Int. J. Electrochem. Sci., 6 **(2011)** 532 - 547

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

In Vitro Study of DNA Interaction with Trichlorobenzenes by Spectroscopic and Voltammetric Techniques

Hao-Yu Shen [*], *Xiao-Li Shao, Hua Xu, Jia LI, Sheng-Dong Pan*

Biological and Chemical Engineering School, Ningbo Institute of Technology, Zhejiang University; Ningbo, 315100, China
[*]E-mail: hyshen@nit.zju.edu.cn

Received: 20 January 2011 / *Accepted:* 5 February 2011 / *Published:* 1 March 2011

In vitro studies on the interactions between native Herring Sperm DNA (HS-DNA) and 3 kinds of trichlorobenzenes (TCBs), *i.e.* 1,3,5-, 1,2,4-, 1,2,3-TCB, have been investigated by spectrophotometric, spectrofluorometric, melting temperature (T_m), viscosimetric and cyclic voltammetric techniques. The interaction constants between any of the TCBs and HS-DNA were found be at ~10^4 L·mol^{-1}. The thermodynamic studies suggested that the interaction processes were endothermic disfavored ($\triangle H>0$) and entropy favored ($\triangle S>0$), which indicated that the TCBs might interact with HS-DNA by a non-traditional intercalation mode of binding *via* hydrophobic force.

Keywords: trichlorobenzenes (TCBs), Herring Sperm DNA (HS-DNA), interaction, non-traditional intercalation mode, hydrophobic force.

1. INTRODUCTION

Trichlorobenzenes (TCBs) are cyclic aromatic compounds formed by the addition of 3 atoms of chlorine to the benzene ring. There are 3 isomers: 1,2,3-trichlorobenzene (1,2,3-TCB), 1,2,4-trichlorobenzene (1,2,4-TCB) and 1,3,5-trichlorobenzene (1,3,5-TCB). As a result of widespread use in industry and agriculture, TCBs are produced in large amounts as solvents and precursors of herbicides, insecticides, *etc*. Research results have shown that the bioaccumulation potential of TCBs is very high and not readily biodegradable. They are very toxic to aquatic organisms and may cause long term adverse effects in the aquatic environment [1-4]. Recent reports have shown that TCBs have reproductive and endocrine disrupting effects [1, 3, 4]. TCBs have been included in the EU List of Substances with Suspected Endocrine Effects and ranked as priority pollutants by United States Environmental Protection Agency (USEPA) as well [3, 4]. Although, TCBs are known to be toxic to

liver and blood system, whether or not there are some interactions bewteen TCBs and DNA, especially the mechanism of the interactions bewteen TCBs and DNA, have not been reported.

DNA is known to be a major target for drugs and some harmful chemicals to be attacked. Small moleculars normally interact with DNA *via* non covalent interaction modes, *e. g.*, (i) intercalating between stacked base pairs, (ii) non-covalent groove binding, or (iii) electrostatic interaction with the negatively charged nucleic acid sugar-phosphate structure [5]. Studies on the binding mechanism of some small molecules with DNA have been identified as one of the key topics during the past few decades [6-12]. It is of great help to understand the structural properties of DNA, the mutation of genes, the origin of some diseases, the action mechanism of some antitumour and antivirus drugs. Recently, researches on the interaction between DNA and some harmful chemicals, such as enviromental pollutants, pesticides, *etc*, gradually become a hot topic as a main way for the investigation of DNA damage, as well as understanding of toxic mechanism [6, 9, 12]. Some techniques, including gel electrophoresis [13], footprinting technique, X-ray crystallography [14], NMR [15], fluorescence [16], UV/vis spectroscopy [17], electrochemical [18] *etc*., have been used to investigate this interaction. Among them, spectroscopic and voltammetric techniques have been testified to be of high sensitivity, relatively low cost, direct monitoring and simplicity [16-23].

In this work, the interactions of native Herring Sperm DNA with 1,2,3-TCB, 1,2,4-TCB and 1,3,5-TCB have been investigated by spectrophotometric, spectrofluorometric, melting temperature (T_m), viscosimetric techniques and cyclic voltammetry studies. Some valuable results were obtained. We hope this work will provide some additional useful information for the evaluation of the safety performance of TCBs through understanding their interaction with DNA.

2. EXPERIMENTAL

2.1. Chemicals and reagents

1,2,4-TCB, 1,3,5-TCB, 1,2,3-TCB, were purchased from the National Research Center for Chinese Reference Materials (NRCCRM) with purity higher than 99%. Herring Sperm DNA (HS-DNA) was purchased from Shanghai Bio Life Science and Technology Co., Ltd (Shanghai, China). The stock solution of DNA was prepared by dissolving DNA in 0.01 mol/L of Tris buffer at pH 7.2 (0.01 mol/L of tris(hydroxymethyl)aminomethane (Tris) with NaCl concentration at 0.01 mol/L), dialyzing exhaustively against the same buffer for 24 h, and used within 5 days. A solution of DNA gave a ratio of UV absorbance at 260 and 280 nm more than 1.8, indicating that DNA was sufficiently free from protein. The DNA concentration of the stock solution was determined by UV spectrophotometry, in properly diluted samples, using the molar absorption coefficient 6600 $L \cdot mol^{-1} \cdot cm^{-1}$ at 260 nm; the stock solution was stored at 4 ^oC. An individual stock solution for each compound containing 1×10^{-4} $mol \cdot L^{-1}$ 1,3,5-TCB, 1,2,4,-TCB or 1,2,3-TCB was prepared by dissolving an appropriate amount of the individual TCB in methonal. A fresh working solution was prepared daily by diluting the stock solution with Tris buffer and used for different studies. Other used chemicals were of analytical reagent grade.

2.2. Apparatus

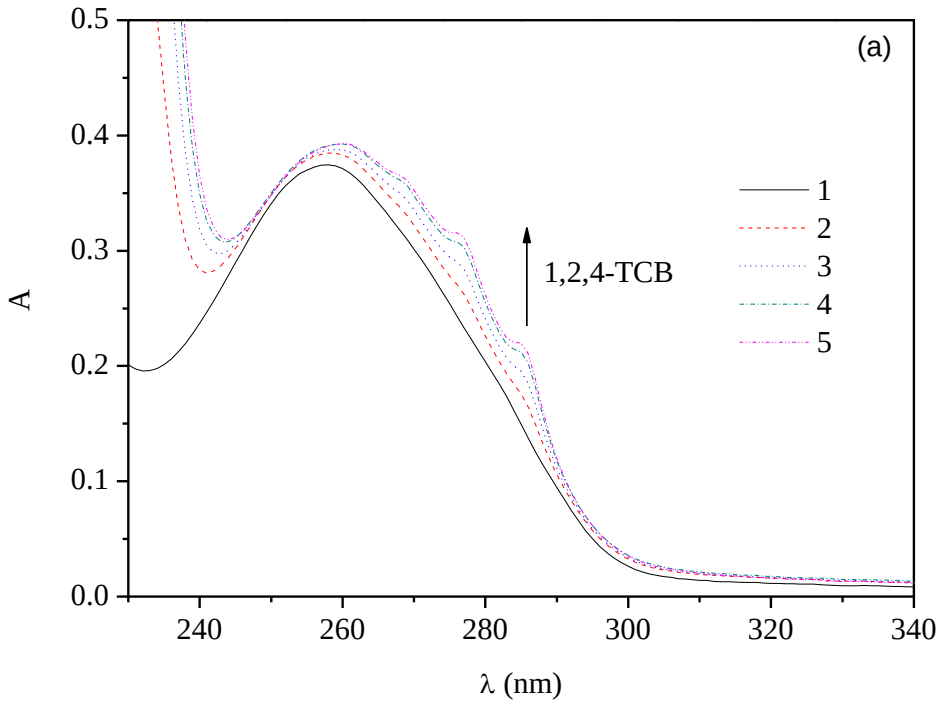
The UV-vis spectra for TCB–DNA interactions were obtained by using a PE λ25 spectrophotometer. For DNA melting studies, the temperature of the cell holder was changed as 30 ^{o}C, 37 ^{o}C, 40 ^{o}C, 45 ^{o}C , 50 ^{o}C , 55 ^{o}C , 60 ^{o}C , 65 ^{o}C , 70 ^{o}C , 75 ^{o}C , 80 ^{o}C, 85 ^{o}C, and 90 ^{o}C. The fluorescence studies were carried out by using an F4500 fluorescence spectrophotometer with methylene blue (MB) as a fluorescent probe. Spectra were recorded at room temperature using a standard quartz cell of 1.0 cm path length, where 3.0 mL of Herring Sperm DNA solution with a concentration of 6.0×10^{-5} mol·L^{-1} was placed. Once the first absorption spectrum was achieved, 10.0 μL of TCB solution was added, which resulted in a new spectral acquisition. This procedure allowed us to increase the TCBs after each addition without any appreciable change in the total volume, thus preserving DNA concentration. Therefore, obtained features for absorption bands over the whole series of spectra were comparable. For viscosity measurements, a Ubbelohde viscosimeter (0.6-0.7 type) was used, which thermostated at 25 ^{o}C by a constant temperature bath. Flow time was measured with a digital stopwatch; the mean values of three replicated measurements were used to evaluate the viscosity (η) of the samples (η was calcalated by the equation $\eta = (t\text{-}t_0)/t_0$, where, t_0 is the flow time of the blank solution; t is the flow time of the DNA solutions with the concentration ratios of [DNA]/[TCB] from 0.05 to 1.20). The data were reported as $(\eta/\eta_0)^{1/3}$ *vs* the [DNA]/[TCB] ratio, where η_0 is the viscosity of the DNA solution alone. The cyclic voltammetry studies were carried out by using LK-2006 electrochemical system (Lanlike Co. Ltd, Tianjing, China). Electrochemical cell consisted of a glass container with a cap having holes for introducing electrodes and nitrogen. The cell was then maintained oxygen free by passing nitrogen over the solution. The reference electrode used was saturated calomel electrode (SCE), while the auxillary and working electrodes were platinum foil and glassy carbon electrode (GCE), respectively. In a typical cyclic voltammetric experiment of the reaction mixture consisted of each of TCB and DNA in Tris buffer, a stream of nitrogen was passed over them and the reaction mixture was thermostated. The three electrodes were connected to a computer controlled potentiostat and required potential scan rate, current sensitivity, initial potential and final potential were given and the resulting current was measured as a function of applied potential.

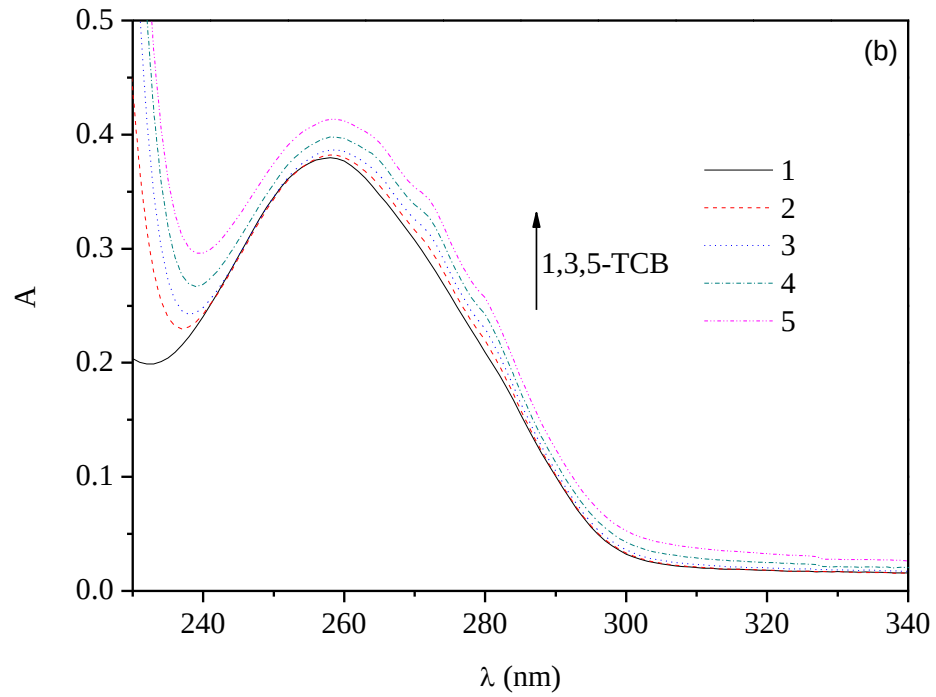
3. RESULTS AND DISCUSSION

3.1. Electronic spectra

The electronic spectra obtained *via* titration of DNA with TCBs solutions were shown in Figure 1. As shown in Fig. 1, the spectra of DNA recorded in the presence of increasing amounts of TCBs represented a slight bathochromic shift (~4 nm) centered at the 258 nm with a pronounced hyperchromic effect (the absorbency at 258 nm increased 0.02~0.05, 4.4~13.9 %). The band at ~ 260 nm of DNA arises because of the π-π* transition of DNA bases. Changes in absorbance and wavelength shifts of this characteristic band reflect the corresponding structural changes of the DNA,

including changes of stacking pattern, disruption of the hydrogen bonds between complementary strands, covalent binding of DNA bases, intercalation between aromatic rings of molecules, *etc* [24-27]. Since only a slight bathochromic shift was obsearved in UV absorption band of DNA, the conformation changes of DNA structure may realized *via* non-traditional intercalating mode with TCBs [5, 28]. This indicated that the conformation of DNA double-helix structure was changed after the TCBs being added.





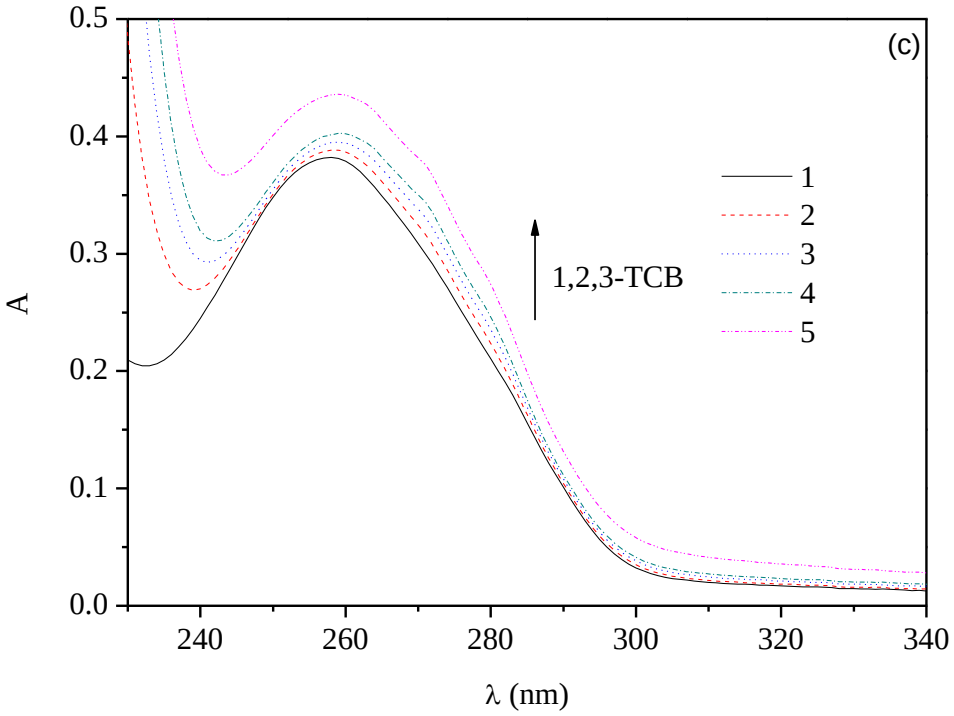


Figure 1. Electronic spectra of Herring Sperm DNA (6.0×10^{-5} mol/L) with 1,2,4-TCB (a), 1,3,5-TCB (b), or 1,2,3-TCB (c) in 0.01 mol/L of Tris buffer at pH 7.2 (1) DNA (c(DNA)＝6.0×10^{-5} mol/L), (2-5) any of TCB-DNA: c(TCB): (2) 1.0×10^{-6} mol/L, (3) 5.0×10^{-6} mol/L, (4) 1.0×10^{-5} mol/L, (5) 5.0×10^{-5} mol/L)

For further investigation the intensity of the interaction between the TCBs and DNA, the interaction constants were evaluated by double reciprocal equation as listed in eq. 1 [8, 12].

$$1/(A-A_0)=1/A_0+1/[K\times A_0\times c(\text{TCB})] \quad (1)$$

Where A_0 and A are the absorbency before and after adding any of TCBs, while c(TCB, mol·L^{-1}) is the concentration of any of TCBs added and K (L ·mol^{-1}) is the interaction constant of TCBs-DNA, respectively.

By plotting of $1/(A-A_0)$ *vs* $1/c$(TCB), K can be obtained from the slope and intercept of resulted curves, shown in Figure 2. The values of K for the TCBs-DNA complexes were summarized in Table 1, which were 5.7×10^4, 1.7×10^4, 1.3×10^4 L·mol^{-1} for 1,2,4-TCB, 1,3,5-TCB, and 1,2,3-TCB, respectively.

To have a better understanding of thermodynamics of the interactions between TCBs and DNA, it is useful to determine the contributions of enthalpy and entropy of the interactions. The thermodynamic parameters describing the interactions can be divided into three kinds of contributions. The first contributions are from the molecular interactions between the TCBs and DNA binding sites because of hydrogen bonding and hydrophobic interactions. The next contributions are from the conformational changes in either the nucleic acid or the TCBs upon binding. Finally, there are contributions that may be coupled processes like ion release, proton transfer, or changes in the hydration water [29].

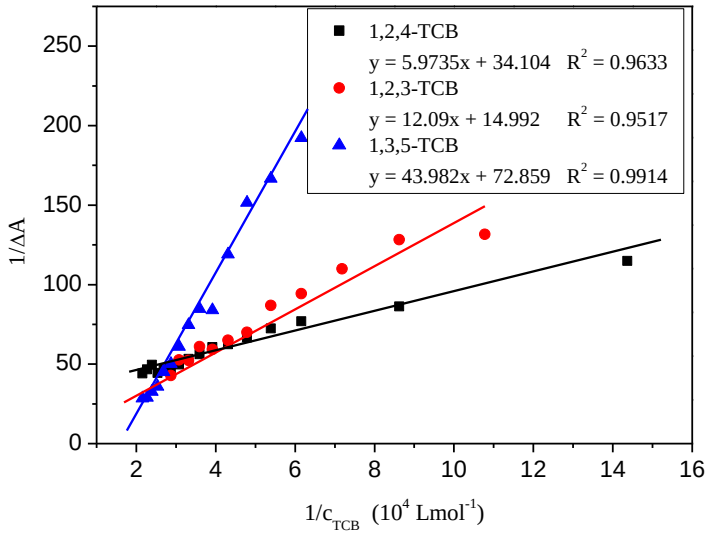


Figure 2. The interaction constant (37 °C) of the interaction between TCBs and DNA (c(DNA) = 6.0×10^{-5} mol/L), c(TCB) = 1.0×10^{-6} ~ 5.0×10^{-5} mol/L)

The evaluation of K for the TCBs-DNA complexes at different temperatures (298~363 K) allows to determine the thermodynamic parameters such as enthalpy (ΔH) and entropy (ΔS) of TCBs-DNA formation by Van't Hoff equation (as listed in eq. 2.) by plotting ln$K$ versus 1/T. The results were listed in Table 1. The values of the free energy (ΔG) of the interaction between TCBs and DNA at different temperature (298~363 K) were all negative, which revealed that the processes of the interaction between TCBs and DNA were spontaneous.

$$\ln K = -(\triangle H - T\triangle S)/RT = -\triangle H/RT + \triangle S/R \qquad (2)$$

Table 1. The thermodynamic parameters of TCB-DNA interactions

TCB	△H (kJ·mol^{-1})	△S (J·mol^{-1}·K^{-1})	K (L ·mol^{-1})		
			Electronic spectra	Fluorescence studies	Electrochemical method
1,2,4-	19.62 ± 0.06	119.45 ± 0.11	5.7×10^4	7.4×10^4	1.5×10^4
1,3,5-	26.59 ± 0.08	146.22 ± 0.07	1.7×10^4	9.4×10^3	1.1×10^4
1,2,3-	9.64 ± 0.05	95.49 ± 0.08	1.3×10^4	3.2×10^4	1.5×10^4

As listed in Table 1, the $\triangle H$ and $\triangle S$ values of the TCBs-DNA complexes were 19.62 ± 0.06 kJ·mol^{-1} and 119.45 ± 0.11 J·mol^{-1}·K^{-1} for 1,2,4-TCB, 26.59 ± 0.08 kJ·mol^{-1} and 146.22 ± 0.07 J·mol^{-1}·K^{-1} for 1,3,5-TCB, 9.64 ± 0.05 kJ·mol^{-1} and 95.49 ± 0.08 J·mol^{-1}·K^{-1} for 1,2,3-TCB, respectively. These thermodynamic results suggested that the interaction processes were endothermic disfavored ($\triangle H$>0) and entropy favored ($\triangle S$>0). As proposed by P. D. Ross [30], when $\triangle H$<0 or $\triangle H\approx0$, $\triangle S$

>0, the mainly acting force is electrostatic; when $\triangle H$<0, $\triangle S$ < 0, the mainly acting force is van der Waals or hydrogen bond and when $\triangle H$>0, $\triangle S$ >0, the mainly force is hydrophobic. For all the binding systems of the TCBs with DNA, the $\triangle H$ and $\triangle S$ values were positive. Therefore, in the cases of the present systems, we presumed that hydrophobic interaction might be the main acting force in the binding of the TCBs and DNA.

From the thermodynamic data, it was quite clear that the interaction processes were endothermic disfavored but entropy favored ($\triangle H$>0, $\triangle S$>0). The value of *K*, the interaction constants of TCBs-DNA, was ~10^4 for any of the TCBs, which was at least 100 times smaller than reported examples of traditional intercalating mode, such as daunomycin [31], cryptolepine [32], and chlorobenzylidine [33]. These results furtherly illuminated that the interactions between DNA and TCBs did not follow the traditional intercalating mode, while the conformation changes of DNA structure may be realized *via* an entropy driven non-classical intercalation interaction. The mainly force is hydrophobic.

3.2. Fluorescence studies

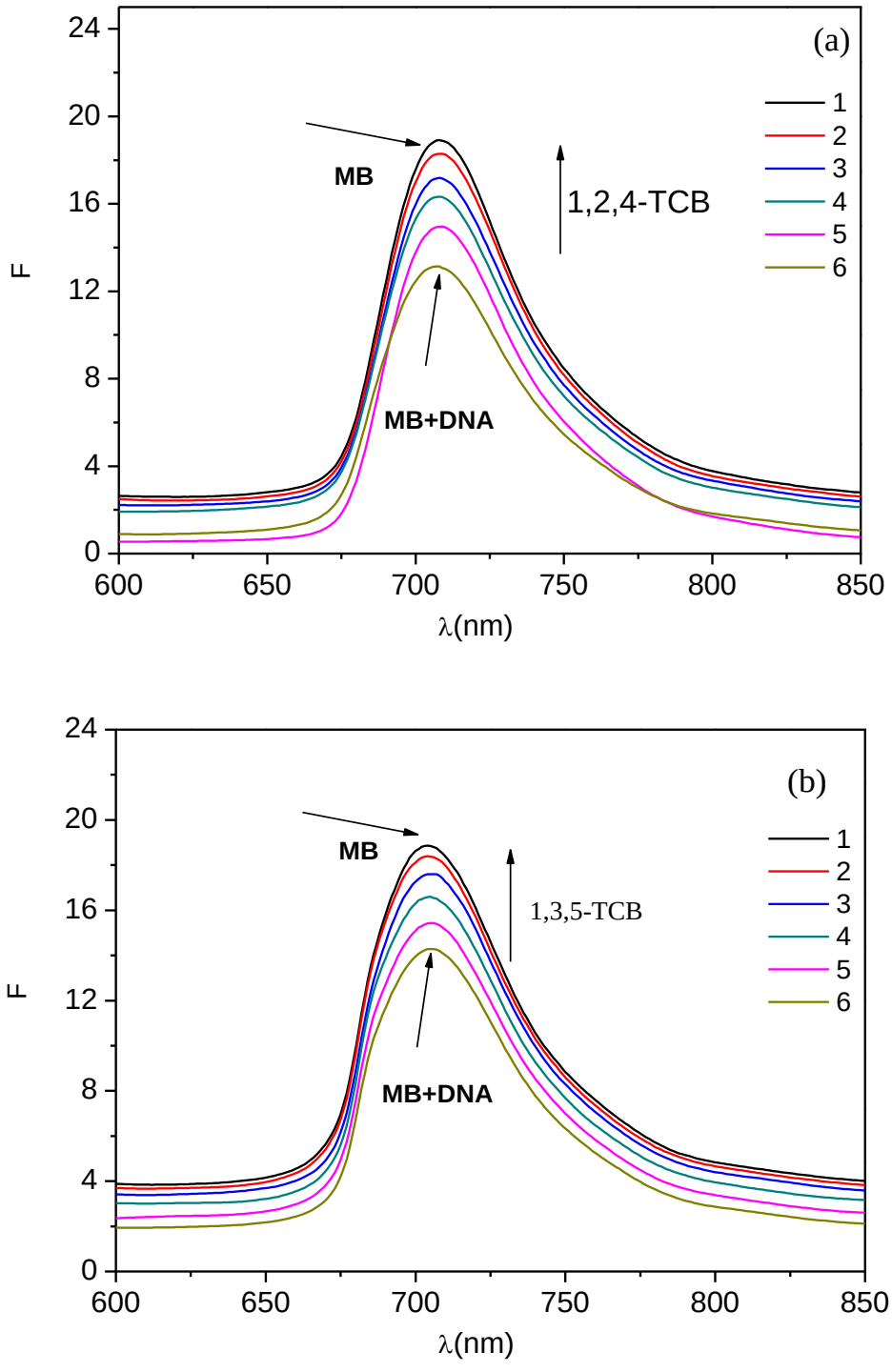
The fluorescence studies of the interactions between TCBs and DNA were carried out by using methylene blue (MB) as a fluorescent probe. Traditionally, the standard method for fluorescence enhancement of DNA is based on ethidium bromide (EB) usage. Recently, due to carcinogenic properties of EB, the methylene blue (MB) replaced EB and has become a safe reagent in nucleic acid chemistry. MB is a phenothiazinium dye that can interact with DNA not only by intercalation, but also by non-intercalation mode base on the concentration of MB used. These results have been testified by several spectroscopic methods [9]. The fluorescence spectra of MB, MB-DNA complex before and after addition each TCB were recorded and shown in Figure 3.

As shown in Figure 3, by addition of DNA, the fluorescence of MB was quenched in some extent. This emission-quenching phenomenon was due to the changes in the excited-state electronic structure in consequence of electronic interactions of MB-DNA complex [5]. It should be noted that the effect of the TCBs on pure MB spectrum has been carefully checked, and no variation of the absorption and emission of spectrum was detected. By adding each TCB to the DNA-MB solution, the fluorescence of MB was increased (Figure 3). The increase of the fluorescence intensity should be due to the fact that MB was released after the addition of TCBs. The formation of TCBs-DNA complex prevents MB binding to DNA. By using this phenomenon, the formation constant of each TCB to DNA was measured based on the recorded fluorescence data using the modified Benesi-Hildebrand equation, as described in eq. 3 [34]:

$$\frac{1}{\mathrm{F\text{-}F_0}} = \frac{1}{\{\mathrm{KLQ[MB\text{-}DNA]_0}\}[\mathrm{TCB}]_0} + \frac{1}{(\mathrm{LQ[MB\text{-}DNA]_0})} \qquad (3)$$

where F_0 and F represent the fluorescence signals of MB-DNA in the absence and presence of TCBs; $[\mathrm{MB\text{-}DNA}]_0$ and $[\mathrm{TCB}]_0$ represent the initial concentration of MB-DNA complex and any of

TCBs, *L* is the instrumented constant, *K* is formation constant of the TCBs-DNA complexes, and *Q* is the quantum yield for the TCBs-DNA complex. By plotting of $1/(F\text{-}F_0)$ *vs* $1/[TCB]_0$, the *K* can be obtained from the slope and intercept of the resulted curves, shown in Figure 4. Formation constants for each TCB with DNA were summarized in Table 1. The results showed that the values of *K* were 7.4×10^4, 9.4×10^3, 3.2×10^4 $L\cdot mol^{-1}$ for 1,2,4-TCB, 1,3,5-TCB, and 1,2,3-TCB, respectively. The results were consistent with those obtained *via* electronic spectra method. These furtherly illuminated that the interaction between DNA and TCBs might be realized *via* the non-classical intercalation mode.



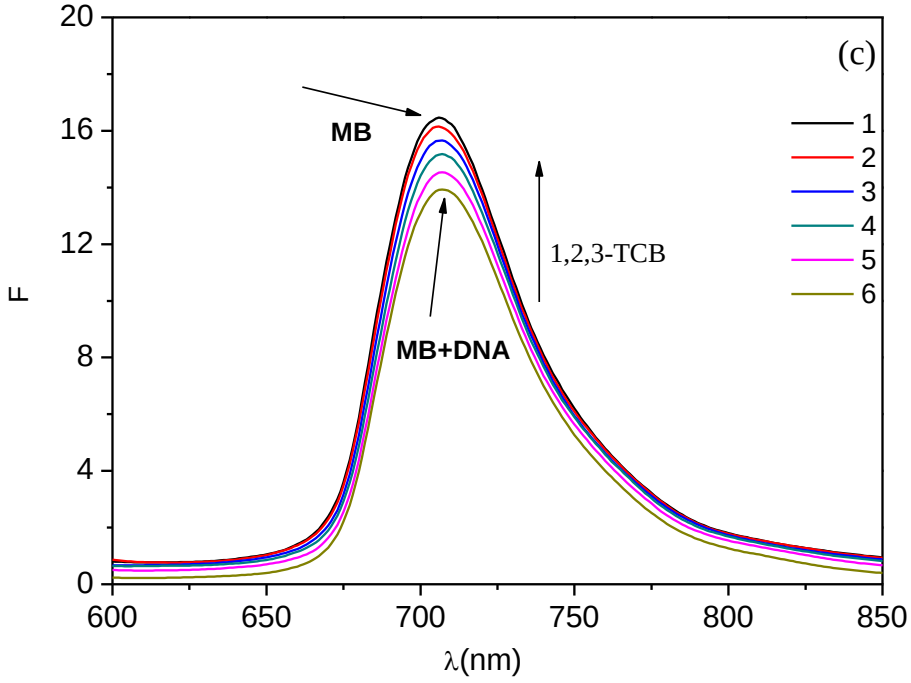


Figure 3. Fluorescence spectra of the MB–DNA complexes in the presence of the increasing each TCB concentrations ((1) MB: *c*(MB): 1.0×10^{-5} mol/L, (2-4) any of TCBs+MB+DNA: *c*(TCB): (2) 1.0×10^{-6} mol/L, (3) 5.0×10^{-6} mol/L, (4) 1.0×10^{-5} mol/L, (5) 5.0×10^{-5} mol/L; (6) MB+DNA: *c*(MB): 1.0×10^{-5} mol/L; *c*(DNA)＝6.0×10^{-5} mol/L) in 0.01 mol/L of Tris buffer at pH 7.2 at room temperature

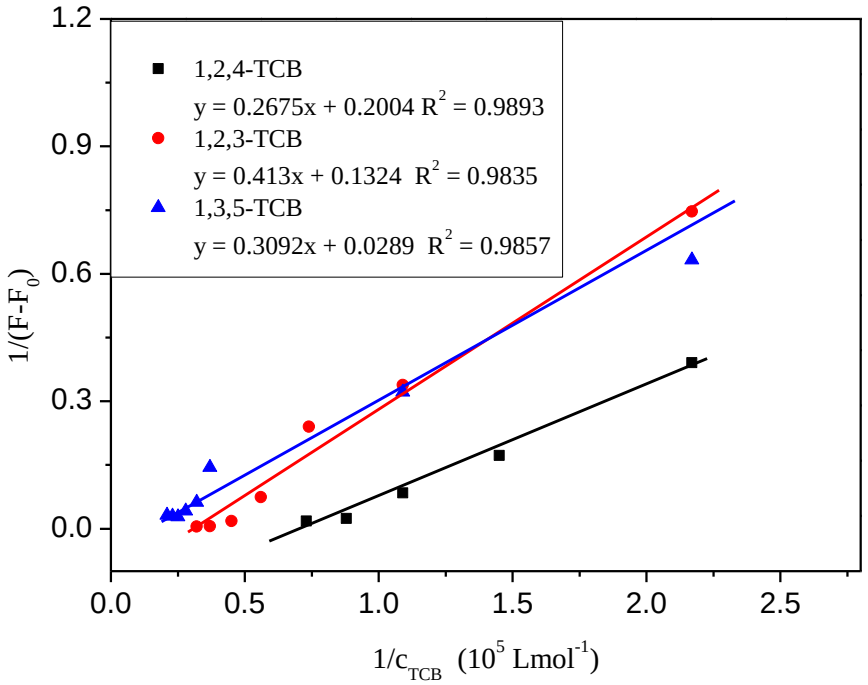


Figure 4. The formation constant of TCB-DNA obtained *via* fluorescence data using the modified Benesi-Hildebrand equation (*c*(MB): 1.0×10^{-5} mol/L; *c*(DNA): 6.0×10^{-5} mol/L, *c*(TCB): 1.0×10^{-6} ~ 5.0×10^{-5} mol/L)

3.3. Viscosity measurements

Spectrophotometric and spectrofluorometric methods can provide necessary but not sufficient clues to support binding modes, whereas hydrodynamic measurements which are sensitive to the length change are regarded as the most critical tests of a binding model in solution [28]. Thus, to further clarify the interaction between TCBs and DNA, we carried out viscosity measurements. A classical intercalation mode is known to cause a significant increase in the viscosity of a DNA solution due to an increase in lengthening the DNA helix, while a non-classical intercalation or a groove mode would reduce the DNA viscosity. The viscosity measurements were taken by varying the concentration ratio of DNA and each TCB. The values of relative specific viscosity $(\eta/\eta_0)^{1/3}$ *vs* [DNA]/[TCB] were plotted in the absence and presence of each TCB in Tris buffers (Figure 5). As it was observed from Figure 5, the relative specific viscosity of DNA exhibited a dependence on the concentration of TCBs, which decreased with the value of [DNA]/[TCB], indicating non-classical intercalation mode of binding that may be realized *via* hydrophobic interaction between the TCBs and DNA.

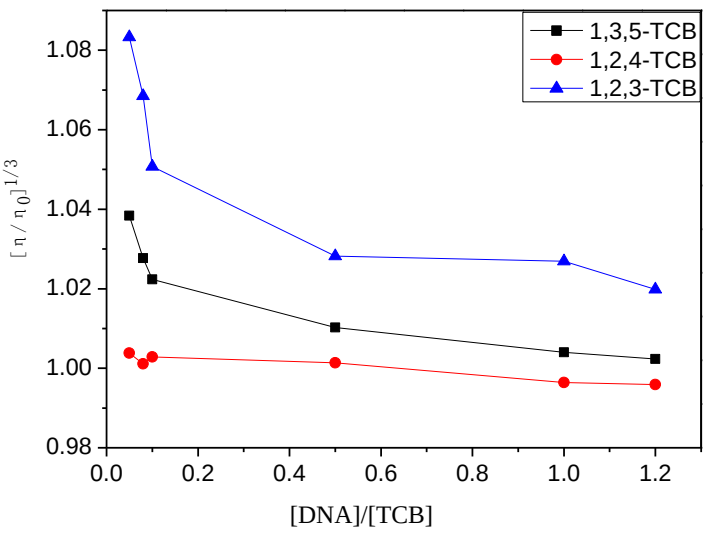


Figure 5. Effect of increasing amounts of TCBs on the viscosity of DNA (6.0×10^{-5} mol/L) in 0.01M Tris buffer (pH 7.2)

3.4. DNA denaturation temperature

Additional evidence for the binding mode between the TCBs and DNA was obtained from DNA melting (T_m) studies by investigating the UV-vis spectra of TCBs-DNA at different temperatures (30-90 ^oC).

The changes in the absorbance at 260 nm as a function of temperature (30-90 ^oC) for HS-DNA in the absence and presence of TCBs were measured. f_{ss} was calculated as eq. 4. [34]:

$$f_{ss}=(A-A_0)/(A_f-A_0) \qquad (4)$$

where A_f and A_0 were the maximum (double strand DNA fully separated to be single strand DNA) and minimum (double strand DNA) absorbances at 260 nm, respectively. *A* was the absorbances at 260 nm at any temperature, f_{ss} was the value of the hyperchromic effect of DNA. The DNA denaturation temperature (T_m) was defined as the temperature when f_{ss} was 0.5. By plotting of f_{ss} *vs* temperature (shown in Figure 6), the T_m can be obtained.

It has been reported that the intercalation of small molecules into the double helix is known to increase T_m significantly, while the interactions realized *via* non-traditional intercalation, or groove binding or electrostatic interaction mode are known to have little effect on T_m [35, 36].

As shown in Figure 6, T_m was increased to be 70.8 ^oC, 71.6 ^oC , and 72.4 ^oC for 1, 2, 4-TCB, 1, 3, 5-TCB, and 1, 2, 3-TCB, with the ratios of the TCBs to DNA at 1.0, respectively. These results showed that T_m of the system with the TCBs added did not increase as much as that of previously observed for daunomycin [31], cryptolepine [32], and chlorobenzylidine [33], which were proved their interaction with DNA to be intercalative mode. The results furtherly supported that the binding modes of these TCBs with DNA were non-traditional intercalated. The small increase of T_m might be due to the interaction of the TCBs with DNA *via* hydrophobic interaction, which subsequently lead the conformation of DNA being changed in some degree and the stabilization of the DNA-TCBs systems being increased.

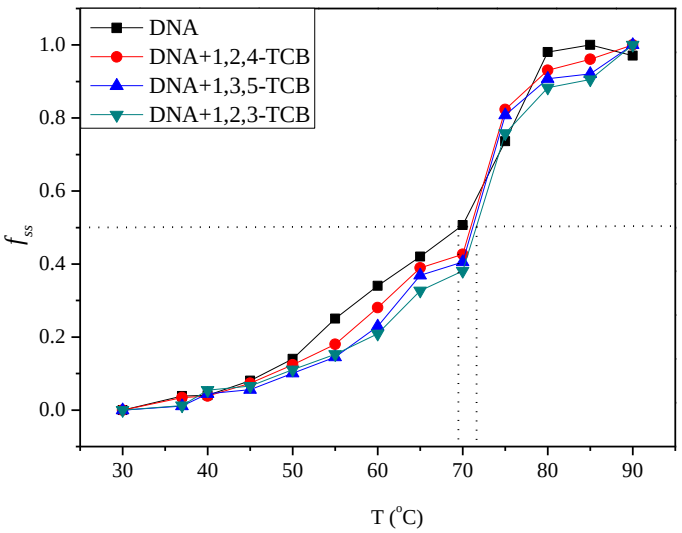


Figure 6. The relationship beween f_{ss} of DNA and *T* before and after adding TCBs to the DNA solution

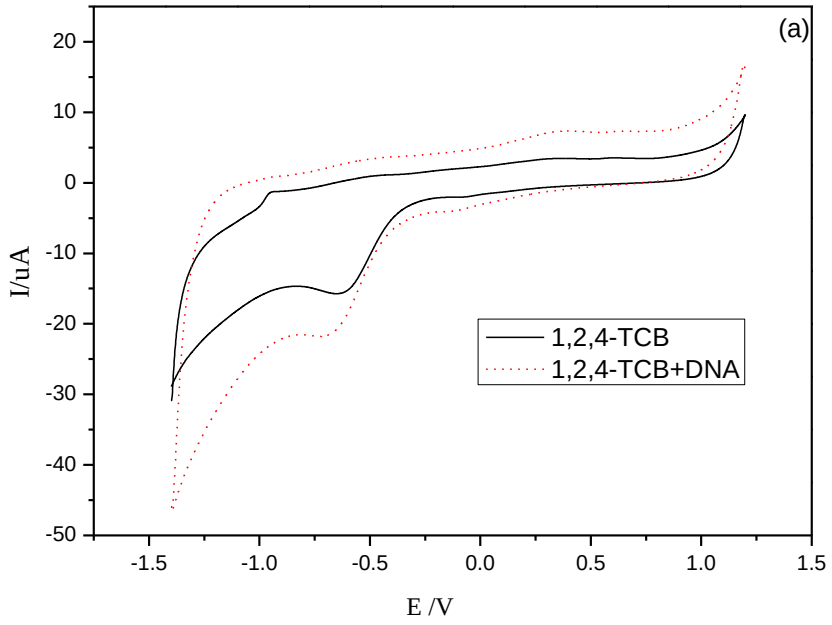
3.5. Cyclic voltammetric studies

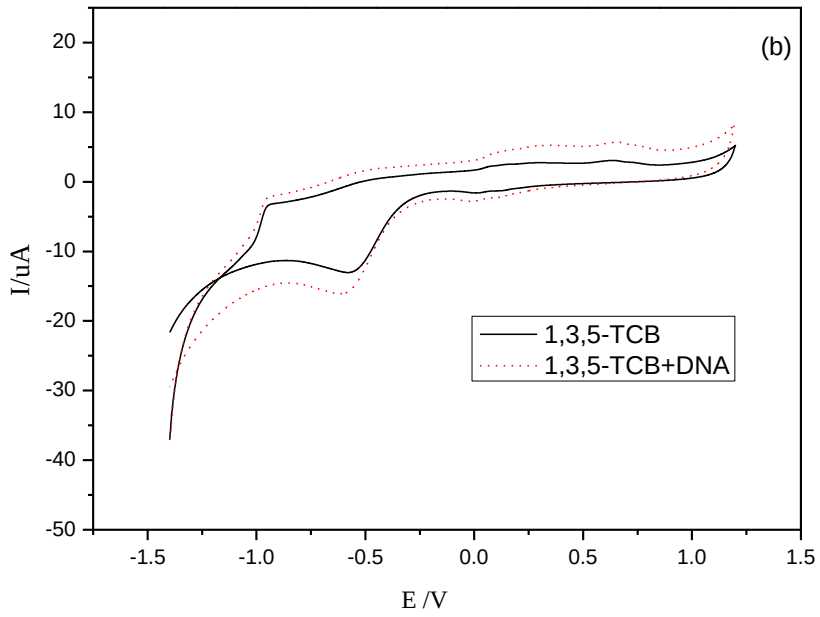
Typical cyclic voltammetric curves of the TCBs with concentration at 1.0×10^{-5} mol·L^{-1}, in absence or presence of HS-DNA (5.0×10^{-5} mol·L^{-1}) in 0.05 mol·L^{-1} Tris (pH 7.0) with a scan rate of 100 mV ·s^{-1} were shown in Figure 7.

A pair of redox peaks for any of the 3 TCBs in absence of HS-DNA appeared using a bare GCE in the range of -1.5 to +1.5 V (vs. SCE). The cathodic peak potential (E_{pc}) was at -0.66 V for

1,2,4-TCB, -0.58 V for 1,3,5-TCB, and -0.63 V for 1,2,3-TCB, with a scan rate of 100 mV $\cdot s^{-1}$, respectively, while the anodic peaks for all the 3 TCBs were not obvious. The reduction peak current of any of the 3 TCBs was obviously much higher than the oxidation peak current. These results indicated that the electrochemical processes of the 3 TCBs at a bare GCE were quasireversible.

As shown in Figure 7, when HS-DNA was added into the TCBs solutions, increases in reduction peak currents of TCBs (from -15.72 μA to -21.77 μA for 1,2,4-TCB, -13.04 μA to -16.07 μA for 1,3,5-TCB, and -17.19 μA to -26.10 μA for 1,2,3-TCB, respectively) with a slight negative shift in peak potential values (from -0.66 V to -0.73 V for 1,2,4-TCB, -0.58 V to -0.62 V for 1,3,5-TCB, and -0.63 V to -0.71 V for 1,2,3-TCB, respectively) were observed. These results indicated that some electrochemical-active complexes have been formed between TCBs and DNA.





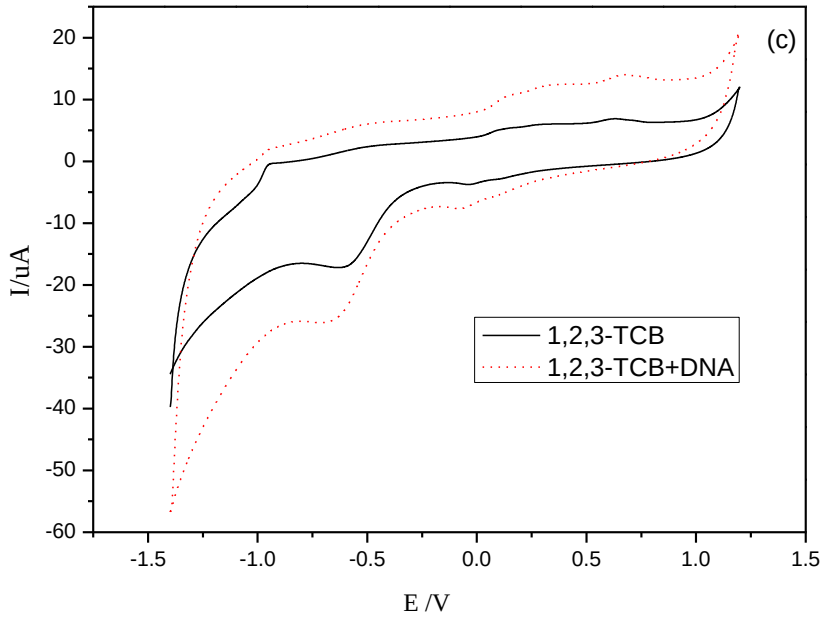


Figure 7. Cyclic voltametry (CV) of TCBs, TCBs-DNA system ((a) 1,2,4-TCB (b) 1,3,5-TCB, and (c) 1,2,3-TCB, *c*(MM): 1.0×10^{-5} mol·L^{-1}, *c*(TCB): 1.0×10^{-5} mol·L^{-1}, 0.05 mol·L^{-1} Tris (pH 7.0), with a scan rate of 100 mV ·s^{-1})

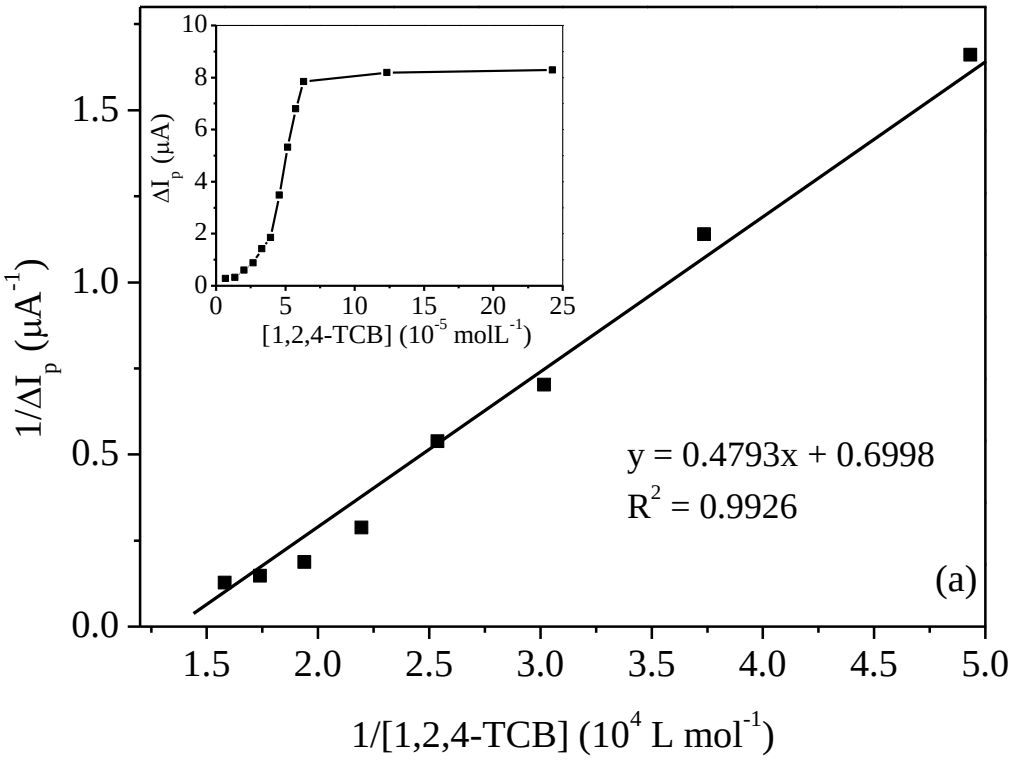
For further investigation the intensity of the interaction between the TCBs and DNA, cyclic voltametric experiments of TCBs with the varied concentrations in absence and presence of HS-DNA (*c*(DNA)＝5.0×10^{-5} mol·L^{-1}) were carried out. The results showed that the difference of the reduction peak current in absence and presence of DNA ($\triangle I_p$) gradually increased with the concentration of the TCBs and finally reached to a flatform (shown in Figure. 8 (insert, (a) for 1,2,4-TCB, (b) for 1,3,5-TCB, and (c) for 1,2,3-TCB), which was the typical phenomenon for an adsorption controlled quasireversible electrochemical process.

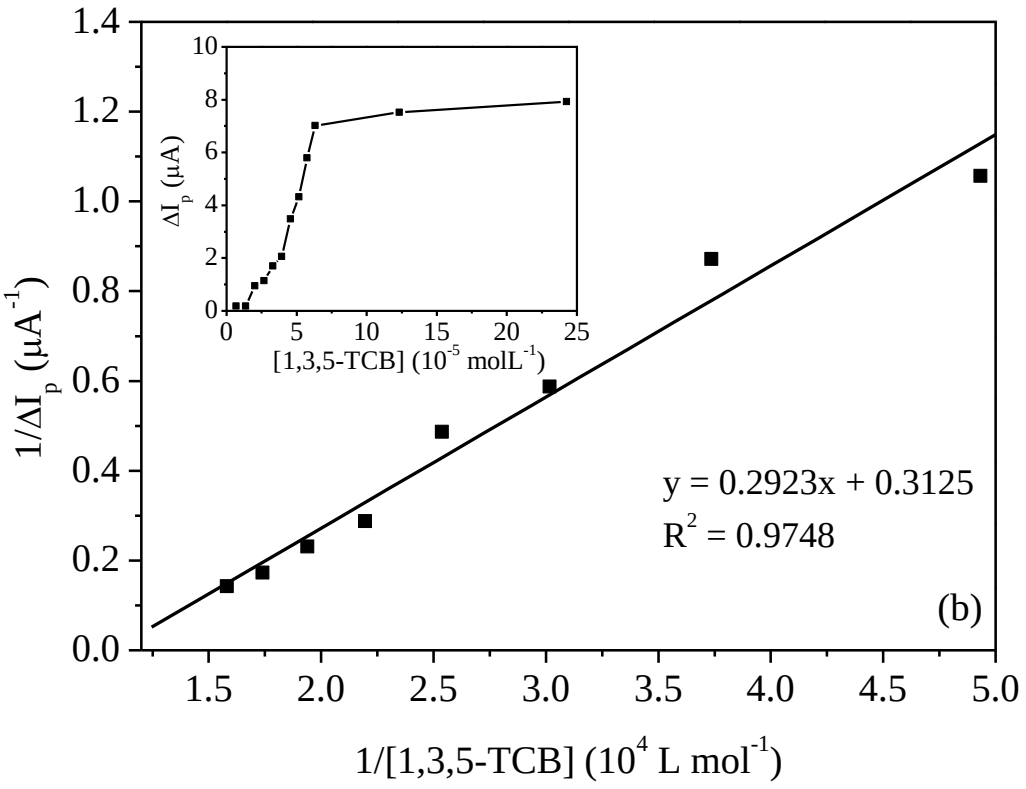
Thus, the interaction constants were evaluated by double reciprocal equation as listed in eq. 5 [37].

$$\frac{1}{\Delta I_p} = \frac{1}{\Delta I_{pmax}} + \frac{1}{K\Delta I_{pmax}} \times \frac{1}{[TCB]^s} \qquad (5)$$

Where $\triangle I_p$ and $\triangle I_{pmax}$ are the difference and the maxium difference of the reduction peak current in absence and presence of DNA, respectively, while *c*(TCB, mol·L^{-1}) is the concentration of any of TCBs added, and *K* (L ·mol $^{-1}$) is the interaction constant of TCBs-DNA, *s* is the binding number for TCBs to DNA. Taken different values of *s* (*s* = 1, 2 .... n), by plotting of $1/\triangle I_p$ *vs* $[1/c(\mathrm{TCB})]^s$, *K* can be obtained from the slope and intercept of resulted curves. In the present work, when *s* = 1, a good linear relationship between $1/\triangle I_p$ *vs* [1/*c*(TCB)] can be obtained, as shown in Figure 8. The *K* values for the TCB-DNA complexes were 1.5×10^4, 1.1×10^4, 1.5×10^4 L·mol^{-1} for

1,2,4-TCB, 1,3,5-TCB, and 1,2,3-TCB, respectively, which were consisted with the findings obtained *via* spectrophotometric, spectrofluorometric methods as shown in Table 1.





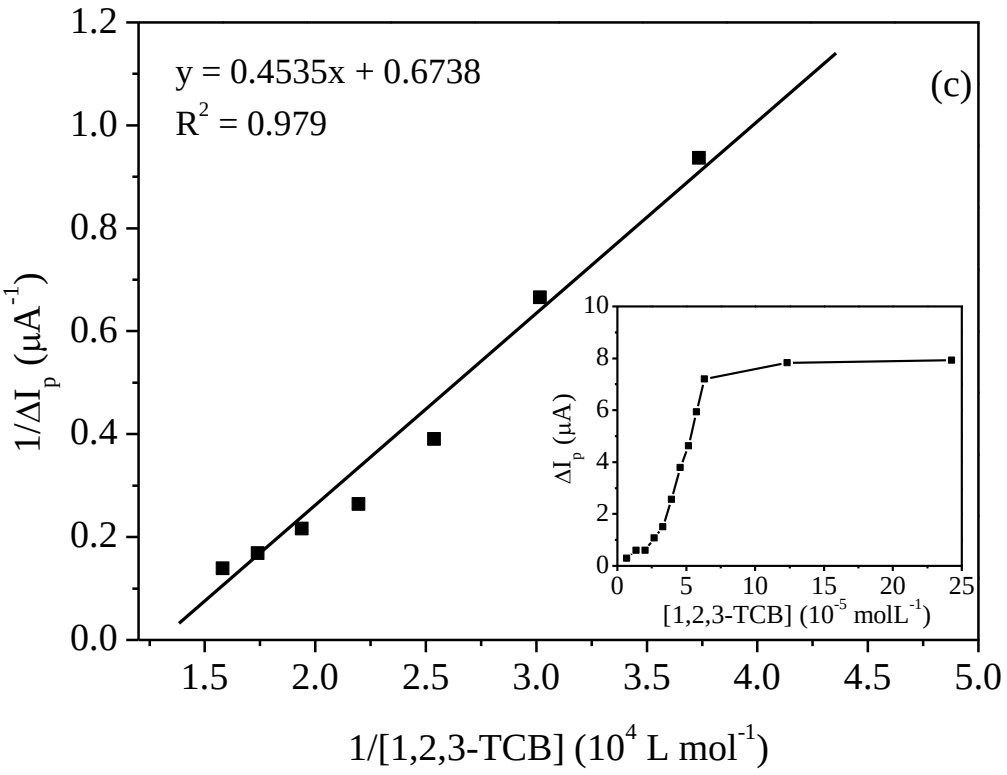


Figure 8. Relationship curves between ΔI_p^{-1} and $[TCBs]^{-1}$ (insert: Relationship curves between ΔI_p and [TCBs]; $\triangle I_p$: the diference of peak currents before and after addition of DNA; [TCB] the equilibrium concentration of 1,2,4-TCB (a), or 1,3,5-TCB (b), or 1,2,3-TCB (c)

4. CONCLUSIONS

In summary, spectrophotometric, spectrofluorometric, melting temperature (T_m), viscosimetric and electrochemical studies showed that TCBs might interact with native Herring Sperm DNA (HS-DNA) by a non-traditional intercalation mode *via* hydrophobic force. The thermodynamic studies suggested that the interaction processes were endothermic disfavored ($\triangle H$>0) and entropy favored ($\triangle S$>0), with the interaction constants between any of the TCBs and HS-DNA at ~10^4 L·mol^{-1}.

References

1. OSPAR Commission, OSPAR background document on trichlorobenzenes, 2005
2. J. Thornton. *Risk Assess. Global. Poll.* 6 (2000) 318
3. R. Barro, S. Ares, C. Garcia-Jares, M. Llompart, R. Cela. *J. Chromatogr. A.,* 1045 (2004) 189
4. A. Tor. *J. Chromatogr. A.,* 1125 (2006) 129
5. E. C. Long, J. K. Barton. *Acc. Chem. Res.,* 23 (1990) 271
6. A. Hartwig. *Chemico-Biological Interact.,* 184 (2010) 269
7. P. G. Mantle, V. Faucet-Marquis, R. A. Manderville, B. Squillaci, A. Pfohl- Leszkowicz. *Chem. Res. Toxicol.,* 23 (2010) 89
8. K. J. Jang, G. Y. Yeo, T. S. Cho, G. H. Eom, C. Kim, S. K. Kim. *Biophys. Chem.,* 148 (2010) 138

9. S. Kashanian, S. Askari, F. Ahmadi, K. Omidfar, S. Ghobadi, F. A. Tarighat. *DNA Cell Biol.* 27(10) (2008) 581
10. C. Y. Qiao, S. Y. Bi, Y. Sun, D. Q. Song, H. Q. Zhang, W. H. Zhou. *Spectrochim. Acta A.,* 70 (2008) 136
11. F. A. Tanious, D. Y. Ding, D. A. Patrick, R. R. Tidwell, W. D. Wilson. *Biochem.,* 36 (1997) 15315
12. F. Ahmadi, F. Bakhshandeh. *DNA Cell Biol.,* 28(10) (2009) 527
13. A. Z. Li, J. L. Qi, H. H. Shih, K. A. Marx. *Biopolymers,* 38 (1996) 367
14. J. K. Barton. *Comments Inorg. Chem.,* 3 (1985) 321
15. K. Sandstrom, S. Warmlander, M. Leijon, A. Graslunda. *Biochem. Biophys. Res. Commun.,* 304 (2003) 55
16. W. Zhong, J. H. Yu, Y. Liang. *Spectrochim. Acta* A., 59 (2003) 1281
17. J. Pastor, J. G. Garcia-Navio, J. J. Vaquero, J. Alvarez-Builla, F. Gago, B. Pascual- Teresa, M. Pastor, M. M. Rodrigo. *J. Org. Chem.,* 62 (1997) 5476
18. M. T. Carter, M. Rodriguez, A. J. Bard. *J. Am. Chem. Soc.,* 111 (1989) 8901
19. S. Riahi, A. Mashhadi, S. Eynollahi, M. R. Ganjali, P. Norouzi. *Int. J. Electrochem. Sci.,* 5 (2010) 955
20. H. Y. Shen, H. M. Zheng, N. Zhu, Y. Q. Liu, J. Gao, J. Li. *Int. J. Electrochem. Sci*., 5 (2010) 1587
21. J. B. Raoof, M. S. Hejazi, R. Ojani, E. H. Asl. *Int. J. Electrochem. Sci.,* 4 (2009) 1436.
22. H. Ilkhani, M. R. Ganjali, M. Arvand, P. Norouzi. *Int. J. Electrochem. Sci.,* 5 (2010) 168
23. S. Riahi, S. Eynollahi, M. R. Ganjali, P. Norouzi. *Int. J. Electrochem. Sci.,* 5 (2010) 355
24. T. M. Kelly, A. B. Tossi, D. J. McConnel, T. C. Streakas. *Nucleic Acids Res.,* 13, (1985) 6017
25. S. A. Tysoe, R. J. Morgan, A. D. Bakerand, T. C. Streakas. *J. Phys. Chem*., 97, (1993) 1707
26. C. J. Burrows, J. G. Muller. *Chem. Rev.,* 98, (1998) 1109
27. S. R. Chowdhury, K. K. Mukherjea, R. Bhattacharyya. *Trans. Met. Chem.,* 30, (2005) 601
28. N. Zhao, X. M. Wang, H. Z. Pan, Y. M. Hua, L. S. Ding. *Spectrochim. Acta A.,* 75 (2010) 1435
29. S. Bhattacharya, M. Banerjee. *Chem. Phys. Lett.,* 396 (2004) 377
30. P. D. Ross, S. Subramanian. *Biochem.,* 20 (1981) 3096
31. J. B. Chaires, N. Dattagupta, D. M. Crothers. *Biochemistry,* 21(1982) 3933
32. K. Bonjean, M. C. de Pauw-Gillet, M. P. Defresne. *Biochemistry,* 37 (1998) 5136
33. W. Zhong, J. Yu, Y. Liang, K. Fan, L. Lai. *Spectrochim. Acta A.,* 60 (2004) 2985
34. G. C. Catena, F. V. Bright. *Anal. Chem.,* 61 (1989) 905
35. D. Zeynep, P. Ralph, A. R. S. Jan, N. Sukunath, R. Clemens. *J. Am. Chem. Soc.,* 126 (2004) 4762
36. Y. Q. Li, J. White, S. David, S. Gary, S. Michael. *Biotechnol. Prog.,* 17 (2001) 348
37. Z. S. Yang, D. P. Zhang, H. Y. Long, Y. C. Liu. *J. Electroanal. Chem.,* 624 (2008) 91