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Spectroscopic Study on the Interaction of Three Water-Soluble Porphyrins with Calf Thymus DNA

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ABSTRACT

Porphyrins and their metal derivatives are strong DNA binders with association constant of 10^4 to $10^5 M^{-1}$. Some of these compounds have been used for radiations sensitization therapy of cancer and are targeted to interact with cellular DNA. Binding of porphyrins to DNA changes the spectral and other physico chemical characteristics of porphyrins. The mode of binding can be extracted from inspection of spectral changes of porphyrin. Hence, Uv-Vis spectroscopy is one of the most usual techniques for such investigations. More over, measuring of these spectral changes at various molar ratio of DNA/porphyrin can let to calculate the binding constant and stoichiometry. In the present work , the interaction of three water soluble porphyrins, tetra (p-trimethyle) ammonium phenyl porphyrin iodide (TAPP) as a cationic prophyrin , tetra sodium meso– tetrakis (p– sulphonato phenyl) porphyrin (TSPP) as an anionic porphyrin and manganese tetrakis (p– sulphonato phenyl) porphyinato acetate (MnTSPP) as a metal porphyrin with Calf thymus DNA have been comprehensively studied at 1mM phosphate buffer, pH 7.0 and various temperatures using Uv-Vis absorption spectroscopy. The results represent a bath chromic effect without any shift in maximum wavelengths, which can be related to the external binding mode without stacking. The absorption data at all wavelength regions were analysis at various molar ratios of DNA/ porphyrin for estimation of binding characteristic using SQUAD software. The result confirms the formation of 1:1 complex between DNA and porphyrins. The MnTSSP has the highest affinity respect to TSPP and TAPP that can be represent the formation of axial bond between phosphate group of nucleotide and Mn in the central core of MnTSSP. However, the higher affinity of TAPP as a cationic porphyrin respect to TSPP as an anionic can be related to the role of electrostatic interactions. The results represent that the process is endothermic and essentially entropy driven.

Keywords: DNA; Porphyrin; SQUAD; Thermodynamic parameters; Optical absorption.

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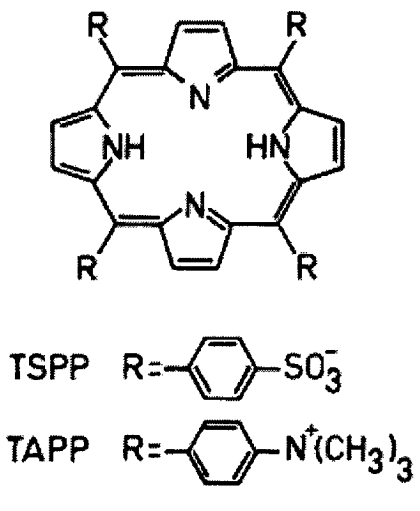
INTRODUCTION

Porphyrins have attracted considerable attention due to their remarkable ability to from complexes with DNA and cleave nucleic acids [1-4]. Since the molecular recognition of DNA is of fundamental importance to life, analyzing the interaction of small molecules with DNA continues to be an important area of research. Potential applications of these system include photodynamic therapy of cancer (PDT) [5-9] molecular biology applications such as DNA foot printing [10], design of telomerase inhibitors [11] stabilizing DNA /RNA hybrids [12], DNA triplexes [13] or quadruplexes [14], specific sensing of DNA quadruplexes [13], etc. Development in this area is predicated upon a detailed understanding of the porphyrin-nucleic acid binding mechanism. One of the most important groups of those reactive molecules is porphyris and their metal derivatives [15]. These compounds are widely used as probes for nucleic acid structure and dynamics and have possible medical applications [16]. The binding strength of porphyrin to DNA is one of the important parameters on its efficacy [17].The thermodynamic parameters of binding can also help as to obtain more insights into the molecular nature of interactions. In order to shed more light on the effect of peripheral groups and metal ions of porphyrin core into DNA binding behavior of metallo porphyrins we have chosen to investigate. These compounds are widely used as probes for nucleic acid structure and dynamics and have possible medical applications. The binding strength of porphyrin to DNA is one of the important parameters on its efficacy.Thethermodynamic parameters of binding can also help as to obtain more insights into the molecular nature of interactions. This paper reports a compnehensive thermodynamic study on interaction of three water – soluble porphyrins tetra(p– trimethyle) ammonium phenyl porphyrin iodide (TAPP) as a cationic porphyrin tetra sodium meso–tatrakis (p-sulphonato phenyl) porphyrin (TSPP) as an anionic porphyrin and manganese tetrakis (p– sulphonato phenyl) porphinato acetate (MnTSPP) as a metal porphyrin (Scheme 1,2) with DNA at various temperatures. The interaction process has been followed using Uv–Vis optical absorption spectroscopy. . The binding constant and stoichiometry were determined by analysis of optical absorption spectra of porphyrin at various DNA concentrations using SQUAD software.

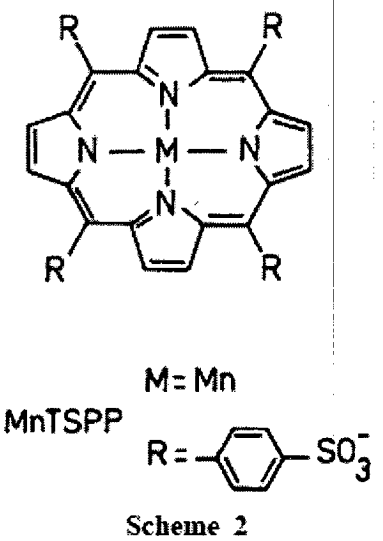
EXPERIMENTAL

DNA from Calf–Thymus was obtained from Sigma – Chemical Co. TSPP and TAPP were prepared by methods described previously [18]. TSPP was metallated according to the literature method [19]. These complexes were characterized by Uv–Vis spectroscopy and elemental analysis [20]. The spectral characteristics of the isolated materials were compared to the literature values and found to be in excellent agreement. All of the chemicals, which have been used for these syntheses, were of analytical grade and purchased form Sigma Chemical Co. All solutions were prepared using double–distilled water. Porphyrin stock solution was made by dissolving the solid porphyrin in buffer solution. Phosphate buffer, 1 mM, pH, 7.0, was used as buffer. Porphyrin stock and working solutions were stored at room temperature in the dark to avoid undesired photochemical reactions.

Uv-Vis measurements were performed on a Cary 100 Spectrophotometer using 1 cm quartz cuvettes. To prepare the DNA stock solution, about 2 mg of DNA was dissolved in 1 ml of the phosphate buffer at 4°C for 48 h.with occasional stirring to ensure the formation of a homogenous solution. The DNA concentrations were determined using molar



Scheme 1



Scheme 2

extinction coefficients of $\varepsilon_{258\ nm}$ = 6700 M-1 cm-1. In all experiments, the porphyrins and DNA – solutions were freshly prepared before spectral analysis and were protected from direct sun lights until they were inserted into the cell compartments. To observe the salt effect on the porphyrin absorption, the titrations were made by addition of aliquots of the NaCl solution into cuvette containing the porphyrin solution of appropriate concentration. The obtained spectra were corrected with respect to dilution effect. The titration of porphyrin solution as a function of DNA concentration was performed at pH 7.0, 1 mM phosphate buffer and at 20, 25, 30, 35, 40 and 45 °C.

RESULTS AND DISCUSSION

In order to identify the solution properties of these porphyrins we employed Uv-Vis spectroscopy. Optical absorption spectrum of MnTSPP shows 4 distinct picks at 563, 466, 400 and 379 nm. The Soret band appears at 466nm. The molar absorption of the Soret band is $1.82 \times 10^5\ M^{-1}\ cm^{-1}$. The Soret band maximum obeys Beer's law over an extended concentration range between 1.0×10^{-5} to 2.0×10^{-4} M, in phosphate buffer 1mM, pH, 7.0.After the upper limit of this concentration range a negative deviation has been observed that corresponds the self association of this porphyrin due to increasing of concentration . In spite of MnTSPP, the spectrum of TSPP just shows a distinct Soret band at 413 nm. The molar absorptive of this band is $1.80 \times 10^5\ M^{-1}, cm^{-1}$. The Soret band maximum obeys Beer's law in a concentration rang between 1.0×10^{-5} M to 1.0×10^{-4}M in 1 mM phosphate buffer, pH. 7.0. The observed positive deviation from linearity that represents the self aggregation was occurred after this range. The spectral feature of TAPP at the same condition shows a Soret band at 412 nm, with a shoulder. The molar absorptivity of Soret band was $3.02 \times 10^5\ M^{-1}\ cm^{-1}$. The Soret band obeys Beer's law in the concentration rang from 1.50×10^{-5} to 1.10×10^{-4} M. A negative deviation was observed after this range. The results in this part represent the higher tendency of TAPP for concentration self-aggregation respect to others. However, the less tendency of MnTSPP may be related to Mn. It seems that metallation of TSPP with Mn inhibited the intermolecular interactions which responsible of self-aggregation.

EFFECT OF SALTS

The effect of NaCl on the absorption spectrum of TAPP, TSPP and Mn TSPP are shown in Figs 1, 2 and 3, respectively. As the concentration of NaCl increases, the absorbance at all of the spectral regions of studied porphyrins has been significantly decreased. The decreasing of the absorbance for MnTSPP spectra is accompanying with red shift and disappearing of Q-bands which represents the formation of well define aggregates. These results also represent the strong electrolyte effect on aggregation state of MnTSPP. The bath chromic shift of spectra with breading of spectral bandwidth which have been observed for TSPP and TAPP can be related to formation of ill-define aggregate in the presence of salt. However, the tendency of TSPP for formation of aggregate is more than TAPP.

INTERACTION OF PORPHYRINS WITH DNA

With respect to our previous discussion, we can conclude that in homogenous aqueous solution at low ionic strength these studied porphyrins exist mainly as monomer. So, we conducted the titration of porphyrin solution at fixed concentration and varying [DNA] at pH, 7.0 and 1mM phosphate buffer as a low ionic strength medium. Figs 4, 5, and 6 show a representative titration spectrum of TAPP, TSPP and MnTSPP upon increasing concentration of DNA, at 25 °C. In all of the spectral regions, the intensity of Soret band decrease as DNA concentration increased. The absorption data were analyzed in order to calculate the binding parameters using SQUAD program. This program is designed to calculate the best values for the stability constants of the proposed equilibrium model by employing a non – linear least square approached. The results represent the formation of 1:1 complex model between studied porphyrins and DNA at all temperatures with sum of squares of reduced error between $10^{-3} - 10^{-4}$. Hence a simple equilibrium between free porphyrin and DNA pair base is existing. The estimated equilibrium constants for the formation of base-pair porphyrin complex at various temperatures are listed in Tables 1, 2 and 3.

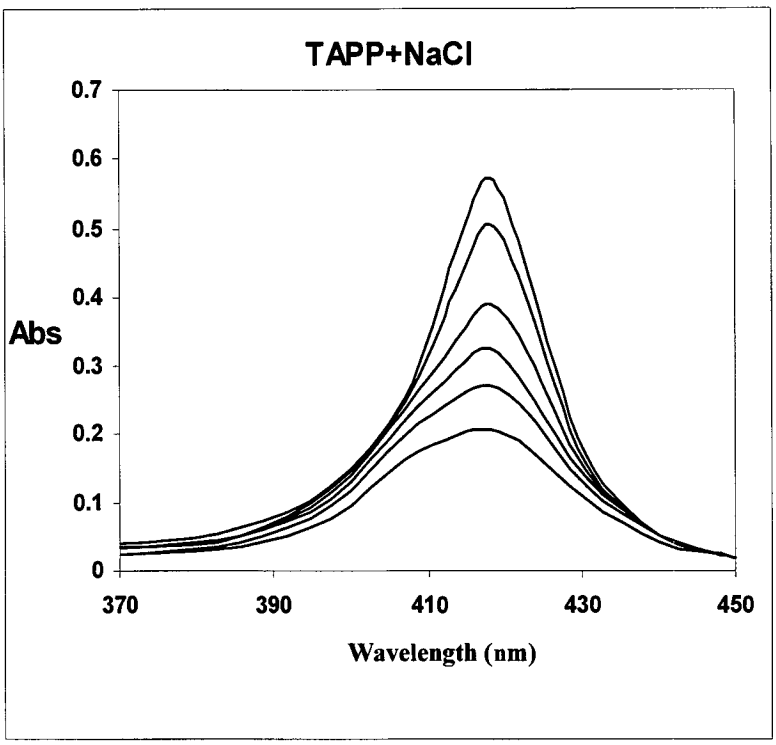


Fig.1. Corrected absorption spectra of **TAPP** upon titration with **NaCl** in phosphate buffer, pH7.0 at 25 °C.

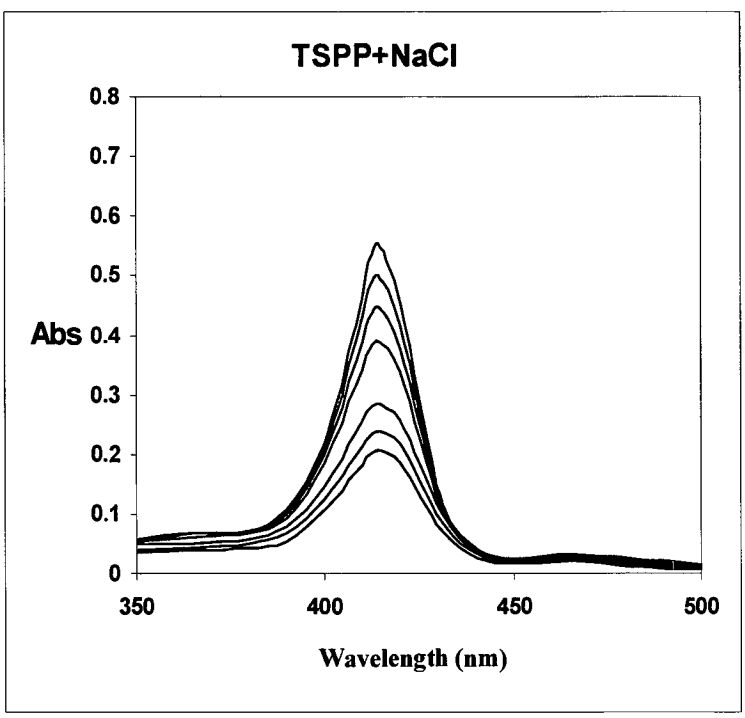


Fig.2. Corrected absorption spectra of **TSPP** upon titration with **NaCl** in phosphate buffer, pH7.0 at 25 °C.

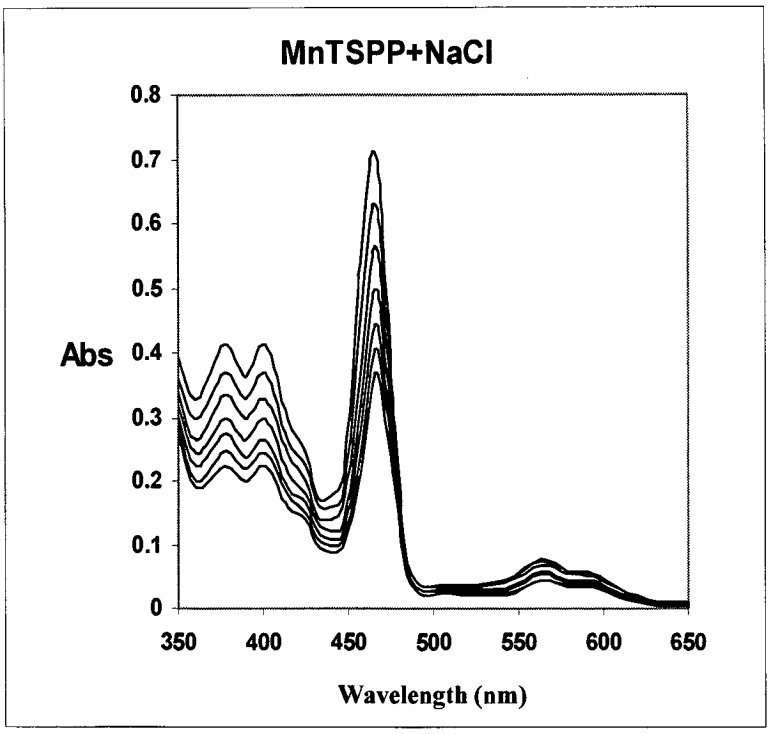


Fig.3. Corrected absorption spectra of **MnTSPP** upon titration with **NaCl** in phosphate buffer, pH7.0 at 25 °C.

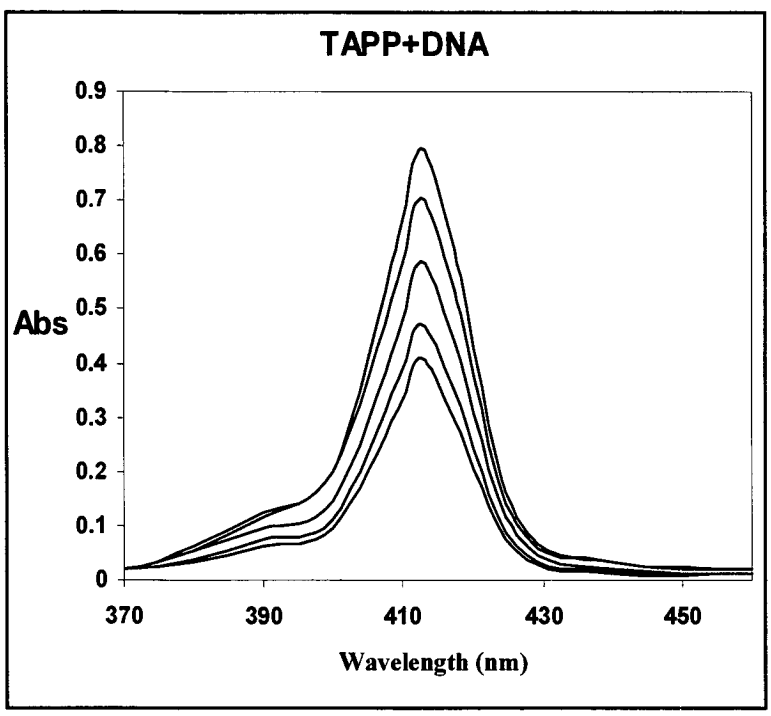


Fig.4. Corrected absorption spectra of **TAPP** upon titration with **DNA** in phosphate buffer, pH7.0 0 at 25 °C.

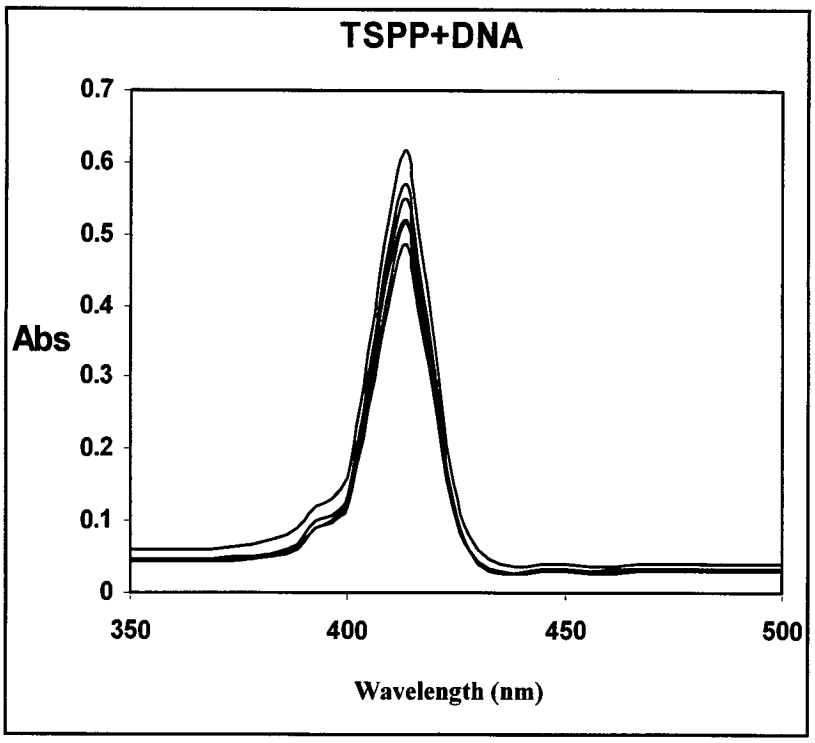


Fig. 5. Corrected absorption spectra of **TSPP** upon titration with **DNA** in phosphate buffer, pH7.0 0 at 25 °C.

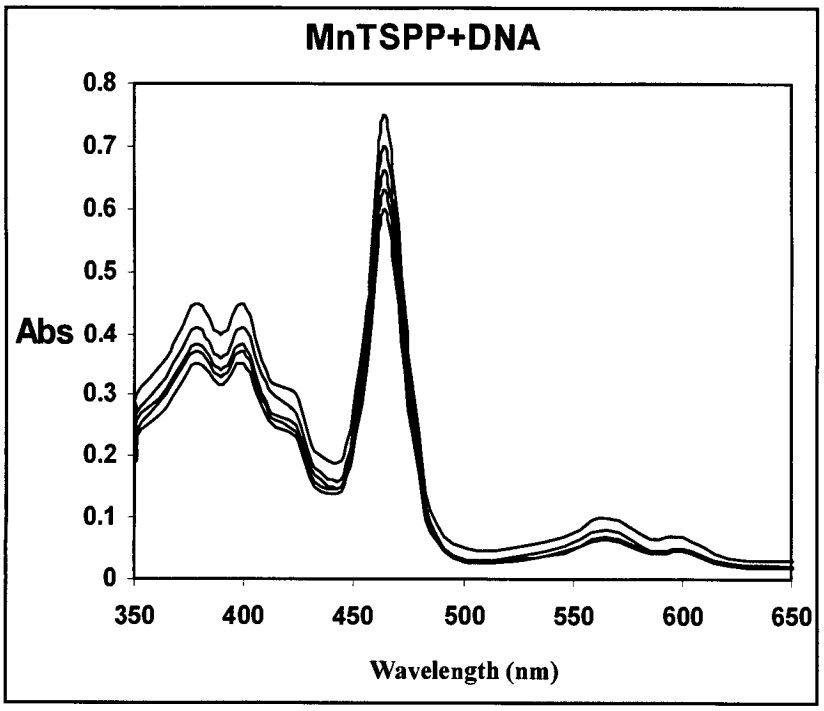


Fig. 6. Corrected absorption spectra of **MnTSPP** upon titration with **DNA** in phosphate buffer, pH7.0 0 at 25 °C.

Table 1. Thermodynamic parameters for binding of **TAPP** to **DNA** in 1mM phosphate buffer, pH7.0 at various temperatures.

$t^{o}c$	$(K \pm \Delta K)\times 10^{-4}$	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} KJmol^{-1}$	$\Delta H^{o} \pm \Delta\Delta H^{o} KJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	5.754± 1.026	-26.717± 0.061	57.777± 0.117	288.228± 0.208
25	7.080± 1.023	-27.691± 0.057	57.777± 0.117	286.661± 0.191
30	10.715± 1.030	-29.196± 0.076	57.777± 0.117	286.898± 0.254
35	16.982± 1.028	-30.858± 0.072	57.777± 0.117	287.636± 0.117
40	24.547± 1.023	-32.318± 0.060	57.777± 0.117	287.706± 0.138
45	33.884± 1.030	-33.688± 0.079	57.777± 0.117	287.490± 0.254

Table 2. Thermodynamic parameters for binding of **TSPP** to **DNA** in 1mM phosphate buffer, pH7.0 at various temperatures.

$t^{o}c$	$(K \pm \Delta K)\times 10^{-4}$	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} KJmol^{-1}$	$\Delta H^{o} \pm \Delta\Delta H^{o} KJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	9.333± 1.023	-27.897± 0.056	45.519± 0.055	250.438± 0.191
25	12.302± 1.031	-29.057± 0.072	45.519± 0.055	250.129± 0.242
30	14.135± 1.033	-30.071± 0.083	45.519± 0.055	249.349± 0.274
35	21.878± 1.026	-31.507± 0.063	45.519± 0.055	249.963± 0.205
40	28.841± 1.028	-32.666± 0.077	45.519± 0.055	249.673± 0.227
45	40.738± 1.035	-34.175± 0.093	45.519± 0.055	250.492± 0.249

Table 3. Thermodynamic parameters for binding of **MnTSPP** to **DNA** in 1mM phosphate buffer, pH7.0 at various temperatures.

$t^{o}c$	$(K \pm \Delta K)\times 10^{-4}$	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} KJmol^{-1}$	$\Delta H^{o} \pm \Delta\Delta H^{o} KJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	1.023± 1.030	-22.508± 0.032	105.551± 0.017	436.836± 0.109
25	1.622± 1.028	-24.035± 0.030	105.551± 0.017	434.634± 0.101
30	3.631± 1.026	-26.469± 0.028	105.551± 0.017	435.494± 0.093
35	7.081± 1.023	-28.619± 0.026	105.551± 0.017	435.405± 0.083
40	14.125± 1.033	-30.878± 0.083	105.551± 0.017	435.667± 0.265
45	28.841± 1.035	-33.259± 0.074	105.551± 0.017	436.304± 0.193

CONCLUSION

The energetic of DNA–porphyrin equilibrium can be conveniently characterized by three thermodynamic parameters , standard Gibbs free energy , ΔG°, can be calculated from the equilibrium constant ,K, of the reaction using the familiar relationship, $\Delta G^{\circ} = -RT\ln K$, in which R and T referring to the gas constant and the absolute temperature, respectively. The van't Hoff equation (1)

$$\frac{d\ln K}{d(\frac{1}{T})} = \frac{-\Delta H^{\circ}}{R} \qquad (1)$$

Gives a linear plot of lnK versus $\frac{1}{T}$, if the heat capacity change for the reaction is essentially zero. The ΔH° can be calculated from the slope of the straight line, $\frac{-\Delta H^{\circ}}{R}$ and the standard entropy from its intercept, $\Delta S^{\circ}/R$ or by equation (2)

$$\Delta S^{\circ} = \frac{\Delta H^{\circ} - \Delta G^{\circ}}{T} \qquad (2)$$

The Van 't Hoff plots for binding of these porphyrins to DNA in the phosphate buffer are shown in Fig (7). All of the thermodynamic parameters with their uncertainly for Interaction of TAPP, TSPP, and MnTSPP were calculated, and reported in Tables 1,2 and 3, respectively. The negative slopes of the lines in van't Hoff plots Fig (7) represent the endothermicity of the reaction. The high correlation coefficient of the lines indicate the little value of heat capacity change of reaction .With respect to the values of ΔG° , the binding affinity of TAPP is more thane TSPP at all studied temperatures. This can be related to the role of electrostatic interactions in the formation of

complex. The strong attractive electrostatic interaction between TAPP as a cationic porphyrin with DNA chains with negative charge density, in comparison to TSPP as an anionic porphyrin, is responsible for this observation. However, MnTSPP has the highest affinity to DNA with respect to other porphyrins. This can be related to the special role of Mn in formation of complex with DNA. Probably, the axial ligation of Mn with phosphate group increases its affinity to DNA. Comparison of enthalpy and entropy values represents the essential role of entropy in reaction driven. In conclusion the formation process in essentially entropy driven and the metal in the central core of porphyrin have an important enhancing role in the interaction with DNA.

ACKNOWLEDGEMENT

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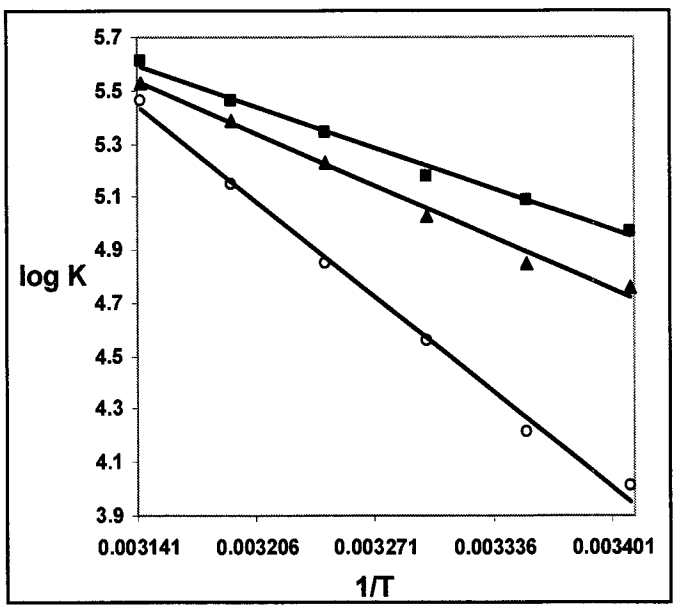

Fig.7. The Van't Hoff plots for binding of **TAPP** (▲), **TSPP** (■) and **MnTSPP** (○) to **DNA** in 1mM phosphate buffer, pH7.00 at various temperatures.

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