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Electrochemical and Spectroscopic Studies on the Interaction between (1,10-Phenanthroline-N,N′)-nickel(II) phthalate and DNA

SHU-SHENG ZHANG*, SHU-YAN NIU, LONG WANG, JUN WAN and XUE-MEI LI
College of Chemistry and Molecular Engineering
Qingdao University of Science and Technology
Qingdao, shandong 266042, P.R. China
Fax: (86)(532)4023927; Tel: (86)(532)4022750,
E-mail: shuszhang@126.com

The crystal structure of ([Ni(phen)](phth)$(H_2O)_3 \cdot H_2O$ (1) $[Ni(phen)]^{2+}$) (phen = 1,10-phenanthroline, phth = phthalate) was determined by X-ray crystallography. The interaction between $[Ni(phen)]^{2+}$ and salmon sperm DNA in 0.2 mol·L^{-1} Britton-Robinson buffer solution (pH 6.50) was studied by cyclic voltammetry, ultraviolet spectroscopy and fluorescence spectroscopy. All the experimental results indicate that $[Ni(phen)]^{2+}$ can bind to DNA mainly by intercalative binding mode. The binding ratio of the DNA-$[Ni(phen)]^{2+}$ association complex is calculated to be 1 : 1 and the binding constant is 8.28×10^2 L mol^{-1}.

Key Words: (1,10-Phenanthroline-N,N4)-nickel(II) phthalate, Salmon sperm DNA, Cyclic voltammetry, Intercalative binding.

INTRODUCTION

Deoxyribonucleic acid (DNA) is an important genetic material in organisms and it is the basis of gene expression. It plays an important role in the process of storing, copying and transmitting gene messages. The recognition that DNA serves as a target for natural and artificial molecules in the inhibition of cellular disorders and in the therapy of certain diseases is of paramount importance in inorganic biochemistry. The binding of small molecules, especially transition metal complexes, to DNA and molecular identification are important research subjects in life science[1, 2]. It is generally accepted that there are three kinds of binding models for small molecules to DNA, namely intercalative binding, groove binding and electrostatic binding. In these binding models, the intercalative binding one is thought to be the strongest binding because it is a type of binding in which the intercalative molecule surface is sandwiched between the aromatic, heterocyclic base pairs of DNA[3]. For a small molecule, the part of the molecule intercalating into a DNA strand is of interest because it can provide useful information for the design of new and efficient compounds for disease diagnosis and as chemotherapeutic agents.

Metal complexes, especially transition metal complexes, are an important kind of DNA target chemical compounds. Many articles on the interaction between transition metal complexes and DNA have been published since the 1980s. Gradually this research has become a field of general interest. These investigations have contributed to the understanding of the mode of interaction between transition metal complexes and DNA. What is more, this research is helpful for the design and synthesis of anticancer and antiviral drugs. Furthermore, it was reported[4] that transition metal complexes have the activity of chemical nucleases; they have the ability of specifically splitting DNA.

1,10-Phenanthroline is of considerable interest as a ligand as it plays an important role in many fields. The interactions between nickel(II) coordination compounds of 1,10-phenanthroline and DNA have not been reported much in literature. In 1998, Yang reported studies of the interaction between $[Ni(phen)_3]^{2+}$ and DNA by UV/Vis and fluorescence spectroscopy[5, 6]. This was significant for further research to design new and efficient antitumour drugs. In addition, in 2003, Zhuang reported studies by UV/Vis and fluorescence spectroscopy on the interaction between two kinds of nickel(II) coordination compounds of 1,10-phenanthroline and DNA for further research to design new nucleic acid fluorescence probes[7]. Electrochemical studies of the interaction between nickel(II) coordination compounds of 1,10-phenanthroline and DNA have not been reported in literature. In this paper, we describe the synthesis and crystal structure of the complex $[Ni(phen)](phth)(H_2O)_3 \cdot H_2O$ (1) and our studies of the interaction of 1 with DNA by cyclic voltammetry, UV spectroscopy and fluorescence spectroscopy. The experimental results have proved that $[Ni(phen)](phth)(H_2O)_3 \cdot H_2O$ could interact with DNA mainly by intercalative binding. This conclusion would surely provide detailed insight into the action mechanism of 1 with DNA for us and is significant for further research to design new and efficient antitumour drugs.

EXPERIMENTAL

A CHI 832 electrochemical analyzer (Shanghai Chenhua Instrument Company, China) was used; the three-electrode system was composed of a glassy carbon electrode (GCE) as working electrode, an Ag/AgCl electrode as the reference electrode and a platinum electrode as auxiliary electrode; UV/Vis spectra were recorded on a Cary 50 UV/Vis spectrophotometer (Varian Company, Australia) and fluorescence spectra on a Shimadzu Hitachi F-4500 fluorescence spectrophotometer (Hitachi, Japan); A pHS-25 pH-meter was used (Shanghai Leici Instrument Factory, China).

Salmon sperm DNA (10 mg mL^{-1}) was purchased from the Shanghai Huashun Biologic Engineering Company. Its purity was measured by $A_{260}/A_{280} > 1.8$. Its concentration was determined by the ultraviolet absorption at 260 nm ($\varepsilon = 6600$ L mol^{-1} cm^{-1}), used without further purification; 1.07×10^{-2} mol L^{-1} $[Ni(phen)]^{2+}$ was prepared by dissolving 0.0507 g $[Ni(phen)](phtp)(H_2O)_3 \cdot H_2O$ in 10 mL doubly deionized water; 0.2 mol·L^{-1} B-R, pH 6.50, was used as the buffer solution. The other reagents were all of analytical reagents grade prepared with doubly deionized

water. All chemicals were of analytical reagent grade and used directly without further purification.

Preparation of [Ni(phen)](phth)$(H_2O)_3 \cdot H_2O$[15]

Nickel(II) phthalate was prepared by mixing aqueous solutions of nickel(II) sulfate and phthalate acid disodium salt according to the literature method. To a warm solution of 1,10-phenanthroline (1.0 g, 5.5 mmol) in H_2O (50 mL), was added Ni(*o*-ph$(COO)_2$) (1.0 g, 4.5 mmol) with stirring and the mixture was refluxed for 30 min. The blue-green solution was filtered and the filtrate was left to stand undisturbed. Upon slow evaporation at room temperature, a blue-green crystalline solid appeared several weeks later and was separated by filtration.

Electrochemical behaviour of the interaction between $[Ni(phen)]^{2+}$ and DNA

Appropriate amounts of $[Ni(phen)]^{2+}$ were added to 0.2 mol L^{-1} BR buffer solution (5 mL). The cyclic voltammograms of the solutions were recorded on the CHI 832 electrochemical analyzer. Then different amounts of DNA were added to the solution followed by recording the CV trace. The potential scanning range was from –1.0 V to 0.8 V. The scanning rate was 0.20 V s^{-1}; the sample interval was 0.001 V and the quiet time 2 s.

UV spectroscopic studies of the interaction between $[Ni(phen)]^{2+}$ and DNA

Appropriate amounts $[Ni(phen)]^{2+}$ and salmon sperm DNA solution were placed in a 10 mL colorimetric tube, then diluted to the desired scale with B-R buffer solution. The solution was set for 12 min at room temperature. The UV spectra were recorded on a Cary 50 probe spectrophotometer in 1.0 cm quartz cuvettes. The range of the scanning wavelengths was from 200 nm to 350 nm.

Fluorescence spectroscopic studies of the interaction between $[Ni(phen)]^{2+}$ and DNA

Appropriate amounts of $[Ni(phen)]^{2+}$ and 2.5 mL BR buffer solution were transferred into each of the four 10 mL colorimetric tubes, and then different amounts of DNA solution were added. The mixture was reacted for 12 min at room temperature. The measurements of fluorescence were made by using the above instruments in 1.0 cm quartz cell.

RESULTS AND DISCUSSION

X-ray crystal structure of the title compound

The X-ray structure of the complex $[Ni(C_{12}H_8N_2)(C_8H_4O_4)(H_2O)_3] \cdot H_2O$ is made up of discrete monomeric molecules. A perspective view of the title compound with atomic numbering scheme is shown in Fig. 1. Fig. 2 shows a perspective view of the crystal packing in the unit cell for the complex.

The crystal structure of the complex consists of $[Ni(C_{12}H_8N_2)(C_8H_4O4)(H_2O)_3]$ and one uncoordinated water molecule linked by hydrogen bonds. The coordination model of the nickel(II) atom can be described as NiN_2O_4 chromophore. The nickel(II) ion has a distorted octahedral geometry. Most of the

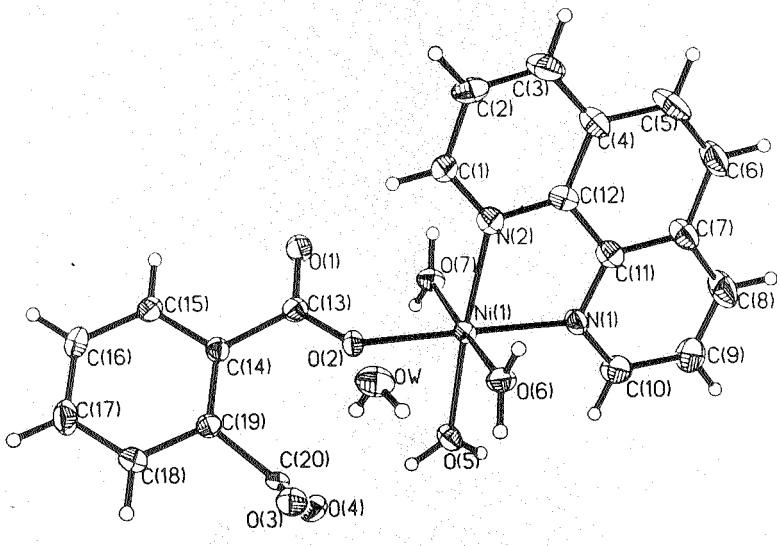

Fig. 1. Molecular structure for $[Ni(C_{12}H_8N_2)(C_8H_4O_4)(H_2O)_3]\cdot H_2O$ with the atomic numbering scheme

o-phthalates reported in literature were involved in coordination as bidentate ligands[8, 9], while the title complex differs from this coordination model. The nickel(II) ion is coordinated with the 1,10-phenanthroline ligand through its tertiary nitrogen atoms, one oxygen atom of the phthalic dianion and three oxygen atoms from water molecules. The ligand 1,10-phenanthroline and nickel(II) atom are coplanar. Two nitrogen atoms and nickel(II) form a five-membered ring and the bond distances of Ni(1)-N(1) and Ni(1)-N(2) are 2.071(6) and 2.082(6) Å, respectively.

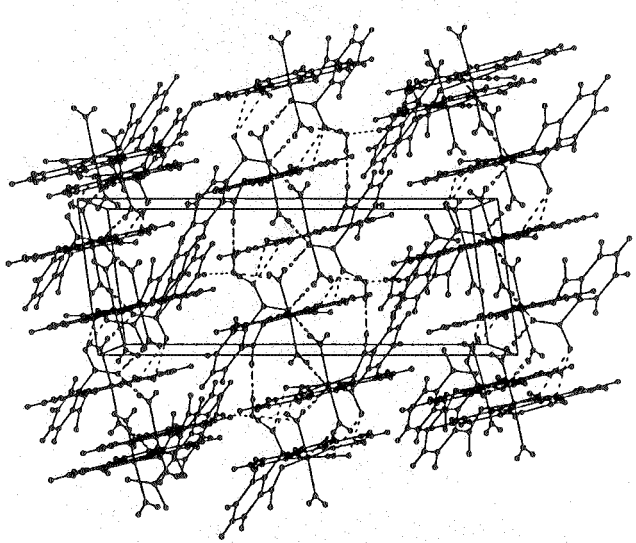

Fig. 2. A view of the crystal packing down the a axis for $[Ni(C_{12}H_8N_2)(C_8H_4O_4)(H_2O)_3]\cdot H_2O$

Electrochemical behaviour of the interaction between $[Ni(phen)]^{2+}$ and DNA

Fig. 3 shows the typical cyclic voltammograms of $[Ni(phen)]^{2+}$ in 0.2 mol L^{-1}

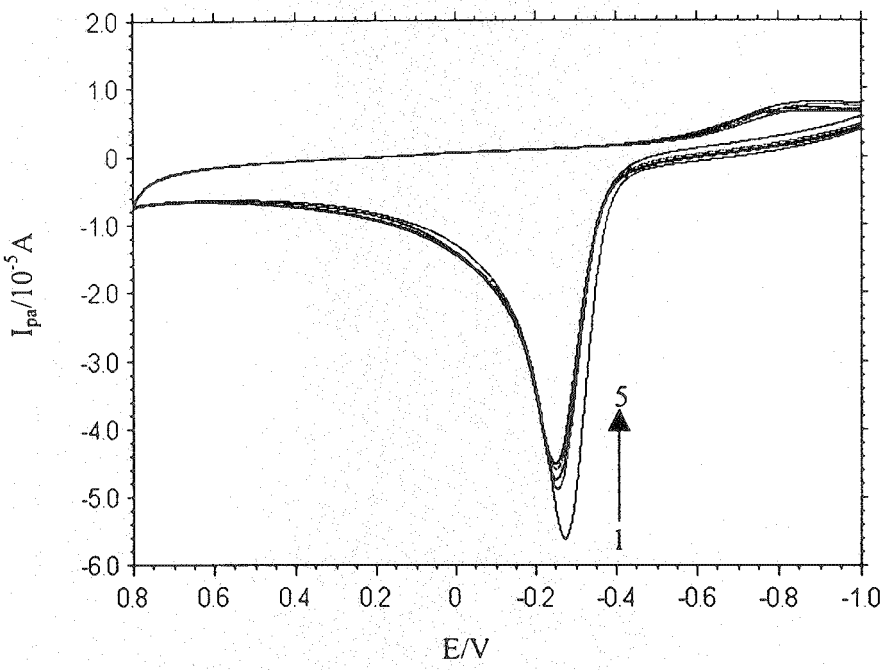

Fig. 3 Cyclic voltammograms of $[Ni(phen)]^{2+}$ with increasing concentrations of DNA $C^{2+}_{[Ni(Phen)]}$: 6.00×10^{-4} mol L^{-1}; C_{DNA}: (1) 0; (2) 3.53×10^{-4}mol L^{-1} (3) 4.40×10^{-4} mol L^{-1}; (4) 5.32×10^{-4}mol L^{-1}; (5) 6.20×10^{-4}mol L^{-1}

Britton-Robinson (B-R) buffer solution (pH 6.50) on the glassy carbon electrode. Curve 1 is the CV of 6.00×10^{-4} mol L^{-1} $[Ni(phen)]^{2+}$ solution in the absence of DNA, in which an anodic peak for $[Ni(phen)]^{2+}$ is observed, with the anodic peak potential (E_{pa}) being –0.272 V.

The curves 2–5 are the CV of $[Ni(phen)]^{2+}$ in different concentrations of DNA; the oxidation peak current (I_{pa}) decreases with increasing concentrations of DNA and the E_{pa} shifts to more positive potential. The shift of $E^{o'}$ and the decrease of peak current are evidence of the formation of a new association complex. Furthermore, no new oxidation-reduction peaks appear after adding DNA, which suggests that $[Ni(phen)]^{2+}$ interacting with DNA forms an electrochemically non-active complex. It has no electrochemical response, which results in a decrease of the equilibrium concentration of $[Ni(phen)]^{2+}$ or the diffusion coefficient; so the peak currents decrease. Among the three kinds of binding modes for small molecules to DNA, Bard has reported[10] that if $E^{o'}$ shifts to more negative value when small molecules interact with DNA, the interaction mode is electrostatic binding. On the contrary, if $E^{o'}$ shifts to more positive value, the interaction mode is intercalative binding. According to Fig. 3 and the structure of $[Ni(phen)]^{2+}$, the initial conclusion can be obtained that the binding mode between $[Ni(phen)]^{2+}$ and DNA is intercalative binding where $[Ni(phen)]^{2+}$ can intercalate into the base pairs of DNA molecules.

Effect of pH on the oxidation peak current of $[Ni(phen)]^{2+}$

The relationship between the oxidation peak current of $[Ni(phen)]^{2+}$ and the pH value was examined in 0.2 mol L^{-1} B-R. It was found that the value of I_{pa}

increased first and then reached a maximum when the pH was 6.50. After that, the I_{pa} decreased slowly. Consequently, 6.50 was chosen as the best pH for the reaction.

Effect of the scan rate on the oxidation peak current of $[Ni(phen)]^{2+}$

Fig. 4 is the plot of I_{pa} vs. $\nu^{1/2}$ (where ν is the scanning rate). I_{pa} is not directly proportional to the square root of the scanning rate in the range from 0.03 V s^{-1} to 0.23 V s^{-1}, indicating that the electro-oxidation process of **1** is controlled not only by the diffusion of **1**, but also by the adsorption effect on the electrode surface[11]. That means **1** has the character of surface adsorption.

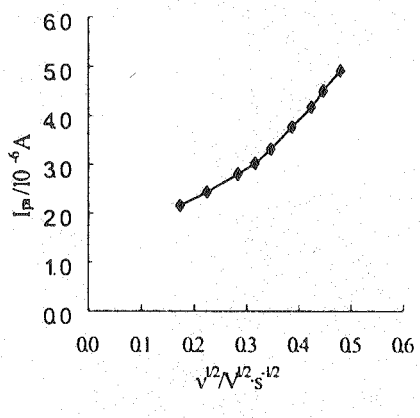

Fig. 4. The relationship between the oxidation peak current of $[Ni(phen)]^{2+}$ and $\nu^{1/2}$ $C^{2+}_{[Ni(Phen)]}$: 6.00×10^{-4} mol L^{-1}

Effect of the reacting time on the interaction of $[Ni(phen)]^{2+}$ with DNA

The relationship between the oxidation peak current of $[Ni(phen)]^{2+}$ and the reaction time was studied. The peak current first decreases with the reaction time and reaches a constant value after about 12 min, indicating that the reaction of $[Ni(phen)]^{2+}$ with DNA has reached the equilibrium state. Consequently, we choose 12 min as the reaction time.

Effect of the concentration of DNA on oxidation peak current of $[Ni(phen)]^{2+}$

An experimental study of the change in the interaction of $[Ni(phen)]^{2+}$ with DNA as concentrations of DNA increased gradually while the concentration of $[Ni(phen)]^{2+}$ was unchanged was done. At the beginning, the peak current decreased with the increase of the concentration of DNA. 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 $[Ni(phen)]^{2+}$ with DNA was saturated.

In addition, the relationship between the variation of the oxidation peak current (ΔI_{pa}) of $[Ni(phen)]^{2+}$ before and after adding DNA and with DNA concentration was studied; it was found that ΔI_{pa} is linear proportional to the concentration of DNA in the range of 3.53×10^{-4} to 6.20×10^{-4} mol L^{-1}, with a regression equa-

tion, $\Delta I_{pa} = 0.1678C_{DNA} + 0.1395$ and a correlation coefficient $\gamma = 0.9919$, so the quantity of DNA can be measured by using the linear relationship between ΔI_{pa} and DNA concentration in this range.

The binding ratio and the binding constant of the DNA – n[Ni(phen)]$^{2+}$ association complex

According to reference 12, it is assumed that DNA and [Ni(phen)]$^{2+}$ only produce a single complex DNA – n[Ni(phen)]$^{2+}$:

$$\text{DNA} + \text{n[Ni(phen)]}^{2+} \rightleftharpoons \text{DNA} - \text{n[Ni(phen)]}^{2+}$$

$$(\text{n} = 1, 2, 3, \ldots \text{or } 1, 1/2, 1/3, \ldots)$$

The equilibrium constant can be expressed as follows:

$$\beta = \frac{[\text{DNA} - [\text{Ni(phen)}]^{2+}]}{[\text{DNA}][\text{Ni(phen)}^{2+}]} \tag{1}$$

and the following equations can be deduced:

$$\Delta I_{pa,\,max} = KC_{DNA} \tag{2}$$

$$\Delta I_{pa} = K[\text{DNA} - \text{n[Ni(phen)]}^{2+}] \tag{3}$$

$$[\text{DNA}] + [\text{DNA} - \text{n[Ni(phen)]}^{2+}] = C_{DNA} \tag{4}$$

$$\Delta I_{pa,\,max} - \Delta I_{pa} = K(C_{DNA} - [\text{DNA} - [\text{Ni(phen)}]^{2+}] \tag{5}$$

$$\Delta I_{pa,\,max} - \Delta I_{pa} = K[\text{DNA}] \tag{6}$$

$$\frac{1}{\Delta I_{pa}} = \frac{1}{\Delta I_{pa,\,max}} + \frac{1}{\beta \Delta I_{pa,\,max}[[\text{Ni(phen)}]^{2+}]^{n}} \tag{7}$$

With different n, there are different relationship curves between ΔI_{pa}^{-1} and $[[\text{Ni(phen)}]^{2+}]^{-n}$. According to equation (7), the relationship curve between ΔI_{pa}^{-1} and $[[\text{Ni(phen)}]^{2+}]^{-n}$, with suitable n, should be a straight line if only one complex was formed when [Ni(phen)]$^{2+}$ was bound to DNA.

From the slope and intercept of the straight line, the binding constant β can be calculated. The dependence of the oxidation peak current for [Ni(phen)]$^{2+}$ in the absence or presence of DNA on the concentration of [Ni(phen)]$^{2+}$ is shown in Fig. 5. By calculating different ΔIpa (the difference of Ipa_1 and Ipa_2) and $[[\text{Ni(phen)}]^{2+}]$ (the equilibrium concentration of [Ni(phen)]$^{2+}$) from Fig. 5, the relationship curve of ΔI_{pa}^{-1} *vs.* $[[\text{Ni(phen)}]^{2+}]^{-n}$ was obtained. As for n = 1, the curve is a straight line ($\gamma = 0.9988$), as shown in Figure 6; it means that [Ni(phen)]$^{2+}$ is bound to DNA to form a 1 : 1 association complex under this case. While for n = 2 and 1/2, the curve bends up and down respectively. From the slope and intercept of the straight line, the binding constant β was calculated to be 8.28×10^2 L mol^{-1}, which was corresponding to the equation

$$\text{DNA} + [\text{Ni(phen)}]^{2+} \rightleftharpoons \text{DNA} - [\text{Ni(phen)}]^{2+}$$

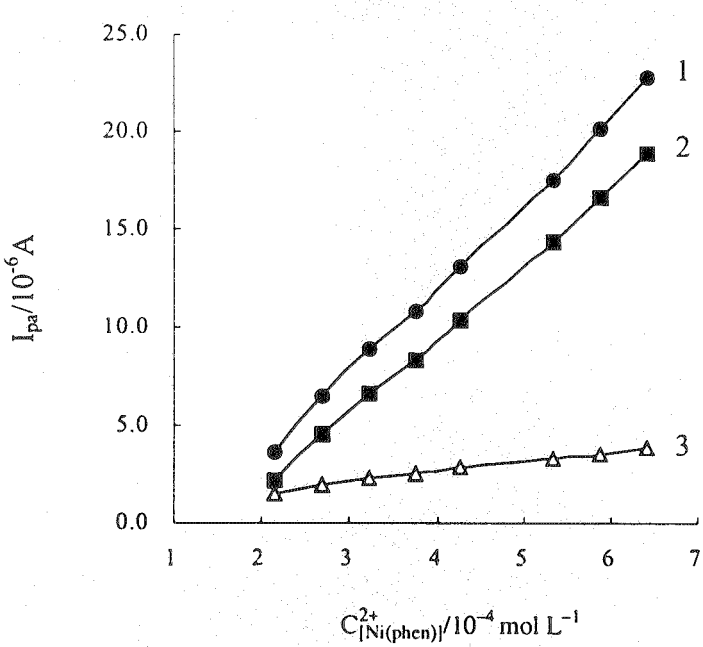

Fig. 5. The relationship between I_{pa1}, I_{pa2}, ΔI_{pa} and $C^{2+}_{[Ni(phen)]}$ (1) C_{DNA}: 0; (2) C_{DNA}: 3.74×10^{-4}mol L^{-1}; (3) $\Delta I_{pa} = I_{pa1} - I_{pa2}$

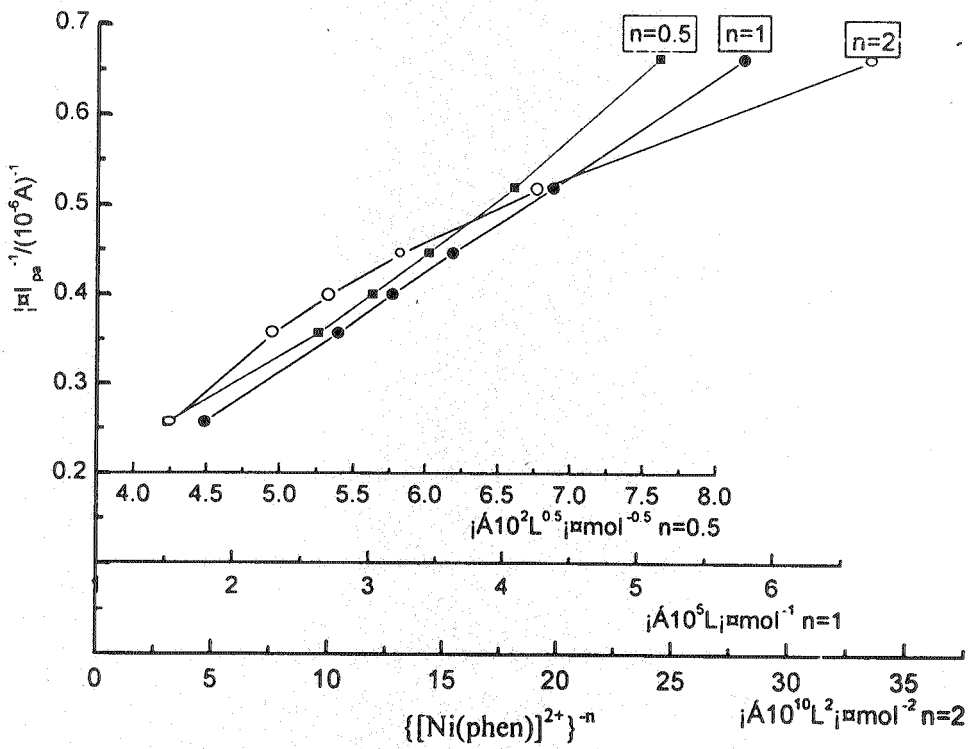

Fig. 6. The relationship between ΔI_{pa}^{-1} and $[Ni(phen)]^{2+}$

UV spectroscopic studies of the interaction between $[Ni(phen)]^{2+}$ and DNA

The variation of the spectra of 1 in the presence of certain concentrations of DNA are shown in Fig. 7. According to reference 13, the hypochromic and bathochromic effects are the identifying marks of the intercalation, both of which are seen in Fig.

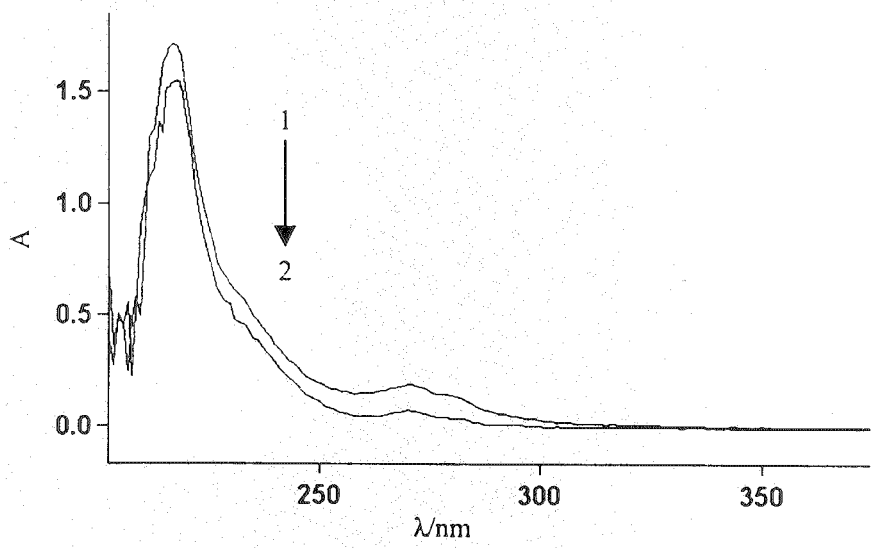

Fig. 7. UV absorption spectra of $[Ni(phen)]^{2+}$ in the absence or presence of DNA. $C^{2+}_{[Ni(Phen)]}$: 6.42×10^{-5} mol L^{-1}; C_{DNA}: (1) 0; (2) 4.68×10^{-5} mol L^{-1}

7. For example, it is observed that all the absorption peaks of **1** at 203.0 nm, 205.0 nm, 217.0 nm decrease and undergo a bathochromic shift with the increase of DNA concentration. What is more, the peaks at 217.0 nm shift to 219.0 nm, which indicates that **1** can interact with DNA via intercalative interaction. The result is consistent with the above electrochemical studies.

Fluorescence spectroscopic studies of the interaction between $[Ni(phen)]^{2+}$ and DNA

Fig. 8 displays the fluorescence features of the $[Ni(phen)]^{2+}$-DNA interaction. It was observed that $[Ni(phen)]^{2+}$ has fluorescence emission at 404 nm, whose fluorescence intensity being weak (curve 1). After adding DNA to the $[Ni(phen)]^{2+}$ solution, the fluorescence intensity of $[Ni(phen)]^{2+}$ was gradually enhanced with increasing concentrations of DNA (curves 2–4). When DNA solution was pipetted

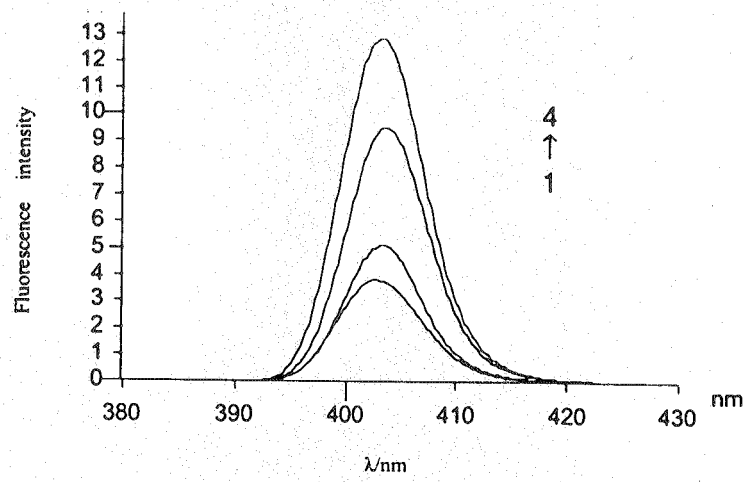

Fig. 8. Fluorescence spectra of $[Ni(phen)]^{2+}$ with increasing concentrations of DNA. $C^{2+}_{[Ni(phen)]}$: 4.28×10^{-4}mol L^{-1}; C_{DNA}: (1) 0; (2) 2.81×10^{-4} mol L^{-1} (3) 4.68×10^{-4} mol L^{-1}; (4) 6.55×10^{-3} mol L^{-1} (excited at 402 nm)

into the $[Ni(phen)]^{2+}$ solution, $[Ni(phen)]^{2+}$ can enter the interior of DNA molecules and intercalate between the base pairs of DNA molecules, then the base pairs of DNA can provide a hydrophobicity microenvironment for $[Ni(phen)]^{2+}$ and lead to fluorescence enhancement, suggesting that the hydrophobicity surrounding of DNA is favourable to enhance the fluorescence quantum yield of the $[Ni(phen)]^{2+}$. Energy transfer from DNA to $[Ni(phen)]^{2+}$ can occur inducing the enhancement of fluorescence[14].

Conclusions

Based on the measurements of cyclic voltammetry, UV spectroscopy and fluorescence spectroscopy, the interaction between $[Ni(phen)]^{2+}$ and salmon sperm DNA was studied. The oxidation peak for $[Ni(phen)]^{2+}$ from CV decreased with increasing concentrations of DNA, the peak potential positively shifted. It was further investigated by UV spectroscopy and fluorescence spectroscopy. The hypochromic effect of the absorption peak and phenomena of bathochromic effect appeared. In addition, the considerable fluorescence enhancement of $[Ni(phen)]^{2+}$ with the addition of DNA was observed. The conclusion can be drawn that $[Ni(phen)]^{2+}$ could interact with DNA mainly by intercalation mode and form a 1 : 1 DNA-$[Ni(phen)]^{2+}$ association complex with the binding constant of 8.28×10^2 L mol^{-1}.

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15. CCDC 220448 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (44)(1223)336033; e-mail: deposit@ccdc.cam.ac.uk).

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