Tambi Richa et al /J. Pharm. Sci. & Res. Vol. 8(1), 2016, 35-39

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Study of Spectral and Thermodynamical Interaction of Calf Thymus Deoxyribonucleic Acid (Ct-DNA) and an Anticancer Analogue Drug 10-Molybdo 2-Vanado Phosphoric Acid

Tambi Richa and Thirunavukkarasu Sivaraman
School of Chemical and Biotechnology,
SASTRA University, Thanjavur-613401,Tamil Nadu, India.

Abstract

In this study, at first the physicochemical properties of 10-molybdo 2-vanado phosphoric acid are investigated under various environmental conditions, such as ionic strength and concentration. The results represent no aggregation behavior over the concentration range of 2.86×10^{-5} to 5.05×10^{-5} M. Second, the interaction of $[H_5PMo_{10}V_2O_{40}]$ with calf thymus deoxyribonucleic acid (ct-DNA) is considered using UV/Vis spectroscopy. The spectral data were analyzed using Origin 6.1 software. In order to determinate the thermodynamic parameters, the titration of $[H_5PMo_{10}V_2O_{40}]$ with DNA was made at several temperatures. The binding constant and the other thermodynamic parameters for this interaction are calculated. The heterogeneous binding mode for the $[H_5PMo_{10}V_2O_{40}]$ complex to DNA was concluded from such analysis. The spectral investigations do not represent any isobestic point, which is consistent with there being more than one binding mode between $[H_5PMo_{10}V_2O_{40}]$ and DNA. Also the results are consistent with outside and groove binding modes. The results show that the driving force for binding is entropy. This result is in agreement with outside binding mode. Third,the melting curves of DNA was studied. The results of the thermal denaturation curves of DNA represent outside binding mode.

Keyword: ct-(DNA)-Thermal denaturation- UV/Vis spectroscopy-10-molybdo 2-vanado phosphoric acid- Ligand Binding

INTRODUCTION

Deoxyribonucleic acid (DNA) contains all of genetic information required for cellular function hence it plays a role in life process since [1]. During cell division the DNA molecule replicates, so that each new cell receives an exact copy of genetic information. Replication is a process for cell pollution. Under various condition, DNA to be damaged. Some of damage may lead to various pathological changes in living organism. Therefore, explorer in case of the binding of drug with DNA is growing in recent years. Polyoxometalates are negatively charged metal oxygen cluster. These compounds have attracted much attention during last decades because of their extensive application to many fields, such as catalysis, analytical chemistry, medicine and materials science [2-5] . Several polyoxometalates had been reported to inhibit the replication of virus and cancer DNA. In situ, a significant antiviral effect of polyoxometalates against replication of retrovirus ,toga virus ,paramyxo virus [6], herpes simplex [7], rauscher leukemia, polio virus, epstein-barr, rabies [8] and anti tumoral effect against various cancer such as AsPC-1 human pancreatic cancer cells [9],Meth A sarcoma, OAT lung cancer, Co-4 [10], MX-1 murine mammary cancer cell line and MM46 adenocacinoma [11,12] are investigated. In this paper we studied the interaction of $[H_5PMo_{10}V_2O_{40}]$ with DNA by UV/Vis spectroscopy method.

METHODS

Chemicals and Preparations

Preparation of 10-molybdo 2-vanado phosphoric acid , $[H_5PMo_{10}V_2O_{40}]$, was based on a literature procedure [13], potassium dihydrogen phosphate, potassium monohydrogen phosphate were obtained from Merck Chemical Co. and were in analytical grades and used as received. Calf thymus DNA (ct-DNA) was purchased from Sigma Chemical Co. Stock solution of ct-DNA was prepared by dissolution in 5 mM phosphate buffer and 24h stirred overnight , was stored below 4°C in the dark for short periods only. The base-pairs concentration of ct-DNA was determined by its known absorbance measurements using $\varepsilon = 1.32\times10^4$ $L.mol^{-1}.Cm^{-1}$ (i.e. reported in molar base pair) at the absorption maximum of 260 nm [14, 15]. Phosphate buffer solution was used to control the pH of the media (pH 7.0) and measurements were performed on a Metrohm-691 pH-meter. All other reagents were analytical reagent grade and used without further purification. Double distilled water was used throughout the experiments.

Optical Absorption

The absorbance monitoring was performed on a GBC UV/Vis Cintra 101 Spectrophotometer (Victoria, Australia) equipped with thermostat cell compartment and UV-Lite software. The UV/Vis titration experiments were made by addition of the polyoxomolybdate solution into a 1.4 mL cuvette containing the DNA solution of appropriate concentration. The titration experiments were performed at various temperatures with precision of ±0.1 °C.

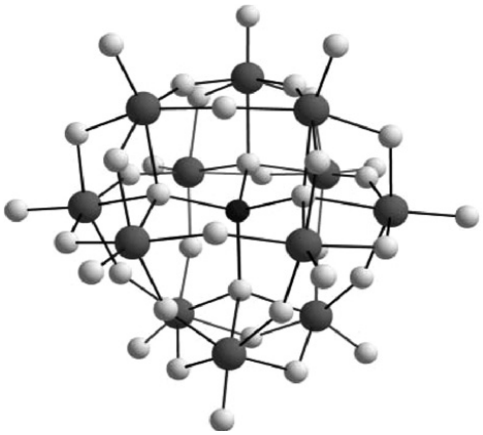
Thermal Denaturation of ct-DNA

The melting curves of both free ct-DNA and POMo–DNA complex in phosphate buffer were obtained by measuring the of ct-DNA absorbance at 260 nm as a function of temperature. Melting temperatures were measured in phosphate buffer solutions pH 7.0 containing 200 µM ct-DNA. The temperature was scanned from 24 to 86ºC.

RESULTS AND DISCUSSION

Solution properties of $[H_5PMo_{10}V_2O_{40}]$

In order to identify the solution properties of $[H_5PMo_{10}V_2O_{40}]$, we employed UV/Vis spectroscopy. Figure 1 show the structure of $[H_5PMo_{10}V_2O_{40}]$. The optical absorption spectrum of $[H_5PMo_{10}V_2O_{40}]$ shows three bands in 209, 223 and 306 nmWe choices 306 nm for our study. Table 1 summarizes the molar absorptivity of this bands in various temperature. In 25°C, the band maximum of $[H_5PMo_{10}V_2O_{40}]$ obeys Beer's law over concentration range between 2.86×10^{-5} to 5.05×10^{-5} M in 5 mM phosphate buffer, pH 7.0. From this observation we can conclude that $[H_5PMo_{10}V_2O_{40}]$ does not show concentration dependent aggregation.



- - heteroatom
- - metal
- – oxygen

Fig. 1. Structure of POMo

Table 1. UV-vis spectral characteristics of $[H_5PMo_{10}V_2O_{40}]$ in aqueous solution

C / ° Temprature	$M^{-1} cm^{-1} / _{306}\varepsilon$	$M^{-1} cm^{-1} / _{223}\varepsilon$	$M^{-1} cm^{-1} / _{209}\varepsilon$
25	16652	54453	65684
30	16712	51219	73384
35	17200	62310	88749
40	18645	65532	75324
45	22253	63811	67511
50	23431	71883	82139

Effect of ionic strength

The effect of NaCl on the UV/Vis spectrum of $[H_5PMo_{10}V_2O_{40}]$ (1×10^{-4}M) in water is shown in Fig.2 and the data concerning these spectral changes are presented in Table 2 that obtained by Origin 6.1 software. As the concentration of NaCl increases, the band width at half height, $W_{1/2}$, increase and the wavelength of maximum absorption, λ_{max} don't show considerable changes. Also, the absorption spectrum of $[H_5PMo_{10}V_2O_{40}]$ shows no significance electrolyte effect, no new band appears even in high concentration of salt. This result means that $[H_5PMo_{10}V_2O_{40}]$ does not form well defined aggregates (i.e. H or J type) even at high concentrations of salt.

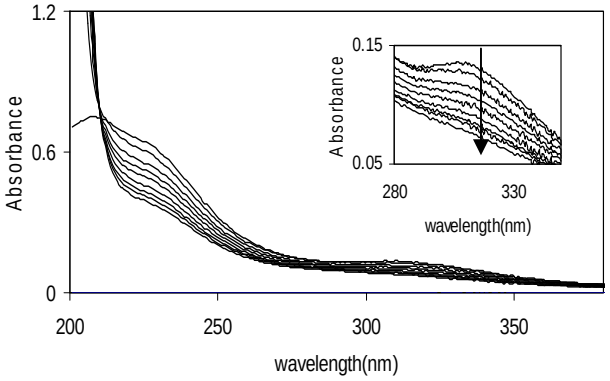


Fig. 2. Absorption spectra of $[H_5PMo_{10}V_2O_{40}]$ solution (1×10^{-4}) upon addition of NaCl solution (0.45, 0.83, 1.15, 1.42, 1.66, 1.87 and 2.34 M) in 5 mM phosphate buffer, pH 7and at 25 °C.

Table 2. UV-vis spectral characteristics of of $[H_5PMo_{10}V_2O_{40}]$ solution (1×10^{-5}) upon increasing the NaCl concentration

[NaCl] / M	A_{max}	λ_{max}(nm)	$W_{1/2}$(nm)
0.45	0.20	304.22	94.62
0.83	0.17	303.46	95.50
1.15	0.16	299.94	98.48
1.42	0.16	298.47	98.80
1.66	0.14	294.25	102.86
1.87	0.12	293.64	105.20
2.34	0.11	292.72	108.35

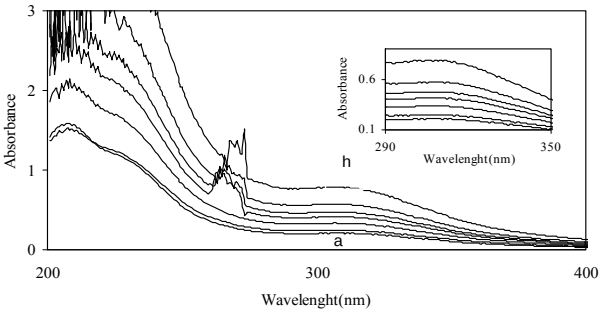


Fig. 3. The titration absorption spectra of $[H_5PMo_{10}V_2O_{40}]$-DNA complex with various mole ratios of [POMo]/[DNA] in 5mM phosphate buffer, pH 7 in 25°C . Mole ratios: 0, 1.46, 2.18, 2.91, 3.64, 5.38 and 11.64 respectively.

Binding of $[H_5PMo_{10}V_2O_{40}]$ to ct-DNA

Optical absorption

The interaction of $[H_5PMo_{10}V_2O_{40}]$ with calf thymus DNA was studied by UV/Vis technique. The experiments carried out at six temperatures as 25, 30, 35, 40, 45 and 50 °C. Because both DNA and POMo have same absorbance wavelength , we have to use differential absorbance titration method. The titration was performed in a fixed concentration of DNA and varying concentration of POMo in 5 mM phosphate buffer pH 7.0. Figure 3 shows a typical titration spectra of DNA upon increasing addition of POMo

at 25 °C. We analyzed the UV/Vis data by Origin 6.0 software and found the binding of POMo to DNA produces hyperchroism, to increase the band weidth at half height, $W_{1/2}$, without change in wavelength of maximum absorption. These effects are particularly pronounced for outside binders.

Thermodynamic investigation of binding

The binding constant at any specified temperature was determined by following equation [16]:

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \quad \text{eq (1)}$$

Where ε_a is the appearance molar absorption coefficient, ε_f is molar absorption of free POMo and K_b is binding constant. The ratio of slope to intercept of linear plot of $\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)}$ vs. $[DNA]$ is K_b. We calculated K_b for binding at various temperatures. The thermodynamic parameters such as standard Gibbs free energy change, ΔGº, and standard molar enthalpy change, ΔH º, and standard molar entropy change, ΔSºcan determine by the alues at various temperatures. The standard Gibbs free energy change is usually calculated from the equilibrium constant (K) of the reaction, by the following relationship:

$$\Delta G^o = -RT \ln K_b \quad (2)$$

Since the activity coefficients of the reactions are not known, the usual procedure is to assume them unity and to use the equilibrium concentrations instead of the activity. Therefore, it would be appropriate to adjust the terminology of apparent equilibrium constant K′, and Gibbs free energy ΔGº′. Apparent standard enthalpies per mole of cooperative unit can be obtained from the dependence on temperature of the apparent binding constant K′, by van't Hoff equation:

$$\partial \ln K' = -(\Delta H^{o\prime}/R)\, \partial(1/T) \quad (3)$$

This is the so-called van't Hoff enthalpy. The apparent standard entropy change, ΔSº′, can be derived from the Eq. (4)

$$\Delta S^{o\prime} = (\Delta H^{o\prime} - \Delta G^{o\prime})/T \quad (4)$$

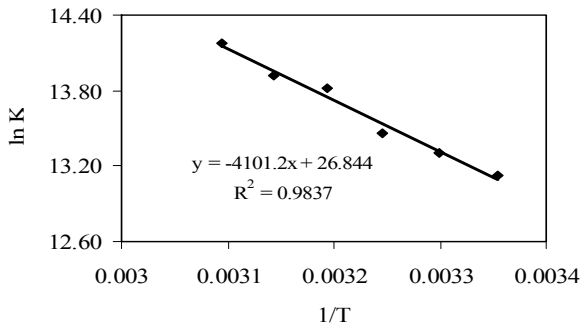


Fig. 4. The van't Hoff plot $[H_5PMo_{10}V_2O_{40}]$ binding to DNA

The van't Hoff plot for interaction of POMo complex with ct-DNA is shown in Fig.4. The calculated thermodynamic parameters for binding of POMo to ct-DNA are listed in Tables 3. It can be seen that the ct-DNA-binding process was endothermic for $[H_5PMo_{10}V_2O_{40}]$ and has the large positive entropy value.

Table 3. Thermodynamic parameters and affinity constants for binding of $[H_5PMo_{10}V_2O_{40}]$ to ct-DNA at 5mM phosphate buffer, pH 7.0 and various temperatures

T (K)	ln K	ΔG_b^0 (KJ/mol)	ΔH_b^0 (KJ/mol)	ΔS_b^0 (J/mol.K)
298.15	13.12	-32.52 ± 2.26	34.10 ± 2.27	223.46 ± 0.04×10^{-2}
303.15	13.30	-33.53 ± 2.26	34.10 ± 2.27	223.09 ± 0.04×10^{-2}
308.15	13.46	-34.48 ± 2.26	34.10 ± 2.27	222.54 ± 0.04×10^{-2}
313.15	13.82	-35.97 ± 2.26	34.10 ± 2.27	223.74 ± 0.04×10^{-2}
318.15	13.92	-36.82 ± 2.26	34.10 ± 2.27	222.91 ± 0.04×10^{-2}
323.15	14.17	-38.08 ± 2.26	34.10 ± 2.27	223.34 ± 0.04×10^{-2}

Analyzing of binding data

For ligand binding to macromolecule we writhe following equilibrium:

$$P + L \Leftrightarrow P - L$$

Where P is macromolecule and L is ligand. For difference titration

$$A^{\lambda} = A^{\lambda}_{poly,f} + A^{\lambda}_{poly,b} \quad (5)$$

Where A^{λ} is total absorption, $A^{\lambda}_{poly,f}$ and $A^{\lambda}_{poly,b}$ are absorption for free and binding ligand respectively. We can writhe

$$A^{\lambda} = \varepsilon_f [poly]_f + \varepsilon_b [poly]_b \quad (6)$$

Where f and b indices indicate free and binding ligand. For saturated complex we have:

$$A^{\lambda} = \varepsilon_{f,sat} [poly]_{f,sat} + \varepsilon_{b,sat} \times n[DNA]_{total} \quad (7)$$

In this time, the concentration of free ligand is equal with:

$$[poly]_{f,sat} = [poly]_{total} - [poly]_b = [poly]_{total} - n[DNA]_{total} \quad (8)$$

From (7) and (8) we obtained:

$$A^{\lambda}_{sat} = \varepsilon_{f,sat}([poly]_{total} - n[DNA]) + \varepsilon_{b,sat} n[DNA]_{total} \quad (9)$$

And in finally

$$A^{\lambda} = \varepsilon_{f,sat}[poly]_{total} + (\varepsilon_{b,sat} - \varepsilon_{f,sat}) n[DNA]_{total} \quad (10)$$

From $\frac{A^{306}}{[DNA]_{total}}$ versus $\frac{[poly]_{total}}{[DNA]_{total}}$ we obtained $\varepsilon_{f,sat}$. In here, n is the number of ligand that binding to macromolecule. From $\varepsilon_{apparence}$ we calculated n and by these parameters, we evaluated K_b for various temperatures and listed in Table 4. Also, we shown scattchard and binding isotherm diagrams in 25 °C in Figure 5 a, b. These curves reperesent the binding process has one step and positive cooperartivity.

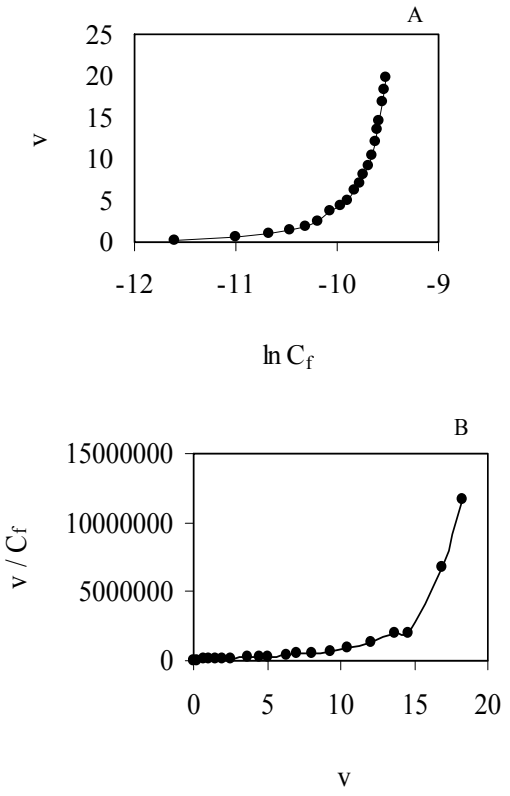


Fig. 5. The (A) binding isotherm and (B) scattchard diagrams in 25°C

Table 4. Thermodynamic parameters and affinity constants for binding of $[H_5PMo_{10}V_2O_{40}]$ to ct-DNA calculated with equation (10)

t (°C)	n	K	K_b
25	19.8	139777.9	500000
30	20	643347.4	600000
35	24	1389978	700000
40	25	1766596	1000000
45	26	1224762	1111111
50	28	-	1428571

Thermal Denaturation oF ct-DNA

We have characterized the stability of DNA as a function of POM by UV-Vis. Melting temperatures were measured in phosphate buffer solutions pH 7.0 containing 200 µM ct-DNA. In the wavelength of 260 nm (of DNA) the temperature was scanned from 24 to 86ºC at the speed of 0.4°C/Min. The melting temperature (T_m) was taken as the midpoint of the hyperchromic transition. The melting temperature (T_m) of ct-DNA is sensitive to its double helix stability and the binding of compounds to ct-DNA alters the T_m values depending on the strength of interactions[17]. Therefore, it can be used as an indicator of binding properties of polyoxomolybdate to ct-DNA and their binding strength. The obtained results of such studies for $[H_5PMo_{10}V_2]$–DNA complex are shown in Fig.6. Very small changes of T_m value confirmed outside binding. We studied T_m value in presence of different concentration of POMo. The obtained results of such studies for $[H_5PMo_{10}V_2]$–DNA complex are summarized in Table 5.

Table 5. Melting pont of DNA temperature changes upon increasing the mole ratios of $\frac{[H_5PMo_{10}V_2O_{40}]}{[DNA]}$

$\frac{[H_5PMo_{10}V_2O_{40}]}{[DNA]}$	0	0.024	0.047	0.088
$T_m(K)$	338.72	338.2	342.8	338.2

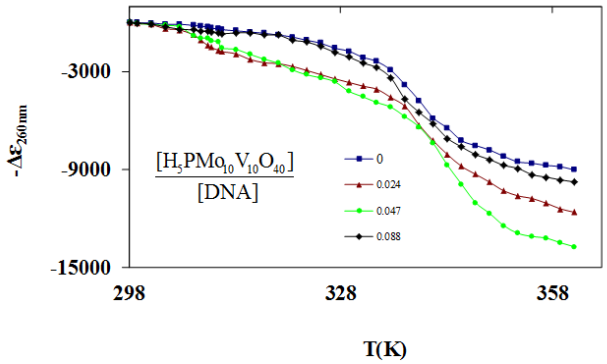


Fig.6. Melting profiles (λ_{max} = 260 nm) for the free ct-DNA in the absence of ligand and for the different mole ratios of ct-DNA and $[H_5PMo_{10}V_2O_{40}]$

Determination of thermodynamic parameter

Values of ΔH_m were obtained from melting curves data and following equation:

$$-\Delta\varepsilon_{260}(T)=\frac{(a_N+b_NT)+(a_D+b_DT)\exp[\frac{\Delta H_m}{R}(\frac{1}{T}-\frac{1}{T_m})]}{1+\exp[\frac{\Delta H_m}{R}(\frac{1}{T}-\frac{1}{T_m})]} \quad (11)$$

Where $-\Delta\varepsilon_{260}(T)$ is negative changes of difference molar absorption coefficient between any temperature and 25 °C, (a_N+b_NT) is the thermal dependence of $-\Delta\varepsilon_{N,260}$ (for native DNA in 260 nm), (a_D+b_DT) is thermal dependence of $-\Delta\varepsilon_{D,260}$ (for denaturated DNA in 260 nm), T_m is melting point of DNA and ΔH_m is change in molar enthalpy of denaturation. Table 6 show these data for different mol ratio of $\frac{[POMo]}{[DNA]}$. we obtained these values by fitting data in equation (11) by Sigma Plot software.

ΔC_p (the change in excess heat capacity upon DNA denaturation) for DNA was estimated from ΔH_m versus T_m. The value of ΔC_p is $24.433\ \text{kJ.mol}^{-1}.K^{-1}$.

Table 6. the curve fitting parameter for deaturation of ct-DNA

$\frac{[H_5PMo_{10}V_2O_{40}]}{[ct-DNA]}$	0	0.024	0.047	0.088
$(a_N \times 10^4)$ $M^{-1}.cm^{-1}$	1.39±1.02	3.87±1.12	4.04±1.31	1.28±1.31
$(b_N \times 10^1)$ $M^{-1}.cm^{-1}.K^{-1}$	-4.62±3.30	-12.95±3.62	-13.48±4.19	-4.32±4.24
$(a_D \times 10^4)$ $M^{-1}.cm^{-1}$	1.74±3.84	3.83±2.82	3.84±6.61	1.40±3.16
$(b_D \times 10^1)$ $cm^{-1}.K^{-1}$	-7.36±1.09	-14.35±7.95	-14.53±1.87	-6.59±1.07
T_m (K)	338.72±0.01	338.19±0.01	342.79±0.01	338.19±0.01
ΔH_m (kJ/mol)	314.90±1.69	378.00±2.79	402.20±3.68	277.50±1.53

Magnitude of this value represent that the unnatural process is cooperative and hydrophobic. With T_m, ΔH_m and ΔC_p by using equation (12) we estimated $\Delta G_{m,D}$ for denaturation:

$$\Delta G = \Delta H_m(1-\frac{T}{T_m}) - \Delta C_p[(T_m - T) + T\ln\frac{T}{T_m}] \quad (12)$$

Figure 7 show $\Delta G_{m,D}$ versus $T(K)$. According to this Figure, all of curves are coincide and have a maximum that represent maximum of stability.

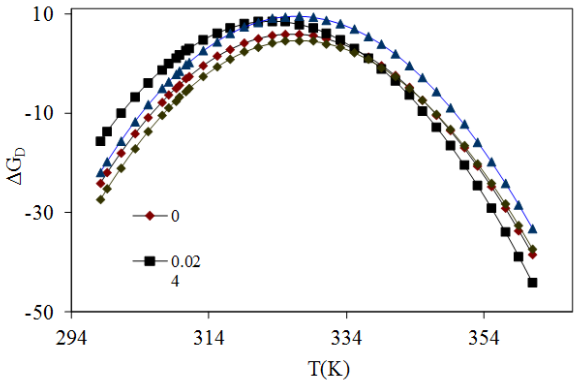


Fig.7. DNA stability curves at phosphate buffer 5 mM, pH=7 as a function of $[H_5PMo_{10}V_2O_{40}]$ concentrations.

Denaturation is always endothermic and increase disorder. For values of T that ΔS is zero, ΔG is maximum. Table 7 show values of T_S for different mol ratio of $\frac{[POMo]}{[DNA]}$.

Table 7. T_s value for DNA in addition of POMo

$\frac{[H_5PMo_{10}V_2O_{40}]}{[DNA]}$	0	0.024	0.047	0.088
$T_S(K)$	323.15	325.15	327.15	327.15

CONCLUSION

$[H_5PMo_{10}V_2O_{40}]$ does not show concentration dependent aggregation over an extended concentration range "2.86×10^{-5} to 5.05×10^{-5} M" in 5 mM phosphate buffer, pH 7.0. Addition of NaCl shows no significance electrolyte effect and no new band appears even in high concentration of salt. This result suggests that $[H_5PMo_{10}V_2O_{40}]$ does not form well defined aggregates (i.e. H or J type) even in high concentrations of salt. $[H_5PMo_{10}V_2O_{40}]$ binds to external region of ct-DNA. The ct-DNA-binding process was endothermic for $[H_5PMo_{10}V_2O_{40}]$ and has a large positive entropy value. The small change of melting temperature (Tm) of ct-DNA upon addition of POMo represents the existence of outside binding mode.

ACKNOWLEDGMENTS

We are grateful to Payame Noor and Yazd universities.

REFERENCES:

[1] Dahm, R.: Friedrich Miescher and the discovery of DNA. Dev. Biol., 278, 274 (2005)
[2] Wang, Y; Mendoza, S.; Icaifer, A. E.: Inorg. Chem., 37, 317 (1998)
[3] Yamase, T. :Photo- and Electrochromism of Polyoxometalates and Related Materials. Chem. Rev. 98, 307(1998)
[4] Rhule, J. T.; Hill, L. C.; Judd, D. A.: Polyoxometalate in medicin. Chem. Rev., 98, 327 (1998)
[5] Sadakane, M.; Steckhan, E. :Electrochemical Properties of Polyoxometalates as Electrocatalysts. Chem. Rev. 98, 219(1998)
[6] Raynaud, N.; Jasmin, C.; Raynaud, M.: Study of antivirus activity of mineral condensed polyanions. II. Effect on vesicular stomatitis virus Rev. Eur. Etud. 17, 295(1972)
[7] Sarafianos, S. G.; Kortz, U.; Pope, M. T.; Modak, M. J.: Mechanism of polyoxometalate-mediated inactivation of DNA polymerases: an analysis with HIV-1 reverse transcriptase indicates specificity for the DNA-binding cleft. Biochem. J. 319, 619 (1996)
[8] Rhule, J. T.; Hill, L. C.; Judd, D. A. :Chem. Rev. 98, 327 (1998)
[9] Ogata, A.; Mitsui, S.; Yanagie, H.; Kasano, H.; Hisa, T.; Yamase, T.; Eriguchi, M. :A novel anti-tumor agent, polyoxomolybdate induces apoptotic cell death in AsPC-1 human pancreatic cancer cells. Biomed. Pharmacother. 59, 240 (2005)
[10] Yamase, T.: Polymeric Materials Encyclopedia 1, 365 (1996)
[11] Yamase, T. Mol. Eng. 1993, 3, 241.
[12] Yamase, T.: Medical chemistry of polyoxometalates. Part 1. Potent antitumor activity of polyoxomolybdates on animal transplantable tumors and human cancer xenograft. Inorg. Chim. Acta. 151, 15(1988)
[13] Tsigdinos, G. A.; Hallada, C. J. : Molybdovanadophosphoric acids and their salts. I. Investigation of methods of preparation and characterization. Inorg. Chem. 7, 437(1968)
[14] Pasternack R.F.; Bustamante C.B.; Collings P.J.; Giannetto A.; Gibbs E.J. : Porphyrin assemblies on DNA as studied by a resonance light-scattering technique. J. Am. Chem. Soc. 115, 5393 (1993)
[15] Pasternack R.F.; Collings J.P.: Resonance light scattering: a new technique for studying chromophore aggregation. Science 269, 935(1995)
[16] Pyle, A. M.; Rehman, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K.: Mixed-ligand complexes of ruthenium(II): factors governing binding to DNA. J. Am. Chem. Soc., 111, 3051(1989)
[17] Fiel R.J.; Howard, C.; Mark, E. H.; Dattagupta, N.: Interaction of DNA with a porphyrin ligand: evidence for intercalation. Nucleic Acids Res. 6, 3093(1979)