

## Electrochemical Study on the Interaction between the {2-[(2-Hydroxy-5-sulfo-benzylidene)amino]acetyl amino}acetic Copper(II) Complex and DNA

S.Y. NIU, G.F. JIE, SHU-SHENG ZHANG\*, Y. LI, K. JIAO and F. YANG†

*College of Chemistry and Molecular Engineering*

*Qingdao University of Science and Technology, Qingdao, Shandong-266 042, P.R. China*

*E-mail: shushzhang@126.com*

A voltammetric study of the interaction between CuL (L = {2-[(2-hydroxy-5-sulfo-benzylidene)amino]acetyl amino} acetic acid) and DNA at glassy carbon electrode in 0.1 mol L<sup>-1</sup>, pH 6.90, Na<sub>2</sub>HPO<sub>4</sub>-NaH<sub>2</sub>PO<sub>4</sub> buffer solution is described. After adding DNA to CuL solution, the oxidation peak of CuL decreases. The oxidation peak potential of CuL in a pH 6.90 phosphate buffer solution in the presence of DNA shifts negatively, which indicates that the binding mode of CuL to DNA is electrostatic binding. Experimental results indicate that CuL can bind to DNA to form a 1:1 association complex with the binding constant of  $7.45 \times 10^4$  mol<sup>-1</sup> L.

**Key Words:** Copper(II) complex, DNA, Cyclic voltammetry, UV spectroscopy, Electrostatic binding.

### INTRODUCTION

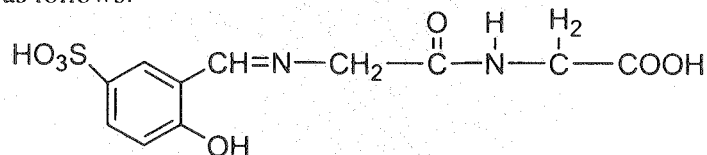
The binding of small molecules, especially the transitional metal complexes, to DNA and molecular identification are important research subjects in life science. There are many articles on the interaction between small molecules and DNA since the 1960s. Gradually this research has become a field of general interest<sup>1</sup>, because it is helpful to understand the way of the interaction between small molecules and DNA. What is more, this is very important to expound the interaction mechanism of anticancer drugs, the external selection of drugs and carcinogenesis of the carcinogenic compounds. It has been reported that many metal complexes are anti-carcinogenic. Among these compounds, people have paid much attention to the complexes such as Fe[EDTA]<sup>2-</sup>, Cu(phen)<sub>2</sub><sup>2+</sup>, RuNi binuclear complex, dicyclopentadienyl iron etc., which have the ability of splitting DNA and distinguishing DNA<sup>2-4</sup>. Furthermore, copper complexes including Cu(II)<sub>2</sub>(salicylate)<sub>4</sub> and Cu(II)<sub>2</sub>(3,5-DIPS)<sub>4</sub> which have the SOD-mimetic activity lead to the conclusion that copper complexes might have anticancer activity<sup>5</sup>. These complexes decrease tumour growth, metastasis and increase the survival of tumour-bearing mice. An interaction mechanism of copper complexes as anticancer<sup>6</sup> has been suggested to involve glutathione oxidation and accumulation of H<sub>2</sub>O<sub>2</sub>.

In recent years, there has been an explosion in the research effort directed towards the design and synthesis of model compounds that can cleave DNA. These compounds have become the indispensable tools for analyzing DNA

†Department of Chemistry, East China Normal University, Shanghai-200 062, P.R. China.

structure, sequencing DNA molecules, isolating and cloning genes. Copper complexes appear to be promising chemical/artificial nucleases<sup>7</sup>. Studies on metal complexes of nitrogen-containing ligands, especially heteroaromatic nitrogen bases are of much interest.

In this paper, CuL is a kind of copper complex with Schiff base {2-[(2-hydroxy-5-sulfo-benzylidene)amino]acetyl amino} acetic acid as the ligand. The structure of this ligand is as follows:



It was newly synthesized by the Department of Chemistry in East China Normal University and there has been no report about it; so we have conducted experiments on the interaction of CuL with DNA by electrochemical and spectroscopic methods. The experimental results have proved that CuL can interact with DNA mainly by electrostatic binding. This conclusion would surely bring detailed insight into the interaction mechanism of CuL with DNA and provide useful message for designing novel and efficient drugs as disease diagnosis and chemotherapeutic agents.

## EXPERIMENTAL

The three-electrode system was composed of a glassy carbon electrode (GCE) as the working electrode, Ag/AgCl as the reference electrode and a platinum electrode as the auxiliary electrode; Cary50 UV/Vis spectrophotometer was produced by Nicolet Company of United States; 510 P FTIR spectrometer was produced by Nicolet Company of the United States; pH-25 pH-meter was produced by Shanghai Leici Instrument Factory of China.

Salmon sperm DNA was purchased from Shanghai Huashun Biologic Engineering Company. Its concentration was determined by the ultraviolet absorption at 260 nm ( $\epsilon = 6600 \text{ mol}^{-1} \text{ cm}^{-1}$ ), used without further purification. CuL solution:  $3.56 \times 10^{-3} \text{ mol}^{-1} \text{ L}^{-1}$ ,  $0.1 \text{ mol}^{-1} \text{ L}^{-1}$ , pH 6.90;  $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$  buffer solution was used as buffer solution. The other reagents were all analytical reagents prepared with doubly deionized water.

**Electrochemical studies of the interaction between CuL and DNA:** Different quantities of CuL were added to 5 mL of  $0.1 \text{ mol L}^{-1}$  pH 6.90  $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$  buffer solution. The cyclic voltammograms (CV) of the solutions were recorded on CHI832 electrochemical analyzer (Shanghai Chenhua Instrument Co., China). Then different amounts of dsDNA (or ssDNA) were added to the solution followed by recording the CV curves. The potential scanning range is from  $-0.8$  to  $0.8$  V. The scanning rate is  $0.1 \text{ V s}^{-1}$ . The sample interval is  $0.001 \text{ V}$  and the quiet time is 2 s.

**UV studies of the interaction between CuL and DNA:**  $80 \mu\text{L}$  of  $3.56 \times 10^{-3} \text{ mol L}^{-1}$  CuL and different volumes of  $4.68 \times 10^{-2} \text{ mol L}^{-1}$  DNA solution were in turn added to 10 mL colorimetric tubes respectively. The UV spectra of CuL and DNA were recorded on a Cary 50 spectrophotometer in 1 cm quartz cells. The range of the scanning wavelengths is from 200 to 400 nm.

## RESULTS AND DISCUSSION

## Electrochemical studies of the interaction between CuL and DNA on the glassy carbon electrode

There are sharply oxidation peaks for CuL from cyclic voltammograms in the buffer solution of  $0.1 \text{ mol L}^{-1} \text{ Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$ ,  $0.2 \text{ mol L}^{-1} \text{ B-R}$ ,  $0.05 \text{ mol L}^{-1} \text{ tris-HCl}$  and  $0.1 \text{ mol L}^{-1} \text{ NaOAc-HOAc}$ . Among them, the peaks in  $0.1 \text{ mol L}^{-1} \text{ Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$  are the best. Therefore, the  $0.1 \text{ mol L}^{-1} \text{ Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$  solution was chosen as the buffer solution.

The CV of CuL before and after adding DNA were recorded to test whether CuL interacted with DNA. The cyclic voltammograms of CuL on the glassy carbon electrode in  $0.1 \text{ mol L}^{-1} \text{ pH } 6.90 \text{ Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$  buffer solution are shown in Fig. 1. The curve 1 was the cyclic voltammogram of  $5.68 \times 10^{-5} \text{ mol L}^{-1} \text{ CuL}$  solution in the absence of DNA, in which the observed oxidation peak potential ( $E_{pa}$ ) was  $0.129 \text{ V}$ . The curves 2–5 were the cyclic voltammograms of CuL in the presence of different concentrations of DNA. It can be observed that the peak currents of CuL were greatly decreased with increasing concentrations of DNA and the peak potential shifted to more negative value. No new redox peaks appeared. So CuL interacting with DNA formed no-electrochemical active complex, which resulted in the decrease of the equilibrium concentration of CuL as well as the peak current. It is generally accepted that there are three kinds of binding modes for small molecules to DNA, which refer to the intercalative binding, the groove binding and the electrostatic binding. Bard *et al.*<sup>8</sup> have reported that if the peak potential shifted to more negative value when small molecules interacted with DNA, the interaction mode was the electrostatic binding. On the contrary, if the peak potential shifted to more positive value, the interaction mode was the intercalative binding. According to Fig. 1, the initial conclusion can be drawn that the major interaction mode of CuL with DNA is "electrostatic binding", in which CuL binds to DNA through its cationic group to  $\text{PO}_4^{3-}$  in backbone of DNA.

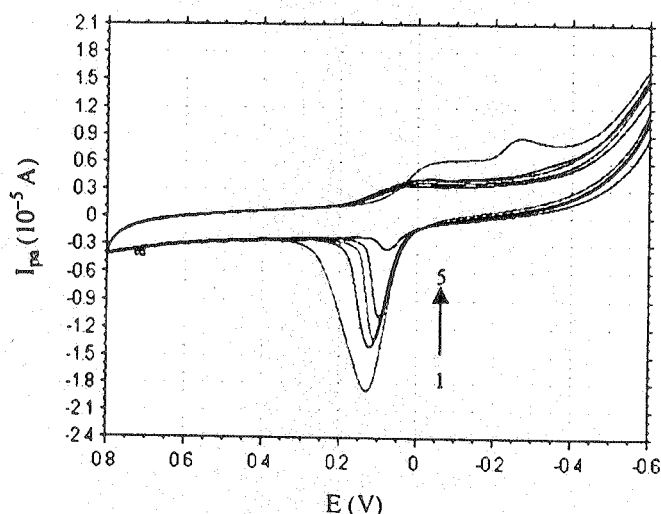


Fig. 1. Cyclic voltammograms of CuL with increasing concentrations of DNA  $C_{\text{CuL}}$ :  $5.68 \times 10^{-5} \text{ mol L}^{-1}$ ;  $C_{\text{DNA}}$ : (1) 0; (2)  $4.68 \times 10^{-5} \text{ mol L}^{-1}$ ; (3)  $9.36 \times 10^{-5} \text{ mol L}^{-1}$ ; (4)  $2.81 \times 10^{-4} \text{ mol L}^{-1}$ ; (5)  $3.28 \times 10^{-4} \text{ mol L}^{-1}$

**Effect of pH on the  $I_{pa}$  of CuL:** Fig. 2 showed the relationship between the pH value and the  $I_{pa}$  of CuL. During the experiment, the value of  $I_{pa}$  increased firstly and then reached a maximum when pH is 6.90. After that, it decreased slowly. Consequently, 6.90 was chosen as the best pH of the reaction.

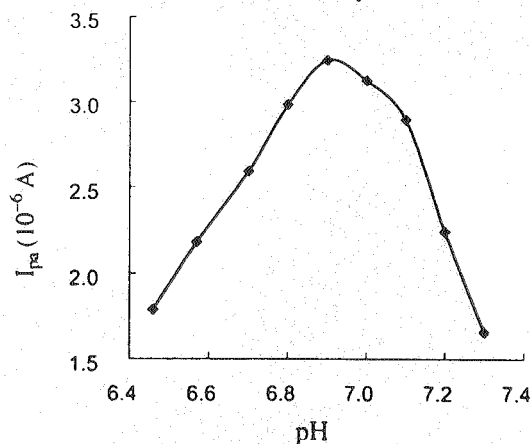


Fig. 2. The relationship between pH and the  $I_{pa}$  of CuL  $C_{CuL}$ :  $6.40 \times 10^{-5}$  mol L $^{-1}$

**Effect of reaction time on the oxidation peak current of CuL:** 5  $\mu$ L of  $4.68 \times 10^{-2}$  mol $^{-1}$  L DNA was added to 90  $\mu$ L of  $3.56 \times 10^{-3}$  mol $^{-1}$  L CuL solution, and then diluted to 5 mL with 0.1 mol L $^{-1}$  Na $_2$ HPO $_4$ -NaH $_2$ PO $_4$  buffer solution, and the relationship between the oxidation peak current of CuL and the reaction time after adding DNA at room temperature was experimented, as shown in Fig. 3. The oxidation peak current of CuL becomes smaller and smaller with the increase of reaction time and reaches a constant value when the reaction time is 6 min, indicating that the reaction of CuL with DNA has reached the equilibrium state. Consequently, 6 min was chosen as the reaction time.

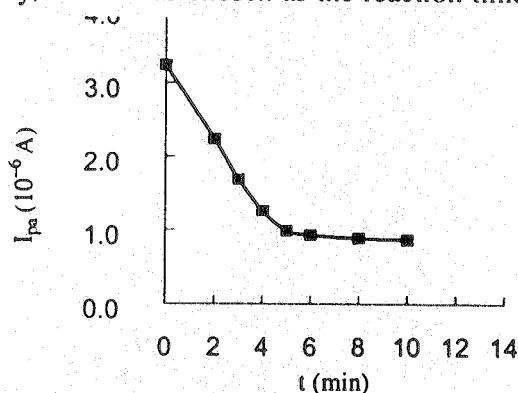


Fig. 3. Effect of time on the  $I_{pa}$  of CuL  $C_{CuL}$ :  $6.41 \times 10^{-5}$  mol L $^{-1}$ ;  $C_{DNA}$ :  $4.68 \times 10^{-5}$  mol L

**Effect of the scanning rate on the oxidation peak current of CuL:**  $I_{pa}$  of CuL is directly in proportion to the square root of the scanning rate in the range from 0.01 to 0.25 V s $^{-1}$ , with a regression equation  $I_{pa} = 12.345^{1/2} - 0.5564$  and a correlation coefficient  $R^2 = 0.9988$ . Fig. 4 is the plot of  $I_{pa}$  vs.  $v^{1/2}$  ( $v$  is the scanning rate), which is a straight line, indicating that the electrooxidation process of CuL is controlled by the diffusion of CuL.

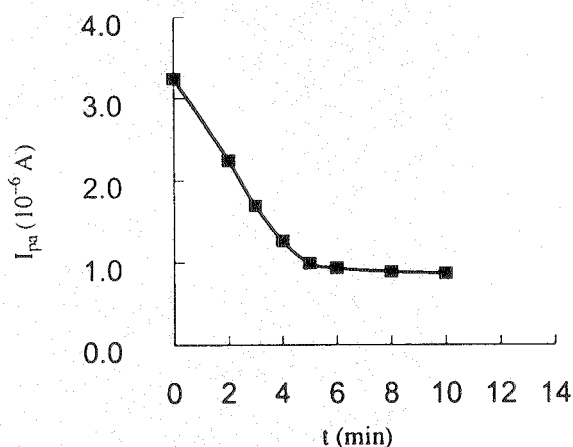


Fig. 4. The relationship between the  $I_{pa}$  of CuL and the scan rate  $C_{CuL} : 6.40 \times 10^{-5} \text{ mol L}^{-1}$

#### Effect of the concentration of DNA on the oxidation peak current of CuL

The experiment that the concentrations of both dsDNA and ssDNA increased gradually while the concentration of CuL was unchanged was done. Fig. 5 showed the relationship between the oxidation peak current of CuL and DNA concentration. At the beginning, the peak current decreased obviously. When the concentration of DNA increased to a certain degree, the peak current reached a constant value. Eventually, the peak current decreased no longer, suggesting that the interaction of CuL with DNA was saturated.

According to literature<sup>9</sup>, for the intercalative binding in which the intercalator can provide a planar aromatic heterocyclic molecular surface for efficient intercalation into dsDNA strand, the reductive effect of dsDNA concentration on the peak current is very obvious, while ssDNA has almost no reductive effect on it<sup>10</sup>. According to Fig. 5, the concentration of both dsDNA and ssDNA had obvious reductive effect on the peak current of CuL, and there was no obvious difference between their effect, indicating that the binding mode of CuL to DNA is not intercalative binding, CuL bind to DNA through "electrostatic binding".

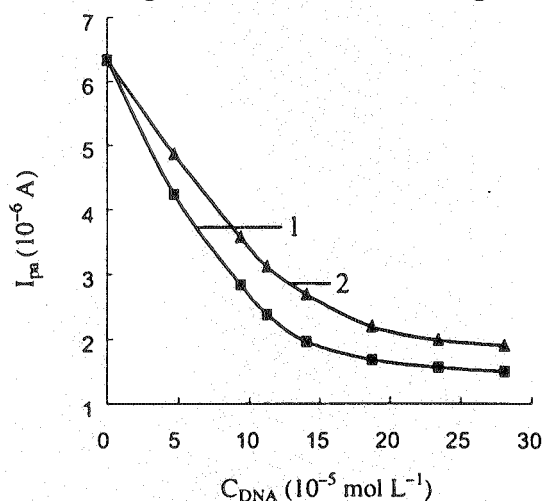
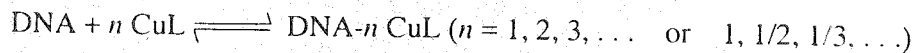


Fig. 5. Effect of the concentration of dsDNA (curve 1) and ssDNA (curve 2) on the  $I_{pa}$  of CuL  $C_{CuL} : 1.42 \times 10^{-4} \text{ mol L}^{-1}$

### Binding ratio and binding constant of DNA-CuL complex

According to reference 11, it is assumed that DNA and CuL only produce one single complex DNA-*n* CuL:



The equilibrium constant can be expressed as follows:

$$\beta = \frac{[\text{DNA} - n \text{CuL}]}{[\text{DNA}][\text{CuL}]^n} \quad (1)$$

and the following equations can be deduced:

$$\Delta I_{\text{pa, max}} = K C_{\text{DNA}} \quad (2)$$

$$\Delta I_{\text{pa}} = K[\text{DNA} - n \text{CuL}] \quad (3)$$

$$[\text{DNA}] + [\text{DNA-}n \text{CuL}] = C_{\text{DNA}} \quad (4)$$

$$\Delta I_{\text{pa, max}} - \Delta I_{\text{pa}} = K(C_{\text{DNA}} - [\text{DNA} - n \text{CuL}]) \quad (5)$$

$$\frac{1}{\Delta I_{\text{pa}}} = \frac{1}{\Delta I_{\text{pa, max}}} + \frac{1}{\beta \Delta I_{\text{pa, max}} [\text{CuL}]^n} \quad (6)$$

$$\frac{1}{\Delta I_{\text{pa}}} = \frac{1}{\Delta I_{\text{pa, max}}} + \frac{1}{\beta \Delta I_{\text{pa, max}} [\text{CuL}]^n} \quad (7)$$

With different *n*, there are different relationship curves between  $\Delta I_{\text{pa}}^{-1}$  and  $[\text{CuL}]^{-n}$ . According to eqn. (7), the relationship curve between  $\Delta I_{\text{pa}}^{-1}$  and  $[\text{CuL}]^{-n}$ , with the suitable *n*, should be a straight line if only one complex is formed when DNA is bound to CuL. From the slope and intercept of the best line, the binding constant can be calculated.

The dependence of the oxidation peak current for CuL in the absence or in the presence of DNA on the concentration of CuL is shown in Fig. 6. By calculating different  $\Delta I_{\text{pa}}$  (the difference of  $I_{\text{pa}1}$  and  $I_{\text{pa}2}$ ) and  $[\text{CuL}]$  (the equilibrium concentration of CuL) from Fig. 6, the relationship curve of  $\Delta I_{\text{pa}}^{-1}$  vs.  $[\text{CuL}]^{-n}$  was

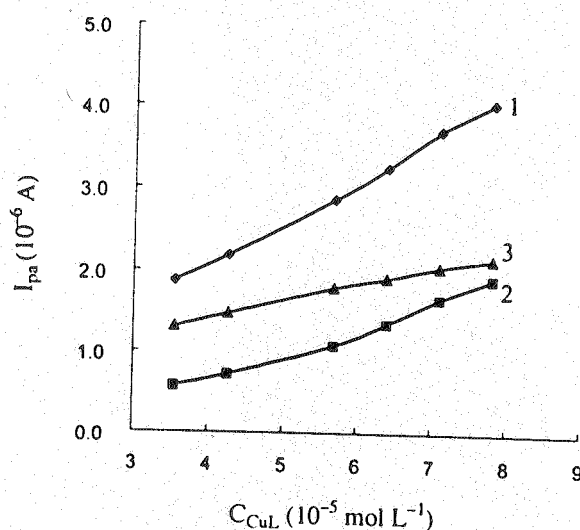


Fig. 6. The relationship between  $I_{\text{pa}1}$ ,  $I_{\text{pa}2}$ ,  $\Delta I_{\text{pa}}$  and  $C_{\text{CuL}}$ : (1)  $C_{\text{DNA}} = 0$ ; (2)  $C_{\text{DNA}} = 4.68 \times 10^{-5} \text{ mol L}^{-1}$ ; (3)  $\Delta I_{\text{pa}} = I_{\text{pa}1} - I_{\text{pa}2}$

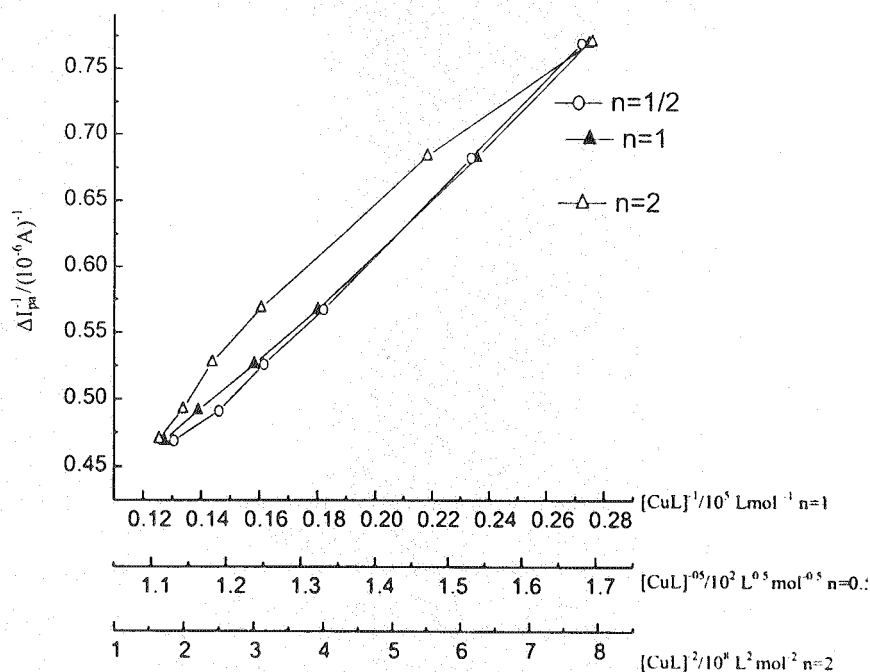


Fig. 7. The relationship curve of  $\Delta I_{pa}^{-1}$  vs.  $[CuL]^{-n}$

obtained. As for  $n = 1$ , the curve was a straight line ( $\gamma = 0.9997$ ), shown in Fig. 7. While for  $n = 0.5$  and  $2$ , the curve bended up and down respectively. From the slope and intercept of the straight line, the binding constant was calculated to be  $7.45 \times 10^4 \text{ L mol}^{-1}$ , which was corresponding to the equation



This means that CuL is bound to DNA to form a 1 : 1 association complex. In the three kinds of interaction mode, the most possible mode for forming a 1 : 1 association complex is "electrostatic binding"<sup>12</sup>. In addition, the effect of the salt concentration of solution on binding constant was also investigated. It was observed that the binding constant increased with the decrease of the salt concentration. The salt effect is an important evidence for "electrostatic binding". Thus, the major interaction mode of CuL with DNA is mainly "electrostatic binding".

#### Absorption spectra of CuL in the absence or presence of DNA

Hypochromism and red shift of the absorption bands were used to characterize the binding mode of small molecules to DNA<sup>13</sup>. In  $0.1 \text{ mol L}^{-1}$  phosphate buffer solution, the variation of CuL spectra in the presence of DNA are shown in Fig. 8.

It was observed that the absorbance of CuL at 222.1, 236.0 and 348.0 nm in UV region all greatly decreased with increasing DNA concentrations, but no obvious red shift was observed. It is recognized that the red shift of the absorption band is an important evidence for the intercalation of small molecules into DNA base stack<sup>14, 15</sup>, while the phenomena of hypochromic effect without shift are evidence for "electrostatic binding"<sup>14</sup>. Therefore, it can be deduced that CuL interact with DNA mainly by "electrostatic binding", which is consistent with the above electrochemical studies.

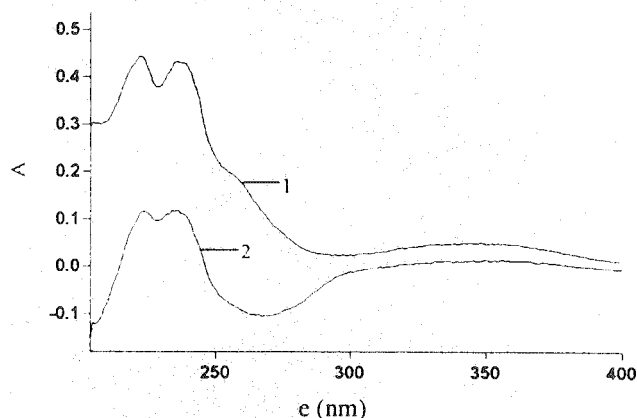


Fig. 8. UV absorption spectra of CuL: (1)  $C_{\text{CuL}}: 2.85 \times 10^{-5} \text{ mol L}^{-1}$ ; (2)  $C_{\text{CuL}}: 2.85 \times 10^{-5} \text{ mol L}^{-1}$ ;  $C_{\text{DNA}}: 3.74 \times 10^{-5} \text{ mol L}^{-1}$

### Conclusions

The interaction between CuL and salmon sperm DNA was studied by cyclic voltammetry and UV spectroscopy. The oxidation peak current for CuL decreased with increasing concentrations of DNA. In addition, the absorbance of CuL decreased greatly with no shift of absorption peaks in the presence of DNA. The conclusion can be drawn that CuL could interact with DNA mainly by "electrostatic binding" and form a 1 : 1 DNA-CuL association complex with a binding constant of  $7.45 \times 10^4 \text{ mol L}^{-1}$ .

### ACKNOWLEDGEMENTS

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