Thermodynamic study of interaction between phthalocyanines with calf thymus DNA

M. Aghaie[2], F. Naderi[1, 3], M.Monajjemi[1], H. Aghaie [1,*], N.Safari[4],P.R.Jamaat[4]

1 Science & Research Campus, Islamic Azad University, P.O.Box14515-775, Tehran, Iran
2 Faculty of Chemistry, North Tehran Branch, Islamic Azad University,Tehran,Iran
3 Shahriyar-Shahre Ghods Branch, Islamic Azad University,Iran
4 Chemistry Department, Shahid Beheshti University, Tehran, Iran

ABSTRACT

The experimental determination of formation constant for interaction of two water soluble phthalocyanines, Cobalt (II) 4,4',4'',4'''-tetrasulpho phthalocyanine (CoTsPc) and Manganese(II) 4,4',4'',4'''-tetrasulpho phthalocyanine (MnTsPc) , with calf thymus DNA have been studied by UV-Vis spectrophotometric method at 1mM phosphate buffer , pH 7.4 and at 5 different temperatures 20,25,30,35 and $40^o C$. The changes of Uv-Vis absorption spectra during the titration of these phthalocyanines with calf thymus DNA revealed a large hypochromicity (up to 45%) of the phthalocyanines maximum bands, usually considered as proof of phthalocyanine intercalation in to DNA. The calculated formation constants, K_s , according to the SQUAD software were in the range of 10^4-10^5 M^{-1}.By using the Van't Hoff equation. The values of enthalpy and entropy changes associated to the (CoTsPc +DNA) and (MnTsPc+DNA) were determined .Then by using ΔG^0=-RTlnK, we calculated the values of ΔG^0 at considered temperatures. Our results showed strong electrolytic effects on phthalocyanines behavior and increasing NaCl concentration induced self-aggregation of the CoTsPc and MnTsPc.

Key words: DNA; Phthalocyanine; SQUAD; Formation constant

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* . Corresponding author; Fax: +982144804172.
E – Mail address:hn_aghaie@yahoo.com

INTRODUCTION

The quantitative determination of nucleic acids is of great importance in fundamental research and in clinical diagnosis[1].The quantitative aspects of the interaction of DNA and drug can be studied by the use of a variety of methods which utilize either thermodynamic or kinetic approaches. A variety of methods have been developed which facilitate experimental calculations of formation constants for the interaction between DNA and drug. These include spectrophotometry, DNA footprinting, equilibrium dialysis, thermal denaturation and ultracentrifugation [2, 3, 4, and5]. Generally, it may be study the DNA behavior in solutions phases. DNA in this conditions reacts as a donor or as an acceptor molecule via different sites along its long axis. The different behaviors of DNA depend on the type of DNA [6-9]. DNA is a macro-molecule consisting of repeated stacks of bases formed by either Adenine-Thymine AT (TA) or Guanine-Cytosine GC (CG) pairs coupled via hydrogen bonds and held in double-helix structure by a sugar-phosphate backbone.

Owing to the central role of DNA in replication and transcription, it has a major target for antibiotics and anticancer and the effects of nucleic acid binding drugs are known for various diseases such as cancer, malaria and AIDS [10].

Phthalocyanines are large, planar, and hydrophobic molecules. Since their accidental synthesis in Scotland, by Imperial Chemical Industries, in 1928, phthalocyanines (Pc) have enjoyed considerable industrial importance for use in dyestuffs, paints, colors for metal surfaces, fabrics and plastics. In recent years there has been considerable interest in developing their use in electrochromic devices, organic catalysis, electro catalysis, photo catalysis, photovoltaic devices, lithium batteries, fuel cells, pollution control (especially desulphurization), etc[11,12].

Phthalocyanines (Ptc) represent an interesting family of compounds used now for the photodynamic therapy (PDT) of malignant tumors [13].They can initiate DNA oxidation acting as photosensitizers for the generation of singlet molecular oxygen.

Phthalocyanines have attracted large attention because of their role in the human body, ability to accumulate in many kinds of cancer cells, as well as magnetic and optical properties. These features make them useful in cancer medicine and photodynamic therapy [14].

Some of phthalocyanines are known to dimerize and further agglomerate in aqueous solutions.

A series of water soluble phthalocyanines can be derived from phthalocyanines precursors insoluble in water by introducing ionic group such as $-COO^-$, $-SO_3^-$, $=N-CH_3^+$ or $-N(CH3)_3^-$. Then chemical, spectral, and redox properties of the compounds and their metal complexes will change [15, 16].

The DNA binding mechanism is very dependent upon not only the sequence of the DNA strands but also the structure perturbation of the phthalocyanine molecules [14, 17, 18].

The minor groove of DNA has been discussed as a target for the design of drugs [19].Molecular recognition of DNA is one of the most fundamental processes in nature, and analyzing the interaction of small molecules with DNA continues to be an important area of research. Three main types of noncovalent small molecule – DNA interactions are: intercalation, groove binding and simple electrostatic attraction. In scheme 1, one small molecule binds to DNA by a novel mode of interaction to indicate intercalation with one strand of duplex DNA but not the other [20].

Space-filling representation of a continuous helix as observed in the d(CGATCG) a CuTMPyP4 crystal. The DNA is in blue with phosphorus atoms in gold to highlight the phosphodiester backbone. Porphyrin molecules are in red. Cytidine residues from adjacent stacks in the crystal are in purple. The asymmetric unit contains one strand of DNA and oneCuTMPyP4molecule.

This representation was generated with INSIGHTII (1991).
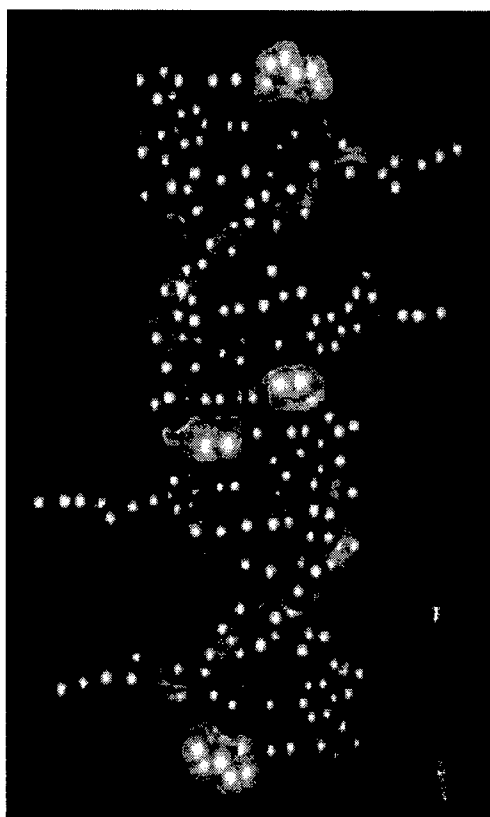
Scheme 1

This work reports a thermodynamic study on interaction of Cobalt (II) 4,4',4",4"'-tetrasulpho phthalocyanine (abbreviated to CoTsPc hereafter) with calf Thymus DNA and Manganese(II) 4,4',4",4"' -tetrasulpho phthalocyanine (abbreviated to MnTsPc hereafter), with the same DNA (Scheme 2).

M:indicates Co or Fe
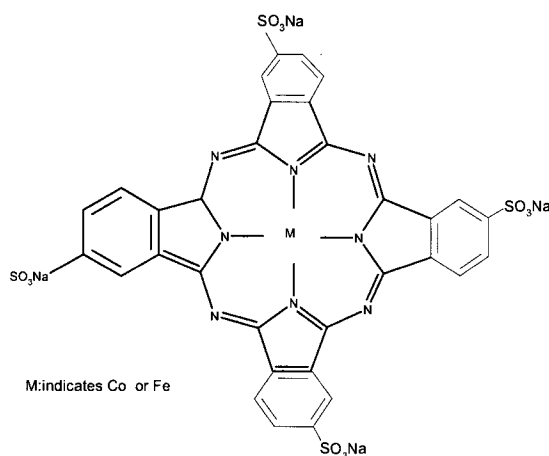
Scheme 2

Materials and Methods

Deoxyribo nucleic acid, DNA from calf – thymus was purchased from Sigma Company. Highly purified phthalocyanines were prepared by Chemistry Department of Shahid Beheshti University of Iran. All considered solutions were prepared using double- distilled water. Phosphate buffer, 1mM, pH, 7.4, was used as buffer. All of the work solutions were made by dissolving the solid compounds in buffer solution. The phthalocyanines solutions were freshly prepared before spectral analysis and their concentration range were between $0.20 \times 10^{-5} - 5.00 \times 10^{-5}$ M. DNA solutions were prepared in a cold room ($4^o C$) by stirring them for 2 days in order to ensure the homogeneity of solutions. The prepared DNA solutions were stored in refrigerator at 4-5°C.

The calf thymus DNA concentration determination was based upon the reported ε_{258} value of 6700 $M^{-1}cm^{-1}$ and Beer-Lambert's equation , A = εbc where A is the absorbance, ε is the molar extinction coefficient, b is the length of cell and c is the concentration. The concentration ranges were between $0.50 \times 10^{-5} - 5.00 \times 10^{-5}$ M.

The titration of considered phthalocyanine solutions as a function of DNA concentration was performed at 20,25,30,35 and $40^o C$. Spectrophotometric measurements were performed on a UV-Vis Shimadzu 2101 PC, using 1.00 cm quartz cuvttes in the spectral range 200-800 nm with thermostat cell compartment that control the temperature around the cell within $\pm 0.1^o C$.

The stoichiometry of complexes and formation constant were determined by analyzing the optical absorption of considered phthalocyanines at various DNA concentrations using SQUAD software.

1 Phthalocyanines

Figure 1 shows that the maximum band obeys Beer's law over concentration range between $0.20 \times 10^{-5} - 5.00 \times 10^{-5}$ M. Figure 2 shows absorption spectra of CoTsPc, and MnTsPc. The band of CoTsPc consists of two components 631.5 and 670.0 nm. The bands of MnTsPc are 637.8 and 722.0 nm. All

phthalocyanine solutions were titrated with NaCl, 5M, in phosphate buffer, pH 7.4, at 25° C. The hypochromisity of 30 –50% can be related to formation of agglomerate in present of salt. The UV-Vis spectra are shown in figures 3, and 4.

2 Interaction with DNA

All phthalocyanine solutions were titrated with a stock solution of calf thymus DNA. It can be assumed that the concentration change due to the adding titrant is negligible because the total volume change during the titration is less than 4%. Maximum bands respect to CoTsPc, were shifted hypochromicity of 15-30%, while in MnTsPc, Maximum bands were shifted hypochromicity of 20-45 %.

The values for hypochromicity suggest that the interactions were via both the intercalative type of binding and external binding. The representative Uv-Vis spectra are shown in figures 5 and 6.

Standard Gibbs free energy change (ΔG^{o}) is calculated according to equation1:

$$\Delta G^{\circ}=-RT\ln K \qquad (1)$$

where K is the association formation constant, T is temperature in Kelvin and R is gas constant. According to the Van't Hoff equation (2):

$$d\ln K/d(1/T)=-\Delta H^{\circ}/R \qquad (2)$$

a linear plot of lnK versus $\dfrac{1}{T}$ is observed, if the heat capacity change for the reaction is essentially zero

$$\ln K=(-\Delta H^{0}/R)(1/T)+constant \qquad (3)$$

The standard entropy change, ΔS°, of the reaction is calculated from equation (4):

$$\Delta S^{\circ}=(\Delta H^{\circ} - \Delta G^{\circ})/T \qquad (4)$$

The formation constants for considered reactions "phthalocyanine+DNA"; at different temperatures and their thermodynamic parameters as ΔG°, ΔH° and ΔS° were calculated (table 1).

3 SQUAD program

In order to analysis the spectral data at various concentration of DNA in titration experiments, the 50 wavelengths were selected. The values of absorbance of these selected wavelengths at various DNA concentrations were analyzed in order to calculate equilibrium formation constants using SQUAD program .Input data were absorbance at 50 different wavelengths of 15 solution spectra. These 15 spectra are corresponding to 15 various concentrations of DNA .The outputs are the logarithm of equilibrium formation constants, $\log K_{ij}$, for the following reaction:

$$iDNA + jPhthalocyanine \rightleftharpoons (DNA)_i (phthalocyanine)_j \qquad (5)$$
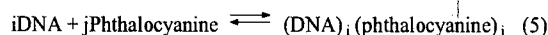
$$K_{ij}= [(DNA)_i (phthalocyanine)_j]/ ([DNA]^i [phthalocyanine]^j) \qquad (6)$$

The estimated formation constants for the formation of 1:1 complexes between (DNA +CoTsPc) and (DNA +MnTsPc) at various temperatures are listed in tabel1.

Conclusion

Adding 5M, NaCl, to the phthalocyanines solutions results a decrease in the absorbance of solutions. Increasing the NaCl concentration induces self -aggregation of phthalocyanines.

Using SQUAD program we analyzed the absorbance data obtained from titration in order to calculate the binding parameters. SQUAD program refine stability constants by employing a non-linear least square approach. The results represent the formation of 1:1 complex model between phthalocyanines and DNA.

In figure 7, a linear plot of lnK versus 1/T for binding of these phthalocyanines to DNA, in 1mM phosphate buffer, pH 7.4, are shown. The negative slopes in the plots represent the endothermicity of reaction and the high correlation coefficients of the lines indicate that the heat capacity of the components is temperature independent.

Acknowledgment

Table 1. Thermodynamic parameters for binding of CoTsPc, MnTsPc to DNA in 1mM phosphate buffer, pH7.4 at various temperatures

phthalocya nines	$\theta °C$	$K \times 10^{-4}$	$\Delta G°$ (kJmol^{-1})	$\Delta H°$(kJmol^{-1})	$\Delta S°$ (Jmol^{-1}K^{-1})
CoTsPc	20	3.1659	-25.2567	53.6577	269.1946
	25	4.5789	-26.6019	53.6577	269.1922
	30	7.0326	-28.1298	53.6577	269.7922
	35	10.6862	-29.6656	53.6577	270.3988
	40	19.0308	-31.6495	53.6577	272.4164
MnTsPc	20	5.0367	-26.3883	65.1343	312.2043
	25	6.8912	-27.6155	65.1343	311.0846
	30	10.0901	-29.1304	65.1343	310.9511
	35	15.3737	-30.5974	65.1343	310.6663
	40	28.6444	-32.7141	65.1343	312.4652
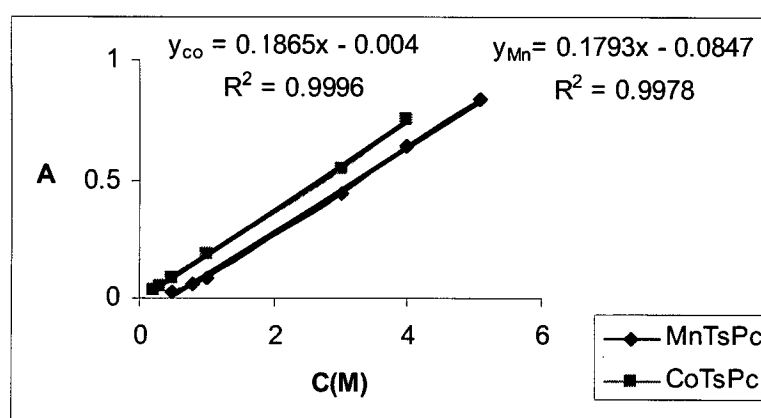
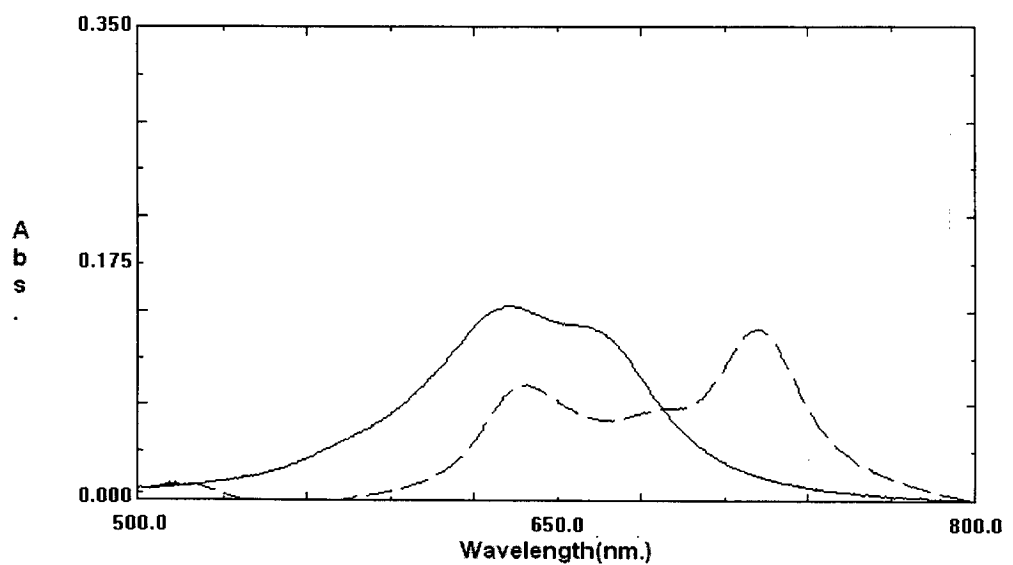**Fig .1.** Absorbance as a function of concentration of CoTsPc and MnTsPc at $25°C$.

Fig.2. Absorption spectra of CoTsPc (solid), MnTsPc (dotted) and their maximum bands.
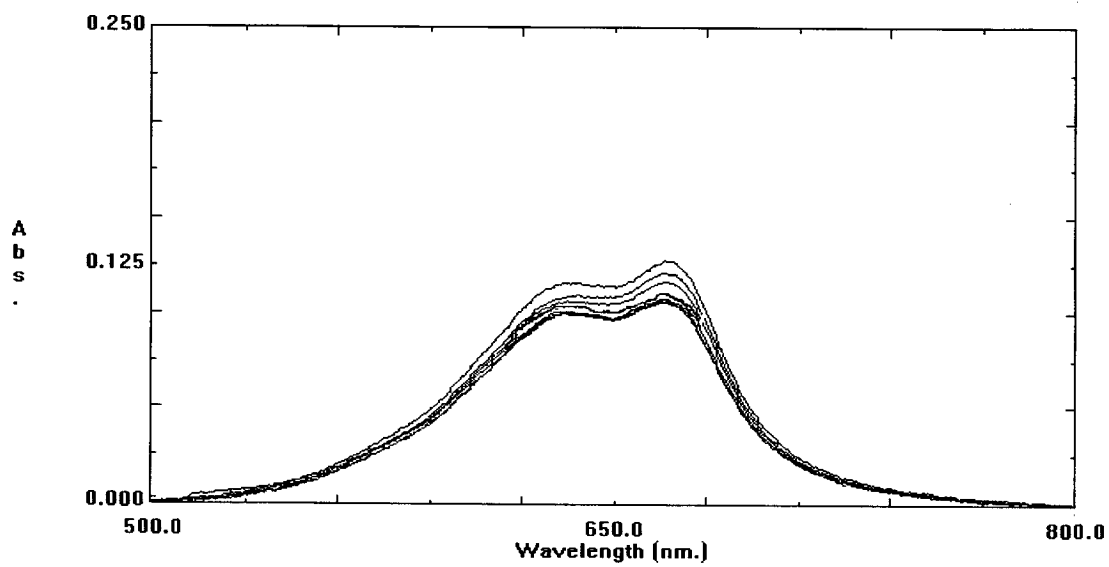

Fig.3. Absorption spectra of CoTsPc upon titration with NaCl in phosphate buffer pH 7.4 at 25^0C.
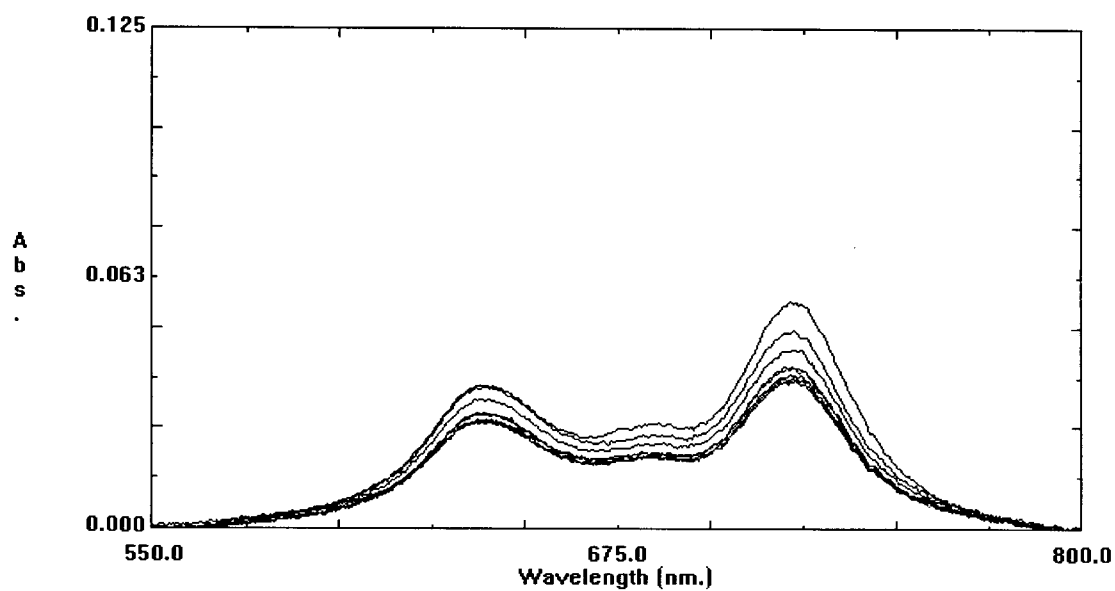
Fig.4. Absorption spectra of MnTsPc upon titration with NaCl in phosphate buffer pH 7.4,   at 25 °C.
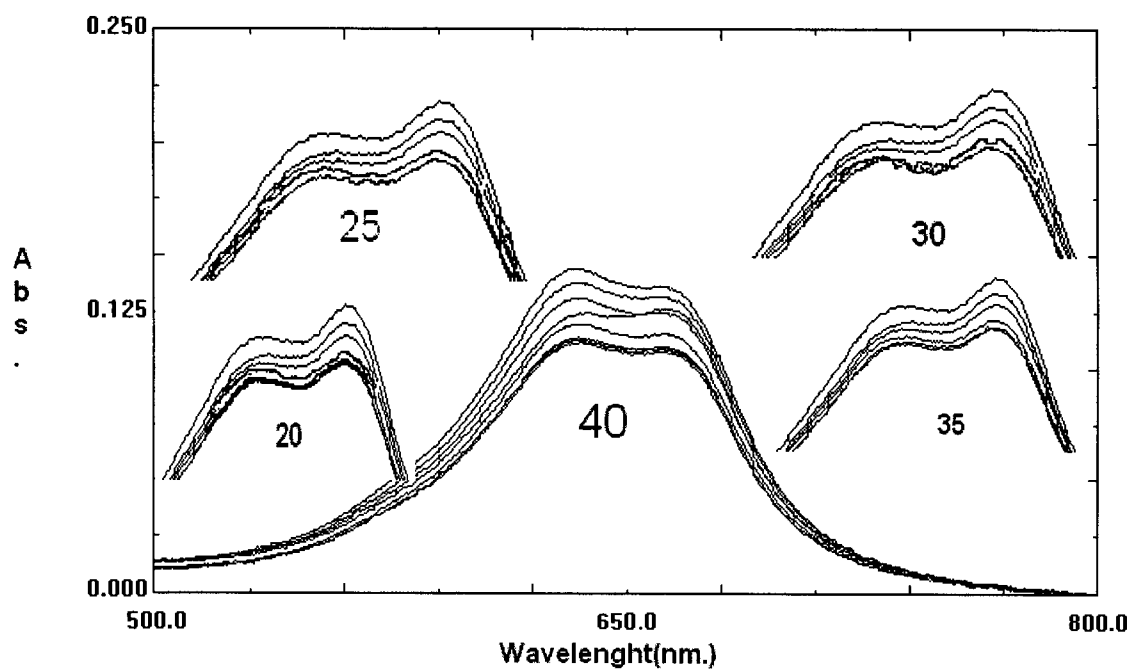

Fig.5. Absorption spectra of CoTsPc upon titration with DNA in phosphate buffer pH 7.4 ,at 20°,25°,30°,35° and $40^o C$.
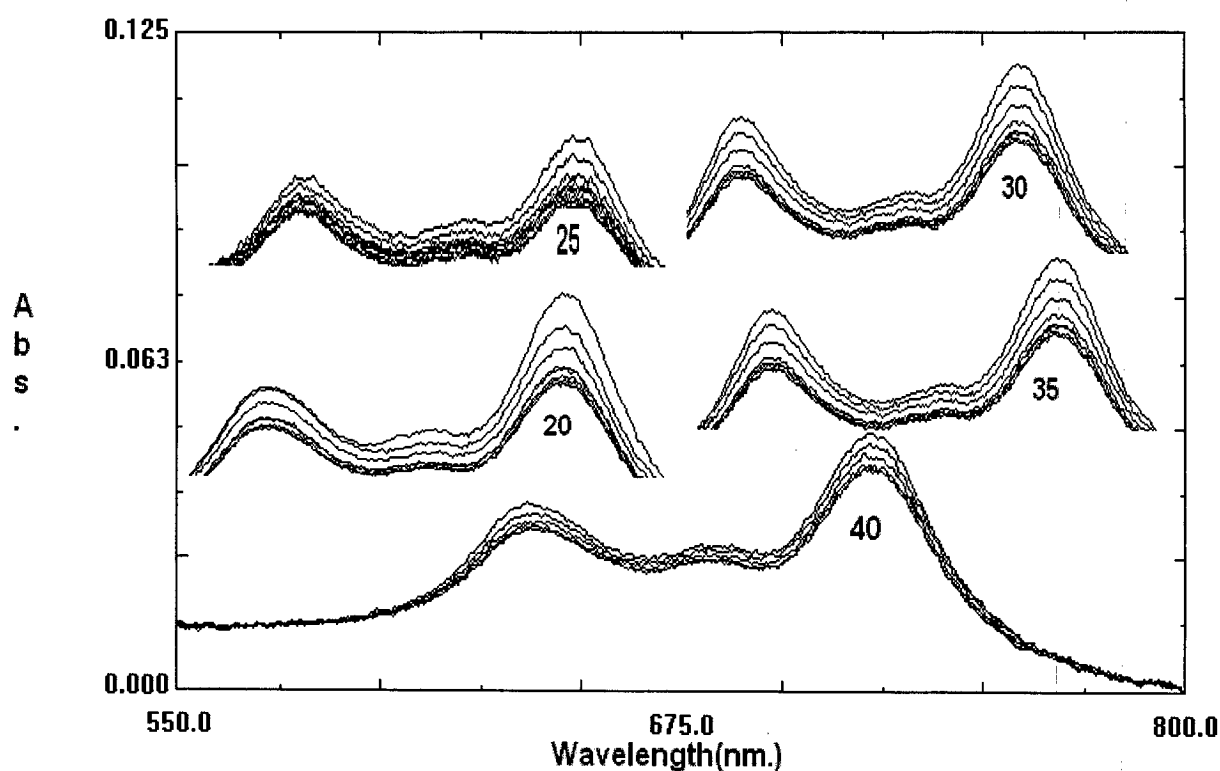
Fig.6. Absorption spectra of MnTsPc upon titration with DNA in phosphate buffer pH 7.4, at 20°, 25°, 30°, 35° and $40^o C$.

$$y_{Co} = -6453.9x + 32.475$$
$$R^2 = 0.9978$$

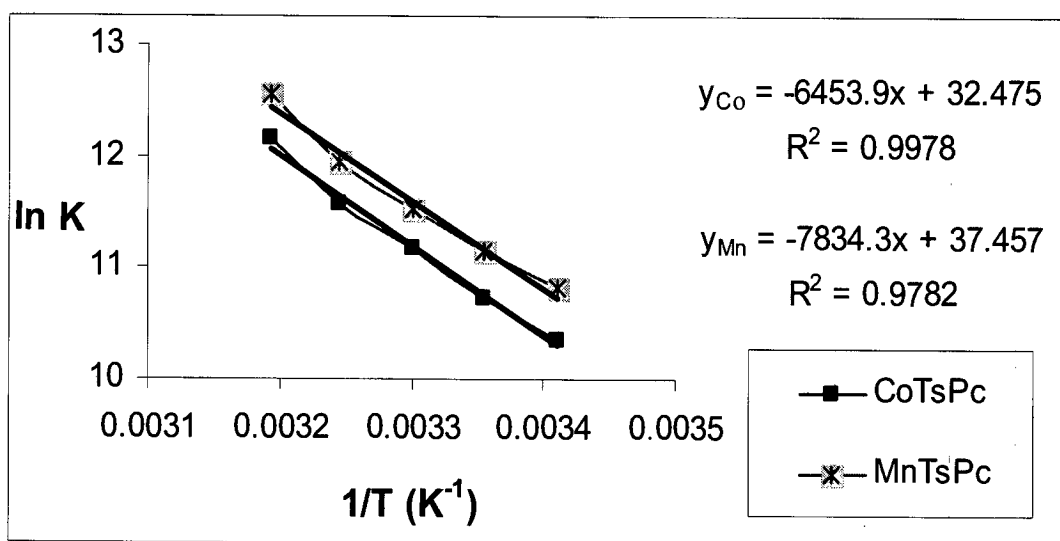$$y_{Mn} = -7834.3x + 37.457$$
$$R^2 = 0.9782$$

—■— CoTsPc

—✕— MnTsPc

Fig.7. A linear plot of lnK versus 1/T for binding of our Phthalocyanines to DNA, in the phosphate buffer, pH 7.4.

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