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DNA binding studies of 2-*tert*-butylhydroquinone (TBHQ) food additive

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ABSTRACT

The interaction of native calf thymus DNA with 2-*tert*-butylhydroquinone (TBHQ) in 10 mM Tris–HCl aqueous solutions at neutral pH 7.4, has been investigated by spectrophotometric, spectrofluorometric, voltammetric and viscosimetric techniques. It is found that TBHQ molecules could intercalate between base pairs of DNA as are evidenced by: hyperchromism in UV absorption band of DNA, sharp increase in specific viscosity of DNA and decrease in the fluorescence of TBHQ solutions in the presence of increasing amounts of DNA. Also peak current decrease, positive shift in the cyclic voltammetry (CV) and differential pulse voltammetry (DPV) are other evidences to indicate that, TBHQ is able to be intercalated in the DNA base pairs.

1. Introduction

Food additives have been extensively applied in recent decades in food industry throughout the world. 2-*tert*-butylhydroquinone (TBHQ) is a highly effective preservative for unsaturated vegetable oils, many edible animal fats and meat products. It does not cause discoloration even in the presence of iron, and does not change flavour or odour of the material it is added to. Metabolically, TBHQ is formed from 3-*tert*-butyl-4-hydroxyanisole (BHA), another widely used food additive, by *O*-demethylation (Okubo et al., 2003). It is also used industrially as a stabiliser to inhibit autopolymerisation of organic peroxidase. In high doses, it has some negative health effects on lab animals, such as precursors to stomach tumours and damage to DNA. A number of studies have shown that TBHQ caused DNA cleavage in vitro (Okubo et al., 1997) and the formation of 8-hydroxydeoxyguanosine in calf thymus DNA (Nagai et al., 1996) due to the generation of reactive oxygen species (ROS) such as superoxide anion and hydrogen peroxide.

Small molecules can react with DNA via covalent or non covalent interactions, with interest generally focusing on the latter. There are several sites in the DNA molecule where such binding can occur: (i) between two base pairs (full intercalation), (ii) in the minor groove, (iii) in the major groove, (iv) on the outside of the helix and (v) electrostatics binding (Ni, Lin, & Kokat, 2006; Cao & He, 1998). Some interactions can cause the damage to dsDNA and influence the duplication of dsDNA (Sun et al., 2006). In the study presented here, we investigated the interaction of native calf thymus DNA with TBHQ in 10 mM Tris–HCl aqueous solutions at neutral pH 7.4 and in vitro condition using spectroscopic (UV/VIS, fluorescence), viscosimetric and voltammetric procedures.

2. Experimental

2.1. Chemical and materials

The highly polymerised calf thymus DNA (CT DNA) and Tris–HCl were purchased from Sigma Co. All solutions were prepared using double distilled water. Tris–HCl buffer solution was prepared from (Tris–(hydroxymethyl)-amino-methane–hydrogen chloride) and pH was adjusted to 7.4. The stock solution of DNA was prepared by dissolving of DNA in 10 mM of the Tris–HCl buffer at pH 7.4 and dialysing exhaustively against the same buffer for 24 h and used within 5 days. A solution of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm more than 1.8, indicating that DNA was sufficiently free from protein (Liu et al., 2002). The concentration of the nucleotide was determined by UV absorption spectroscopy using the molar absorption coefficient ($\varepsilon = 6600\ M^{-1}\ cm^{-1}$) at 260 nm. The stock solution was stored at 4 °C. A TBHQ stock solution (1×10^{-3} M) was prepared by dissolving an appropriate amount of compound in Tris–HCl buffer/DMSO (90:10%). It has been verified that the low DMSO percentage added to DNA solution would not interfere with the nucleic acid (Zhou & Yang, 2006).

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2.2. Instrumentation

2.2.1. UV–VIS absorption spectra

The UV–VIS spectra for DNA–TBHQ interactions were obtained using a Cary (UV 100 Bio) spectrophotometer. Solutions of DNA and TBHQ were scanned in a 0.5 cm (1 ml) quartz cuvette. The spectra were recorded by progressive addition of TBHQ to the pure DNA solution.

2.2.2. Viscosity measurements

For viscosity measurements a viscosimeter (SCHOT AVS 450) was used, which thermo stated at 25 °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 ($\acute{\eta}$) of the samples. The data were reported as $\acute{\eta}/\acute{\eta}^{\circ}$ versus the [TBHQ]/[DNA] ratio, where $\acute{\eta}^{\circ}$ is the viscosity of the DNA solution alone (Vijayalakshmi et al., 2000).

2.2.3. Fluorescence quenching

All fluorescence measurements were carried out with a JASCO spectrofluorometer (FP6200). The excitation wavelength that 285 nm was used for the Fluorescence measurements and the emission spectra were recorded between 300 and 450 nm. The excitation and emission slits were both 5 nm, and the scan speed was 125 nm min^{-1}. Two millilitre of the TBHQ (10^{-5} M) was placed in a 1-cm thermo stated quartz fluorescence cuvette and titrated with 20 μ aliquots of 0.2 mM DNA With continuous stirring. After each titration, the solution was mixed thoroughly and was allowed to be equilibrated thermally for 5 min prior to the fluorescence measurements. The fluorescence data for a control system, the TBHQ titrated with 20 μ aliquots of the buffer, were also measured under the same condition and was used to correct the observed fluorescence and the dilution effects fluorescence titration experiments were performed by keeping the TBHQ concentration constant and stoichiometrically varying the DNA concentration (Wu et al., 2005;. Gopala Krishna, Vijay Kumar, Khan, Rawal, & Ganesh, 1998). The titration data was fitted into the Stern–Volmer Eq. (1) (Lakowicz, 2006):

$$F_0/F = 1 + K_{SV}[\mathrm{DNA}]$$

where F_0 and F are the fluorescence intensities of the probe in the absence and presence of DNA, respectively, and K_{SV} is Stern–Volmer quenching constant which is a measure of the efficiency of fluorescence quenching by DNA.

2.2.4. Electrochemical experiments

The cyclic voltammetric, linear sweep voltammetry and differentials pulse voltammetry (DPV) measurements were performed using an AUTOLAB model (PG STAT C), with a three-electrode system: a 0.10-cm-diameter Glassy carbon (GC) disc as working electrode, an Ag/AgCl electrode as reference electrode, and a Pt wire as counter electrode. Electrochemical experiments were carried out in a 25-mL voltammetric cell at room temperature. All potentials are referred to the Ag/AgCl reference. Their surfaces were freshly polished with 0.05 mm alumina prior to each experiment and were rinsed using double distilled water between each polishing step. The supporting electrolyte was 0.01 M of Tris–HCl buffer solution (pH 7.4) which was prepared with double distilled water. Before experiments, the solution was deaerated via purging with pure nitrogen gas for 1 min, and during measurements a stream of nitrogen was passed over the solution. The current–potential curves and experimental data were recorded on software GPES (Ni et al., 2006; Kashanian, Gholivand, Ahmadi, & Ravan, 2008).

3. Results and discussion

3.1. Effect of the TBHQ on UV spectra of DNA

"Hyperchromic" and "hypochromic" effects are the spectra features of DNA concerning its double helical structure (Zhou, Xi, & Yang, 2007). The spectral change process reflects the corresponding changes in DNA in its conformation and structure after TBHQ binding to DNA. Hypochromism results from the contraction of DNA in the helix axis, as well as from the conformational change of DNA; in contrast, hyperchromism derives from damage of the DNA double-helix structure (Zhou et al., 2007; Shi et al., 2006). As shown in Fig. 1, the absorption spectra of DNA increase with increasing the TBHQ concentration. This is a typical "hyperchromic" effect, which suggests that the DNA double-helix structure is damaged after the TBHQ bound to DNA through intercalation mode.

3.2. Viscosity study

To further clarify the nature of the interaction between the TBHQ and DNA, viscosity measurements were carried out by varying the concentration of the added TBHQ to DNA solution. In Fig. 2, the specific viscosity of the DNA sample clearly increases with the addition of the TBHQ. The viscosity studies provide a strong argument for intercalation. The viscosity increase of DNA is ascribed to the intercalative binding mode of the TBHQ, due to effective DNA length increase (Lepecq & Paoletti, 1967). So, we think the viscosity increase of the DNA caused by the addition of the TBHQ can provide further support for the intercalative mode of binding of the TBHQ (Zhou et al., 2007).

3.3. Effect of temperature on quenching efficiency

A variety of molecular interactions can result in quenching, including excited-state reactions, molecular rearrangement, energy transfer, ground-state complex formation, and collisional quenching. Quenching normally refers to nonradiative energy transfer from excited species to other molecules. In fact, two quenching processes are known: static and dynamic. Dynamic quenching or collisional quenching requires contact between the excited lumophore and the quenching specie, the quencher. The rate of quenching is diffusion controlled and depends on temperature and viscosity of the solution. The quencher concentration must be high enough that the probability of collision between the analyte and quencher is significant during the lifetime of the excited species. As mentioned above the other form of quenching is static quenching in which the quencher and the fluorophore in

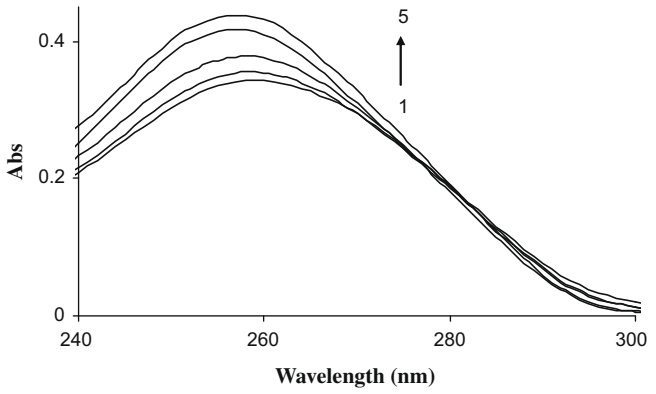

Fig. 1. UV/VIS spectra of DNA (5.0×10^{-5}M) with TBHQ in 0.01 M Tris–HCL buffer (pH 7.4) with r_i = [TBHQ]/[DNA] = 0.0, 0.2, 0.4, 0.6 and 0.8.

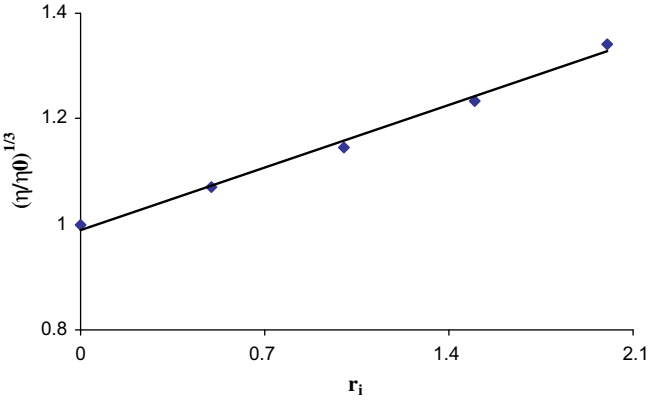

Fig. 2. Effect of increasing amounts of TBHQ on the viscosity of CT-DNA (5×10^{-5} M) in 0.01 M Tris–HCL buffer (pH 7.4), (r_i = 0.0, 0.5, 1, 1.5 and 2).

ground state form a stable complex. Fluorescence is only observed from the unbound fluorophore. The lifetime is not affected in this case; measurement of the lifetime provide a means of distinguishing between dynamic and static quenching (Ingle & Crouch, 1988). Dynamic and static quenching can also be distinguished by their differing dependence on temperature (Baguley & Bret, 1984) (Fig. 3a). Dynamic quenching depends upon diffusion. Since higher temperatures lead to larger diffusion coefficients, the K_{sv} can be increased by rising the temperature. In contrast, increased temperature is likely the result of decrease in complexes stability, and thus lower values of the static quenching constants were resulted. By

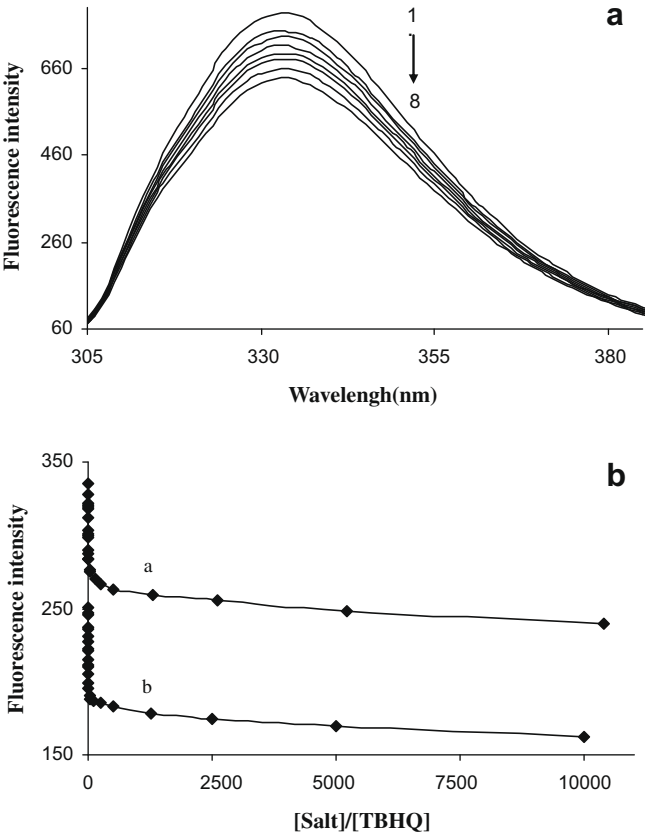

Fig. 3. (a) Emission spectra of the TBHQ in the presence of the increasing DNA concentrations in 0.01 M Tris–HCL buffer (pH 7.4) at room temperature. r_i = [DNA]/[TBHQ] = 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.7 and 0.9. (b) Effect of ionic strength on the TBHQ-DNA spectra.

Table 1
The K_{SV} values of TBHQ-DNA at different temperatures.

Temperature (°C)	$K_{sv} \times 10^4$	R^2
25.0	2.43	0.9912
30.0	3.23	0.9885
37.0	3.88	0.9882
40.0	4.49	0.9860

using Eq. (1), the K_{sv} of TBHQ fluorescence by DNA at different temperatures (25, 30, 37 and 40 °C) was obtained and the results are shown in Table 1. These results show that the probable quenching mechanism of TBHQ fluorescence by DNA is a dynamic quenching procedure, because the K_{sv} has been increased by temperature rising (Kashanian et al., 2008).

3.4. Effect of ionic strength on the spectrum of TBHQ–DNA

Monitoring the change of ionic strength is an efficient method to recognise the binding modes between molecules and DNA. Increasing the concentration of cation will increase the complexation probability between the cation and DNA phosphate backbone. Due to a competition for phosphate anion, the addition of the cation will weaken the surface-binding interactions which include electrostatic interaction and hydrogen binding between DNA and molecules (Lepecq & Paoletti, 1967; Wu, Xie, Wu, & Hong, 2008).

In order to prove whether TBHQ provides an electrostatic binding or other kinds of binding with DNA, TBHQ solution was titrated with NaCl in the absence and presence of DNA (2×10^{-4} M). TBHQ was titrated with NaCl with the increasing concentration of the salt from10^{-6} up to 1 M^{-1}. As it is elucidated from Fig. 3b, the slopes of the curves in the absence and presence of DNA are the same. It is deduced that NaCl has no effect on DNA binding with TBHQ. Therefore the results indicated that TBHQ does not give an electrostatic or outside binding with DNA. It should be mentioned that the decrease in the fluorescence intensity is due to salt interaction with TBHQ.

3.5. Electrochemical behaviour of TBHQ in the absence and presence of DNA

Recently, the electrochemical techniques extensively were used as a simple and rapid method to study DNA interaction with different compounds (Kashanian et al., 2008). The electrochemical behaviour of TBHQ is well known, and was strongly influenced by the electrode material. A well-defined and sensitive peak was observed from the solutions of the TBHQ with a GC electrode rather than the Pt one. Therefore a GC electrode was used in this investigation. When CT-DNA is added to a solution of TBHQ both the anodic and cathodic peak current heights of the TBHQ decreased in the same manner of increasing additions of DNA, (Fig. 4a). Also during DNA addition the anodic peak potential (E_{pa}), cathodic peak potential (E_{pc}), and $E_{1/2}$ (calculated as the average of E_{pc} and E_{pa}) all showed positive shifts. These positive shifts are considered as evidences for intercalation of TBHQ into the DNA, because this kind of interaction is due to hydrophobic interaction. From the other point of view, if a molecule binds electrostatically to the negatively charged deoxyribose-phosphate backbone of DNA, negative peak potential shifts should be detected. Therefore, the positive shift in the CV peak potentials of TBHQ is indicative of intercalative binding mode of the TBHQ with DNA (Ni et al., 2006).

3.6. Measurement of the stoichiometry of the DNA–nTBHQ

To determine the composition of the supramolecular complex and the equilibrium constant of the binding reaction (Feng, Li, &

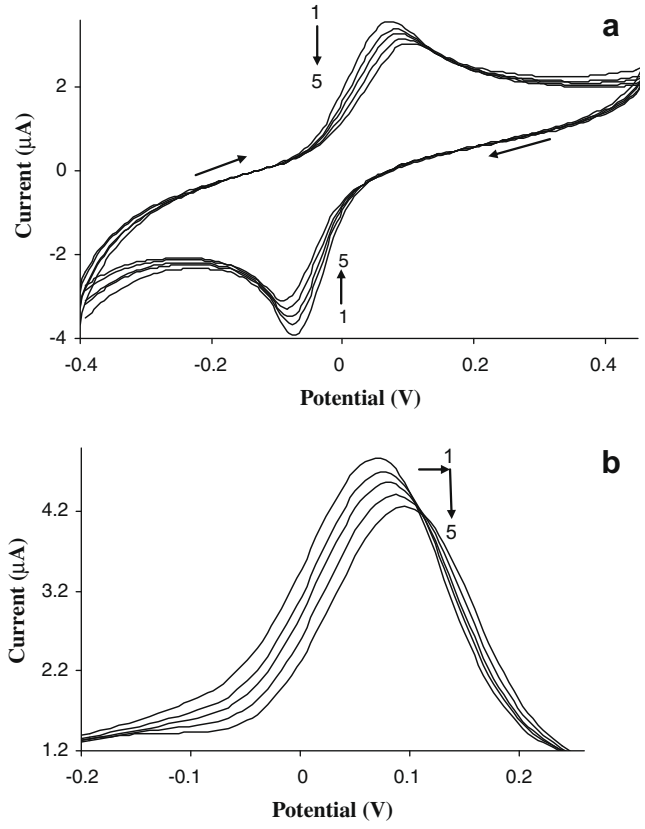

Fig. 4. Cyclic (a) and differential pulse (b) voltammograms for TBHQ ($C_{TBHQ} = 10^{-4}$ mol/L) at the presence of different concentrations of DNA, C_{DNA} = 0.00, 0.99×10^{-7}, 1.98×10^{-6}, 2.94×10^{-6} and 3.9×10^{-6} mol/L for curves 1–5 respectively.

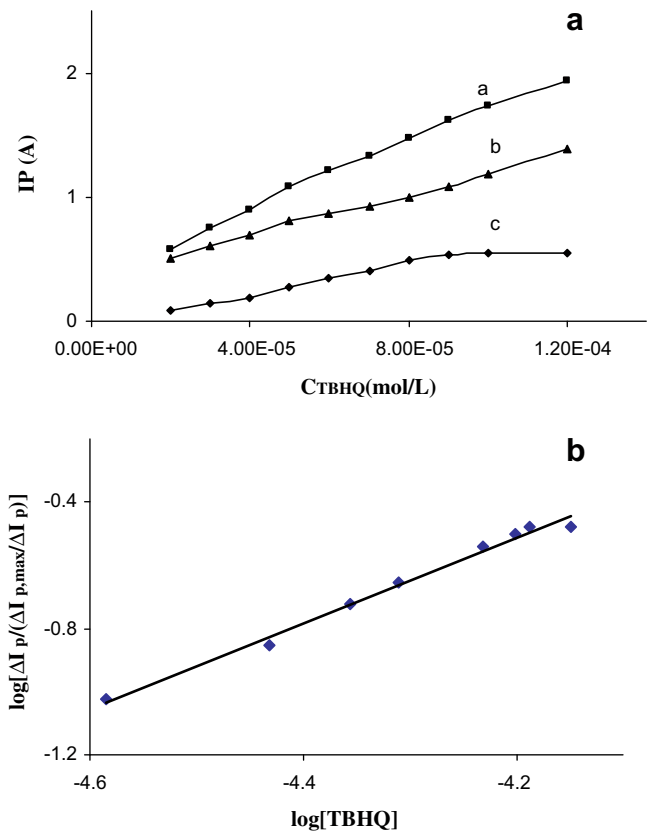

Fig. 5. (a) Relationship between I_p and C_{TBHQ}, [a] $C_{DNA} = 0$; [b] $C_{DNA} = 5 \times 10^{-6}$ M; [c] = a–b. (b) Relationship between $\log[\Delta I_p/(\Delta I_{p,max} - \Delta I_p)]$ and log[TBHQ].

Jiang, 1997) the linear sweep voltammetry was used as a suitable procedure. It is assumed that TBHQ and DNA produce only a single complex DNA–*n*TBHQ. The binding number and the equilibrium constant of the binding reaction can be deduced as follows:

$$\mathrm{DNA} + n\mathrm{TBHQ} \leftrightarrow \mathrm{DNA} - n\mathrm{TBHQ}$$

The binding constant is:

$$\beta = \mathrm{DNA} - n\mathrm{TBHQ}/[\mathrm{TBHQ}]^n\ [\mathrm{DNA}]$$

Because of:

$$[\mathrm{DNA}] = \mathrm{C_{DNA}} - [\mathrm{DNA} - n\mathrm{TBHQ}]$$
$$\Delta I_{p,max} = k\mathrm{C_{DNA}}$$
$$\Delta I_p = K[\mathrm{DNA} - n\mathrm{TBHQ}]$$

Therefore:

$$\Delta I_{p,max} - \Delta I_p = K[\mathrm{DNA}]$$

And the following equations can be deduced:

$$1/\Delta I_p = 1/I_{p,max} + 1/\beta\Delta I_{p,max} \times 1/[\mathrm{TBHQ}]^n$$

or

$$\log[\Delta I_p/\Delta I_{p,max} - \Delta I_p)] = \log\beta + n\log[\mathrm{TBHQ}]$$

Where $\Delta I_{p,\,max}$ represents the maximum difference of the peak currents before and after DNA addition. If DNA and TBHQ form a single complex, the plot of $\log[\Delta I_p/(\Delta I_{p,max} - \Delta I_p)]$ versus log[TBHQ] is linear. In Fig. 5a, curve a shows the relationship between the I_p (peak current) and the concentration of TBHQ. Curve b typically represents I_p changes at C_{DNA} (5×10^{-6} M) on varying amounts of TBHQ concentration. Curve c is the relationship between ΔI_p, which is $I_{pa} - I_{pb}$, and the concentration of TBHQ. When the concentration of TBHQ is over 9.0×10^{-5} mol/L, ΔI_p tends to be a stable value, which is indicative of no free DNA in the solution. Fig. 5b shows the dependence of $\log[\Delta I_p/(\Delta I_{p,max} - \Delta I_p)]$ on log[TBHQ], by showing a fine straight line. From the slope and the intercept of the straight line the values of n and β were calculated to be 1 and 1.07×10^5, respectively. So we can claim that the binding ratio of DNA and TBHQ is 1:1. In Fig. 5a and b, C_{TBHQ} and [TBHQ] represent the added concentration and the equilibrium concentration of TBHQ, respectively. [TBHQ] in Fig. 5b can be obtained from the data of Fig. 5a. We can take any one concentration C_{TBHQ} from the abscissa of Fig. 5a. Then $I_{pa,1}$ and $I_{pb,1}$ corresponding to C_{TBHQ} from curve (a) and curve (b) in Fig. 5a, respectively which can be found. Hence, $\Delta I_{p,1} = \Delta I_{pa,1} - \Delta I_{pb,1}$. A straight line paralleling with the abscissa at $\Delta I_{pb,1}$, on curve b maybe drawn, which intersects curve a at I'_1, hence $I'_{pa,1} = I_{pb,1}$. The concentration C_{TBHQ} corresponding to $I'_{pa,1}$ could be considered to the equilibrium concentration [TBHQ] at C_{TBHQ} (Jiao, Li, Sun, & Wang, 2005).

Also the TBHQ interaction with DNA at the GC electrode was followed by DPV (Fig. 4b). The voltammograms indicate that the peak current of TBHQ sample decreased as DNA was added and again positive E_p shift was observed. This shift is indicative of the intercalation mode of TBHQ binding to DNA, which involves the hydrophobic interactions in the inside of the DNA molecule, as opposed to the interactions which involve the external part of the DNA and ionic sites (Ni et al., 2006; Carter et al., 1989). On this basis, our DPV observations showed that the TBHQ intercalates into the DNA, and this result supports the CV and fluorescence findings.

4. Conclusion

According to the results arise from, UV and fluorescence spectroscopy, viscosity, cyclic voltammetry (CV) and differential pulse

voltammetry (DPV) we conclude that, TBHQ binds to CT-DNA with a high affinity through intercalation mode, which could cause damage in CT-DNA. Our result also concurs with the other data reported by Nagai et al., 1996, indicating the formation of 8-hydroxydeoxyguanosine in calf thymus DNA due to its interaction with TBHQ. Combining our result and that of the other researchers, we could say that it is the time to stop using the TBHQ as a food additive in the food industry.

At the end it should be noted that, the TBHQ concentration which was used in this study (1×10^{-5} M), is much less than that of currently is used as a food additive. So it is worthy to make a thorough analysis on its widespread usage in food industry.

References

Baguley, B. C., & Bret, M. L. (1984). Quenching of DNA-ethidium fluorescence by amsacrine and other antitumor agents: A possible electron-transfer effect. *Biochemistry, 23*, 937–943.

Carter, M. T., Rodriguez, M., & Bard, A. J. (1989). Voltammetric studies of the interaction of metal chelates with DNA. 2. Tris-chelated complexes of cobalt(111) and iron(11) with 1,10-phenanthroline and 2,2′-bipyridine. *Journal of the American Chemical Society, 111*, 8901–8911.

Feng, Q., Li, N. Q., & Jiang, Y. Y. (1997). Electrochemical studies of porphyrin interacting with DNA and determination of DNA. *Analytica Chimica Acta, 344*, 97–104.

Gopala Krishna, A., Vijay Kumar, D., Khan, B. M., Rawal, S. K., & Ganesh, K. N. (1998). Taxol–DNA interactions: Fluorescence and CD studies of DNA groove binding properties of taxol. *Biochimica et Biophysica Acta, 1381*, 104–112.

Ingle, J. D., & Crouch, S. R. (1988). Spectrochemical analysis. Printed in United States of America, pp. 343–344.

Jiao, K., Li, Q. J., Sun, W., & Wang, Z. J. (2005). Voltammetric detection of the DNA interaction with toluidine blue. *Electroanalysis, 11*, 997–1002.

Kashanian, S., Gholivand, M. B., Ahmadi, F., & Ravan, H. (2008). Interaction of diazinon with DNA and the protective role of selenium in DNA damage. *DNA and Cell Biology, 27*, 325–332.

Lakowicz, J. R. (2006). *Principles of fluorescence spectroscopy*. Printed in Singapore, p. 11.

Lepecq, J. B., & Paoletti, C. (1967). A fluorescent complex between ethidium bromide and nucleic acids: Physical—chemical characterization. *Journal of Molecular Biology and Biotechnology, 27*, 87–106.

Liu, G. D., Liao, J. P., Fang, Y. Z., Huang, S. S., Sheng, G. L., & Yu, R. Q. (2002). Interaction of bis(ethylene)tin(bis(salicydene)ethylenediamine) with DNA. *Journal of Analytical Science, 18*, 391–395.

Nagai, F., Okubo, T., Ushiyama, K., Satoh, K., & Kano, I. (1996). Formation of hydroxydeoxyguanosine in calf thymus DNA treated with *tert*-butylhydroquinone, a major metabolite of butylated hydroxyanisole. *Toxicology Letters, 89*, 163–167.

Okubo, T., Nagai, F., Ushiyama, K., & Kano, I. (1997). Contribution of oxygen radicals to DNA cleavage by quinone compounds derived from phenolic antioxidants, *tert*-butylhydroquinone and 2,5-di-*tert*-butylhydroquinone. *Toxicology Letters, 90*, 11–18.

Okubo, T., Yokoyama, Y., Kano, K., & Kano, I. (2003). Cell death induced by the phenolic antioxidant *tert*-butylhydroquinone and its metabolite *tert*-butylquinone in human monocytic leukemia U937 cells. *Food and Chemical Toxicology, 41*, 679–688.

Shi, S., Liu, J., Li, J., Zheng, K. C., Huang, X. M., Tan, C. P., et al. (2006). Synthesis, characterization and DNA-binding of novel chiral complexes delta- and lambda-[Ru(bpy)2L]2+ (L = *o*-mopip and *p*-mopip). *Inorganic Biochemistry, 100*, 385–395.

Sun, W., You, J. Y., Hu, X., & Jiao, K. (2006). A simple linear sweep voltammetric method for the determination of double-stranded DNA with malachite green. *Chemical Papers, 60*(3), 192–197.

Vijayalakshmi, R., Kanthimathi, M., Subramanian, V., & Unni Nair, B. (2000). Interaction of DNA with [Cr(SchiÀ base)(H_2O)$_2$]ClO_4. *Biochimica Biophysica Acta, 1475*, 157–162.

Wu, J., Du, F., Zhang, P., Ahmed Khan, I., Chen, J., & Liang, Y. (2005). Thermodynamics of the interaction of aluminum ions with DNA: Implications for the biological function of aluminum. *Journal of Inorganic Biochemistry, 99*, 1145–1154.

Wu, F. Y., Xie, F. Y., Wu, Y. M., & Hong, J. I. (2008). Interaction of a new fluorescent probe with DNA and its use in determination of DNA. *Journal of Fluorescence, 18*, 175–181.

Cao, Y., & He, X. (1998). Studies of interaction between safranine T and double helix DNA by spectral methods. *Spectrochemical Acta, Part A, 54*, 883–892.

Zhou, C.-Y., Xi, X.-L., & Yang, P. (2007). Studies on DNA binding to metal complexes of Sal2trien. *Biokhimiya, 72*, 45–51.

Ni, Y., Lin, D., & Kokat, S. (2006). Synchronous fluorescence, UV–visible spectrophotometric, and voltammetric studies of the competitive interaction of bis(1,10-phenanthroline)copper(II) complex and neutral red with DNA. *Analytical Biochemistry, 352*, 231–242.

Zhou, Q., & Yang, P. (2006). Crystal structure and DNA-binding studies of a new Cu(II) complex involving benzimidazole. *Inorganica Chimica Acta, 359*, 1200–1206.