1.1. Introduction

In the present thesis author included detailed study of Drug-DNA interaction that comprises stability of synthetic and natural DNA on binding with various drug, and also incorporated vibrational dynamics of polypeptides such as the tripeptide (glycyl-methionyl-glycine). The present chapter provides basic information to the next five chapters, which include the work on theoretical analysis of Dipyrandium and Thionine binding with synthetic polynucleotide [poly d(AT)] and natural DNA (CP-DNA, ML-DNA, HT-DNA); and vibrational dynamics of a tripeptide glycyl-methionyl-glycine. The concluding chapter of the thesis provides all pros and cons of the present work and their significance in the present scenario.

1.2. Drug-DNA interaction: Significance and future potential

The study of drug-DNA interactions begins around 1960s but the binding of steroid-diamines and aromatic molecules to synthetic and natural nucleic acids has received considerable attention over the past several years (Araya et al., 2007; Bruylants et al., 2005; Gabbay and Glaser, 1971; Gonzalez-ruiz et al., 2011; Islam et al., 2009; Kunwar et al., 2011; Lane, 2001; Mahler and Dutton, 1964; Mahler et al., 1966; 1968. Paul et al., 2010). Molecular interaction of the drug with deoxyribonucleic acid (DNA) is a field of high topical interest and may have a great importance to its biological activity. The molecules can interact with DNA in a variety of ways such as surface
binding to their minor or major grooves, intercalation between adjacent base pairs, or electrostatic binding. There are number and variety of techniques devoted to the study of drug-DNA interactions which are increasing continuously.

DNA is a molecule of great biological significance in which all the information governing life processes of the organism are stored (Figure 1.1). The total DNA content of a cell is termed as genome which is unique for each and every organism. A segment of DNA that code for a polypeptide is known as gene which contains the information necessary to produce a functional product, usually a protein. DNA has two main functions in the cell viz. transcription and translation. In transcription process, information is retrieved from the DNA by RNA and utilized to synthesize proteins in the body. During replication, DNA undergoes self replication process and regenerates two identical strains. DNA present in the form of a double helix, where each strand is composed of a combination of four nucleotides, adenine (A), thymine (T), guanine (G) and cytosine (C). Within a strand these nucleotides are linked via phosphodiester linkages. The two strands are held together primarily via Watson Crick hydrogen bonds where A forms two hydrogen bonds with T and C forms three hydrogen bonds with G. Specific recognition of DNA sequences by proteins/ small molecules is achieved via the combination of hydrogen bond acceptor/donor sites available on the major groove or minor groove of DNA (Figure 1.2).
Transcription and replication plays a vital role in survival and proliferation of the cell as well as for smooth working of all body processes. DNA starts transcribing or replicating only when it receives a signal, which is often in the form of a regulatory protein binding to a particular region of the DNA. Therefore, if the binding specificity and strength of this regulatory protein can be imitated by a small molecule, then DNA function can be artificially modulated, inhibited or activated by binding this molecule instead of the protein. Hence, this synthetic/natural small molecule can act as a drug when activation or inhibition of DNA function is required to cure or control a disease. DNA activation would produce more quantities of the required
protein, or could induce DNA replication; depending on which site the drug is targeted. DNA inhibition would restrict protein synthesis, or replication, and could induce cell death (Sheikh et al., 2004). Regions of DNA involved in vital processes such as origin of replication, promotion of transcription, etc., are of particular interest as targets for a wide range of anticancer and antibiotic drugs (Chaires, 1997, 1998; Haq, 2002; Hurley, 2002.)

1.2.1. Mode of DNA binding with drugs/small molecules

Drugs and/or small molecules bind to DNA both covalently as well as non-covalently. Covalent binding in DNA is irreversible and invariably leads to complete inhibition of DNA processes and subsequent cell death. One of the drug, anticancer antibiotic, covalently bind to DNA is cis-paltin. Non-covalently bound drugs mostly classified into two classes viz. minor groove binders and intercalators. Drugs with ability to minor groove binding (minor groove binders) are usually crescent shaped, which complements the shape of the groove and facilitates binding by promoting van der Waals interactions. Moreover, these drugs can form hydrogen bonds to bases, typically to N3 of adenine and O2 of thymine. Most of the minor groove binding drugs bind to A/T rich sequences. This preference in addition to the designed propensity for the electronegative pockets of AT sequences is probably due to better van der Waals contacts between the ligand and groove walls in this region, since A/T regions are narrower than G/C groove regions and also because of the steric hindrance in the latter, presented by the C2 amino group of the guanine base (Sheikh et al., 2004). Another group of Non-covalently bound drugs,
intercalators, introduce strong structural perturbations in DNA and contain planar heterocyclic groups which stack between adjacent DNA base pairs. The complex is thought to be stabilized by \( n-n \) stacking interactions between the drug and DNA bases. Non-covalent binding is reversible and is typically preferred over covalent adduct formation keeping the drug metabolism and toxic side effects in mind. However, the high binding strength of covalent binders is a major advantage. Some of the minor groove binders and intercalators are netropsin, berenil, distamycin, mithramycin, hoechst 33258, pentamidine, nogalamycin and menogaril. As the number of DNA–drug complex structures that have been solved by X-ray crystallography and NMR analysis is increasing, it is becoming possible to identify structural features and their energetic consequences that guide observed properties like affinity and sequence selectivity.

In drug-DNA interaction, energetics of DNA recognition is not fully understood at a molecular level, due mainly to the strong electrostatic interactions prevalent in the system and van der Waals interactions (Jayaram and Beveridge, 1996; Jayaram et al., 2002). A detailed account and quantification of these contributions can help in addressing issues of both practical and fundamental interest (Lazaridis, 2002). The fundamental interest lies in tackling challenges involved in predicting ligand binding free energies qualitatively. Among the structural and energetic angles, binding of small molecules to DNA and proteins differs significantly. Protein–drug binding has been explained by various popular models such as lock and key model,
induced fit model etc., and it is also believed that hydrophobic effects play an important role in the binding process. However, a straightforward extrapolation of these interaction models to DNA–drug systems is not feasible since there is no formal active site in DNA, unlike enzymes/proteins. Certain base sequence dependent chemical, structural, and conformational characteristics of DNA double helix, nonetheless, carry sufficient information for recognition by regulatory proteins as well as small molecules (Saenger, 1983; Sinden, 1994), which bind non-covalently or introduce small covalent modifications. Fairly strong and specific binding is observed between some ligands and DNA and this is attributed to various factors like hydrogen bonding etc.

For understanding of DNA–drug interaction, experimental studies plays basic role. The necessary information on binding constants and corresponding free energy, entropy, enthalpy, and heat capacity changes on complex formation can be obtained by thermodynamic studies (Chaires, 1997; Cooper, 1999). This information enormously used to authenticate investigation based on theoretical predictions and structural analysis. Along with initial studies focused on thermodynamics of drug-DNA complex formation, Breslauer and his colleagues recommended significant observation of enthalpy–entropy compensation in drug-DNA systems and moreover contributed considerably to developing thermodynamic profiles of these complexes (Zaunczkowski et al. 1987; Chalikian and Breslauer, 1998; Pilch et al., 1999). Among the range of previous studies in the field of DNA–drug complexes modeling, some of the
initial studies focused on netropsin (Zakrzewska et al., 1984; Caldwell and Kollman, 1986). These studies proposed models to explain the accurate functions played by formal charges and hydrogen bonds along with snug fit in the netropsin–DNA complex. Basically molecular mechanical calculations based on energy minimization protocols were employed to broaden the horizons and study different classes of DNA-binding molecules, such as minor groove binding netropsin, bisintercalating drug, triostin (Singh et al., 1986), and the covalent binder acridine (Rao and Singh, 1986). Along with this, the era of free energy calculations and various components contributing to it became a key area of interest (Tembe and McCammon, 1984; Jorgensen and Ravimohan, 1985; Beveridge and DiCapua, 1989). In this regard, results of relative binding free energies calculation for distamycin and its analog was in good agreement with experimental one (Singh et al., 1994). However, computation of absolute binding free energies is a quite complicated assignment and remains semi-quantitative at present scenario. Out of these some successful attempts have been reported in which the theoretically determined absolute binding free energy is in correspondence with experiment (Singh and Kollman, 1999; Pineda and Zacharias, 2002; Spackova et al., 2003). These theoretical studies on drug-DNA interactions have resulted in important contributions to the understanding of binding in a few DNA–drug complexes. Since these studies typically focused on a single DNA–drug complex (Srivastava et al., 2004 Singh and Kollman, 1999; Pineda and Zacharias, 2002; Spackova et al., 2003), the need to examine a series of complexes persists to facilitate origin of a set of general principles.
1.2.2. DNA-interactive agents

The polynucleotide, DNA, is also one of the receptors molecule with which various drugs or small molecules can interact. These DNA-interactive drugs/agents are generally very toxic to normal cells. Thus, these drugs are reserved only for life-threatening diseases such as cancers. There are four general types of compounds that interact with DNA:

1. Intercalators
2. Groove binders
3. Alkylating agents
4. DNA cleaving agents

In general aromatic or hetero-aromatic molecules bind to DNA by intercalating between the base pairs of the double helix. The principal driving forces for intercalation are stacking and charge-transfer interactions, while hydrogen bonding and electrostatic forces also play a role in stabilization (Neidle and Abraham, 1984). Additional interactions may further stabilize the complex but in many cases, no covalent bond is formed. The result of intercalation is that the DNA double helix becomes geometrically distorted and the translation of the genetic code may be unable to properly function. Intercalation, first described in 1961 by Lerman, is a noncovalent interaction in which the drug is held rigidly perpendicular to the helix axis (Lerman, 1961). This causes the base pairs to separate vertically, thereby distorting the
sugar-phosphate backbone and decreasing the pitch of the helix. Intercalation is energetically favored because the energy that holds the intercalated molecule must be greater than normal base stacking. It is cleared that intercalation does not disrupt normal DNA hydrogen bonding. However, it can destroy the regular helical structure by unwinding the DNA at the binding site and importantly, interferes with DNA-binding enzymes like polymerases. Some examples of the intercalators are echinomycin, triostin A, acridine, cis-Platin, daunomycin, adriamycin, actinomycin D, ethidium and propidium.

Groove binders are highly sequence-specific that interact with DNA groove, which are recognized by these molecules. The grooves of DNA (major and minor) differ in hydrogen-bonding, electrostatic potential and degree of hydration. Most of the large protein molecules exhibit high specific binding with major groove region of the DNA and small molecules prefer the minor groove. Minor-groove binding molecules have aromatic rings linked by bonds with torsional freedom. Because groove-binding agents can extent many nucleic acid base pairs, they can have highly sequence-specific recognition. Sequence-specific DNA-binding proteins generally interact with the major groove of B-DNA, because it exposes more functional groups that identify a base pair. However there are some known minor groove DNA-binding ligands such as netropsin, distamycin, pentamidine etc. (Zimmer and Wahnert, 1986; Dervan, 1986)

Alkylation reaction mostly represents an irreversible binding to DNA basically with guanine. Alkylating agents add methyl or other alkyl groups
onto molecules where they do not belong. This alkylation proceeds with
different mechanism. In the first mechanism an alkylating agent attaches alkyl
groups to DNA bases and results in DNA fragmented by repair enzymes in
their attempts to replace the alkylated bases. In second mechanism alkylating
agents caused DNA damage due to formation of cross-bridges and bonds
between atoms in the DNA. Cross-linking prevents DNA replication and finally
transcription. In third type of mechanism, alkylating agents causes the
mispairing of the nucleotides leading to mutations. There are six groups of
alkylating agents viz. nitrogen mustards, ethylenimes, alkylsulfonates,
triazenes, piperazines, and nitrosureas. Cyclosporamide is a classical example
alkylating agent and is one or the most widely used agents.

DNA cleaving agents (strand breaker) initially intercalate into DNA, and
can react in such a way as to generate radicals depending on the local
environmental and cellular metabolism. These radicals typically extract
hydrogen atoms from the DNA sugar-phosphate backbone, leading to scission
of DNA strand. Hence, these DNA-interactive compounds are metabolically
activated radical generators. The anticancer drug bleomycin acts as a strand
breaker and other examples are anthracycline, tirapazamine and enediyne.
Bleomycin is actually a mixture of several glycopeptide antibiotics isolated
from a strain of the fungus *Streptomyces verticillus*. Bleomycin cleaves
double-stranded DNA selectively at 5'-GC and 5'-GT sites in the minor groove
by a process that is both metal ion and oxygen dependent (Povirk, 1989;
1.2.3. Thermodynamics of Drug-DNA Interactions

Nucleic acid-binding drugs are designed to transform gene activity and/or inhibit protein translation. Therefore, these biomolecules represent a major target in drug development strategies designed to manufacture next generation therapeutics for diseases. In order to optimize the clinical efficacy of drugs and also to discover or design new drugs, it is essential to understand and characterize the molecular basis of drug-DNA interactions, including structural details and thermodynamics of binding.

Thermodynamic study of drug-DNA interaction determines the forces involve during the binding of drugs to its target DNA. For the calculation of observed free energy change (ΔG_{obs}) for a drug-DNA interaction, the following relationship is used:

$$
\Delta G_{\text{obs}} = -RT\ln K_B
$$

Where, \( R = \) universal gas constant

\( T = \) temperature

\( K_B = \) equilibrium binding constant (The value of \( K_B \) is dependent on pH, salt concentration, and other experimental parameters)

Isothermal titration calorimetry directly measures the enthalpy of binding (ΔH_{B}) and equilibrium binding constant (\( K_B \)), and the entropy change
associated with binding ($\Delta S_b$) can also be calculated by using following equation:

$$\Delta G_{\text{obs}} = \Delta H_B - T\Delta S_B$$

To determine $K_B$ and $\Delta G_{\text{obs}}$ experimentally, a number of methods are available. These methods include isothermal titration calorimetry and spectroscopic techniques. In addition to $K_B$ and $\Delta G_{\text{obs}}$, it is also important to determine $\Delta H_B$ and $\Delta S_B$ for a binding interaction. Isothermal titration calorimetry is the only experimental method available to directly measure $\Delta H_B$. The driving forces involve in drug-DNA interaction depends on the nature of reaction. When enthalpy is favorable, the driving forces are hydrogen bonding, van der Waals interactions, and electrostatic interactions. When entropy is favorable, binding is driven by hydrophobic interactions, whereas unfavorable entropic changes are due to loss of conformational degrees of freedom.

Change in heat capacity ($\Delta C_p$) due to binding of drugs can also be calculated by isothermal titration calorimetry, by determining the dependence of $\Delta H$ on temperature. The heat capacity change can also be estimated from change in accessible surface area ($\Delta ASA$), and one can correlate calculated heat capacity values to experimentally-determined values. There are number of reports on energetic contributions to the binding free energy for drug-DNA interactions in which free energy was calculated by the data obtained from isothermal titration calorimetry (Chaires, 1998a; 1998b; Haq and Ladbury, 2000; Haq et al., 2000; Haq, 2002). It is required to keep experimental
conditions in mind as the observed binding constant and $\Delta G_{\text{obs}}$ is dependent on these conditions such as buffer, ionic strength, temperature and concentration. For drug-DNA interactions following five free energy components have been identified:

1. Unfavorable contribution from conformational changes in the drug or DNA due to binding ($\Delta G_{\text{conf}}$).

2. Unfavorable contribution due to loss of rotational and translational degrees of freedom upon binding ($\Delta G_{r+t}$).

3. Contribution from hydrophobic transfer of the unbound drug to the DNA binding site ($\Delta G_{\text{hyd}}$). This value can be determined from heat capacity change.

4. Contribution from coupled polyelectrolyte effects due to binding of cationic ligands ($\Delta G_{\text{pe}}$). Binding constant and observed $\Delta G$ are dependent on salt concentration. $\Delta G_{\text{pe}}$ can be determined experimentally by measuring the binding constant at different salt concentrations.

5. Contribution due to non-covalent interactions, such as hydrogen bond formation and van der Waals interactions ($\Delta G_{\text{mol}}$).

These free energy terms can be experimentally determined by isothermal titration calorimetry, in combination with recognized empirical relationships from other experimental data. This information is used to modify drug structure to improve binding affinity and specificity of the drug.
Differential scanning calorimetry is also used to study the binding of various drugs to synthetic and natural DNA (Marky, et al., 1983a; Marky, et al., 1983b; Leng, et al., 1998; 2003). UV melting profiles and DSC have been used to measure the binding affinity of a new bisintercalating antibiotic, while other methods were not successful. DSC was also used to characterize the binding of echinomycin to DNA, a bisintercalator that is difficult to study due to its poor solubility (Leng et al., 2003). Thermodynamic parameters for some of the intercalators and minor groove binders are given in table 1.1. On addition of drug to DNA, the transition midpoint (T_m) increases, and when drug concentration is high enough to saturate the binding sites on the DNA, McGhee (McGhee and von Hippel, 1974) showed that:

\[
\frac{1}{T_m^0} - \frac{1}{T_m} = \frac{R}{\Delta H_{DNA}} \ln \left[ \frac{1}{1 + \frac{1}{K_h L}} \right]
\]

where \(T_m^0\) and \(T_m\) are the melting temperature of drug-free and drug-bound DNA, respectively.

1.2.4. Heat capacity changes associated with Nucleic acid folding transitions

Besides the changes in free energy (\(\Delta G\)), enthalpy (\(\Delta H\)), and entropy (\(\Delta S\)) during the nucleic acid structures modification, heat capacity (\(C_p\)) are also changes significantly and affect the overall energetics of folding. Heat capacity is defined as the amount of heat required to increase the temperature of a substance by a unit degree and can be presented by:
\[ C_p = \left[ \frac{\partial q}{\partial T} \right]_p \]

**Table 1.1.** Thermodynamic parameters for intercalators and minor groove binders

<table>
<thead>
<tr>
<th>Drugs</th>
<th>T</th>
<th>( \Delta C_p )</th>
<th>( \Delta G_{\text{obs}} )</th>
<th>( \Delta H_B )</th>
<th>T( \Delta S_B )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intercalators:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethidium</td>
<td>25</td>
<td>-140</td>
<td>-6.7</td>
<td>-9.0</td>
<td>-2.3</td>
<td>Chaires, 1998b</td>
</tr>
<tr>
<td>Actinomycin</td>
<td>10</td>
<td>-364</td>
<td>-8.5</td>
<td>-2.7</td>
<td>+5.8</td>
<td>Chaires, 1998b</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>20</td>
<td>-150</td>
<td>-8.9</td>
<td>-7.4</td>
<td>+1.5</td>
<td>Chaires, 1998b</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>20</td>
<td>-160</td>
<td>-7.9</td>
<td>-9.0</td>
<td>+1.1</td>
<td>Chaires, 1998b</td>
</tr>
<tr>
<td>Propidium</td>
<td>25</td>
<td>-150</td>
<td>-7.5</td>
<td>-6.8</td>
<td>+0.7</td>
<td>Chaires, 1998b</td>
</tr>
<tr>
<td><strong>Minor Groove Binders:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Netropsin</td>
<td>25</td>
<td>-213</td>
<td>-8.7</td>
<td>-5.8</td>
<td>+2.9</td>
<td>Haq, 2002</td>
</tr>
<tr>
<td>Distamycin</td>
<td>20</td>
<td>NA</td>
<td>-10.1</td>
<td>-12.3</td>
<td>-2.2</td>
<td>Chaires, 1998b</td>
</tr>
<tr>
<td>Berenil</td>
<td>25</td>
<td>-146</td>
<td>-8.0</td>
<td>+0.6</td>
<td>+8.6</td>
<td>Haq, 2002</td>
</tr>
<tr>
<td>Hoechst 33258</td>
<td>25</td>
<td>-330</td>
<td>-7.7</td>
<td>+4.4</td>
<td>+12.1</td>
<td>Haq et al., 1997</td>
</tr>
</tbody>
</table>

NA=Not available
Heat capacity is related to other fundamental thermodynamic parameters also. Accordingly, different expressions relating $C_P$ to these other parameters may be selected as a function of experimental ease or to highlight some physical aspect of the system. For example, the most commonly employed relationships of $C_P$ are

$$C_P = \left[ \frac{\partial H}{\partial T} \right]_P = \left[ \frac{\partial S}{\partial T} \right]_P$$

Hence, $C_P$ can be calculated as the temperature dependence of the entropy or enthalpy. Changes in heat capacity ($\Delta C_P$), associated with the folding or unfolding of a biopolymer can be expressed as:

$$\Delta C_P = \left[ \frac{\partial \Delta H}{\partial T} \right]_P = \left[ \frac{\partial \Delta S}{\partial T} \right]_P$$

The above equation demonstrates the most common experimental method for determining $\Delta C_P$, by measuring $\Delta H$ as a function of temperature. The same equation also implies that $\Delta H$ and $\Delta S$ for a given transition change together and in the same direction as a function of the $\Delta C_P$. Additionally, to describe $\Delta C_P$ with respect to $\Delta H$ and $\Delta S$, we can also calculate change in heat capacity with respect to $\Delta G$ that may be calculated as:

$$\Delta C_P = \left[ -T \frac{\partial^2 \Delta G}{\partial T^2} \right]_P$$

Change in heat capacity is the second derivative of $\Delta G$ with respect to temperature. Therefore, folding transitions associated with large $\Delta C_P$ values
should exhibit significant curve in a plot of $\Delta G$ versus temperature. In terms of physical origins, the heat capacity changes of a nucleic acid in solution derives from a combination of solvent interactions (both solute–solvent and solvent–solvent) and internal solute effects, such as conformational entropy, electrostatics, vibrational modes, and others (Sturtevant, 1977).

To find the impact of heat capacity changes on nucleic acid folding we must go through the basics of heat capacity and its relation with the change in temperature. In the absence of a $\Delta C_p$, the free energy associated with nucleic acid unfolding is linear with temperature, as expressed by the Gibbs equation:

$$\Delta G = \Delta H - T\Delta S$$

However, introduction of a $\Delta C_p$ introduces curvature in the temperature-dependent stability profile, as reflected by the fact that $C_p$ is the second derivative of $G$ with respect to temperature. In the case of nucleic acid folding transitions it is highly likely that $\Delta C_p$ itself significantly depends on temperature. To accommodate a temperature-dependent $\Delta C_p$, the Gibbs law can be modified:

$$\Delta G = \left(\frac{T_{ref} - T}{T_{ref}}\right)\Delta H_{ref} + \int_{T_{ref}}^{T} \Delta C_p dT - \int_{T_{ref}}^{T} \Delta C_p d(lnT)$$

where $\Delta H_{ref}$ is the change in enthalpy upon unfolding at an arbitrary reference temperature ($T_{ref}$) which is often set at the high-temperature melting midpoint ($T_M$) for convenience. Two related consequences of the temperature-
dependent curvature in $\Delta G$ imparted by $\Delta C_P$ are i) that there exists a
temperature of maximum stability, $T_{\text{max}}$, and ii) that unfolding can in principle
be induced by sufficiently deviating in temperature either above or below $T_{\text{max}}$. The phenomenon of cold unfolding or cold denaturation has been
sufficiently demonstrated for proteins and linked to the presence of a large, positive $\Delta C_P$ for unfolding (Privalov, 1990).

Since temperature-dependent changes in $\Delta H$ and $\Delta S$ largely offset one
another in $\Delta G$, the zero $\Delta C_P$ approximation is usually adequate for routine
stability estimations of short stretches of duplex at 37°C. The same may not
be true for applications that require high-precision estimates of $\Delta G$ for
hybridization of short duplexes. The temperature dependence of $\Delta C_P$
conferred by linked single strand equilibria further complicates the prediction
of folding energetic at temperatures distant from those at which parameter
data are collected. Predicted values of $\Delta H$ and $\Delta S$ are less accurate than those
for $\Delta G$ and vary especially between oligomeric and polymeric duplexes;
inclusion of $\Delta C_P$ within stability calculations can reconcile these differences. A
more recent study found that explicit consideration of $\Delta C_P$ was necessary to
calculate adequate differential thermodynamic parameters between various
duplexes in an effort to understand the physical basis of their different
stabilities (Tikhomirova et al., 2004; Mikulecky and Feig, 2006). Change in
heat capacity for nucleic acid structural changes have been obtained by
different methods. One of the methods is purely computational method with
hydration of solvent accessible surfaces. Some of the major experimental
approaches used to determine $\Delta C_P$ are van’t Hoff method, differential scanning calorimetry (DSC) method and isothermal titration calorimetry (ITC) method.

1.2.5. Thermodynamics in Drug Design

Drugs that interact with DNA have clinical significance especially for the treatment of solid tumors, lymphoma, and leukemia. The interruption of DNA replication is effective against rapidly reproducing cancer cells. More efficient and targeted DNA-binding drugs can be achieved by designing new compounds with multiple binding moieties that bind to specific DNA sequences. Application of calorimetric methods to study the thermodynamics of nucleic acid-folding transitions has gradually been improved in recent years. These improvements escort to the production of high-precision microcalorimeters. Therefore, the amount of high-resolution structural results about nucleic acids has developed and clarified the physico-chemical interactions, which force the formation of nucleic acid structures (Mikulecky and Feig, 2006; Gill et al., 2010). DSC monitors the excess $C_p$ of a nucleic acid in solution, and the temperature is increased or decreased with a constant rate. Measuring the differential heat flow between the sample and a reference accomplishes the excess $C_p$. When sample and reference are scanned simultaneously, folding and unfolding reactions occur in the nucleic acid as a consequence of absorbed or released heat. This differential heat is gained with subtraction from the reference thermal profile. Peaks obtained from types of DSC thermograms represent folding transitions, and melting points of
the transition occur near the jump point of the curve. The melting point corresponds to a single molecular transition, where \( \Delta C_p \) is equal to zero (Mikulecky and Feig, 2006). Peak results are fitted in such ways that accommodate Limited \( \Delta C_p \) or ignore them. The later leads to \( \Delta H_{VH} \). The ratio of \( \Delta H_{cal} \) to \( \Delta H_{VH} \) could be used for study of intermediate folding states, which occur near the melting point (Pilch, 2000; Breslauer et al., 1992). Therefore, the DSC calorimeter is ideal for determining \( \Delta C_p \) and measuring \( C_p \) directly (Mikulecky and Feig, 2006).

Latest drug discovery efforts have been dominated by structure-based design concepts in which lead compounds are sought by attempting to match their shapes with the complementary shapes of active sites of receptors using known structures obtained by X-ray crystallography. However, it was formerly noted that thermodynamic studies are an essential and necessary complement to structural studies in drug design (Henry, 2001). Structural data alone, even when coupled with the most sophisticated current computational methods, cannot fully define the driving forces for binding interactions. Thermodynamics provides quantitative data of use in elucidating these driving forces and for evaluating and understanding at a deeper level the effects of substituent changes on binding affinity (Weber and Salemme, 2003; Chaires, 2008).
1.3. Previous research work done

Ligands that can bind to nucleic acids have been the subject of much research for the past five decades because DNA/RNA has been presumed to be the intracellular targets for various small ligands and the ligand-nucleic acid interaction creates the potential for regulating gene expression resulting in therapeutic treatment for tumors and other bacterial and viral diseases.

Single strand nucleic acids do not form unique structure but rather partially stacked helixes or random coils (Fresco and Klempere,r 1959; Saenger, 1984), with the degree of stacking being strongly temperature, ion-strength or pH dependent (Fresco and Klempere,r 1959; Saenger, 1984). Among those single nucleic acids, poly(A) and poly(U) have attracted much attention due to their ability to form higher order or other secondary nucleic acid structures, and the importance of their biological functions in cell growth. It is known that poly(A) structure play an important role in gene expression in eukaryotic cells. All mRNAs in eukaryotic cells contain a poly(A) tail at the 3’ end that is important for the maturation and stability of mRNA and for the initiation of translation (Zarudnaya and Hovorun, 1999; Munroe and Jacobson, 1990). Post-translational polyadenylation of mRNA is catalyzed by the enzyme poly(A) polymerase. Recent studies have found that neoPAP, a human polyadenylic polymerase, is significantly over-expressed in human cancer cells (Topalian et al., 2001; 2002). Thus, the new cancer therapeutic agents can be developed, ones that bind to the poly(A) tail of mRNA and thereafter interfering with mRNA processing by polyadenylic polymerase.
However, very few molecules are known to bind to a poly(A) single structure or to stabilize the poly(A)•poly(A) duplex at neutral pH. Of this small number of ligands, berberine (Yadav et al., 2005), coralyne (Xing et al., 2005, and palmatine (Giri et al., 2006) have been studied.

The various conformations that DNA can adopt exhibit different binding properties upon interacting with small ligands. As discussed previously, the small ligands bind to duplex DNA/RNA mainly via intercalation and groove binding. Since the major groove is larger than the minor groove, the majority of groove binders selectively bind to the minor groove because their charges and shapes are complementary. In addition to the groove size, the binding of small ligands to the duplex is also sequence dependent. For example, Hoechst 33258 prefers AT-rich duplexes (Harshman and Dervan, 1985), with imidazole-pyrrole-pyrrole (Bremer et al., 2000) recognizes the GC base pair. For most drug-DNA interactions, these small molecules essentially act as a rigid body, with little conformational change upon binding. However, the DNA/RNA duplex conformation may be changed upon binding. In describing the B-form DNA minor groove recognition, the lexitropson family of compounds, typically netropsin and distamycin, have been studied extensively due to their inherent high affinity for DNA duplex. Because of the changes in the minor groove width and the unusually flexible TpA base step, the particularly binding preference for these compounds is 5'-AATT and 5'-ATAT rather than 5'-TTAA and 5'-TATA (Abu-Daya et al., 1995; Lane et al., 1993). Structural analyses obtained through NMR and crystallographic studies have
indicated that the recognition of a ligand by DNA arises from H-bonded contacts with A/T, electrostatic interaction with the backbone of the DNA, and the van der Waals interaction with the floor and the walls of the groove. It has also been observed that the stacking interaction between the O4’ atom of sugar and the pyrrole ring of the ligand contributes to the stability of the complex formation (Pelton and Wemmer, 1988). In addition to the structural findings, binding of lexitropsin with the DAN minor groove is also accompanied by high binding affinity, favorable enthalpy change and binding entropy (Marky and Breslauer 1987).

The thermodynamics of drug-DNA interaction has been the focus of much past research; however, the recent introduction of the high sensitive micro-calorimetric technique has made this study in this yield more straightforward. Consistent with other minor groove binding ligands, the interaction between lexitropson and DNA are characterized by enthalpy-entropy compensation mechanisms. Studies have shown that netropsin has a binding affinity of 4.3×10^7 M\(^{-1}\) at 1:1 binding to the A\(_3\)T\(_2\)T\(_3\) oligomer, with the entropy change contributing more to the binding free energy than enthalpy (Rentzeperis et al., 1995). However, significant differential effects have been observed in the binding of netropsin to poly(dA)•poly(dT) and poly(dA-dT)\(_2\) duplex systems, suggesting that conformational change plays an important role in the binding (poly(dA).poly(dT) experiences a conformational change) (Marky and Kupke, 1989). Additional classic B form DNA minor groove binders such as berenil, Hoechst 33258, and propamidine have also been studied,
results indicating binding affinities in the range of $10^5 - 10^7 \text{M}^{-1}$ and the heat capacity changes ($\Delta C_p$) in the range of -150 ~ -400 cal/mol.K. The $\Delta C_p$ value for minor groove binders is typically higher than for intercalators, for which this value is usually less than -200 cal/mol.K (Lane and Jenkins, 2000). Recently, the study developed in Arya’s laboratories has found B-form DNA recognized by aminoglycosides, which were previously thought to have no effect on B-form DNA. This recognition, specifically dual recognition, was achieved by synthesizing the neomycin-Hoechst 33258 conjugate (Willis and Arya, 2006) or the neomycin-neomycin dimer (Arya et al., 2004) and then binding them to the B-form duplex, in particular the AT-rich sequences. It was found that this binding affinity is significantly higher than the classic B-form DNA binders mentioned previously. These findings open the door for the development of compounds that target the B-form DNA duplex. Recognition of A-form nucleic acid, in particular the RNA duplex, by small ligands, however, exhibits a higher binding affinity than the B-form DNA systems. A good example is the 16S A site rRNA interaction with aminoglycosides (Kaul and Pilch, 2002). As discussed above, this system was found to achieve a high binding affinity in the nanomolar range, but different from the B-form DNA-minor groove binder system, this binding is predominately enthalpy driven and the binding site is within the major groove of the RNA. Very few small molecules, however, have been found to be able to bind to the DNA•RNA hybrid duplex. Recent report has shown that ethidium bromide is the preferred catalyst for this hybrid recognition (Ren and Chaires, 2001). The binding affinity is in low micromolar range. Aminoglycosides, were also found
by Arya to bind to this hybrid in the 100 nM range. The methidium-Neomycin conjugate was subsequently synthesized to achieve a high binding affinity and specificity of drug binding to DNA•RNA hybrid. Initial results have shown the powerfulness of this conjugate, which binds to the DNA•RNA hybrid specifically at an affinity of approximately 0.1 nm.

To date, the only full and detailed conformational information about the DNA triplex comes from NMR studies. In addition to the conformational change, the stability of the triplex has found to be much lower than its corresponding duplex because of the charge neutralization resulting from the high electronegative potential formed when three backbones are associated with one another. Bringing in a positive charge on the third strand (Plum and Breslauer, 1995), replacing the phosphodiester with a peptide at the backbone (Cherny et al., 1993), introducing the ionic strength of the solution (Cheng and Pettitt, 1992), and applying polycations (aminoglycosides) (Arya et al., 2001), all have been shown to increase the stability of the triplex effectively. Even though the formation of triplex is slow and less stable than the formation of duplex, this structure has received much attention as an antigene strategy. Triplex forming oligonucleotide has served as a potential genetic drug regulating the gene expression after binding. Triplex formation also performs numerous functions in biological systems. The replication of polynucleotide is inhibited by the third-strand binding. In addition, HIV DNA has been reported to be inhibited from integrating into host genomes (Bouziane et al., 1996). Cooney and his coworkers have demonstrated the
specific inhibition of transcription by means of triplex formation at the poly(purine).poly(pyrimidine) sites in the promoter regions (Cooney et al., 1988). Because of the numerous activities the triplex may exhibit in the biological systems, increasing its stability has been the focus of much research, with many endeavors being conducted using small ligands. Intercalators such as acridines, ethidium, proflavine, and ruthenium complexes (Wilson et al., 1994; Cassidy et al., 1994; Choi et al., 1997; Mergny, et al., 1991), as well as DNA minor groove-binding ligands such as berenil, netropsin, and Hoechst 33258 (Durand et al., 1994a; Park and Breslauer, 1992; Durand et al., 1994b), have been shown to bind to DNA triplex. However, those ligands do not bind to the triplex selectively, and some of them even destabilize it. Benzo(e)pyridoindole, the first triplex-specific ligand described (Mergny et al., 1992), binds to the triplex through an intercalation mechanism, increasing the melting temperature of triplex over the duplex significantly. Aminoglycosides have been shown in Arya’s laboratories to stabilize triplex nucleic acids both selectively and significantly. Among these aminoglycosides, neomycin exhibits the most potent effect, while, neomycin is the first groove-binding ligand to result in triplex stabilization effect. Molecular modeling has suggested that neomycin binds within the Watson-Hoogsteen groove (Arya et al., 2003) formed between the third strand and the pyrimidine strand.

The formation of a tetraplex or a quadruplex DNA structure has been suggested as a potential chemotherapeutic target of a new antitumor
compound. This structure is formed from the tandemly repeating guanine clusters, present primarily in the single stranded segment at the 3’ ends of chromosomal DNA and characterized by a stacked arrangement of several G4 planar tetrads in which each G interacts with the adjacent one via forces of the Hoogsteen bonds. These G-rich sequences occur frequently in human genomes and in many other organisms (Huppert and Balasubramanian, 2005; Todd et al., 2005). The formation of G-tetraplex has been reported to exhibit many biological functions. For example, G-tetraplex has been found to exist at the promoter region of oncogenes such as c-myc (Simonsson et al., 1998). Of the many G-rich sequences in the organisms, telomere has been the primary focus over the past few years due to its important biological role and its potential as an antitumor target. Targeting the G-tetraplex with small ligands to improve its stability has been the subject of much recent research. One of the first studies involved the binding of ethidium bromide to the G-tetraplex d(T_4G_4)_4 (Guo et al., 1992). Arya’s laboratory has studied the binding of aminoglycosides, specifically neomycin, to the G-tetraplex d(T_2G_20T_2)_4 using sodium and a pH of 6.8.

A huge number of studies have been reported on vibrational dynamics of biopolymer system having different confirmations (Prasad et al., 1996; Gupta et al., 2008; Srivastav et al., 1997; Krishnan and Gupta, 1970a, 1970b; Singh and Gupta, 1971; Dwivedi and Gupta, 1972; Gupta et al., 1973; Burman et al., 1995a, 1995b; Gupta et al., 1995). The analysis in most of these cases is based on separation of the vibrational spectrum into group and
skeletal vibrations. The former are taken from computationally fitted IR and Raman data and the latter by using the two-parameter Tarasov model (Wunderlich and Bu, 1987) and fitting to low temperature heat capacities. The vibrational dynamics of poly(L-glutamine) was interpreted by LaVerne and coworkers from the dispersion and dispersion profile of the normal modes of poly(L-glutamine) as obtained by Higg’s method for infinite systems (LaVerne et al., 2009). Additionally, the dispersion curves give information of the degree of coupling and the dependence of the frequency of a given mode on the sequence length of ordered conformation. These curves also facilitate a correlation of the microscopic properties, such as specific heat, free energy and enthalpy.

1.4. Methods used in previous studies and in present work

Advancement in the innovation of nucleic acid binding drugs obviously relies on the availability of analytical methods or procedures that judge the efficiency and nature of interactions between nucleic acids and their putative ligands. A range of novel methods for these studies have emerged in current years, and methods for studying DNA/Drug interactions highlights new and non-conventional methods for exploring nucleic acid/ligand interactions. The structure of these systems can be studied experimentally as well as theoretically. From the conventional UV-VIS spectrophotometry to the improved gel mobility electrophoresis assay or the powerful HPLC-MS, the number of novel specific assays and methodologies is overwhelming (Tian et al., 2005). Her, I will describe some of the most useful techniques used for
drug-DNA interaction studies. Vibrational spectroscopy such as Infrared spectroscopy (IR) has been widely used for the structural analysis of DNA because it can distinguish among A-, B-, and Z-forms of DNA, triple stranded helices, and other structural motifs. It has also been a useful tool to study interactions of nucleic acids with drugs and the effects of such interactions in the structure of DNA, providing some insights about the mechanism of drug action. A major advantage is that samples can be analyzed in different aggregation states as solids or crystals, and also in solution. In addition, small quantities of sample are needed and collection of spectra is very fast. The region of interest in IR studies dealing with DNA in aqueous solutions is between 1800 and 800 cm\(^{-1}\). Due to interfering absorption bands of water at 1650 cm\(^{-1}\) and below 950 cm\(^{-1}\), spectra are generally recorded in D\(_2\)O, where these bands move to 1200 cm\(^{-1}\), and below 750 cm\(^{-1}\). Fourier transform IR (FTIR) has been used alone or supporting other techniques to determine drug binding sites and sequence preference, as well as conformational changes due to drug-DNA interaction (Jangir et al., 2010; Mandeville et al., 2010; Neault and Tajmir-Riahi, 1996). Raman spectroscopy also depends on the vibrational frequencies of characteristic groups and has been used sometimes in conjunction with infrared spectra to study DNA-drug interactions.

Nuclear Magnetic Resonance (NMR) spectroscopy is based on the fact that atomic nuclei endowed with a property called nuclear spin will align with an applied magnetic field. The degree of this alignment depends not only on the strength of the magnetic field, but also on the type of nucleus and its
chemical environment. Every nucleus with spin gives rise to a signal or peak which represents a transition between a ground and an excited state. Each magnetically active nucleus is characterized by different parameters such as chemical shift, multiplicity, J-couplings and relaxation data that can be used to obtain detailed structural information about the molecule under study. Among the atomic nuclei available for the study of DNA (\(^1\)H, \(^{13}\)C, \(^{15}\)N and \(^{31}\)P), \(^1\)H is the most common, but \(^{31}\)P NMR is especially useful for studying the effects of ligand binding on the phosphate groups of DNA. NMR experiments are very versatile and the information can be obtained at different temperatures, solvents, pH values, ionic strengths and dielectric constants. DNA-binding drugs specific for the minor groove of DNA generally prefer AT-rich, rather than GC-rich regions of DNA. These drugs are usually planar with crescent shapes. The DNA minor groove possesses an electrostatic potential minimum attractive to many such ligands. Many anti-tumour drugs bind to the major groove, and they usually do it covalently through N-7 of guanine but their modes of interaction have been studied with techniques different from NMR (González-Ruiz et al., 2011).

The interaction of drugs with DNA can be also detected by UV-VIS absorption spectroscopy on the basis of absorption properties of the drug or the DNA molecules. The UV-VIS absorption spectrum of DNA exhibits a broad band (200-350 nm) in the UV region with a maximum absorbance at 260 nm. This maximum absorbance is a result of the chromophoric groups present in purine and pyrimidine moieties responsible for the electronic transitions. The
application of this versatile and simple technique allows estimating the molar concentration of DNA on the basis of the measurement of the absorbance value at 260 nm. In practice, the molar concentration of DNA is evaluated in terms of the concentration of base pairs. The absorbance ratios \( A_{260}/A_{280} \) can also characterize the DNA molecules (Paul et al., 2010). Slight changes in the absorption maximum as well as the molar absorptivity can be appreciated with the variations in pH or ionic strength of the media. The \( \varepsilon \) values (\( \lambda_{\text{max}} = 260 \text{ nm} \)) of free oligonucleotides are higher than the ones corresponding to the same oligonucleotides in single strand DNA (ss-DNA) and double strand DNA (ds-DNA) because base-base stacking results in a hypochromic effect. This behavior can be exploited to verify denaturation of DNA by measuring its absorbance values before and after denaturing treatment. The hypochromic effect can also be employed to verify the existence of drug-DNA interactions, due to the fact that the monitoring of the absorbance values allows studying the melting behaviour of DNA. Melting temperature \( (T_m) \) is the temperature value corresponding to the conversion of 50 % of the double strands into single strands. An increase in the absorbance value with the increase of temperature is observed because the \( \varepsilon \) (260 nm) of ss-DNA is higher than the \( \varepsilon \) (260 nm) of ds-DNA. When a drug–DNA interaction exists, \( T_m \) is shifted to values different from native ds-DNA. The magnitude of the shift depends on the type of interaction. Thus, for intercalating agents the increase observed in the \( T_m \) value is higher than in the case of agents interacting through the DNA minor or major grooves (González-Ruiz et al., 2011). The changes in the \( T_m \) value can be followed by other techniques such
as fluorescence, circular dichroism, NMR or calorimetry, but UV-Vis absorption spectrometry is the most frequently employed method due to its good sensitivity, versatility and simplicity. Drug-DNA interactions can be resolved by comparison of UV-VIS absorption spectra of the free drug and drug-DNA complexes, which are usually different.

Circular dichroism (CD) spectroscopy is valuable techniques to explore non-covalent drug-DNA interactions, which affect the electronic structure of the molecules and also modify their electronic spectroscopic behavior. Circular dichroism spectroscopy allows to rapidly characterize drug-DNA complexes using a small amount of sample. Circular dichroism is defined as the difference in absorption of left and right circularly polarised light. Circular dichroism provides additional structural details of the complex. When electromagnetic radiation reaches DNA, the macromolecules present a certain degree of alignment in the direction of the electric field vector, and this molecular alignment is measured by the light polarised absorbance. When a drug binds to DNA, its spectrum will be modified if this binding causes changes in DNA conformation. When a drug binds to DNA, an induced CD spectrum is observed because of the interaction with DNA. This may result from either a geometric change in the drug or from coupling between its electronic transitions and those of the DNA. Particularly the electronic CD has become very significant for qualitative characterization of conducting biopolymers.
Differential scanning calorimetry (DSC) is a technique capable to study thermally induced transitions and particularly, the conformational transitions of biological macromolecules. There are number of literature support applications of this technique (Robertson and Murphy, 1997; Privalov and Potekhin, 1986; Biltonen and Freire, 1978; Freire, 1995; Lopez and Makhatadze, 2002; Jelesarov and Bosshard, 1999; Cooper and Johnson, 1994; Hinz and Schwarz, 2001). DSC is used to understand the molecular mechanism and energetics of drug-DNA interactions. Statistical mechanical theories were developed by Crothers and McGhee allowing interpretation and calculation of DNA melting curves in the presence of ligands (Spink and Wellman, 2001; Crothers, 1971; McGhee, 1976). The combination of DSC and temperature dependent UV melting methods allowed the first direct determination of the binding enthalpy for echinomycin (Leng et al., 2003). DSC was also applied to understand interaction of distamycin with ColE1 DNA, a well characterized plasmid (Ueta et al., 2001). DSC measurements were conducted to characterize the thermally induced denaturation of the 20-bp duplex with the specific goal of elucidating the thermal and thermodynamic consequences of modifying and constraining DNA via a single, site-specific interstrand cross-link of antitumor cis-platin or its clinically ineffective trans isomer (Hofr and Brabec, 2001). The interaction of other drugs, like chlorobenzylidin (Zhong et al., 2003), phenoxazone (Veselkov et al., 2003) or CP-31398 (Rippin et al., 2002), an anticancer drug, with DNA has been also investigated by differential scanning calorimetry.
In the present work, we have attempted to understand the effect of drugs/small molecules binding on synthetic and native deoxyribonucleic acids through the theoretical analysis. We used amended Zimm and Bragg theory, initially considered for helix coil transitions in polypeptides, to explain lambda point anomalies in heat capacities and order-disorder transition in drug bounded and unbounded DNAs (Zimm and Bragg, 1959). The effect of drugs or small molecules binding is reflected in the change in nucleation parameter, which is an inverse measure of binding strength. Our study is aimed at providing such a theoretical protocol for complementing experimental techniques and facilitating a minute study of the structure-energy relationships in DNA-drug complexes.

Theoretically the vibrational dynamics of peptides can be studied by normal mode analysis. Out of various techniques described above the vibrational spectroscopy provide important information about confirmation of peptides. Infrared (IR) spectroscopy is an established research tool which has renovate in recent years due to the introduction of Fourier-transform techniques. The quantitative interpretation of IR spectra has been improved by the computer assisted data analysis. The IR spectrum characterizes the vibrational modes of the molecule. The frequencies in the IR spectra can be assigned empirically to different vibrational modes of the molecule or groups within it but assignment of modes which are mixtures of group modes may not be straight forward. In such cases information on the normal modes of vibration can be obtained from the knowledge of the geometry of the
molecule and interatomic force constants. The spectral characteristics can be used to relate observed spectra to details of molecular structure and dynamics (Painter et al., 1982; Bower and Maddams, 1989). Therefore, it may be concluded that the study of normal mode is vital to understand dynamical and thermodynamical behavior of conducting peptides.

Computer aided modeling and graphic visualization have emerged as powerful tools in conformational studies of the peptides and polymers. Programmes such as ACD, Chemdraw, Discovery Studio etc. are available for modeling of peptides and long chain biopolymers that involve large number of variables (Brooks et al., 1983; Jorgensen and Rives, 1988; Weiner et al., 1986). These software packages enable us to determine structure, stretching, bending and torsion angles between the atoms of molecule or peptides.

1.5. Chapter wise presentation of the work done

There are total six chapters in the present thesis which compiled all the methodology and results of the present work including conclusion. Chapter 1 consists of detailed introduction of the thesis and chapter 2 comprises complete experimental and theoretical approaches used in the next three chapters.

The third chapter of this thesis deals with theoretical analysis of steroid diamine (Dipyrandium), binding with DNA duplex by using an amended Zimm and Bragg theory, to explain the melting behavior and heat capacity of DNA with and without dipyrandium binding. The experimental models of Marky and
coworkers (1983) have been used for the study. Chapter 4 of the thesis deals with the similar work done on theoretical analysis of tricyclic heteroaromatic molecule (Thionine) binding with natural DNAs of varying base composition by using an amended Zimm and Bragg theory, to explain the melting behaviour and heat capacity of DNAs with and without thionine binding. We used experimental models of Paul et al. for implementing this study (Paul et al., 2010).

The fifth chapter of this thesis reported vibrational dynamics of tripeptide, glycyl-methionyl-glycine. This work includes normal mode analysis and force constant evaluation. The calculated frequencies are found to be in reasonably good agreement with the IR spectra. Chapter 6 presented the conclusion of the entire work. This is the last chapter which includes all the pros and cons of the present work.
References:


