)-N(3)-Cu(1)	125.09(15)	C(4)-N(4)-C(5)	107.57(19)
C(1)-N(1)-Cu(1)	128.32(14)	N(3)-C(4)-N(4)	110.96(19)
N(1)-C(3)-N(2)	111.9(2)	N(2)-C(2)-C(1)	106.2(2)

Symmetry transformations used to generate equivalent atoms: $1: -x+2, -y, -z

Cyclic Voltammetry of the $Cu(Im)_4^{2+}$ at the Bare Glassy Carbon Electrode

Cyclic voltammogram (CV) of $Cu(Im)_4^{2+}$ was recorded to test whether $Cu(Im)_4^{2+}$ interacted with DNA. To prevent DNA from acid or basic denaturing, buffers with pH values ranging from 5 to 10 are available [10]. The cyclic voltammograms of 10 $mmol \cdot l^{-1}$ $Cu(Im)_4^{2+}$ was run at the bare glassy carbon electrode in 0.5 $mmol \cdot l^{-1}$ Tris-HCl buffer (pH 7.40). Instrument parameters are initial potential: -0.4 V; high potential: + 0.2 V; low potential: – 0.4 V; scan rate: 0.1 V s^{-1}; sample interval: 0.1 V and quiet time: 2 s.

The curve a in Fig. 2 is the cyclic voltammogram of $Cu(Im)_4^{2+}$ at the GCE. The oxidation peak and reduction peak potential separation was about 90 mV. The ratio of the oxidation peak current (I_{pa}) to the reduction peak current (I_{pc}) is equals approximately to 1. Therefore, this oxidation-reduction process is quasi-reversible [11]. The curve b is the cyclic voltammo-gram of $Cu(Im)_4^{2+}$ in the presence

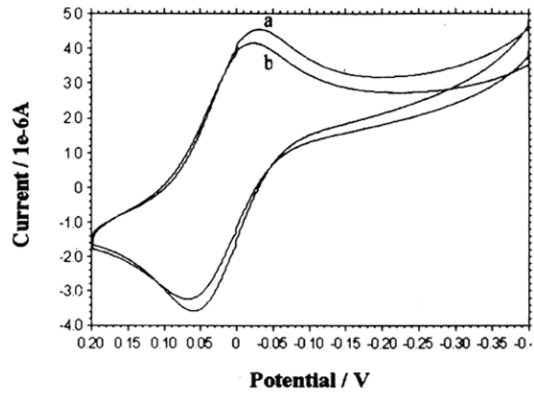

Fig. 2: Cyclic voltammetry of $Cu(Im)_4^{2+}$, Tris-HCl buffer solution, pH 7.40, a. 1×10^{-4} $mol \cdot L^{-1}$ $Cu(Im)_4^{2+}$, b. $1+4.68\times10^{-4}$ $mol \cdot L^{-1}$ DNA.

of DNA at the GCE. The results depicted an obvious decrease of the peak current with a positive shift of the formal potential after the interaction between the two studied species. There was no new redox peaks after the addition of DNA. So $Cu(Im)_4^{2+}$ interacting with DNA forms an electrochemically non-active complex [12]. In the presence of DNA, the equilibrium concentration of $Cu(Im)_4^{2+}$ decreases which results in a decrease of peak current. We deduce initially that $Cu(Im)_4^{2+}$ could interact with DNA[10]. Bard has reported [13] that if the formal potential shifts negatively when the small molecule acts with DNA, the interaction is electrostatic effect. On the contrary, if the formal potential shifts positively, the interaction is intercalation. According to Fig. 2, the initial conclusion can be obtained that $Cu(Im)_4^{2+}$ can intercalated into DNA.

The influence of DNA concentration on the peak current of $Cu(Im)_4^{2+}$ was also tested. Fig. 3 shows the relationship between the largest peak current density and the concentration of DNA. The amount of DNA was increased gradually while the concentration of $Cu(Im)_4^{2+}$ was kept constant.

Increasing amounts of DNA were added into the solution whose concentration of $Cu(Im)_4^{2+}$ was

Table-2: Hydrogen bond distances (nm) of the title compound

D	H	A	Symm.	D-H	H...A	D...A	D-H...A
N(2)	H(2A)	O(1)	1-x, -y, -z	0.08600	0.23318	0.31364	155.87
N(2)	H(2A)	O(2)	1-x, -y, -z	0.08600	0.21381	0.28793	144.10
N(4)	H(4A)	O(2)	1-x, -y, 1-z	0.08600	0.20331	0.27805	144.82
C(4)	H(4B)	O(1)	--	0.09300	0.23898	0.30354	126.40

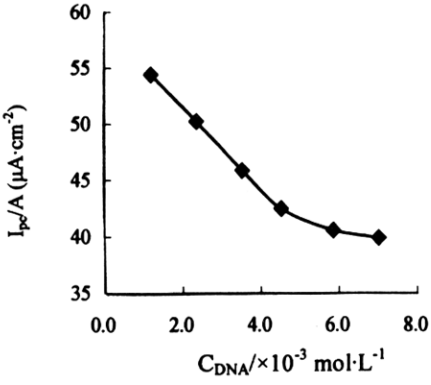

Fig. 3: The relationship curve of I_{pc} vs. C_{DNA}

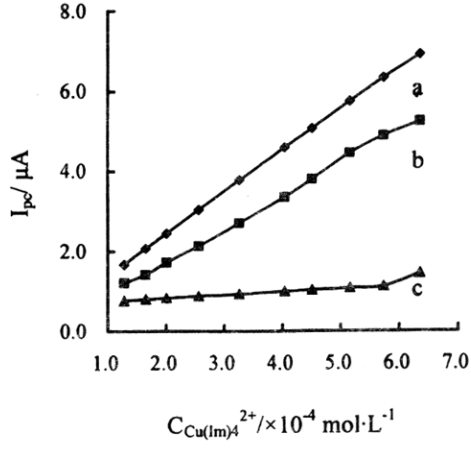

Fig. 4: The relationship curve of i_1, i_2 andΔi vs. $C_{Cu(Im)4}^{2+}$, a. $C_{DNA} = 0$, b. $C_{DNA} = 5.6\times10^{-5}$ $mol \cdot L^{-1}$, c.$\Delta I = I_1 - I_2$

unchanged. At the beginning, the peak current decreased obviously. Then the change of the peak current was small when the concentration of DNA reached at a certain degree. Eventually, the peak current did not no longer decrease. Obviously, at the situation, the interaction of $Cu(Im)_4^{2+}$ and DNA was saturated.

The relationship between peak current and diffusion coefficient is as follows [14].

$$I_p = 269\ n^{3/2} A\ D^{1/2} v^{1/2} C_0$$

The relationship between the largest peak current density and the diffusion coefficient can be deduced. The diffusion coefficient was decreased from 3.00×10^{-7} $cm^2 \cdot s^{-1}$ to 0.04×10^{-7} $cm^2 \cdot s^{-1}$ with the addition of DNA. Perhaps the formation of $Cu(Im)_4^{2+}$-DNA complex results in the reduction of diffusion velocity.

Linear Sweep Voltammetry

Keeping the FS DNA concentration constant, the concentration of $Cu(Im)_4^{2+}$ was varied, the relationship between the largest peak current and $C_{Cu(Im)4}^{2+}$ can be obtained.

In Fig. 4, curve a shows the relationship between $I_{pc}(I_1)$ and the $Cu(Im)_4^{2+}$ concentration in the absence of DNA. Curve b show the relationship between $I_{pc}(I_2)$ and $Cu(Im)_4^{2+}$ concentration when the DNA concentration is fixed at 5.6×10^{-5} $mol \cdot l^{-1}$. Curve c is the relationship between ΔI which means the (I_1-I_2) value and the concentration of $Cu(Im)_4^{2+}$.

It is assumed that DNA and $Cu(Im)_4^{2+}$ only produce a single complex DNA-$mCu(Im)_4^{2+}$ according to the reference [9].

$$DNA + mCu(Im)_4^{2+} \rightleftharpoons DNA\text{-}mCu(Im)_4^{2+}$$

The equilibrium constant (β) is as follows:

$$[DNA\text{-}mCu(Im)_4^{2+}$$

$$\beta_S = \frac{[DNA - mCu(Im)_4^{2+}]}{[Cu(Im)_4^{2+}]^m \{DNA]} \qquad (1)$$

And the following equations can be deduced:

$$\Delta Imax = K' C_{DNA} \qquad (2)$$

$$\Delta I = K'[DNA\text{-}mCu(Im)_4^{2+}] \qquad (3)$$

$$[DNA] + [DNA\text{-}mCu(Im)_4^{2+}] = C_{DNA} \qquad (4)$$

$$\Delta I_{max} - \Delta I = K'(C_{DNA} - [DNA\text{-}mCu(Im)_4^{2+}] \qquad (5)$$

$$\Delta I_{max} - \Delta I = K'[DNA] \qquad (6)$$

Introducing Eqs. 3 and 6 into Eq. 1 leads to :

$$\lg[\Delta I/(\Delta I_{max}-\Delta I)] = \lg\beta_s + ml\ g[Cu(Im)_4^{2+}] \qquad (7)$$

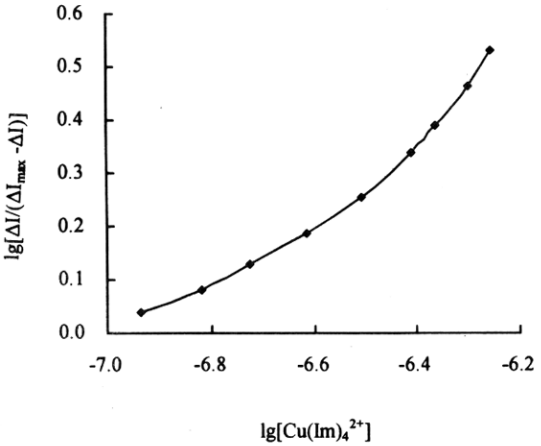

Fig. 5: The relationship curve of $lg[\Delta I/(\Delta I_{max}-\Delta I)]$ vs. $lg[Cu(Im)_4^{2+}]$

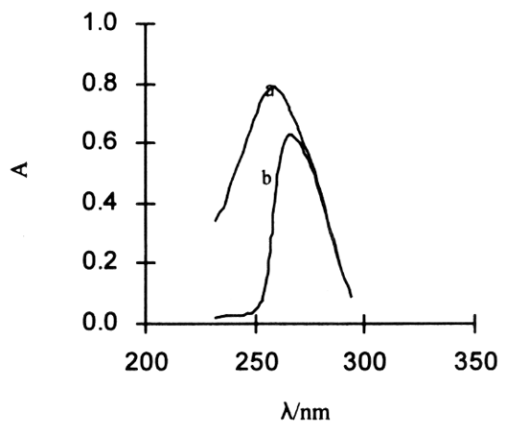

Fig. 6: Absorption spectra of DNA, a. $C_{Cu(Im)4}^{2+}=0$ $C_{DNA}=4.68\times10^{-5}$ mol·L^{-1}, b. $C_{Cu(Im)4}^{2+}=1.48\times10^{-4}$ mol·L^{-1} $C_{DNA}=4.68\times10^{-5}$ mol·L^{-1}

If DNA and $Cu(Im)_4^{2+}$ form a single complex, then the plot $lg[\Delta I/(\Delta I_{max}-\Delta I)]$ vs. $lg[Cu(Im)_4^{2+}]$ is a straight line with the slope of m.

However, for $Cu(Im)_4^{2+}$, the plot in Fig. 5 showed two straight lines in different concentration ranges of $Cu(Im)_4^{2+}$, which implied that DNA and $Cu(Im)_4^{2+}$ form two kinds of complex. Two values of m (m≈0.5, m≈1) match the two kinds of complex.

Absorption Spectra

The change of the absorption spectrum of FS DNA with the addition of $Cu(Im)_4^{2+}$ complex ion is shown as in Fig. 6. The curve a represents the ultraviolet spectrogram of DNA in the absence of $Cu(Im)_4^{2+}$. Curve b shows the ultraviolet spectrogram of DNA at $C_{Cu(Im)4}^{2+} = 1.48\times10^{-4}$ mol·L^{-1}. 5 mmol·L^{-1} Tris-HCl buffer solution (pH 7.40) was used as solvent. From the Fig.6 we can see DNA has a maximum absorption at 259 nm.

Hypochromicity was observed after the interaction of DNA and the $Cu(Im)_4^{2+}$ complex ion, and the maximum absorption of DNA shifted bathochromically about 8 nm. If DNA and the $Cu(Im)_4^{2+}$ had not interacted with each other, the curve a and curve b would have been overlapped. However, the result of the experiment shows that the shapes of two curves did not change while the curve b shows the hypochromic effect. According to the literatures[15-17], hypochromic effect, bathochromic effect and the formation of isobestic point are the identifying mark of the intercalation.

Therefore, the conclusion can be made preliminarily that the $Cu(Im)_4^{2+}$ can bind to DNA by intercalation.

The influence of $Cu(Im)_4^{2+}$ on the absorption spectrum of EB-DNA

Although ethidium bromide does not have a requirement for any particular base in binding to DNA, it does show a definite binding preference in forming complexes with C-G, dC-dG and other pyrimidine-purine sequence dinucleotides, as indicated by the visible absorbance, fluorescence, and proton magnetic resonance data [18]. We have therefore examined the binding interactions of EB with DNA in the presence of $Cu(Im)_4^{2+}$, in the hope of providing information about the similarities or differences in the nature of the binding sites of these complexes to DNA.

Absorption spectra for EB are shown in Fig.7. EB had a maximum absorption at 479 nm in 0.5 mmol·L^{-1} Tris-HCl buffer solution (pH 7.40). There were hypochromic effect and bathochromic effect in the presence of DNA. This phenomenon resulted from the intercalation between EB and DNA. It is generally recognized that the strong mode of binding of EB to double-stranded nucleic acids results in the intercalation of the planar phenanthridinium ring

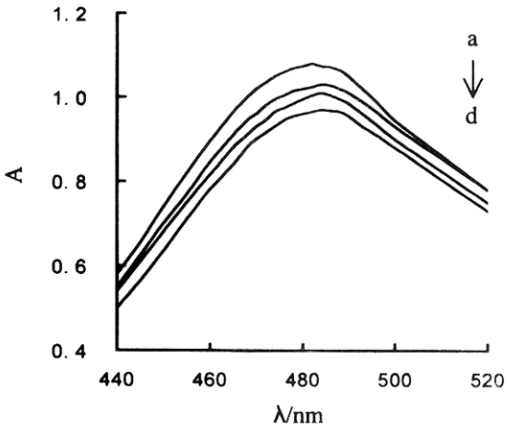

Fig. 7: Absorption spectra of EB, a. C_{EB} = 3×10^{-5} mol·L^{-1}, b. C_{EB} = 3×10^{-5} mol·L^{-1} C_{DNA}= 4.68×10^{-5} mol·L^{-1} $C_{Cu(Im)4}^{2+}$ =2.12×10^{-4} mol·L^{-1}, c. C_{EB} = 3×10^{-5} mol·L^{-1} C_{DNA}= 4.68×10^{-5} mol·L^{-1} $C_{Cu(Im)4}^{2+}$ = 1.06×10^{-4} mol·L^{-1}, d. C_{EB} = 3×10^{-5} mol·L^{-1} C_{DNA} = 4.68×10^{-5} mol·L^{-1}

between adjacent base pairs on the double helix [18,19]. The maximum absorbance of EB increased after the addition of $Cu(Im)_4^{2+}$, and the peak which had bathochromic shifted back to 479 nm. Therefore, it is conceivable that $Cu(Im)_4^{2+}$ and EB do appear to compete for the same binding sites.

The competitive-binding studied of ethidium bromide and $Cu(Im)_4^{2+}$ indicates that these two intercalating drugs bind to DNA in a competitive manner. The DNA-bound ethidium bromide is effectively displaced by the addition of $Cu(Im)_4^{2+}$. It is therefore quite likely that ethidium bromide and $Cu(Im)_4^{2+}$ preferentially intercalate at the same sequences along the DNA double helix.

The competive-binding behavior observed for the EB-DNA interaction in the presence of $Cu(Im)_4^{2+}$ provide further evidence of the specificity of the interaction of the coordination complex with DNA and also illustrates the effect that the coordination complexes with planar ring have on the binding of the DNA.

Experimental

Materials

Fish sperm DNA (FS DNA) was purchased from Shanghai Huashun Biochemistry Technology Company. Its concentration was determined by the ultraviolet absorption at 259 nm. It was used without further purification. Ethidium Bromide (EB) (also from Shanghai Huashun Biochemistry Technology Company) was diluted to the needed concentration with a 5 mmol·L^{-1} Tris-HCl buffer solution (pH 7.40). Metal-free distilled water used for all solutions was prepared by SZ-93 automatic double pure water cooling unit (from Shanghai Yarong Biochemisry Intruments). All chemicals were of analytical reagent grade and used directly without further purification.

Instrumentation

The UV-visible spectra were recorded at room temperature with a Cary 50 UV-vis spectrophotometer (Australian Varian Company) with solutions in 10 mm quartz cuvettes. Cyclic Voltammetric and linear sweep voltammetric experiments were carried out on CHI832 Electrochemical system (Shanghai CH Instruments Company) which is connected with a cell using a three-electrode system: a glassy carbon electrode (GCE) as working electrode, a saturated calomel electrode (SCE) as reference electrode, and a platinum wire as counter electrode. The surface area of the glassy carbon electrode was about 0.07 cm^2. The pH of all samples was monitored by a pHS-25 pH meter, of Shanghai Leici Instrument Factory, China.

Synthesis of Tetraploid(imidazole) Copper(II) Terephthalate

According to the method of Jun Wan [9], copper(II) terephthalate was prepared by mixing aqueous solutions of copper(II) sulfate and terephthalic acid disodium salt. $Cu(p\text{-}ph(COO)_2)\cdot2H_2O$ (1.0 g, 3.8 mmol·L^{-1}) was added to a warm solution of imidazole (1.0 g, 15 mmol·L^{-1}) in H_2O (50 mL) with stirring and the mixture was refluxed for 30 min. The blue solution formed was filtered and the filtrate was left to stand undisturbed. Upon slow evaporation at room temperature, a blue crystalline solid appeared several weeks later and was separated by filtration. Its crystal structure was determined by X-ray crystallography.

Conclusions

It was observed that the presence of DNA in $Cu(Im)_4^{2+}$ solution reduces the equilibrium concentration of $Cu(Im)_4^{2+}$ and produces electrochemically non-active complexes. DNA interacting with $Cu(Im)_4^{2+}$ forms two kinds of complexes. They are 1

molar $Cu(Im)_4^{2+}$ per 2 molar base pair of DNA and 1 molars $Cu(Im)_4^{2+}$ per molar base pair. It is confirmed that EB and $Cu(Im)_4^{2+}$ bind in a competitive fashion to sites on the nucleic acid double helix. The conclusion can be made that the interaction between DNA and $Cu(Im)_4^{2+}$ complex ion was intercalation.

References

1. T. Uno, K. Hamasaki, M. Tanigawa and S. Shimabayashi. *Inorg. Chem.*, **36**, 1676 (1997)
2. W. S. Zhang, A.L. Li, *Medicinal Chemistry*, Higher Education Press, 479 (1999)
3. J.J. Zhang, G. L. Zou, R. Ou, *Amino Acids and Biotic Resources*, **20**, 50 (1998)
4. Y. Ye, J. M. Hu, Y.E. Zeng, *Chinese Journal of Analytical Chemistry*, **28**, 798 (2000)
5. Y. Ye, J. M. Hu, X.Q. Yin, *Journal of Instrumental Analysis*, *19*, 37 (2000)
6. S. Shawn, W. R. Lee, F. Zhenglai, M. Matthew, B. K. Jenks, *Proceedings of SPIETHE International Society for Optical Engineering*, **4512**, 75 (2001)
7. M. Khan, F. Ismail, N. Y. Ahmad, P. M. Khan and G. Majid, *J. of Inorg. Chem.*, **14**, 29 (1998)
8. M. Benigno, V. Maria V., Fiz Emilio, G. Isabel, C. Alfonso, G. A. Marta and B. Joaquin, *J. of Inorg. Biochem.*, **88**, 101 (2002)
9. J. Wang, S.J. Ye, Y.H. Wen, S.S. Zhang. *Chinese Journal of Chemistry*, **21**, (2003)
10. Qu Feng, N. Q. Li, Y.Y. Jiang. *Analytica Chimica Acta*, **344**, 97 (1997)
11. H.B. Sheng, L.H. Kuai, L.H. Ni, J.Y. Li. *Journal of the Chinese Rare Earth Society*, **15**, 182 (1997)
12. D.J. Liu, Z.X. Wang, S.J. Dong. *Chinese Journal of Analysis Laboratory*, **21**, 54 (2002)
13. M. T. Carter, M. Rodriguez and A. J. Bard, *J. Am. Chem. Soc.*, **111**, 8901 (1989)
14. J. E. B. Randles, *Discussions Faraday Soc.*, (1), 11 (1947) A. Ševčík, *Collection Czechoslov. Chem. Communs*, **13**, 349 (1948)
15. E. C. Long, J. K. Barton, *Accounts Chem Res*, **23**, 273 (1990)
16. R.Y. Zhang, Y. Liu, D.W. Pang, R. X. Cai, P. Yi, *Analytical Sciences*, **18**, 761 (2002)
17. R.K. Roland, K. Burkhard. *Journal of the Chemical Society, Dadton Tremsactions*, **2**, 121 (2002)
18. G. Christian, Reinhardt, Thomas R. Krugh. *Biochemistry*. **14**, 4845 (1978)
19. J. X. Lu, G. Z. Zhang, Z.N. Huang, P. Zhao, *Acta Chimica Sinica*, **60**, 967 (2002)