Asian Journal of Chemistry Vol. 18, No. 2 (2006), 1017–1024

Electrochemical and Spectroscopic Studies on the Interaction between 1-(2-Pyridylazo)-2-naphthol and DNA

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Cyclic voltammetry was used to study the interaction between salmon sperm DNA and 1-(2-pyridylazo)-2-naphthol (PAN) which was dissolved in the mixture of ethanol and water. PAN had irreversible oxidation peaks in 0.2 mol L^{-1} Britton-Robinson (B-R) buffer solution at pH 4.80 on a glassy carbon electrode (GCE). After adding certain concentration of dsDNA, the oxidation peak current of PAN decreased. 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 dsDNA was added to the solution of PAN. Quenching fluorescence phenomenon was observed in EB-DNA system when PAN was added. All the experimental results showed that the binding mode of PAN with dsDNA was intercalative binding. The binding ratio of the DNA-PAN association complex was calculated to be 1 : 2 and the binding constant was 4.56×10^5 L^2 mol^{-2}.

Key Words: 1-(2-Pyridylazo)-2-naphthol, DNA, Cyclic voltammetry, Spectrum methods, Intercalative binding.

INTRODUCTION

The azoic dyestuff, whose molecular structure contains two nitrogen atoms coupling together, is an artificial synthetic chemical compound. The annual output of this compound in the USA is 90,000 tons while 200 tons of the azoic colour has been drained into the public water. There are about 3,000 kinds of azo dyestuff in the globel market, involving more than 200 kinds composed of benzidine[1].

The water-soluble azoic dyestuff can be reduced into aromatic amines by the germs in the gastrointestinal tracts. The non-water-soluble ones, on the other hand, will be absorbed by the liver and in turn transformed into aromatic amine by the enzymes in the liver. The mechanism of this azo-reduction is metabolic activation. Some aromatic amines are mutagens, others are carcinogenic substances still others may have some biotoxin. Developing the mechanism of these aromatic amines which lead to mutation, carcinoma or other biotoxins will not only enhance the knowledge on the toxicological mechanism of these compounds, but also can provide counter-measures against the diseases which may be caused as

a result of these compounds[2]. 1-(2-Pyridylazo)-2-naphthol (PAN) is one of the azo dyestuffs. In this article, the interaction of DNA and the PAN system was studied with cyclic voltammetry (CV) and spectrum methods. The conclusion was obtained that there is intercalative binding between DNA and PAN. The binding number and the binding constant of the DNA-PAN complex were deduced.

EXPERIMENTAL

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

Salmon sperm DNA (10 mg mL^{-1}) was purchased from Shanghai Huashun Biologic Engineering Company. Its purity was measured by $A_{260}/A_{280} > 1.8$. 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}) (also from Shanghai Huashun Biochemistry Technology Company) was diluted to the needed concentration with doubly deionized water. 0.2 mol L^{-1} Britton-Robinson (B-R), pH 4.80, was used as the buffer solution. The other reagents were all of analytical reagents grade prepared with doubly deionized water. 6.00×10^{-3} mol L^{-1} solution of PAN (from Shanghai Chemical Reagent Company of China) was prepared by dissolving 0.0149 g PAN in 10 mL cuvette with the mixture of ethanol and doubly deionized water. Fig. 1 is the structure of PAN.

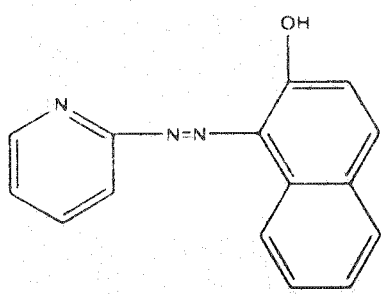

Fig. 1. Structure of 1-(2-pyridylazo)-2-naphthol (PAN)

Electrochemical behaviour of the interaction between PAN and DNA: For the electrochemical experiments, the GCE surface was freshly polished prior to each experiment with 1.0 μm, 0.3 μm and 0.05 μm α-Al_2O_3 paste, washed by doubly deionized water and finally cleaned ultrasonically in water. Required amount of PAN was added to 5 mL of 0.2 mol L^{-1} B-R buffer solution. The cyclic voltammograms of the solutions were recorded on CHI 832 electrochemical analyzer. Then different amounts of DNA were added to the solution followed by

recording the CV figure. The potential scanning range is from –0.4 to 1.6 V. The scanning rate is 0.06 V s^{-1}; the sample interval is 0.001 V and the quiet time 2 s.

UV-Vis spectroscopic studies of the interaction between PAN and DNA: 20 μL of 6.00×10^{-3} mol L^{-1} PAN and 0, 30, 40, 50 μL 4.68×10^{-2} mol L^{-1} DNA solution were in turn added to 10 mL colorimetric tubes respectively, then diluted to the desired scale with B-R buffer solution. The solutions were set for 6 min at room temperature. The UV-Vis spectra were recorded on a Cary50 probe spectrophotometer in 1.0 cm quartz cuvettes. The range of the scanning wavelengths was from 200 to 700 nm.

Fluorescence spectroscopic studies of the interaction between PAN and DNA: 40 μL of 6.00×10^{-3} mol L^{-1} PAN and B-R buffer solution were transferred into each of the three 10 mL colorimetric tubes and then added 0, 30, 40 μL of 4.68×10^{-2} mol L^{-1} DNA solution respectively. The mixture was diluted to the mark and set for 6 min at room temperature.

5 μL 2.50×10^{-2} mol L^{-1} EB solution was transferred into each of the four 10 mL colorimetric tubes and then different amounts of DNA and PAN solution were added, in turn, respectively. The mixture was also diluted to the mark and set for 6 min at room temperature.The measurements of fluorescence were made by using the above instruments in 1.0 cm quartz cell.

RESULTS AND DISCUSSION

Electrochemical studies of PAN on the glassy carbon electrode

The electrochemical activities of PAN were set out separately in the base solution of 0.1 mol L^{-1} NaH_2PO_4-Na_2HPO_4, 0.2 mol L^{-1} B-R, 0.1 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). The peak potential of PAN is about 0.86 V in B-R. In

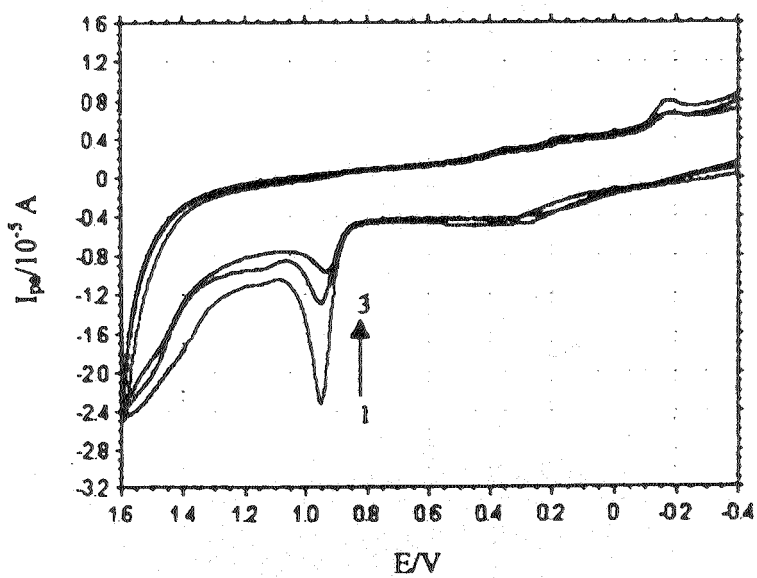

Fig. 2. The cyclic voltammograms of PAN [C_{PAN}: 6.00×10^{-5} mol L^{-1}; C_{DNA}: (1) 0; (2) 1.87×10^{-4} mol L^{-1}; (3) 2.81×10^{-4} mol L^{-1}]

the concentration range from 1.50×10^{-5} mol L^{-1} to 2.50×10^{-4} mol L^{-1}, the peak current is in line with the concentration of PAN. The relationship between I_{pa} and the concentration of PAN shows that I_{pa} is directly proportional to the concentration of PAN with a regression equation: $I_{pa} = 0.29C_{PAN} + 0.5485$ and a correlation coefficient $\gamma = 0.9991$.

The curves 2 and 3 of Fig. 2 are the CV of 6.00×10^{-5} mol L^{-1} PAN in the presence of different concentrations of DNA compared with the curve 1 without DNA. The results show obvious decreases of the peak current with shifts of the oxidative peak potential after adding DNA. No new oxidation-reduction peaks appear. So PAN interacting with DNA forms electrochemically non-active complex. In the presence of DNA, the equilibrium concentration or the diffusion coefficient of PAN decreases, which results in a decrease of the peak current.

Effect of pH on the oxidation peak current of PAN: The relationship between the oxidation peak current of PAN and the pH value was experimented 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 pH was 4.80. After that, I_{pa} decreased slowly, which was shown in Fig. 3. Consequently, 4.80 was chosen as the best pH of the reaction.

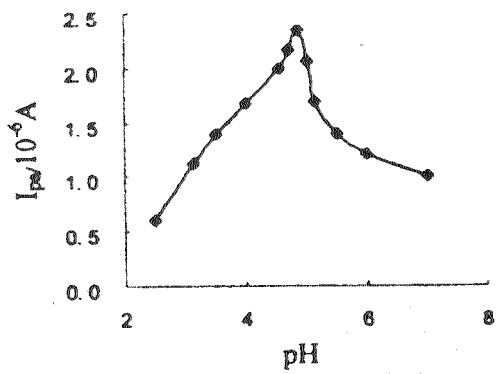

Fig 3. The relationship curve of I_{pa} *vs.* pH of PAN and DNA system [C_{PAN}: 6.0×10^{-5} mol L^{-1}; C_{DNA}: 2.81×10^{-4} mol L^{-1}]

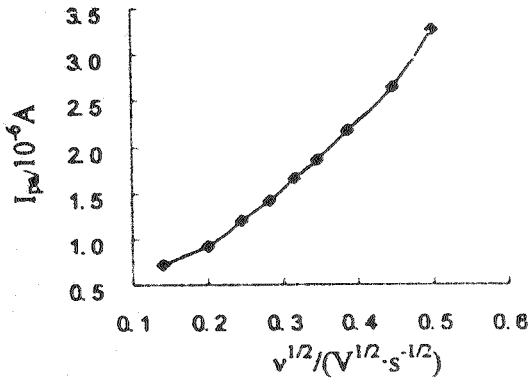

Fig. 4. The relationship curve of I_{pa} *vs.* $v^{1/2}$ of PAN

Effect of the scan rate on the oxidation peak current of PAN: Fig. 4 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 0.02–0.25 V s^{-1}, indicating that the electrooxidation process of PAN is controlled not only by the diffusion of PAN, but also by the adsorption effect on the electrode surface[3]. That means PAN has the character of surface adsorption.

Effect of the reacting time on the interaction of PAN with DNA: The relationship between the oxidation peak current of PAN and the reacting time was studied. The peak current decreases first with the reacting time and reaches a constant value after about 6 min, indicating that the reaction of PAN with DNA has reached the equilibrium state. Consequently, 6 min was chosen as the reaction time.

The binding ratio and the binding constant of the DNA-*n*PAN association complex: According to Qu *et al.*[4], it is assumed that DNA and PAN only produce a single complex DNA-*n*PAN:

$$\text{DNA} + n\text{PAN} \rightleftharpoons \text{DNA-}n\text{PAN}$$

The equilibrium constant can be expressed as follows:

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

$$\Delta I_{pa,\,max} = KC_{DNA} \quad (2)$$

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

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

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

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

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

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

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

In Fig. 5, curve 1 shows the relationship of I_{pa} and C_{PAN} in the absence of DNA. Curve 2 typically represents the current change at $C_{DNA} = 4.68 \times 10^{-4}$ mol L^{-1} on varying the concentrations of PAN. Curve 3 is the relationship between ΔI_{pa} which means the difference of I_{pa1} and I_{pa2} and the concentration of PAN. By calculating different ΔI_{pa} and [PAN] from Fig. 5, the relationship curve of ΔI_{pa}^{-1} *vs.* $[\text{PAN}]^{-n}$ was obtained. As for $n = 2$, the curve is a straight line ($\gamma = 0.9978$), shown in Fig. 6; it means that PAN bound to DNA to form a DNA-2

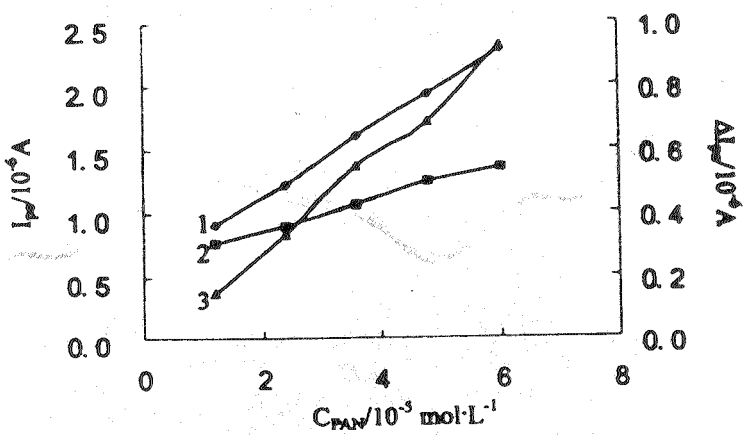

Fig. 5. Relationship curve of I_{pa1}, I_{pa2} and ΔI_{pa} *vs.* C_{PAN}

PAN 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 was calculated to be 4.56×10^5 L^2 mol^{-2}, which was corresponding to the equation

$$DNA + 2\,PAN \rightleftharpoons DNA\text{-}2\,PAN$$

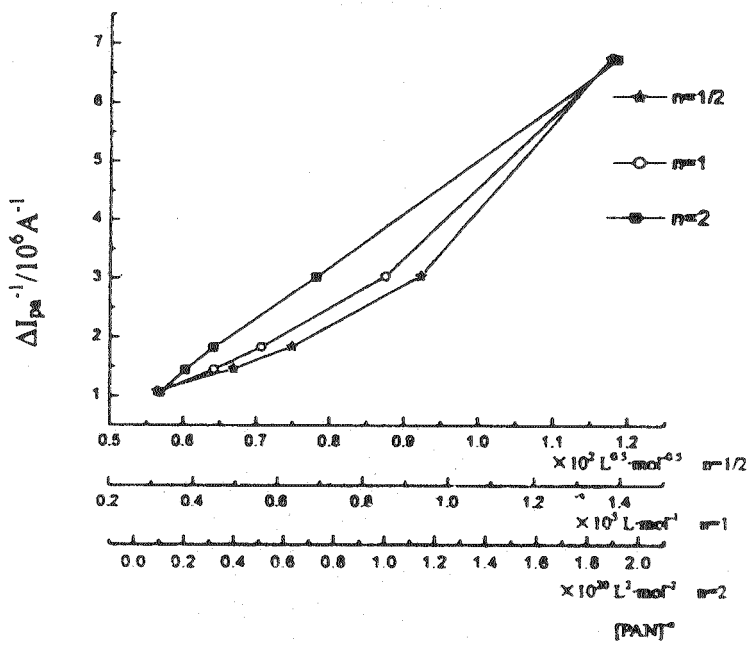

Fig. 6. Relationship curve of ΔI^{-1} *vs.* $[PAN]^{-n}$

UV-Vis spectroscopic studies of the interaction between PAN and DNA

The variations of 1.20×10^{-5} mol L^{-1} PAN spectra in the presence of different concentrations of DNA are shown in Fig.7. Curve 1 is the PAN spectrum in the B-R buffer solution and curves 2 to 4 are the spectra of PAN with different concentrations of DNA. According to Long and Barton[5], hypochromic effect and

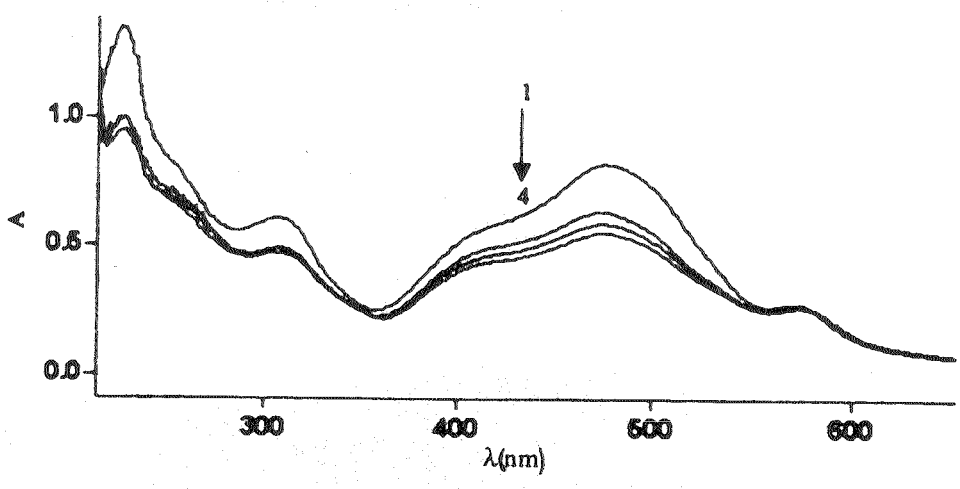

Fig. 7. Absorption spectra of PAN with DNA [C_{PAN}: 1.20×10^{-5} mol L^{-1}, C_{DNA}: (1) 0; (2) 1.40×10^{-4} mol L^{-1}; (3) 1.87×10^{-4} mol L^{-1}; (4) 2.34×10^{-4} mol L^{-1}]

bathochromic effect are the identifying marks of the intercalation, both of which are viewed from Fig. 5. For example, it is observed that all the absorption peaks of PAN at 225, 305, 475 and 571 nm decrease with the increasing of DNA concentration. What is more, the peaks at 225 and 571 nm shift to 2270 and 574 nm respectively, which indicates that PAN can interact with DNA *via* intercalative interaction.

Fluorescence spectroscopic studies of the interaction between PAN and DNA

Fig. 8 displays the fluorescence features of the PAN-DNA interaction. It was observed that PAN has fluorescence emission at 506 nm. Curve 1 is the fluorescence spectrum of PAN. After adding different concentrations of DNA to the PAN solution, the fluorescence intensity of PAN was gradually enhanced with increasing concentrations of DNA (curves 2 and 3). According to Wu *et al.*[6], if DNA solution was pipetted into the PAN solution, PAN 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 PAN and lead to fluorescence enhancement, suggesting that the hydrophobicity surrounding of DNA is favourable to enhance the fluorescence quantum yield of PAN. Energy transfer from DNA to PAN can occur inducing the enhancement of fluorescence. Fig. 8 is also the typical fluorescence spectra of energy transfer for PAN at different concentrations of DNA; the energy transfer from DNA to PAN involves the intercalative binding mode between PAN and DNA.

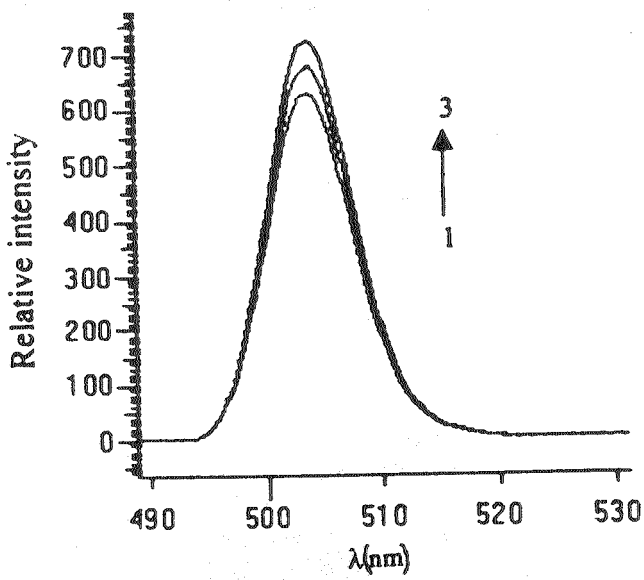

Fig. 8. Fluorescence spectra of PAN with DNA (excited at 506 nm)
[C_{PAN}: 2.40×10^{-5} mol L^{-1}; C_{DNA}: (1) 0; (2) 1.40×10^{-4} mol L^{-1}; (3) 1.87×10^{-4} mol L^{-1}]

The fluorescence spectrum of EB-DNA system in the presence of PAN was examined in the quest of providing information about the similarities or differences of the binding modes of these complexes to DNA, as shown in Fig. 9. EB itself exhibited weak fluorescence emission (curve 1), but when it intercalated

into the base pairs of the DNA double helix, a noticeable increase of EB fluorescence intensity was observed (curve 2). It is generally recognized that if PAN can also bind to DNA through intercalation mode as EB after adding PAN into EB-DNA system, PAN and EB can compete for the same binding sites of DNA and weaken the fluorescence intensity of EB-DNA system[7]. Fig. 9 (curve 2 and 4) showed quenching fluorescence for EB-DNA system when different quantity of PAN was added, indicating that PAN and EB do bind to DNA in a competitive manner. The DNA bound EB was effectively displaced by the addition of PAN. It is therefore quite likely that EB and PAN intercalate at the same sequences along the DNA double helix; so the intercalation mode of PAN with DNA was further confirmed.

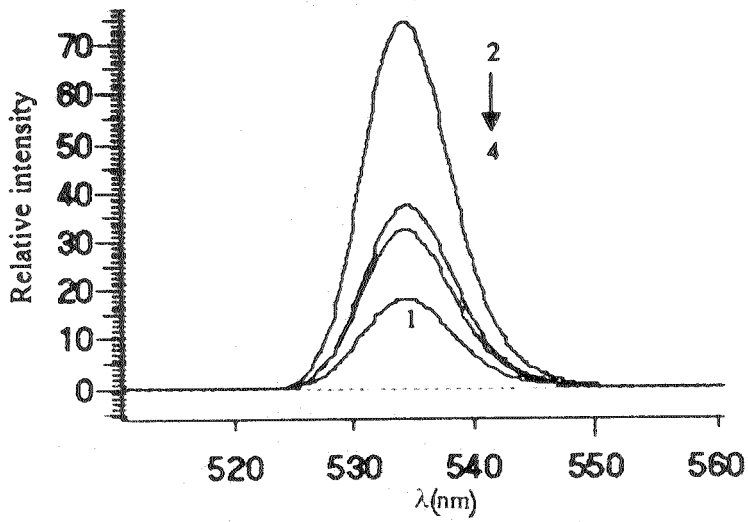

Fig. 9. Effect of PAN on the fluorescence spectra of EB-DNA system (excited at 535 nm) [(1): 1.25×10^{-5} mol L^{-1} EB; (2): (1) + 2.81×10^{-4} mol L^{-1} DNA; (3): (2) + 1.80×10^{-5} mol L^{-1} PAN; (4): (2) + 2.40×10^{-5} mol L^{-1} PAN]

ACKNOWLEDGEMENTS

We thank the National Natural Science Foundation of China (Nos. 20275020 and 20475030), the Program for New Century Excellent Talents in University (No. NCET-04-0649) and the State Key Laboratory Foundation of Electroanalytical Chemistry of Changchun Institute of Applied Chemistry (No. SKLEAC 2004-8).

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(*Received*: 29 March 2005; *Accepted*: 12 December 2005) AJC-4525