Asian Journal of Chemistry Vol. 17, No. 2 (2005), 1250–1258

Electrochemical and Spectroscopic Studies on the Interaction Between DNA and Chromotrope 2B

S.Y. NIU, S.S. ZHANG*, H. Xu and K. JIAO

College of Chemistry and Molecular Engineering,
Quingdao University of Science and Technology, Quingdao, Shandong-266 042, P.R. China
E-mail: zhangshush@public.qd.sd.cn

Differential pulse voltammetry was used to study the interaction of salmon sperm DNA with chromotrope 2B (CT2B). CT2B had irreversible oxidation peaks in 0.2 mol L^{-1} Britton-Robinson (B-R) buffer solution at pH 5.0 on a glassy carbon electrode. After adding certain concentration of DNA, the oxidation peak current of CT2B decreased and the peak potential positively shifted. It was further investigated by UV/Vis spectroscopy and fluorescence spectroscopy. The hypochromic effect of the absorption peak and phenomena of bathochromic effect appeared. In addition, the increases of fluorescence were observed after CT2B was added to DNA. Quenching fluorescence phenomenon was observed in EB-DNA system when CT2B was added. All the experimental results showed that the binding mode of CT2B with DNA was intercalative binding. The binding ratio of the DNA-CT2B association complex was calculated to be 1 : 2 and the binding constant was 2.57×10^5 L^2 mol^{-2}

Key Words: Chromotrope 2B, DNA, Differential pulse voltammetry, UV/Vis spectroscopy, Fluorescence spectroscopy, Intercalative binding.

INTRODUCTION

Deoxyibonucleic acid (DNA) is an important genetic material in organism. It plays an important role in the process of storing, copying and transmitting germ messages. Gradually, the research on the interaction between small molecules and DNA has become a field of general interest[1]. These researches have contributed to the understanding of the ways of interaction between DNA and protein. What is more, these researches are very important to expound the action mechanism of anticancer drugs, the external selection of drugs and carcinogenesis of the carcinogenic compounds[2].

Azoic compound is one of the synthetic dyes which are various, age-old and have maximal dosage. Now, more than three thousand azoic dyes are being used in the world[3], involving textile, papermaking, plastics, medical, food etc., most of which are monozao-dyes. Many azoic compounds have proved to be carcinogenic[4], whose degradable and carcinogenic mechanism is attracting broad

attention and investigations. As reported[3, 5-7], anaerobes can deoxidize certain azoic compounds in the absence of oxygen. The anaerobes' frame is related to degradation and degradable velocity. So the study of the reaction mechanism and kinetics can provide useful messages for bio-degradation and carcinogenic mechanism. In this paper, the interaction of DNA and CT2B system was studied with differential cyclic voltammetry and spectrum methods.

EXPERIMENTAL

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 the auxiliary electrode. Cary 50 probe UV/Vis spectrophotometer (Varian, Australia), Shimadzu Hitachi F-4500 fluorospectrophotometer (Hitachi, Japan), pHS-25 pH-meter (Shanghai Leici Instrument, China), CHI-832 electrochemical analyzer (Shanghai Chenhua Instrument, China).

Ethidium bromide and Salmon sperm DNA (10 mg mL^{-1}) was purchased from Shanghai Huashun Biologic Engineering Company. DNA's purity was measured by $A_{260}/A_{280} > 1.8$ and its concentration was determined by ultraviolet absorption at 260 nm ($\varepsilon = 6600$ L mol^{-1} cm^{-1}), used without further purification. Ethidium bromide (EB) (10 mg mL^{-1}) was diluted to the needed concentration with doubly deionized water. 0.2 mol L^{-1} Britton-Robinson (B-R), pH 5.0, was used as the buffer solution. The other reagents were all analytical reagents grade prepared with doubly deionized water. 6.00×10^{-3} mol L^{-1} solution of CT2B (Shanghai Chemical Reagent, China) was prepared by dissolving 0.0308 g CT2B in 10 mL doubly deionized water.

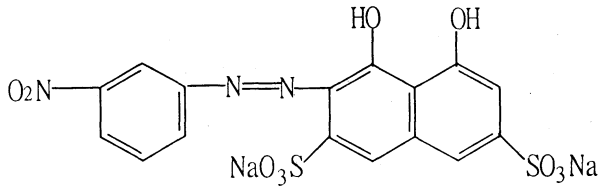

Fig. 1. The structure of CT2B

Electrochemical behaviour of the interaction between CT2B and DNA: Appropriate amounts of CT2B were added to 5 mL of 0.2 mol L^{-1} B-R buffer solution. The differential pulse voltammograms (DVP) of the solutions were recorded on CHI 832 electrochemical analyzer. Then different amounts of DNA were added to the solution followed by recording the DPV figure. The potential scanning range is from –0.4 to 1.2 V at a rate of 0.06 V s^{-1}; the sample interval is 0.001 V and the quiet time 2 s. For the electrochemical experiments, the GCE surface was freshly polished prior to each experiment with 1.0, 0.3 and 0.05 μm Al_2O_3 paste, washed by doubly deionized water and finally cleaned ultrasonically in water.

UV/Vis spectroscopic studies of the interaction between CT2B and DNA: 20 μL of 6.00×10^{-3} mol L^{-1} CT2B and different quantity of 4.68×10^{-2} mol L^{-1} DNA solution were in turn added to 10 mL colorimetric tubes respec-

tively, then diluted to the desired scale with B-R buffer solution. The solutions were set for 8 min at room temperature. The UV/Vis spectra were recorded on a Cary 50 probe spectrophotometer in 1.0 cm quartz cuvettes. The range of the scanning wavelengths was from 300 to 700 nm.

Fluorescence spectroscopic studies of the interaction between CT2B and DNA: Appropriate CT2B and 2 mL B-R buffer solution were transferred into each of the three 10 mL colorimetric tubes and then added different amounts of DNA solution respectively; appropriate EB solution was transferred into each of the four 10 mL colorimeteric tubes, and finally different amounts of DNA and CT2B solution were in turn added respectively. The mixture was diluted to the mark and set for 8 min at room temperature. The measurements of fluorescence were made by using the above instruments in 1.0 cm quartz cell.

RESULTS AND DISCUSSION

The electrochemical studies of CT2B on the glassy carbon electrode

There are electrochemical activities in the base solution of 0.1 mol L^{-1} NaH_2PO_4-Na_2HPO_4, 0.2 mol L^{-1} B-R, 0.05 mol L^{-1} tris-HCl and 0.1 mol L^{-1} HOAc–NaOAc buffer solution. 0.2 mol L^{-1} B-R buffer solution was selected as the base solution because the peak is the best, which is shown in Fig. 2 (curve 1).

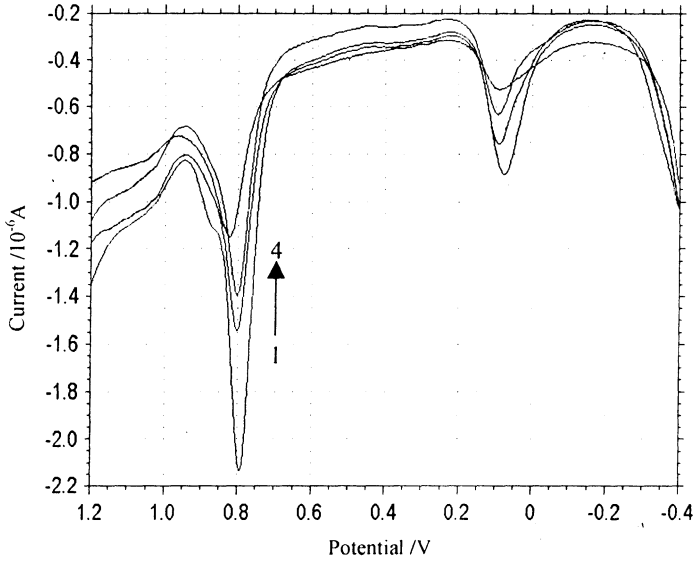

Fig. 2 The differential pulse voltammograms of CT2B with increasing concentrations of DNA: C_{CT2B}: 6.00×10^{-5} mol L^{-1}; C_{DNA}: (1) 0; (2) 9.36×10^{-5} mol L^{-1}; (3) 1.87×10^{-4} mol L^{-1}; (4) 2.81×10^{-4} mol L^{-1}

The relationship between I_{pa} and the concentration of CT2B shows that I_{pa} is directly proportional to the concentration of CT2B with a regression equation: $y = 0.2899x + 0.0486$ and a correlation coefficient $\gamma = 0.9991$, where y is the voltammetric oxidation peak current I_{pa} and x is the concentration of CT2B.

The curves 2–4 of Fig. 2 are the DPV of 6.00×10^{-5} mol L^{-1} CT2B in the presence of different concentrations of DNA compared with the curve 1 without DNA. The results show obvious decrease of the peak current with positive shifts of the oxidative peak potential after adding DNA. No new oxidation-reduction peaks appear. So CT2B interacting with DNA forms electrochemically non-active complex[8]. In the presence of DNA, the equilibrium concentration or the diffusion coefficient of CT2B decreases, which results in a decrease of the peak current. Among three kinds of binding modes for small molecules to DNA, Bard *et al.*[9] has reported that if the oxidative peak potential shifts to more negative value when small molecules interact with DNA, the interaction mode is electrostatic binding. On the contrary, if the oxidative peak potential shifts to more positive value, the interaction mode is intercalative binding. According to Fig. 1 and the structure of CT2B, the initial conclusion can be obtained that the binding mode between CT2B and DNA is intercalative binding, CT2B can intercalate into the base pairs of DNA molecules.

Effect of pH on the oxidation peak current of CT2B

The relationship between the oxidation peak current of CT2B and the pH value was experimented in 0.2 mol L^{-1} B-R. It was found that the value of I_{pa} increased firstly and then reached a maximum when the pH was 5.0. After that, I_{pa} decreased slowly. Consequently, 5.0 was chosen as the best pH of the reaction.

Effect of the scan rate on the oxidation peak current of CT2B

Fig. 3 is the plot of I_{pa} *vs.* $v^{1/2}$ (v is the scanning rate). I_{pa} is not directly proportional to the square root of the scanning rate in the range from 0.02 to 0.25 V s^{-1}, indicating that the electro-oxidation process of CT2B is controlled not only by the diffusion of CT2B, but also by the adsorption effect on the electrode surface[10]. That means CT2B has the character of surface adsorption.

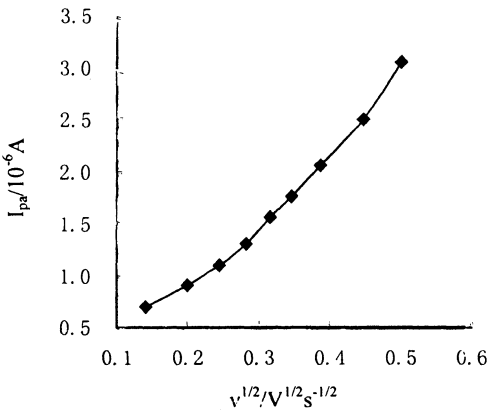

Fig. 3. The relationship curve of I_{pa} and $v^{1/2}$ of CT2B: C_{CT2B}: 6.00×10^{-5} mol L^{-1}

Effect of the reacting time on the interaction of CT2B with DNA

The relationship between the oxidation peak current of CT2B and the reacting time was studied. The peak current decreases firstly with the reacting time and

reaches a constant value after about 8 min, indicating that the reaction of CT2B with DNA has reached the equilibrium state. Consequently, 8 min was chosen as the reaction time.

The binding ratio and binding constant of DNA–nCT2B association complex

According to the reference[11], it is assumed that DNA and CT2B only produce a single complex DNA-nCT2B:

$$DNA + nCT2B \rightleftharpoons DNA\text{-}nCT2B \quad (n = 1, 2, 3 \quad or \quad 1, 1/2, 1/3, \ldots)$$

The equilibrium constant can be expressed as follows:

$$\beta = \frac{[DNA\text{-}nCT2B]}{[DNA][CT2B]^n} \tag{1}$$

and the following equations can be deduced:

$$\Delta I_{max} = K'C_{DNA} \tag{2}$$

$$\Delta I = K'[DNA\text{-}nCT2B] \tag{3}$$

$$[DNA] + [DNA\text{-}nCT2B] = C_{DNA} \tag{4}$$

$$\Delta I_{max} - \Delta I = K'(C_{DNA}\text{-}[DNA\text{-}NCT2B]) \tag{5}$$

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

Introducing eqn. (3) and (6) into eqn. (1) leads to

$$\frac{1}{\Delta I} = \frac{1}{\Delta I_{max}} + \frac{1}{\beta \Delta I_{max} [CT2B]^n} \tag{7}$$

With different n, there are different relationship curves between ΔI^{-1} and $[CT2B]^{-n}$. According to equation (7), the relationship curve between ΔI^{-1} and $[CT2B]^{-n}$, with suitable n, should be a straight line if only one complex was formed when CT2B bound to DNA. From the slope and intercept of the straight line, the binding constant β can be calculated.

In Fig. 4, curve 1 shows the relationship of I_{pa} and C_{CT2B} in the absence of DNA. Curve 2 typically represents the current change at $C_{DNA} = 2.81 \times 10^{-4}$ mol L^{-1} on varying the concentrations of CT2B. Curve 3 is the relationship between ΔI which means the difference of I_{pa1} and I_{pa2} and the concentration of CT2B. By calculating different ΔI and [CT2B] from Fig. 4, the relationship curve of ΔI^{-1} *vs.* $[CT2B]^{-n}$ was obtained. As for n = 2, the curve is a straight line ($\gamma = 0.9988$), shown in Fig. 5; it means that CT2B bound to DNA to form a DNA-2CT2B association complex under this case. While for n = 1/2 and 1, the curve bends up respectively. From the slope and intercept of the straight line, the binding constant β was calculated to be 2.57×10^5 L^2 mol^{-2}, which was corresponding to the equation $DNA + 2CT2B \rightleftharpoons DNA\text{-}2CT2B$.

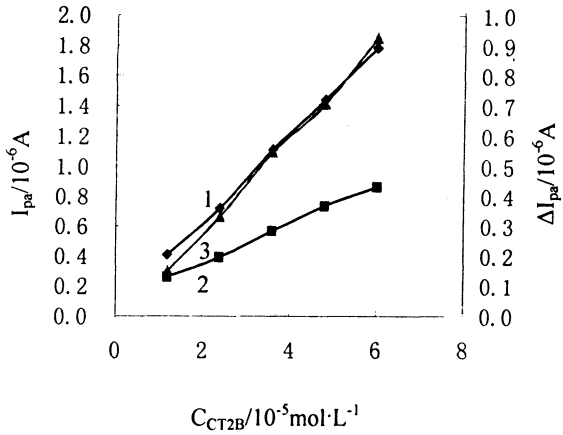

Fig. 4. The relationship curve of I_{pa} and ΔI_{pa} *vs.* C_{CT2B}: (1) C_{DNA}: 0; (2) C_{DNA}: 2.81×10^{-4} mol L^{-1}; (3) $\Delta I_{pa} = I_{pa1} - I_{pa2}$

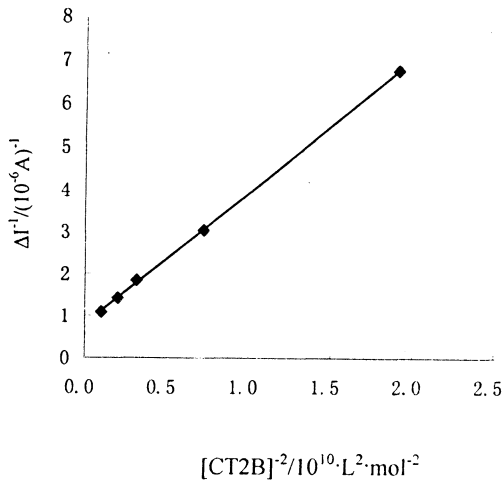

Fig. 5. The relationship curve of ΔI^{-1} *vs.* $[CT2B]^{-2}$

UV/Vis spectroscopic studies of the interaction between CT2B and DNA

The variations of CT2B spectra in the presence of different concentrations of DNA are shown in Fig. 6. Hypochromic effect and bathochromic effect are the identifying marks of the intercalation[12], both of which are shown in Fig. 5. For example, it is observed that all the absorption peaks of CT2B at 311.0, 361.1 and 515.0 nm decrease with the increasing of DNA concentration. What more, the peaks at 311.0 and 515.0 nm shift to 313.0 and 518.0 nm, which indicates that CT2B can interact with DNA *via* intercalative interaction. The result is consistent with the above electrochemical studies.

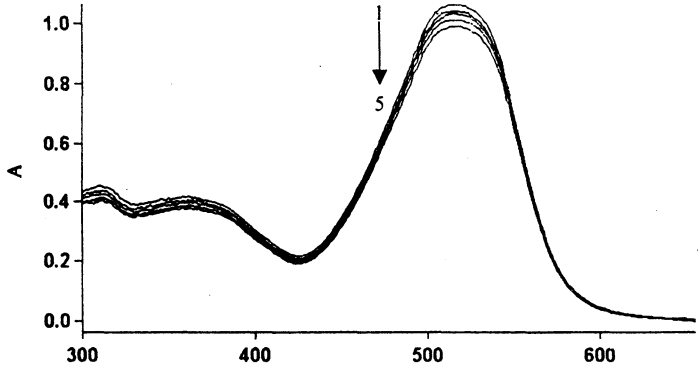

Fig. 6. The UV/Vis spectra of CT2B with different concentrations of DNA: C_{CT2B}: 6.00×10^{-5} mol L^{-1}; C_{DNA}: (1) 0; (2) 4.68×10^{-4} mol L^{-1}; (3) 9.36×10^{-4} mol L^{-1}; (4) 1.40×10^{-3} mol L^{-1}; (5) 1.87×10^{-3} mol L^{-1}

Fluorescence spectroscopic studies of the interaction between CT2B and DNA

It was observed that CT2B has fluorescence emission at 696 nm (Fig. 7, curve 1). After adding DNA to the CT2B solution, the fluorescence intensity of CT2B was gradually enhanced with increasing concentrations of DNA (Fig. 7, curves 2 and 3).

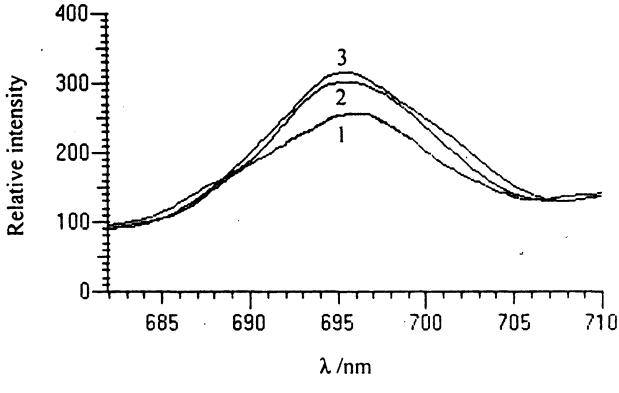

Fig. 7. Fluorescence spectra of CT2B with increasing concentrations of DNA (excited at 300 nm, 2 mL solution). C_{CT2B}: 1.50×10^{-4} mol L^{-1}; C_{DNA}: (1) 0; (2) 4.68×10^{-4} mol $^{-1}$; (3) 9.36×10^{-4} mol L^{-1}

According to literature[13], if DNA solution is pipetted into the CT2B solution, CT2B 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 CT2B and lead to fluorescence enhancement, suggesting that the hydrophobicity surrounding of DNA is favourable to enhance the fluorescence quantum yield of the CT2B. In addition, energy transfer from DNA

to CT2B can occur inducing the enhancement of fluorescence[14]. Fig. 7 also shown the typical fluorescence spectra of energy transfer for CT2B at different concentrations of DNA; the energy transfer from DNA to CT2B involves the intercalative binding mode between CT2B and DNA.

The fluorescence spectrum of EB-DNA system in the presence of CT2B was examined in the hope of providing information about the similarities or differences of the binding modes of these complexes to DNA, as shown in Fig. 8. EB itself exhibited weak fluorescence emission (curve 1), but when it intercalated into the base pairs of the double helix DNA molecules, a noticeable increase of EB fluorescence intensity was observed (curve 2). It is generally recognized that if CT2B can also bind to DNA through intercalation mode as EB after adding CT2B into EB-DNA system, CT2B and EB can compete for the same binding sites of DNA and weaken the fluorescence intensity of EB-DNA system[15]. Fig. 8 (curves 2–4) showed quenching fluoresence for EB-DNA system when different quantity of CT2B was added, indicating that CT2B and EB do bind to DNA in a competitive manner; the DNA bound EB was effectively displaced by the addition of CT2B. It is, therefore, quite likely that EB and CT2B intercalate at the same sequences along the DNA double helix, so the intercalation mode of CT2B with DNA was further confirmed.

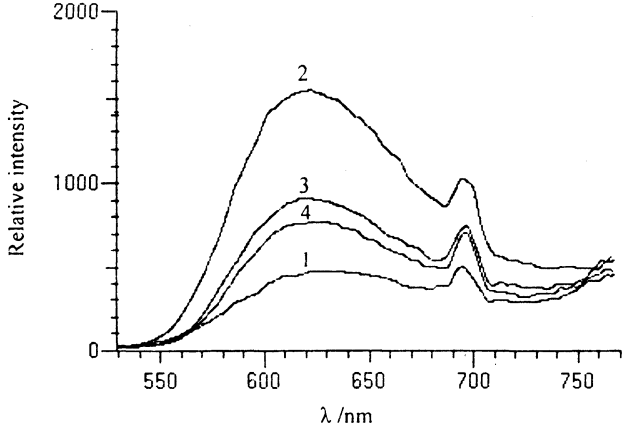

Fig. 8. Fluorescence spectra of EB-DNA system in the presence of CT2B: (1) C_{EB}: 1.27×10^{-4} mol L^{-1}; (2) C_{EB}: 1.27×10^{-4} mol L^{-1}, C_{DNA}: 5.85×10^{-4} mol L^{-1}; (3) C_{EB}: 1.27×10^{-4} mol L^{-1}, C_{DNA}: 5.85×10^{-4} mol L^{-1}, C_{CT2B}: 3.00×10^{-5} mol L^{-1}; (4) C_{EB}: 1.27×10^{-4} mol L^{-1}, C_{DNA}: 5.85×10^{-4} mol L^{-1}, C_{CT2B}: 6.00×10^{-5} mol L^{-1}

Conclusions

Based on the measurements of differential pulse voltammetry, UV/Vis spectroscopy and fluorescence spectroscopy, the interaction between CT2B and Salmon sperm DNA was studied. The peak currents of CT2B from differential pulse voltammetry decreased with increasing concentrations of DNA; the peak

potential shifted to more positive values. In addition, the considerable fluorescence enhancement of CT2B with the addition of DNA and quenching fluorescence for EB-DNA system when CT2B is added were observed. The conclusion can be drawn that CT2B could interact with DNA mainly by intercalation mode and form a DNA-2CT2B association complex with the binding constant of 2.57×10^5 L^2 mol^{-2}.

ACKNOWLEDGEMENT

This work was supported by the National Natural Science Foundation of Chian (No. 20275020) and the State Key Laboratory Foundation of Electroanalytical Chemistry of Changchun Institute of Applied Chemistry (No. SKLEAC 2004–8).

REFERENCES

1. R.-Y. Zhang, D.-W. Pang and R.-X. Cai, *Chem. J. Chinese Univ.*, **20,** 1210 (1999)
2. H.-Z. Huang and X.-R. Yang, *Chinese J. Anal. Chem.*, **30,** 491 (2002).
3. K.-T. Chung and S.E. Stevens, *Crit. Rev. Microbiol.*, **18,** 175 (1992).
4. W.-X. Yang, *Environ. Chem.*, **2,** 1 (1983).
5. K.-T. Chung and S.E. Stevens, *Environ. Toxicol. Chem.*, **12,** 2121 (1993).
6. K.-T. Chung, G.E. Fulk and M. Egan, *Appl. Environ. Microbiol.*, **35,** 558 (1978).
7. J.J. Roxon, A.J. Ryan and S.E. Wright, *Food Cosmet. Toxicol.*, **5,** 367 (1967).
8. D.-J. Liu, Z.-X. Wang and S.-J. Dong, *Chinese J. Anal. Lab.*, **21,** 54 (2002).
9. M.T. Carter, M. Rodriguez and A.J. Bard, *J. Am. Chem. Soc.*, **111,** 8901 (1989).
10. Q.-L. Li, X.-C. Tan and J.-B. Hu, *Chem. J. Chinese Univ.*, **18,** 37 (1995).
11. F. Qu, N.-Q. Li and Y.-Y. Jiang, *Anal. Chim. Acta*, **344,** 97 (1997).
12. E.C. Long and J.K. Barton, *Accounts Chem. Res.*, **23,** 271 (1990).
13. G.-C. Zhao, J.-J. Zhu and H.-Y. Chen, *Chem. J. Chinese Univ.*, **24**, 414 (2003).
14. Y. Cao, Y. Li and X.-W. He, *Chem. J. Chinese Univ.*, **20,** 709 (1999).
15. Y.-M. Song, P.-J. Yang, L.-F. Wang, M.-L. Yang and J.-W. Kang, *Acta Chim. Sinica*, **61,** 1266 (2003).

(*Received*: 6 September 2004; *Accepted*: 31 January 2005) AJC-4076