World Academy of Science, Engineering and Technology
International Journal of Chemical and Molecular Engineering
Vol:9, No:3, 2015

Comparative DNA Binding of Iron and Manganese Complexes by Spectroscopic and ITC Techniques and Antibacterial Activity

Maryam Nejat Dehkordi, Per Lincoln, Hassan Momtaz

***Abstract*—**Interaction of Schiff base complexes of Iron and Manganese: Iron [N, N' Bis (5- (triphenyl phosphonium methyl) salicylidene) -1, 2 ethanediamine) chloride, [Fe Salen]Cl; Manganese [N, N' Bis (5- (triphenyl phosphonium methyl) salicylidene) -1, 2 ethanediamine) acetate, were investigated by spectroscopic and isothermal titration calorimetry techniques (ITC).

The absorbance spectra of complexes have shown hyper and hypochromism in the presence of DNA that is indication of interaction of complexes with DNA. The linear dichroism (LD) measurements confirmed the bending of DNA in the presence of complexes.

Furthermore, Isothermal titration calorimetry experiments approved that complexes bound to DNA on the base of both electrostatic and hydrophobic interactions. More, ITC profile exhibits the existence of two binding phases for the complexes. Antibacterial activity of ligand and complexes were tested in vitro to evaluate their activity against the gram positive and negative bacteria.

***Keywords*—**Schiff base complexes, Linear dichroism (LD), Isothermal titration calorimetry (ITC).

I. Introduction

SCHIFF bases are a significant class of chemical compounds due to the presence of azomethine group on them. Schiff bases have several biological applications such as antibacterial, antifungal, antimicrobial, herbicidal, clinical and analytical activities [1]-[4].

They are prepared by condensation reaction of an aldehyde with imines. Schiff base ligands are converted to the complexes by transition metal ions. By converting the ligand to the complex cytotoxicity effect of both metal ions and Schiff base ligands decrease and the biological activity of the complex increases. Schiff base complexes have been widely studied due to the numerous applications. Chelating ligands containing N, S and O donor atoms show broad biological activity and they are of special interest due to the variety of ways in which they are bound to metal ions. It is known that the existence of metal ions bound to biologically active compounds may enhance their activities [5]-[11]. There has been useful progress in designing and synthesis of new anticancer agents. To obtain more selective agents, exhaustive knowledge of DNA-binding mechanism is required [12]. In previous research, the interaction of a number of iron and manganese derivatives with nucleic acids has been widely investigated [13]-[15]. In continuing the previous research on these compounds, we have focused on the interaction of two bulky Salen-type complexes "Iron [N, N' Bis (5- (triphenyl phosphonium methyl) salicylidene) -1, 2 ethanediamine) chloride, [Fe Salen]Cl and Manganese [N, N' Bis (5- (triphenyl phosphonium methyl) salicylidene) -1, 2 ethanediamine) acetate" with DNA by fluorescence, Linear Dichroism and ITC techniques. It was interesting to us that compare the interaction of two water soluble complexes and estimate their binding modes and the strength of the interaction. Also, we have carried out the antibacterial activity on the ligand and two complexes.

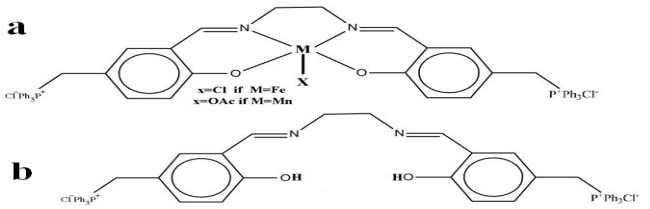

Fig. 1 Iron and manganese complexes (a) and Ligand (b)

II. Experimental

A. Materials and Methods

The substituted phosphonium bulky Salen type ligand and their complexes were synthesized with using standard procedure as stated in our previous work [16]-[18].

Double stranded calf thymus DNA (sodium salt, highly polymerized type I) was purchased from Sigma and dissolved in buffer and filtered with a 0.8 μM Millipore filter before using. The DNA concentration was determined using ε_{260nm}=6600 $M^{-1}cm^{-1}$. The ratio of A_{260}/A_{280} for solution of ct-DNA in buffer was 1.8-1.9 that represents the free protein DNA sample [19], [20]. Other regents were purchased commercially and used without further purification. All experiments were carried out in a 10 mM Tris-HCl aqueous buffer at pH=7.2 and 5 mM NaCl.

Fluorescence measurements were performed on a Varian Cary Eclipse spectrofluorimeter. Emision spectra were recorded at an excitation wavelength of 515 nm and the emission spectra were recorded from 525 nm to 800 nm. Both

Maryam Nejat Dehkordi is with the Department of Chemistry, College of Basic Science, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran (e-mail: mnejatdehkordi@gmail.com).

Per Lincoln is with the Department of Chemical and Biological Engineering, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden (e-mail: Lincoln@chalmers.se).

Hassan Momtaz is with the Department of Microbiology, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran (e-mail: hamomtaz@yahoo.com).

excitation and emission slits were set as 5 nm.

Linear dichroism (LD) was measured using a spectrometer (Applied Photophysics, UK). The wavelength interval was 200 - 600 nm. For LD measurements, DNA solution was 100 µM. The spectrum was measured in a cylindrical flow cell containing 2 mL of DNA solution.

Isothermal titration calorimetry (ITC) measurements were carried out at 25.0 °C using a Microcal (Microcal, Inc., Northampton, MA). The sample cell was loaded with 300 µL of 100 µM of DNA solution and Titration was carried out using a 40 µL syringe filled with 1400 µM of the complex solution and, with stirring at 1000 rpm.

Antibacterial activities

The ligand and their Salen complexes of Iron and Manganese have been tested in vitro to assess their growth inhibitory activity against *Escherichia* coli and *Staphylococcus* bacteria by well diffusion method [21]. The complexes and ligand were dissolved in the buffer Tris and soaked in a filter paper disc of 5 mm diameter and 1mm thickness. The discs were placed on the previously seeded plates and incubated at 37 0C for 24h. Nalidixic acid (NA), Ampicilin (Am), Erythromycin (E) and Vancomycin (V) were used as standard drugs with different concentrations. Also, the buffer was tested against the stated bacteria in the same condition and it was found that the buffer did not have any antibacterial effects.

III. Result and Discussion

A. Fluorescence Study

The Mn & Fe Salen complexes did not show any fluorescence, neither free in aqueous buffer nor in the presence of DNA, making them suitable for a competitive binding study with the fluorescent intercalator EB. To investigate the binding of iron and manganese complexes to DNA by intercalation, an emission quenching experiment has been carried out. The intense decrease of EB-DNA emission due to the addition of complexes was observed (see the inset of Figs. 2 and 3). This phenomenon could be from the competition of the complexes with EB for the same binding sites on DNA resulting release of EB from DNA. To investigate the binding of iron and manganese complexes to DNA by intercalation, an emission quenching experiment has been carried out. The intense decrease of EB-DNA emission due to the addition of complexes was observed (see the inset of Figs. 2 and 3). To determine quantitatively the affinity of the iron and manganese complexes to DNA, the binding constant for them were calculated. In this regard, the experimental data of free and bound EB, in each titration were fitted to McGhee and Von Hipple conditional probability model of excluded site binding according to (1) [22], [23];

$$\theta_{EB}/C_f = K_{EB}(1-s\theta_{EB})[(1-s\theta_{EB})/(1-(s-1)\theta_{EB})]s-1 \quad (1)$$

where KEB is the apparent binding constant of EB and s is the apparent binding site size. The θEB is the ratio of bound EB to the number of DNA base pairs. The values of 5×10^6 μM^{-1} and 4×10^5 M^{-1} were obtained for the iron & manganese complexes respectively that shows the strong affinity of complexes to DNA.

From comparing the inset of fluorescence intensity figures, it found that manganese Salen complex has a sharp decrease in the intensity of DNA-EB and at 10 µM concentration of complex the change on the curve are approximately constant. While for iron complex, the decrease on the intensity has a soft slope relative to manganese complex. It looks like that iron complex interacts with DNA by different mode rather than intercalating mode and consequently extrudes the EB from its binding sites.

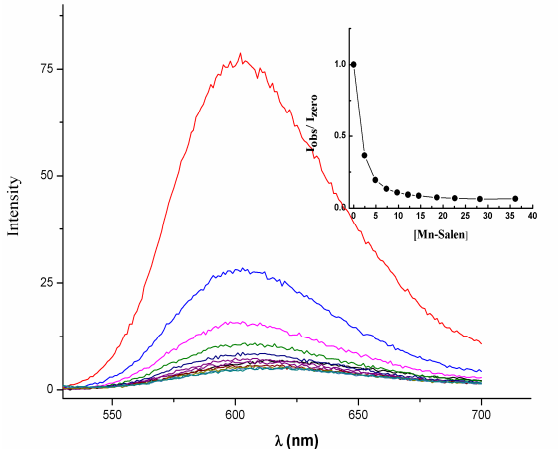

Fig. 2 The emission spectra of DNA-EB (37 µM and EB= 5 µM), λex= 525 nm, λmax-em=600 nm, in the presence of 0–37 µM Mn Salen complex. Intensity decreases by addition complex. Inset: the plot of the relative fluorescence intensity at 600 nm vs total concentration of complex

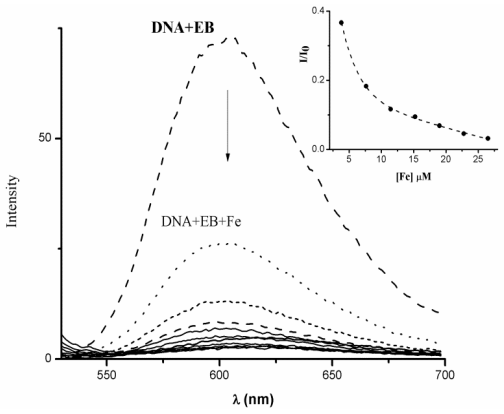

Fig. 3 The emission spectra of DNA-EB (45 µM and EB= 5 µM), λ_{ex}= 525 nm, λ_{max-em}=600 nm, in the presence of Fe Salen complex. Intensity decreases by addition complex. Inset: the plot of the relative fluorescence intensity at 600 nm vs total concentration of complex

B. Isothermal Titration Calorimetry Studies

Isothermal titration calorimetry has become an important tool in direct and reliable measuring of the thermodynamic parameters of the interaction of small molecules with biopolymers [24]. The representative raw ITC profile results from the titration of iron & manganese complex into DNA

solution. Each of the heat burst curves corresponds to a single iron & manganese complexes injection. The areas under these heat burst curves were determined by integration to yield the associated injection heats. These injection heats were corrected by subtracting the corresponding dilution heats derived from the injection of identical amounts of iron & manganese into the buffer alone.

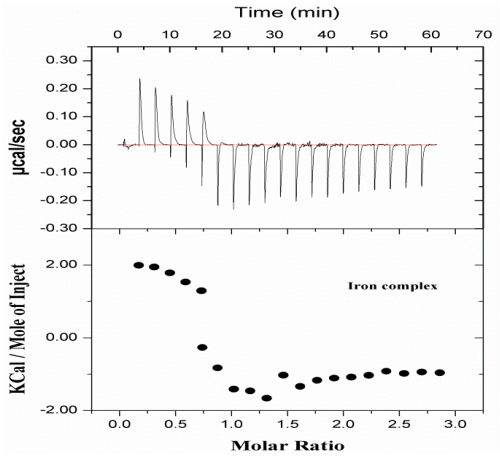

Fig. 4 Calorimetric data (raw) for the titration of DNA 100 µM with iron and Manganese complexes at 25 ^{0}C (top). Binding isotherm (heat change vs [Fe-complex]/[DNA] molar ratio) was obtained from the integration of raw data (bottom)

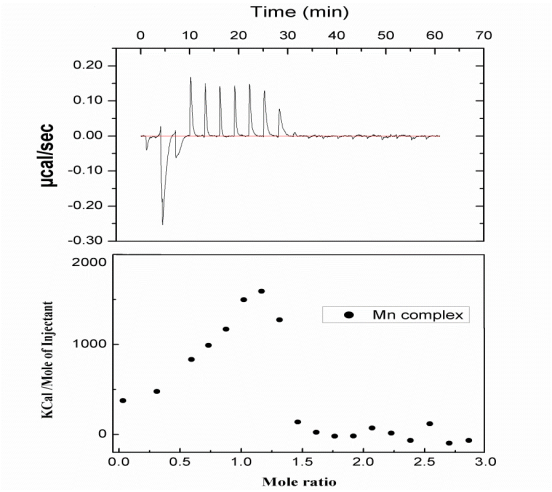

Fig. 5 Calorimetric data (raw) for the titration of DNA 100 µM with iron and Manganese complexes at 25 ^{0}C (top). Binding isotherm (heat change vs [Mn-complex]/[DNA] molar ratio) was obtained from the integration of raw data (bottom)

ITC profile for the binding of the iron & manganese complexes to DNA are shown in Figs. 4 & 5. First, the titration curve displayed that the binding of manganese complex to DNA was endothermic with positive peaks in the plot of power versus time, while iron complex was endothermic at the beginning of titration and after that was exothermic. Therefore, it had the positive and negative peaks in the plots of power versus time. The first binding is overwhelmingly entropy driven, while the second binding is enthalpy driven. It seems that, in the first step entropy term arises from liberation of structured water from interacting surfaces and releasing of counter ions upon binding of positively charged complex to the backbone of DNA helix [25], [26]. On the other hand, in the second step, the process must be enthalpy driven with a decrease in enthalpy due to the effect of more hydrophobic interactions. It is worthy to note that the two binding phases is seen from the ITC plots for two complexes.

With respect to the calorimetric data, experimental data could not be fitted to known binding models. Thus, estimating the thermodynamic parameterers will not be correct. But the binding enthalpy is endothermic, indicating entropy driven process, in agreement with the EB assay results.

C. Linear Dichroism Measurements

Linear dichroism (LD) is defined as the difference in absorbance between light polarized parallel ($A_\parallel$) and perpendicular ($A_\perp$) to a macroscopic orientation axis:

$$LD\ (\lambda) = A_\parallel\ (\lambda) - A_\perp\ (\lambda)$$

To detect an LD signal, a sample with oriented molecules is needed. Large molecules like DNA can be oriented by a flow gradient (flow-LD in a couette cell). DNA in the B form shows the negative LD-signal at 260 nm, due to the base π–π^* transitions. These transition moments will be aligned perpendicular to the orientation axis. The magnitude of this LD signal depends on the degree to which DNA is oriented and reduced by effects such as DNA binding.

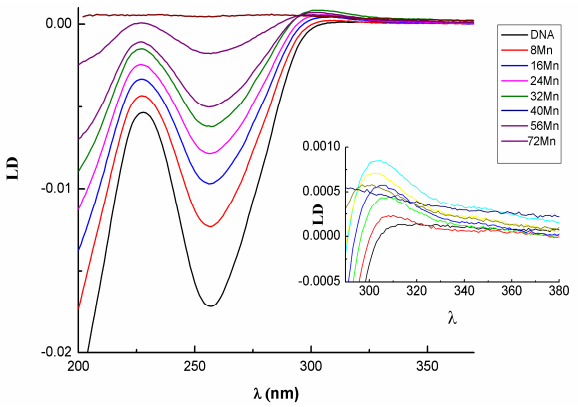

Fig. 6 LD spectra of ct-DNA in the absence (_____) and presence of Mn Salen complex in different concentration

Thus, the LD spectra of Iron & manganese complexes in the presence of DNA were measured as shown in Figs. 6 and 7. The LD signal of DNA arising the bases is significantly reduced by the addition of complexes. The LD signal of DNA in the presence of different concentration of iron & manganese complexes decrease and it disappears in high concentration of complexes. This could be due to either an increase in DNA flexibility or shortening of the DNA by bending, kinking, compaction or aggregation as observed for platinum complexes with DNA before [27].

More, the other reason for decreasing the LD signal of DNA is coordination of Fe^{+3} and Mn^{3+} with donor atom on the base pairs of DNA as explained in our previous works.

Also, wounding or aggregation of DNA may occur in the presence of complexes at high concentration.

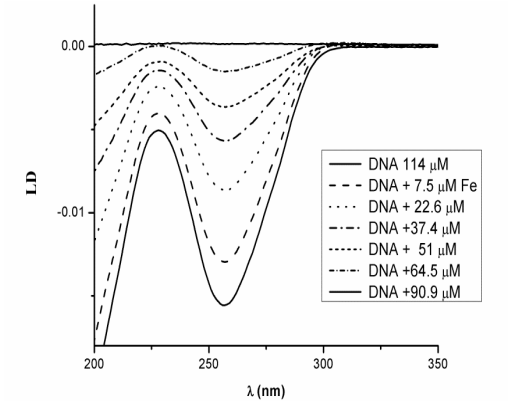

Fig. 7 LD spectra of ct-DNA in the absence (_____) and presence of Fe Salen complex (-------)

D.Antibacterial Screening

The ligand and their complexes (at minimum concentration 2mM) were tested for their in vitro antibacterial activity. They were tested against the bacteria *E. coli, S. aureus*, by diffusion method [21]. The test solutions were prepared in Tris-HCl buffer. The results of the antibacterial activities are summarized in Table I. From the results, it has been observed that complexes have higher antibacterial activity than the ligand against the same microorganism under identical experimental conditions. The antibacterial activity of the complex was increasing with increasing the concentration of complexes. The antibacterial effect of complex was comparable with the standard drugs for gram positive bacteria while, ligand was found to be inactive against *E. coli* even with increasing the concentration of ligand. Morover, the manganese complex was more active than the ligand and iron complex.

Such an enhancement in activity of the complex may be due to the increasing of lipophilic character and efficient diffusion of the metal complex into bacterial cell upon the coordination of ligand to metal on the basis of chelation theory [28].

TABLE I
ANTIBACTERIAL ACTIVITY DATA OF LIGAND AND COMPLEXES,

Microorganism	*S. aureus* (+)	*E. Coli* (-)
NA	8	21
Am	12	22
E	-	7
V	3	9
Ligand	11	-
Fe Salen complex	14	8
Mn Salen complex	16	10
Buffer	-	-

NA (Nalidixic Acid), AM (Ampicilin), V (Vancomycin).

IV. CONCLUSION

This work reports the binding properties of iron and manganese complexes with DNA by spectroscopic and ITC techniques. The binding constants of iron and manganese complexes have been determined by the fluorescence data that indicates strong binding affinity of the complexes to DNA. The decrease on the LD signal of DNA shows that the complexes can bend or aggregate the DNA.

The ITC results confirm two binding phases for the complexes. The binding process for iron complex, is endothermic in the first step (entropy driven) and exothermic in the second step (enthalpy driven). While, for manganese complex whole process of binding is endothermic containing two step of binding.

Therefore, the set of results demonstrated that Iron and manganese complex interact with DNA via electrostatic interaction at the beginning and the surface interactions between the complexes and DNA lead to the coordination of Fe^{3+} and Mn^{3+} with the donor atom on the base pairs of DNA. This behavior was observed before for anticancer drug such as Platinum complexes.

It should be emphasized that the bulky and hydrophobic groups on the ligand have increased the interaction of complexes with DNA and the effect of these groups is straightforward in binding process.

ACKNOWLEDGMENT

The financial support of Research Councils of Islamic Azad University of Shahrekord and Chalmers University are gratefully acknowledged.

REFERENCES

[1] A.A. Jarrahpour, M. Motamedifar, K. Pakshir, N. Hadi, M. Zarei, Synthesis of Novel Azo Schiff Bases and Their Antibacterial and Antifungal Activities", *Molecules*, vol. 9, 2004, pp. 815-824.

[2] T. Gupta, S. Dhar, M. Nethaji, A.R. Chakravarty, "Bis (dipyridophenazine) copper(II) complex as major groove directing synthetic hydrolase", Dalton Trans., 2004, pp. 1896–1900.

[3] B.M. Zeglis, V.C. Pierre, J.K. Barton, "Metallo-intercalators and metallo-insertors", *Chem. Comm*., 2007, pp. 4565–4579.

[4] K.E. Erkkila, D.T. Odom, J.K. Barton, "Recognition and Reaction of Metallointercalators with DNA", Chem. Rev., vol. 99, 1999, pp. 2777–2796.

[5] K. Singh, M.S. Barwa, P. Tyagi," Synthesis and characterization of cobalt(II), nickel(II), copper(II) and zinc(II) complexes with Schiff base derived from 4-amino-3-mercapto-6-methyl-5-oxo1,2,4-triazine", *Eur. J. Med. Chem.*, vol. 42, 2007, pp. 394-402.

[6] P.G. Cozzi," Metal–Salen Schiff base complexes in catalysis: practical aspects", *Chem. Soc. Rev.*, vol. 33, 2004, pp. 410-421.

[7] S. Chandra, J. Sangeetika, "EPR and electronic spectral studies on copper(III) complexes of some NO donor ligands", J. Indian Chem. Soc., vol. 81, 2004, pp. 203-206.

[8] E. Canpolat, M. Kaya, "Studies on mononuclear chelates derived from substituted Schiff-base ligands (part 2): synthesis and characterization of a new 5-bromosalicyliden-p-aminoacetophenone oxime and its complexes with Co(II), Ni(II),Cu(II) and Zn(II)", *J. Coord. Chem*., vol. 57, 2004, pp. 1217-1223.

[9] M. Yildiz, B. Dulger, S.Y. Koyuncu, B.M. Yapici, "Synthesis and antimicrobial activity of bis(imido) Schiff bases derived from thiosemicarbazide with some 2-hydroxyaldehydes and metal complexes", *J. Indian Chem. Soc.*, vol. 81, 2004, pp. 7-12.

[10] G. w. Yang, X. P. xia, H. Tu, C. X. Zhao, *Chinese J. App. Chem*., vol. 12, 1995, pp. 13.

[11] G. w. Yang, X. P. xia, H. Tu, C. X. Zhao, *Chem. Res. App*., vol.7, 1995, pp. 41.
[12] A. Alonso, M.J. Almendral, Y. Curto, J.J. Criado, E. Rodriguez, J.L. Manzano, "Determination of the DNA-binding characteristics of ethidium bromide, proflavine, and cisplatin by flow injection analysis: usefulness in studies on antitumor drugs Anal. Biochem., vol. 355, 2006, pp. 157–164.
[13] A. Trotta, A. Barbieri Paulsen, A. Silvestri, G. Ruisi, M.A. Girasolo, R. Barbieri, *J. Inorg. Biochem*., vol. 88, 2002, pp. 14–18.
[14] A. Silvestri, G. Ruisi, M.A. Girasolo," The dynamics of 57Fe nuclei in FeII–DNA and [FeII(1-methyl-2-mercaptoimidazole)2]–DNA condensates ", *J. Inorg. Biochem*., vol. 92, 2002, pp. 171–176.
[15] F. Capolongo, M. Giomini, A.M. Giuliani, B.F. Matzanke, U. Russo, A. Silvestri, A.X. Trautwein, R. Barbieri," The interactions of Fe^{3+} ions with adriamycin studied by ^{57}Fe Mössbauer and electronic spectroscopies", *J. Inorg. Biochem*., vol. 65, 1997, pp. 115–122.
[16] A. Haikarainen, J. Sipila, P.P. Pietikainen, A. Pajunen, I. Mutikainen, "Synthesis and characterization of bulky salen-type complexes of Co, Cu, Fe, Mn and Ni with amphiphilic solubility properties", *J. Chem. Soc., Dalton Trans.*, 2001, pp. 991–995.
[17] M. N. Dehkordi, A-K Bordbar, P. Lincoln, V. Mirkhani, "Spectroscopic Study on the interaction of ct-DNA with manganese Salen complex containing triphenyl phosphonium groups", Spect. Act. Part A, vol. 90, 2012,) pp. 50– 54.
[18] B. Bahramian, V. Mirkhani, S. Tangestaninejad, M. Moghadam, "Catalytic epoxidation of olefins and hydroxylation of alkanes with sodium periodate by water-soluble manganese (III)salen", *J. Mol. Catal*. A, vol. 244, 2006, pp. 139-145.
[19] C.V. Kumar, E.H. Asuncion, "DNA binding studies and site selective fluorescence sensitization of an anthryl probe", *J. Am. Chem. Soc*., vol. 115, 1993, pp. 8547–8553.
[20] T. Meehan, H. Gamper, J.F. Becker, "Characterization of reversible, physical binding of benzopyrene derivatives to DNA", *J. Biol. Chem*., vol. 257, 1982, pp. 10479–10485.
[21] O. B. Irobi, M. Moo-Young, W. A. Anderson, "Antimicrobial activity of Annatto (Bixa orellana extract)", Int. J. Pharm., vol. 34, 1996, pp.87.
[22] K. Fant, B. Norden, P. Lincoln, "Using ethidium to probe nonequilibrium states of DNA condensed for gene delivery", Biochem., vol. 50, 2011, pp. 1125-1127.
[23] J. D. McGhee, P. H. Von Hipple, "Theoretical aspects of DNA- protein interactions: Co-operative and non-co-operative binding of large ligands to a one-dimensional homogeneous lattice", *J. Mol. Biol*., vol. 86, 1974, pp. 469-489.
[24] R. OBrien, I. Haq, Applications of biocalorimetry: binding, stability and enzyme kinetics, in: J.E. Ladbury, M. Doyle (Eds.), *Biocalorimetry*, John Wiley and Sons Ltd, West Sussex, England, 2004.
[25] J.B. Chaires, "Equilibrium studies on the interaction of daunomycin with deoxypolynucleotides", Biochem., vol. 22, 1983, pp. 4204–4211.
[26] K.J. Breslauer, D.P. Remeta, W.Y. Chou, R. Ferrante, J. Curry, D. Zaunczkowski, J.G. Snyder, L.A. Marky, "Enthalpy-entropy compensations in drug-DNA binding studies", *Proc. Nat. Acad. Sci. USA.,* vol. 84, 1987, pp. 8922–8926.
[27] C. Sanchez-Cano, M. Huxley, C. Ducani, A. E. Hamad, M. J. Browning, C. Navarro-Ranninger, A. G. Quiroga, A. Rodger, M. J. Hannon, "Conjugation of testosterone modifies the interaction of mono-functional cationic platinum(II) complexes with DNA, causing significant alterations to the DNA helix", *Dalton Trans*., vol. 39(47), 2010, pp. 11365-74.
[28] M. V. Angelusiu, S. F. Barbuceanu, C. Draghici, G. L. Almajan, "New Cu(II), Co(II), Ni(II) complexes with aroyl-hydrazone based ligand. Synthesis, spectroscopic characterization and in vitro antibacterial evaluation", *Eur. J. Med. Chem*., vol. 45, 2010, pp. 2055–2062.