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# Recognition and Determination of DNA Using Victoria Blue B as Electrochemical Probe

*Bai Xu, Kui Jiao*, Wei Sun, Xia Zhang*

College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, P. R. China
*E-mail: Kjiao@qust.edu.cn

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The recognition of Victoria blue B (VBB) with DNA was investigated by electrochemical and spectrophotometric method in pH 5.0 B-R buffer solution. In the potential scan range of +0.70~+1.30 V (vs. SCE), VBB had an irreversible oxidative peak at +0.984 V at the scan rate of 100.0 mV/s on cyclic voltammogram. After the addition of double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA) into VBB solution, the peak current decreased significantly and the peak potential shifted negatively. The results demonstrated that VBB binding to DNA was caused by electrostatic interaction. The binding of VBB with dsDNA was stronger than that with ssDNA. By virtue of this difference, VBB can be used as an electrochemical probe to identify ssDNA and dsDNA. The electrochemical behaviours of the VBB in the presence and absence of DNA was carefully studied. Electrochemical parameters such as the diffusion coefficient (D), the electron transfer coefficient (α) and the electrochemical reaction standard rate constant (ks') were calculated. The binding ratio (m) and the binding constant (β) of VBB to dsDNA were further investigated by voltammetric method. UV-Vis spectrophotometry also proved that VBB bound to DNA mainly through electrostatic mode. Based on the decrease of the differential pulse voltammetric peak current of VBB with the increase of dsDNA concentration, a working curve was constructed for dsDNA in the range of 0.10~8.0 mg/L with the linear regression equation as $\Delta i_p(10^{-6}A)=0.072C(mg/L)+0.24$ and the detection limit ($3\sigma$) as 0.069 mg/L. The method was satisfactorily applied to the determination of the DNA content in the artifical samples.

**Keywords:** Victoria blue B; DNA; Electrochemistry; UV-Vis spectrophotometry,;Electrostatic interaction

## 1. INTRODUCTION

In recent decades, the investigation of the interaction between DNA and small molecules has been the focus in the DNA research area. These small molecules studied involve dyes [1-3], drugs [4-6] and

metal coordination compounds [7-9] . The aim of the research is to develop new substances as probes for the determination of DNA in various applications, such as molecular biology, biotechnology, medical diagnostics and forensic analysis. Further more, the investigation of the binding reaction can provide the chemical basis for the carcinogenicity of environmental pollutants and toxic chemicals, or to serve as analogues in the study of protein–nucleic acid recognition, which may be useful in designing new and promising anticancer drugs for clinical use [10]. According to the characteristic of the selected molecules, various methods, such as electrochemical methods, gel electrophoresis, footprinting technique, X-ray crystallography, fluorescent spectroscopy, have been proposed for these researches [11]. Studies on the interaction between DNA and small molecules by electrochemical methods have received much attention, for it can provide much information that other methods can't get. Many papers have been reported for the electrochemical investigation on this respect [12-15] with cyclic voltammetry or other electrochemical methods, due to the characteristics of high sensitivity, good selectivity and seldom disturbed by turbidness or colour of biologic sample. Therefore, electrochemical method is an effective means for investigating the interaction of DNA and small molecules.

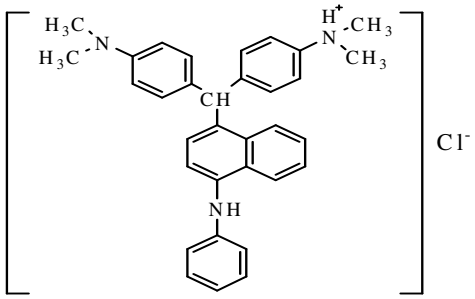



**Figure 1**. The molecular structure of Victoria blue B

Victoria blue B is a cationic dye, which belongs to triphenylmethane dye family and its molecular structure is shown as Figure 1. Su et al.[16, 17] investigated the interaction between DNA and VBB by spectrophotometry and resonance light scattering technique, respectively. To our knowledge, there was no article involving on the interaction of DNA with VBB by means of electrochemical methods, and the electrochemical parameters, the binding ratio and binding constant of VBB to dsDNA had not been reported in the literature. In this paper, the interaction of VBB with DNA was investigated in pH 5.0 B-R buffer solution. VBB has the recognizable characteristic to ssDNA and dsDNA, which can be demonstrated by electrochemical method and UV-Vis spectrophotometry. The results suggested that nonelectroactive complexes were formed between VBB and DNA mainly by eletrostatic interaction. Therefore, the free VBB concentration in the reaction solution decreased, resulting in the decrease of the current of the voltammetric peak of VBB, which was directly proportional to the concentration of dsDNA.

## 2. EXPERIMENTAL

### *2.1. Apparatus and reagents*

All the electrochemical experiments were performed with a CHI 840B electrochemical analyzer (Shanghai CH Instrument Company, China) using a three-electrode system composed of a glassy carbon electrode (GCE, Φ=3 mm) as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. The GCE surface was freshly polished to a mirror prior to each experiment with 0.05 μm α-$Al_2O_3$ paste and then cleaned ultrasonically in ethanol and doubly distilled water for 5 min, respectively. The spectroscopic experiments were performed with a Cary 50 probe UV-Visible spectrophotometer (Varian, Australia). A pHS-25 acidimeter was used for pH measurement.

Native double-stranded fish sperm DNA (fsDNA) was purchased from Beijing Jingke Reagent Company and used as received. 0.5 g/L stock solution of dsDNA was prepared by directly dissolving dsDNA in doubly distilled water, The concentration of dsDNA in nucleotide phosphate was determined spectrophotometrically at 260 nm (the absorption coefficient ε=6600 L/mol·cm, NP). ssDNA was prepared by heating dsDNA solution in a water bath at 100 °C for 8 min, followed by rapid cooling in an ice bath for 5 min. Victoria blue B was obtained from Tianjin Kemiou Reagent Company and $1.0\times10^{-3}$ mol/L VBB solution was prepared before use. 0.20 mol/L pH 5.0 Britton-Robinson buffer solution was used to control the pH of the reaction solution. All the chemical reagents used were of analytical grade and doubly distilled water was used throughout.

### *2.2. Cyclic voltammetry and chronocoulometry*

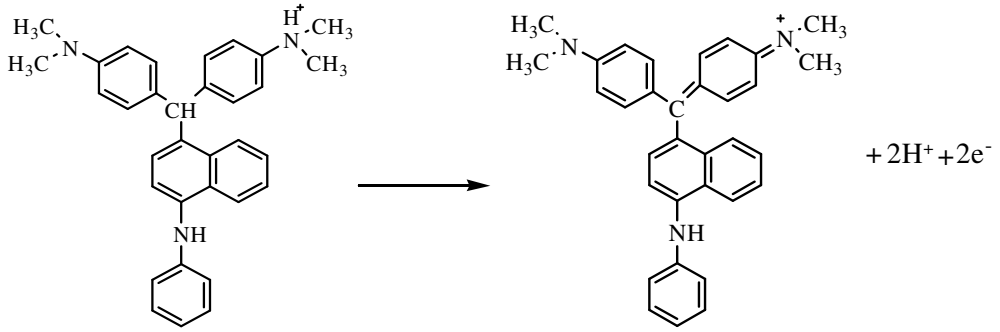
In a 10 ml colorimetric tube were successively added appropriate amount of DNA, VBB and 4.0 ml of 0.20 mol/L pH 5.0 B-R buffer solution. The mixture was diluted to the scale and mixed homogenously. After reaction at ambient temperature for 20 min, the solution was transferred into a 10 ml electrochemical cell and the electrochemical responses were recorded on the CHI 840B electrochemical analyzer. The potential-scanning range of cyclic voltammetry was from +0.70 V to +1.30 V with the scan rate as 100.0 mV/s. For chronocoulometry, the initial potential was at +0.70 V and the final potential at +1.10 V, the potential pulse width as 0.2 s. The quiet time of all electrochemical measurements was fixed at 10 s.

## 3. RESULTS AND DISCUSSION

### *3.1. Electrochemical behaviors of VBB on the GCE*

In 0.20 mol/L pH 5.0 B-R buffer solution, an irreversible anodic peak of VBB was obtained with cyclic voltammetry at the GCE, shown as curve 2 in Figure 2. When the scan rate is 100.0 mV/s, the anodic peak potential ($E_{pa}$) is located at +0.984 V. According to Galus and Adams's [18,19] reports concerning the electrochemical behaviour of triphenylmethane dyes such as crystal violet, brilliant green, malachite green and so on, the values of the electron number (n) of all the electrode reactions

are 2. VBB also belongs to triphenylmethane dye family with the similar structure. So, it is possible that VBB takes place a two electrons electrochemical oxidation process on the GCE with the equation shown as follows:




As shown in Figure 3(a), the dependence of the anodic peak current of VBB on the scan rate was studied. When the scan rate was from 20.0 mV/s to 400.0 mV/s, the peak current was directly proportional to the scan rate with the linear regression equation as $i_p(\mu A)=-55.39v$ (V/s)-0.13,$\gamma=0.997$, indicating that the electrode reaction of VBB was an irreversible process mainly controlled by adsorption.

*3.2. Cyclic voltammograms of the interaction between VBB and DNA*

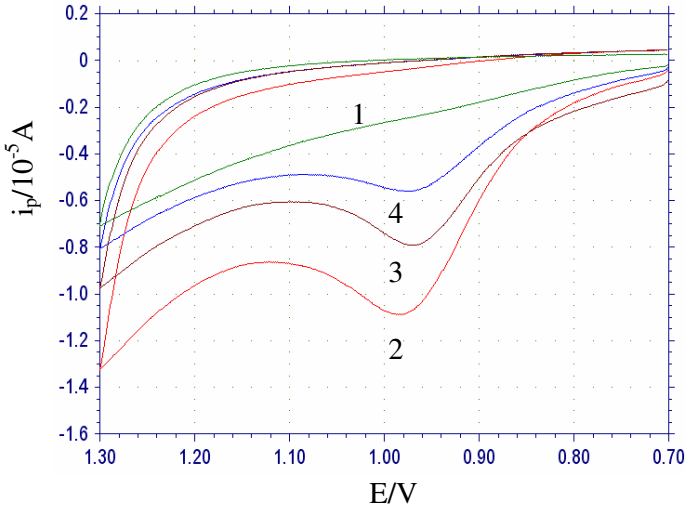



**Figure 2.** Cyclic voltammograms of VBB, VBB-ssDNA and VBB: **1**. 0.20 mol/L pH 5.0 B-R buffer solution; **2**. 1+6.0×$10^{-5}$ mol/L VBB; **3**. 2+8.0 mg/L ssDNA; **4**. 2+8.0 mg/L dsDNA

Cyclic voltammograms of the VBB-ssDNA (curve 3) and VBB-dsDNA (curve 4) interaction system in pH 5.0 B-R buffer solution are shown in Figure 2, respectively. The addition of ssDNA and dsDNA into VBB solution both made the peak current of VBB decrease and the peak potential shift negatively, which suggested that a binding reaction between VBB and DNA took place. According to the literature [20], the peak potential shifted negatively is the characteristic of an electrostatic mode.

On the contrary, if the peak potential shifts positively, the interaction is an intercalative mode. It can be considered that VBB bound to DNA mainly through an eletrostatic mode. The peak current decreased more in the presence of dsDNA than that of ssDNA, showing that VBB exhibited nice discrimination towards dsDNA and ssDNA. It was reasonable to assume that VBB bound to DNA mainly through an electrostatic mode, but can also intercalated into the ordered dsDNA helix at the same time. In contrast, the random, coillike, disordered ssDNA didn't not provide a favorable environment for VBB intercalation.

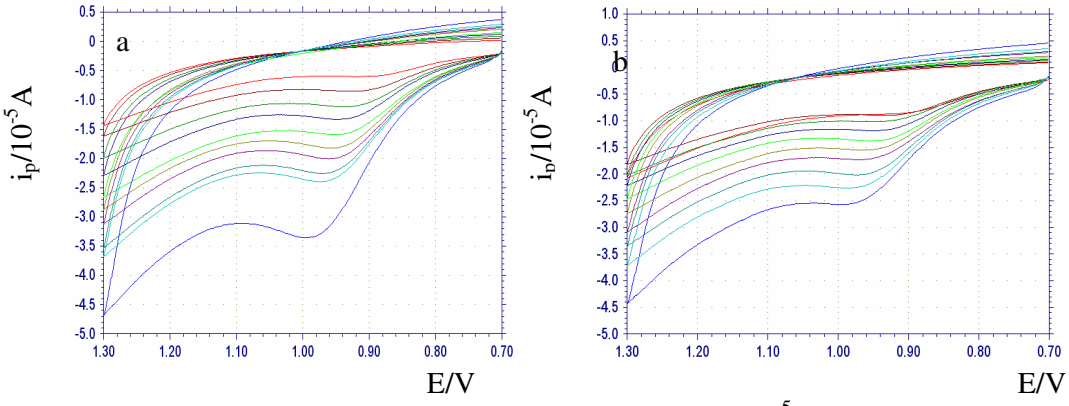



**Figure 3**. Dependence of cyclic voltammetric behavior of $6.0\times10^{-5}$ mol/L VBB in pH 5.0 B-R buffer solution on the scan rate (ν), in the absence (a) and the presence (b) of 8.0 mg/L dsDNA.

As shown in Figure 3(b), the influence of the scan rate on the peak current of VBB-dsDNA was also examined. The peak current was still directly proportional to the scan rate in the range from 20.0 mV/s to 400.0 mV/s, with the linear regression equation as $i_p(\mu A)=-49.08\nu\ (V/s)+1.28$,$\gamma=0.997$. The result indicated that the electro-oxidation process was still mainly controlled by the surface adsorption.

*3.3. Chronocoulometric experiments*

Chronocoulometric method was used to calculate the diffusion coefficients of VBB in the absence and presence of dsDNA. The diffusion coefficient of the redox species can be calculated applying the equation given by Anson [21]:

$$Q_d=2nFAD^{1/2}Ct^{1/2}/\pi^{1/2}+Q_{dl}+Q_{ads}$$

Where n is the number of electron, F ($C\cdot mol^{-1}$) the Faraday constant, A ($cm^2$) the area of the electrode, D ($cm^2\cdot s^{-1}$) the diffusion coefficient of species, C ($mol\cdot cm^{-3}$) the bulk concentration of species, t (s) the potential pulse width, $Q_{dl}$ (C) the double-layer charge (integration of the charging current), $Q_{ads}$ the faradic component due to the oxidation of adsorbed species. The parameter D can be calculated from the slope of $Q_d$ vs. $t^{1/2}$ if the values of A, n and C are known.

Different values of $Q_d$ and $t^{1/2}$ can be obtained from the chronocoulometric experiments before and after the addition of dsDNA. The diffusion coefficient of VBB could be gotten from the slope of $Q_d$ vs. $t^{1/2}$, which was shown in Figure 4. The diffusion coefficients of VBB in the absence and presence of

dsDNA were calculated as $D_f$=8.35×10$^{-4}$ cm$^2$/s and $D_b$=6.11×10$^{-4}$ cm$^2$/s, respectively. The diffusion coefficient decreased after adding dsDNA, but the decrease value was small. Moreover, for an adsorption-controlled process, the diffusion coefficient does not affect the peak current in the cyclic voltammogram, so the decrease of the diffusion coefficient of VBB in the presence of dsDNA was not the main reason for the decrease of the peak current of VBB in the presence of dsDNA.

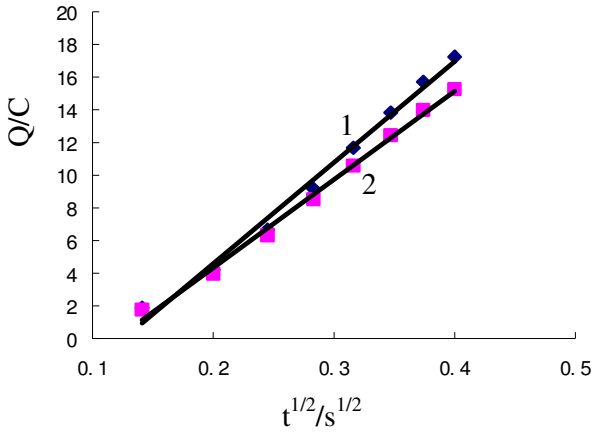



**Figure 4**. Relationship between Q and $t^{1/2}$ : **1.** 6.0×10$^{-5}$ mol/L VBB in pH 5.0 B-R buffer solution; **2.** 1+8.0 mg/L dsDNA

*3.4. Determination of the electron transfer coefficient and the electrochemical reaction standard rate constant*

For an adsorption-controlled irreversible oxidation process, the surface reaction standard rate constant (ks') and the electron transfer coefficient (α) could be calculated from the equation reported by Laviron [22]:

$$E_p = E^{0'} - RT/(1-\alpha)nF\{\ln[ks'RT/(1-\alpha)nF]\} + RT\ln\nu/(1-\alpha)nF$$

Where $E_p$ is the peak potential, $E^{0'}$ the formal potential, R (8.314 J·mol$^{-1}$·K$^{-1}$) the gas constant, T (K) the temperature; n, α, F and ν are of usual meanings mentioned above. There was a linear relationship between the peak potential ($E_p$) and the logarithm of the scan rate (lnν) and the value of α can be obtained from the slope. The value of $E^{0'}$ in above equation can be obtained from the intercept of $E_p$ vs. ν plot on the ordinate by extrapolating the line to ν=0. According to this method, the relationship of $E_p$ with lnν or ν was established and shown in Figure 5. From the slope and intercept, the values of α and ks' were calculated. In the absence of dsDNA, $E^{0'}$=0.93 V, αn=1.42, α=0.71, ks'=0.72 s$^{-1}$ and in the presence of dsDNA, $E^{0'}$=0.92 V, αn=1.38, α=0.69, ks'=0.67 s$^{-1}$were gotten.

The results demonstrated that the electrochemical parameters of VBB had not varied obviously after adding dsDNA. Therefore, the decrease of the oxidative peak current was not caused by the change of the electrochemical parameters. From the results of the above experiments, the formation of an electrochemically non-active complex of VBB with dsDNA resulted in the decrease of the free concentration of VBB in the reaction solution, which caused the decrease of the peak current.

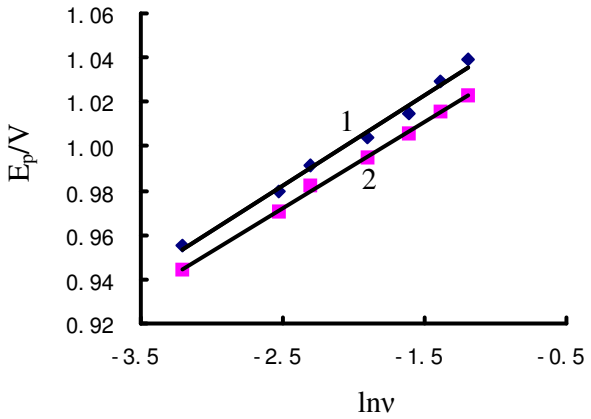



**Figure 5(a)**. Relationship between $E_p$ and lnν: **1**.6.0×$10^{-5}$ mol/L VBB in pH 5.0 B-R buffer solution **2**.1+8.0 mg/L dsDNA

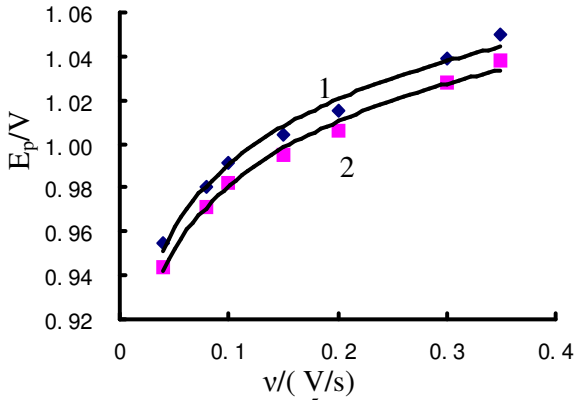



**Figure 5(b)**. Relationship between $E_p$ and ν: **1.** 6.0×$10^{-5}$ mol/L VBB in pH 5.0 B-R buffer solution **2**. 1+8.0 mg/L dsDNA

*3.5. The binding ratio (m) and binding constant (β) of VBB-DNA complex*

According to reference [23,24], the binding ratio (m) and binding constant (β) can be obtained if VBB binds dsDNA to form a simple complex. Assuming that VBB and dsDNA produce only a single complex of dsDNA-mVBB:

$$DNA+mVBB \leftrightarrow DNA\text{-}mVBB$$

The binding constant is deduced as follows:

$$\beta=[DNA\text{-}mVBB]/\,[DNA]\,[VBB]^m$$

Because of

$$[DNA]=C_{DNA}-[DNA\text{-}mVBB]$$

$$\Delta i_p=K[DNA\text{-}mVBB]$$

$$\Delta i_{p,\,max}=KC_{DNA}$$

Therefore

$$\Delta i_{p,\,max}-\Delta i_p=K[DNA]$$

and the following equation can be given:

$$1g[\Delta i_p/(\Delta i_{p,\,max}-\Delta i_p)]=1g\beta+mlg[VBB]$$

or

$$1/\Delta i_p=1/\Delta i_{p,\,max}+(1/\beta\Delta i_{p,\,max})(1/[VBB]^m)$$

Where $\Delta i_p$ is the peak current difference with and without dsDNA, $\Delta i_{p,\,max}$ corresponds to the maximum difference of the peak currents before and after the addition of dsDNA. $C_{VBB}$ and [VBB] correspond to the concentration added and the equilibrium concentration of VBB, respectively.

If dsDNA and VBB form a single complex, the plot of $lg[\Delta i_p/(\Delta i_{p,\,max}-\Delta i_p)]$ vs lg[VBB] is linear. In Figure 6(a), curve a shows the relationship between the current $i_p$ and the concentration of VBB. Curve b typically represents $i_p$ change at $C_{DNA}$=8.0 mg/L on varying the concentration of VBB. Curve c is the differences between curve a and curve b, which represents the relationship between $\Delta i_p(i_{pa}-i_{pb})$ and the concentration of VBB. When the concentration of VBB is over $6\times10^{-5}$ mol/L, $\Delta i_p$ tends to be a stable value. Figure 6(b) shows the dependence of lg $[\Delta i_p/(\Delta i_{p,\,max}-\Delta i_p)]$ on lg [VBB], with a fine straight line. From the slope and the intercept of the straight line the values of m and β were calculated as 2.8 and $7.5\times10^{12}$, respectively. Atentative idea was that the binding ratio of dsDNA and VBB was 1 : 3.

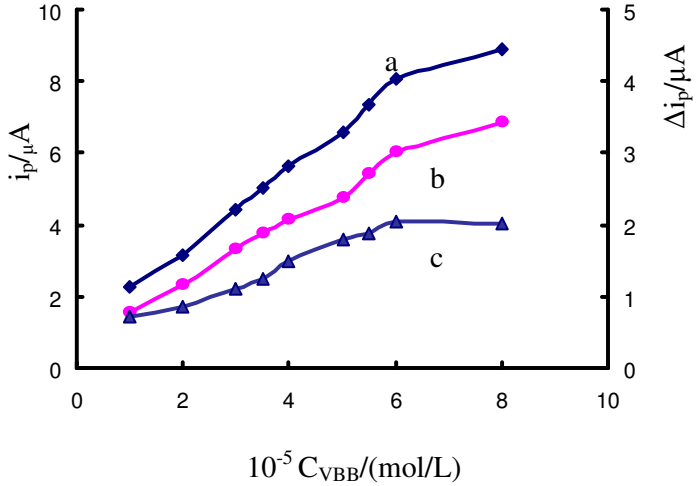



**Figure 6(a)**. Relationship between $i_p$ and $C_{VBB}$ (a, b), and $\Delta i_p$ and $C_{VBB}$ (c) **a.** $C_{DNA}$=0; **b**. $C_{DNA}$=8.0 mg/L; **c**. $\Delta i_p=i_{pa}-i_{pb}$

*3.6. Optimal reaction conditions*

The effect of pH from 3.0 to 8.0 on the interaction was tested. The results showed that the difference in the peak currents reached the maximum at pH 5.0. So pH 5.0 was chosen as the experimental pH. After mixing the two compounds, the decrease value of the VBB peak current reached the largest at

the reaction time of 20 min. So 20 min was selected as the reaction time. The effect of the temperature was tested in the range from 10°C to 40°C. The peak current of VBB varied little with the change of the reaction temperature. Experiments were conducted under the ambient temperature.

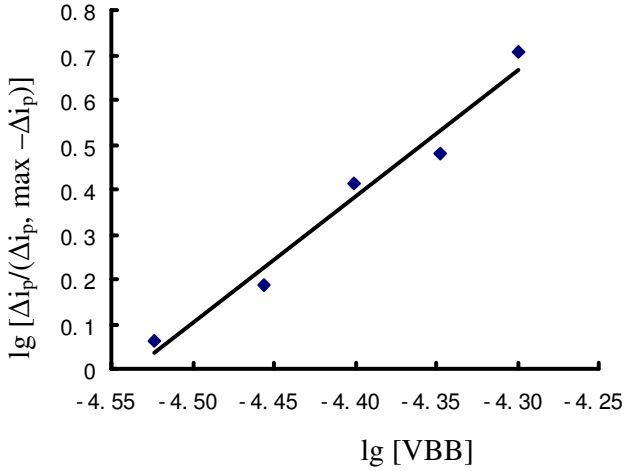



**Figure 6(b)**. Relationship between lg [$\Delta i_p/(\Delta i_{p,\,max}-\Delta i_p)$] and lg [VBB]

*3.7. UV-Vis absorption spectra of VBB interacting with dsDNA*

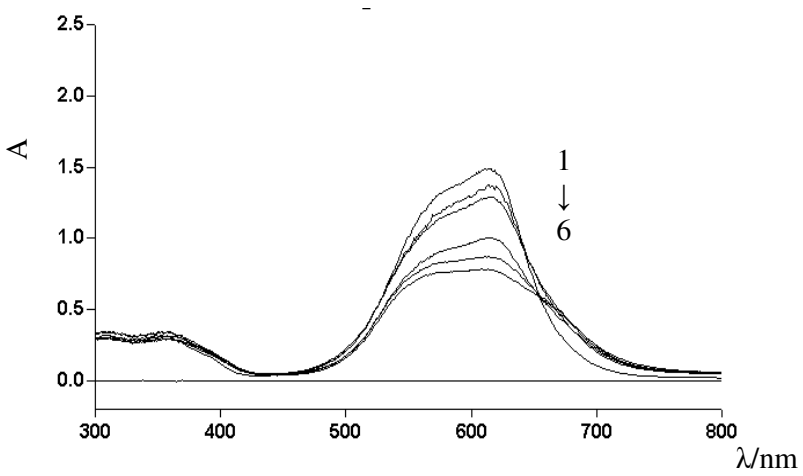



**Figure 7**. UV-Vis absorption spectra of VBB with different amounts of dsDNA
$3.0\times10^{-5}$ mol/L VBB in pH 5.0 B-R buffer solution with 0 (1), 3.5 (2), 4.0 (3), 6.0 (4), 7.0 (5) and 8.0 (6) mg/L dsDNA, respectively

The absorption spectra of VBB in the absence and presence of dsDNA were illustrated in Figure 7. Curve 1 showed that VBB had a maximum absorbance at 613.0 nm. Curve 2-6 showed the absorption spectra of VBB after adding different concentrations of dsDNA. Mixing dsDNA with VBB decreased the absorbance value of VBB obviously, and the more the amount of dsDNA added, the larger the decrease value of the absorbance value appeared. Under the above conditions the maximum absorption wavelength did not shift and there was no new absorption peak. According to Barton's theory [25],the

binding is the typical character of an electrostatic interaction. Spectral experiments further illustrated that VBB had bound with dsDNA to form a supramolecular complex mainly by electrostatic interaction, resulting in the decrease of the free VBB concentration in the solution and so the decrease of the peak absorbance of VBB.

### *3.8. Analytical application*

#### *3.8.1.Calibration curve and detection limit*

Differential pulse voltammetric method (DPV) was chosen for analytical application because it is more sensitive than cyclic voltammetric method. The differential pulse voltammograms of VBB in the absence and presence of dsDNA were shown in Figure 8. After the addition of dsDNA, the peak current decreased obviously. Under the optimum conditions of the standard procedure, by keeping the concentration of VBB at $6.0\times10^{-5}$ mol/L, the calibration curve for detecting dsDNA was obtained. When the concentration of dsDNA was in the range of 0.10~8.0 mg/L, a linear relationship was found with the decrease of the peak current ($\Delta i_p$) and DNA concentration. The linear regression equation was as: $\Delta i_p(10^{-6}A)=0.072C(mg/L)+0.24$, $\gamma=0.9927$, with the limit of detection as 0.069 mg/L, which was given by the equation LOD=3σ, where σ was the standard deviation of the blank measurements (n=11).

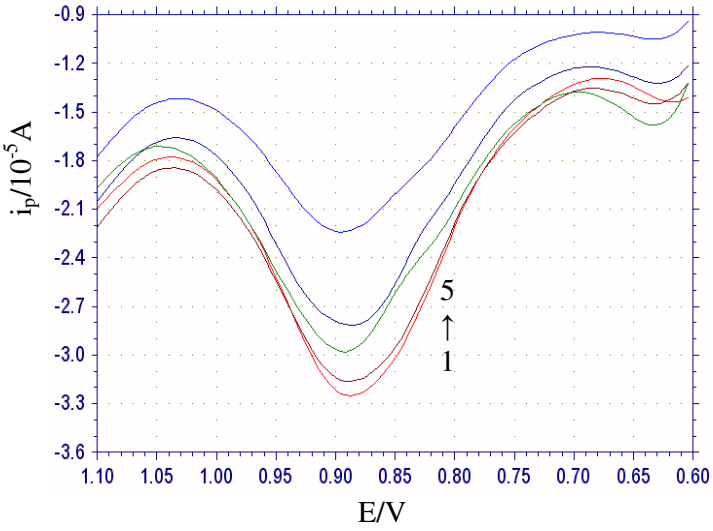



**Figure 8**. Differential pulse voltammograms of VBB-DNA reaction system $3.0\times10^{-5}$ mol/L VBB in pH 5.0 B-R buffer solution with 0 (1), 0.10 (2), 0.50 (3), 1.0(4), 8.0 (5) mg/L DNA, respectively.

#### *3.8.2. Interference test of foreign substances*

By keeping the concentration of dsDNA as 8.0 mg/L, the effects of coexisting substances such as various cationic ions, amino acids and glucose on the determination of dsDNA were tested according to the standard procedure. The results were listed in Table 1. The common coexisting substances such

as $Cu^{2+}$, $Zn^{2+}$, $Mg^{2+}$, glucose, citric acid and so on have little effects on the determination of dsDNA, which showed that the interaction of VBB with dsDNA provided a possibility for detecting the content of dsDNA in practical samples.

**Table 1.** Effect of coexisting substances on the determination of 8.0 mg/L dsDNA

| Coexisting substance | Concentration (μmol/L) | Relative Error (%) | Coexisting substance | Concentration (mg/L) | Relative Error (%) |
|---|---|---|---|---|---|
| $Co^{2+}$ | 5.0 | -3.52 | L-serine | 5.0 | -5.24 |
| $Ca^{2+}$ | 5.0 | -0.45 | L-leucine | 5.0 | - 5.10 |
| $Cu^{2+}$ | 5.0 | - 2.07 | L-tyrosine | 5.0 | +12.3 |
| $Zn^{2+}$ | 5.0 | +3.52 | L-glycine | 5.0 | +5.96 |
| $Mg^{2+}$ | 5.0 | - 1.15 | glucose | 5.0 | +14.00 |
| $Mn^{2+}$ | 5.0 | -0.22 | citric acid | 5.0 | +1.63 |
| $Sn^{2+}$ | 5.0 | -3.1 | L-cysteine | 5.0 | +6.77 |
| CTAB | 5.0 | +20.37 | SDS | 5.0 | +10.80 |

fsDNA (6.0 mg/L), VBB ($6.0\times10^{-5}$mol/L), pH 5.0 B-R buffer solution, CTAB (cetyltrimethylammoniumbromide), SDS (sodium lauryl sulfate)

*3.8.3. Analysis of artificial samples*

To test this new method, four artificial samples, which were prepared by mixing with different kinds of foreign substances, were analyzed according to the general procedure. As shown in Table 2, the results were reproducible and reliable with good recovery.

**Table 2**. Determination results of artificial samples (n=5)

| DNA | Coexisting substance | Added (mg/L) | Found (mg/L) | Recovery (%) | RSD (%) |
|---|---|---|---|---|---|
| (fs)DNA | L-leucine, citric acid, $Sn^{2+}$, $Zn^{2+}$ | 6.00 | 5.97 | 99.5 | 0.43 |
| (fs)DNA | L-serine, citric acid, $Cu^{2+}$, $Mg^{2+}$ | 6.00 | 5.80 | 96.7 | 3.37 |
| (fs)DNA | L-cysteine, L-glycin, $Co^{2+}$, $Mn^{2+}$ | 6.00 | 6.08 | 101.3 | 1.27 |
| (fs)DNA | L-leucine, L-glycin, $Cu^{2+}$, $Zn^{2+}$ | 6.00 | 6.20 | 103.3 | 3.33 |

VBB ($6.0\times10^{-5}$mol/L), $Mg^{2+}$, $Sn^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Co^{2+}$, $Mn^{2+}$ ($5.0\times10^{-6}$mol/L), L-leucine, citric acid, L-serine, L-cysteine, L-glycine (5.0 mg/L)

## 4. CONCLUSIONS

In summary, the interaction between VBB and DNA were studied using electrochemical methods and UV-Vis spectrophotometry. An electrostatic mode had been suggested for the VBB-DNA binding. VBB can bind with dsDNA to form nonelectroactive complexes. After the addition of

dsDNA, the differential pulse voltammetric peak current of VBB decreased, owing to the decrease of the free concentration of VBB. According to this phenomenon, an electrochemical method to determine dsDNA using VBB as probe was established.

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