Chapter-4

Comparative study of the binding mode of 4-aminoquinoline derivatives to calf thymus DNA
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4.1 Introduction

Deoxyribonucleic acid (DNA) plays an important role in all living cells except RNA viruses. DNA carries genetic information from one generation to other. It also instructs the biological synthesis of proteins and enzymes through the process of transcription and translation.[1-4] Interaction studies of some small molecules or drugs with DNA have been widely studied. These studies give an idea to understand the structural properties of DNA, mutation in genes, basic origin of some diseases, the action mechanism of antitumor and antiviral drugs.[5]

Small molecules can react with DNA via covalent or non-covalent interactions. There are several sites in DNA where small molecule or ligand can interact. This includes (i) between two base pairs (full intercalation), (ii) in the minor groove, (iii) in the major groove, (iv) outer surface of the helix (v) electrostatic bindings.[6]

Ligand or small molecules having ability to bind with DNA can artificially modulate and hinder the functioning of DNA. Extreme research has been done to understand how ligand interacts with DNA. Both covalent and non-covalent modes of binding are observed for DNA-drug binding. Covalent binding leads to irreversible changes in the structure of DNA, inhibition of DNA processes and subsequent cell death. Cisplatin [cis-dichlorodiaminoplatinum (II)] an anticancer drug which interacts either intra or inter strand crosslinks via the chloro groups with the nitrogen atoms in the DNA bases.[7-9]

Non-covalent interaction is one of the most studied and explored binding which induces reversible changes in the DNA and are of two types: 1. Intercalation; in which planar heterocyclic compound serves as intercalators and stack between adjacent base pairs of DNA. DNA-intercalator complexes are stabilized by π-π stacking interaction.
Structural perturbations are induced in DNA by intercalators. 2. **Groove binding;** in this binding, small compounds bind to the minor groove of DNA mainly by van der Walls interaction and hydrogen bonding. Minor groove binders are mostly sequence specific and preferably bind to AT rich sequences.\[^{10}\]

The pharmacological properties of quinoline and their derivatives attracted worldwide attention in the last few decades because of its medicinal properties. Quinoline containing compounds have long been used for the treatment of malaria, beginning with quinine which is a 4,6-substituted quinoline. Systematic modification of quinine led to the synthesis of diverse quinoline antimalarial drugs with different substitutions around the quinoline ring.\[^{11-14}\] Literature survey revealed that five to six membered heterocyclic compounds containing one or two heteroatoms fused to a quinoline ring in linear fashion enhances antimalarial, antitumor and anticancer properties.\[^{15}\] Recently, antitumor properties of quinoline against MCF-7 human breast cancer cells with chloroquine being the most apoptosis-inducing agent has been reported.\[^{16}\] All differentiation-inducing quinolines caused growth suppression in MCF-7 and MCF10A cells. The mechanism of action of the differentiation-inducing quinolines has been proposed which involves strong suppression of E2F1 that inhibits growth by preventing cell cycle progression and fosters differentiation by creating a permissive environment for cell differentiation.\[^{17}\] This study proved that anticancer activity of this moiety was due to the intercalation between the quinoline derivative and the base pairs of DNA. It also interferes with normal functioning of the enzyme topoisomerase II that was involved in the breaking and releasing of DNA strands.\[^{18}\] The main reason of the binding of these drugs is the presence of planner fused heterocyclic ring system. Thus, compound having planner medium sized area and hydrophobic nature may act as active scaffold for intercalation. This was a great and extraordinary finding that led to the discovery of new antitumor drugs containing quinoline as a basic scaffold.\[^{19}\]

Quinoline derivatives, such as chloroquine is a wellknown antimalarial drug from past several years. It is also a potential chemosensitizer in cancer in combination with some conventional antineoplastic agents. Recent studies showed that CHQ inhibit the function of membrane-associated proteins belonging to the p-glycoprotein and multi-
drug resistance (MDR) protein families.\textsuperscript{[20,21]} These proteins are the basic inhibitors of chemotherapy in a wide range of cancers. Large numbers of reports published which emphasised that 4-aminoquinolines derivatives can act both as antimalarial as well as anticancer agents. Bis-quinolines comprising rigid linker chains or di-substituted chromophores act as novel bifunctional intercalator.\textsuperscript{[22]}

The DNA binding properties of $\textit{trans}$- and $\textit{cis}$-[PtCl$_2$(NH$_3$)(quinoline)] and quinoline-substituted dinuclear platinum compound $\{\textit{trans}$-PtCl(NH$_3$)(quin)$\}_2$H$_2$N(CH$_2$)$_6$NH$_2$](NO$_3$)$_2$ were reported by Farrell \textit{et al.}, 1997. The dinuclear compounds were also examined for its ability to cause conformational changes in poly[(dG-dC)]poly[(dG-dC)].\textsuperscript{[23]} These compounds exhibited very strong interstrand cross-linking efficiency changes intensities and shapes of both positive and negative bands of the polymer CD spectrum, although the overall behaviour of these changes indicates that the polynucleotide remains in the B conformation. Li \textit{et al.}, 2009 synthesized some novel potential DNA bis-intercalators containing glucose and quinoline hybrids. Preliminary binding experiments suggested that these compounds could bind to calf thymus DNA (CT-DNA) through intercalation.\textsuperscript{[24]} These studies and results clearly indicate that quinoline derivatives could be an active and lead molecules as an anticancer agents.

In this chapter, we investigated binding properties of quinoline derivatives with DNA. Calf-thymus DNA (CT-DNA) is easily available and widely used in diverse biophysical and biochemical studies to understand ligand-macromolecule interactions. The commercial sample of CT-DNA is “highly polymerized”, “polydisperse”, “fibrous preparation”, “containing low amount of RNA and proteins” characterized by water content and UV absorbance per mass.\textsuperscript{[25]} The ligand-CT-DNA interaction was investigated by numerous techniques like molecular spectroscopy method involving UV spectrophotometry\textsuperscript{[26]}, circular dichroism spectroscopy\textsuperscript{[27]} and various other techniques as melting experiments. UV-spectrophotometry, melting temperature and circular dichroism studies have been preferred because small molecule-DNA interaction may be experimentally monitored by changes in the intensity and position of the spectroscopic peak responses of DNA.\textsuperscript{[28-31]}
4.2 Present work

DNA contains the genetic information responsible for the growth and functioning of all living organisms. Conformational changes of DNA directly affect genetic expression, which is closely related to carcinogenesis and anticarcinogenesis. DNA intercalation with compounds plays an important role to exhibit their anti-proliferative activities and apoptosis effects on tumour cells.\textsuperscript{[32]} Active intercalators are required to possess an approximately planar ring structure with a medium-sized planar area and some hydrophobic character.\textsuperscript{[33]} In order to investigate the binding properties of quinoline derivatives to DNA, we carried out a series of spectroscopic studies including UV-vis, Tm and CD. The Intercalation of ligands with DNA is due to the $\pi-\pi$ stacking. This results in hypochromism and peak of intercalated chromophore shifts towards longer wavelength.\textsuperscript{[34]} The Interaction studies of drugs with DNA play central role in modulating the activity of various drugs. These studies are vital for the elucidation of the mechanisms of drug action and designing of more efficient and specifically targeted drugs with lesser side effects.

In this study, the interaction of quinoline derivatives with calf thymus DNA had been investigated. We selected three different scaffolds namely ligands-1, 2 & 3 (figure-4.1) from three different series of synthesized derivatives of 4-aminoquinoline scaffolds which were discussed in chapter-1. Our main aim is to investigate the binding behaviour of different quinoline analogs with calf thymus DNA and compare their binding behaviour. This study would be very useful to predict the anticancer prospect of newly synthesized quinolines.

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{ligands.png}
\caption{Chemical structure of ligands.}
\end{figure}
4.3 Materials and methods

4.3.1 Reagents

The highly polymerised calf thymus DNA, EDTA, Tris-HCl was purchased from the Sigma Chemical, USA. NaCl was purchased from Merck, India. All solutions were prepared using double distilled water. Tris-HCl buffer solution was prepared from (Tris-(hydroxymethyl)-aminomethane-hydrogen chloride) and pH was adjusted to 7.4. The stock solution of DNA was prepared by dissolving of DNA in10 mM of the Tris-HCl buffer, 0.1 mM of EDTA and 10 mM of NaCl at pH 8.0 and dialysing exhaustively against the same buffer for 24 h and used within 5 days. A solution of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm more than 1.8, indicating that DNA was sufficiently free from protein.[35] The concentration of nucleotide was determined by UV absorption spectroscopy using molar absorption coefficient (ε=6600 M⁻¹cm⁻¹) at 260 nm. The stock solution was stored at 4 °C. Ligand stock solution (1x10⁻³ M) was prepared by dissolving an appropriate amount of ligand in tris-HCl buffer (Methanol less than 1%). Concentrations of CT-DNA and ligands were kept constant throughout the experiments.

4.3.2 Apparatus

The absorption spectra were recorded at room temperature by Specord 250-222P145 UV-vis absorption spectrophotometer equipped with a 1.0 cm quartz cell. The CD measurements were carried out on Jasco-810 automatic recording spectrophotometer using 10 mm path length at 20 °C. The spectra were recorded in the range of 200-400 nm (near and middle UV region). Thermal denaturation curves of DNA were recorded by absorbance at 260 nm on a Carry 300 version 9, equipped with a Peltier thermoregulator and an automatic cuvette changer. The sample contained 5 µM drug solution and DNA of 10 µM base pair concentration with initial absorbance of 0.2 at 260 nm in quartz cells of 1cm path length in TE buffer with 10 mM NaCl. The heating rate was 0.5 °C/min in temperature range 30-90 °C and absorbance data were collected at every 0.5 °C.
4.4 Results and discussion

4.4.1 UV-visible spectroscopy

 UV-vis absorption measurement is a simple but effective method in detecting ligand substrate complex formation. In general, when a small molecule interacts with DNA and forms a new complex, change in absorbance and position of the band should occur.\cite{36} If the binding mode is intercalation, the π* orbital of the intercalated molecule can couple with the π orbital of the DNA base pairs thus, decreasing the π-π* transition energy and resulting in the bathochromism. On the other hand, the coupling π orbital is partially filled by electrons, thus decreasing the transition probabilities and concomitantly resulting in hypochromism.\cite{37} Generally, hyperchromism and hypochromism are the spectral features of double helical DNA. Hyperchromism means the breakage of the secondary structure of DNA while in hypochromism DNA-binding mode of molecule is electrostatic or intercalation which can stabilize the DNA duplex. Existence of a red-shift is indicative of the stabilization of DNA duplex.\cite{38}

 Binding properties of quinoline derivatives were determined using various spectroscopic studies. Firstly, UV-visible absorption spectra of CT-DNA was recorded in TEN buffer, pH=8.0 at 288 K which showed single band at 260 nm. Further, UV spectra of all three ligands L-1, L-2 and L-3 were recorded in a similar conditions exhibited two characteristic absorption maxima (\(\lambda_{\text{max}}\)) at different positions between 225-425 nm as shown in figure-4.2. The maxima in the lower wavelength range between 220-280 nm is due to the presence of substituted phenyl ring, hetero-aromatic ring system such as quinoline ring and assigned for charge transfer transitions. The band observed near 346 nm would be due to the n-π* transition of the C=N group as reported earlier.\cite{39} In order to understand the exact mode of interactions of different quinoline derivative with DNA, titration experiments were performed with increasing concentration of ligands-1, 2 & 3 from 0.25 to 1.00 as shown in figure-4.3, 4.4 & 4.5, respectively. All shift values and percentage changes are summarised in table-4.1.

 The stepwise addition of ligand-1 into fixed concentration of calf thymus DNA (15 µM) at physiological pH exhibited a remarkable consistent increase in absorbance intensity of DNA at around 260 nm. The absorption spectrum of ligand-DNA is shown in figure-4.3. It is evident from the spectrum that after addition of ligand-1, absorption
intensity increased at 260 nm and a separate band appeared near 345 nm. The absorbance increased from 0.62 to 1.10 with consistency in the bands. This 77.41% of increase in absorbance suggested the strong interaction between drug and DNA. Another band around 342 nm may assigned to the quinoline ring of ligand-1 which suggested that added ligand did not consumed completely and accumulated intracellularly. The absorption spectra are in well agreement with the earlier reported results.

Figure-4.2: UV absorbance spectra of CT-DNA, L-1, L-2 and L-3 (15 µM, 288 K, pH=8.0).

Similar titration experiment was performed with ligand-2 using identical conditions as mentioned above and shown in figure-4.4. On progressive increase of ligand-2 concentration into fixed concentration of CT-DNA there is a gradual increase at wavelength 260 nm. As we added the ligand (0.25-1.00), absorbance intensity was increasing consistently from 0.61 to 0.95. This 55.73% increase in absorbance clearly revealed the bonding between DNA and L-2. Further, a regular increase in the band at wavelength 334 nm after simultaneous addition of small molecule was due to the incomplete consumption of ligand in binding. Small peak shift in the region 300-400 nm towards the longer wavelength was a characteristic of the formation of quinoline-DNA complex and more and more drug bounded to DNA.
Figure 4.3: UV-absorbance spectrum of CT-DNA in absence and presence of L-1; $C_{CT-DNA} = 15 \mu M$, $C_{CT-DNA}:C_{L-1} = 1.0:0.0-1.0-1.0$, (288 K, TEN buffer, pH=8.0).

Figure 4.4: UV-absorbance spectra of CT-DNA in absence and presence of L-2; $C_{CT-DNA} = 15 \mu M$, $C_{CT-DNA}:C_{L-2} = 1.0:0.0-1.0-1.0$, (288 K, TEN buffer, pH=8.0).
The binding mode of ligand-3 to DNA are characterised classically through electronic absorption titrations. Similar progressive enhancement in absorbance at 260 nm can be observed as already reported in ligand-1 and 2 (figure-4.4). Since the ligand contains the basic planner quinoline ring, it facilitates the classical intercalative interaction. A shift of around 12 nm was also due to the intercalation of quinoline derivative into DNA. Cohen et al., 1965 also observed the shifting in bands towards longer wave length and proposed the formation of quinoline-DNA complex.\[40\] As concentration of ligand increased singlet band was showing slight furcation in band in the range of 300-400 nm. This could be attributed to the fact that one portion of drug is more profound and responsible for binding. Thus we can conclude that all three quinoline ligands are exhibiting different patterns in the absorbance bands, though having the same planner basic skeleton. In this case, absorbance increased from 0.60 to 0.81 which came 35% after DNA: Drug ratio from 1.00:0.00 to 1.00:1.00.

\[\text{Figure-4.5: UV-absorbance spectra of CT-DNA in absence and presence of L-3; C}_{\text{CT-DNA}}=15 \, \mu\text{M, C}_{\text{CT-DNA}:C_{L-3}}=1.0:0.0-1.0:1.0, (288 K, TEN buffer, pH=8.0).}\]

All three ligands contain the basic heterocyclic and planner which might intercalate with DNA helical pair. Variation at the small portion of the ligand is responsible for the
minor groove binding. Previous studies revealed that several low molecular weight drugs such as chloroquine have a bonding affinity to DNA and RNA. It is well documented that chloroquine has been found to have a higher affinity for poly(dG-dC).(dG-dC) than for other synthetic polynucleotides and to inhibit its transition from B-DNA to Z-DNA.\[41\] These data are consistent with the hypothesis that DNA intercalation mediates the antimalarial activity of chloroquine. It is evident from figure-4.3, 4.4 & 4.5 that simultaneous increase in concentration of ligand-1, 2 and 3 into fix CT-DNA increased absorption at 260 nm with slight shift in band position which is a strong indication of DNA-ligand binding. After a certain titration when the ratio of ligand:DNA reaches 1:1, enhancement of absorbance and peak shifting can be compared for all three ligands as shown in figure-4.6. This figure gives a comparative illustration of change in shape and position band of CT-DNA after addition of three different derivatives of quinoline. One of the major reason for this is that in ligands-1 and 2 there is free rotation around the -NH bond while in ligand-3 it is restricted due to the presence of double bond between C and N atom.

![Figure-4.6: UV absorption spectra of CT-DNA in absence (1) \[C_{CT-DNA}C_L=1.0:0.0\] and presence (2-4) of ligands \[C_{CT-DNA}C_L=1.0:1.0\], [288 K, TEN buffer, pH=8.0].](image)
The values of the binding constant, $K_b$, were obtained from the DNA absorption at 260 nm according to the published methods. For weak binding affinities, the data were treated using linear reciprocal plots based on the Equation-1.[42]

$$\frac{1}{A-A_0} = \frac{1}{A_o-A_0} + \frac{1}{K(A_o-A_0)[COM]}$$  \hspace{1cm} (I)$$

where, $A_0$ is the absorbance of DNA at 260 nm in the absence of ligands-1, 2 & 3. $A_o$ is the final absorbance of the ligand-DNA and $A$ is the recorded absorbance at different ligand concentrations. [COM] is the concentration of added ligand. The double reciprocal plot of $1/(A-A_o)$ versus $1/[C]$ was found linear and the bonding constant ($K$) was estimated to be $1.70 \times 10^3$, $1.41 \times 10^3$ & $1.36 \times 10^3$ Lmol$^{-1}$ for ligand-1, 2 & 3, respectively from the ratio of the intercept to the slope.[43]

**Table-4.1:** Thermodynamic parameters of CT-DNA upon interaction with ligand-1, 2 & 3.

<table>
<thead>
<tr>
<th>DNA: DRUG</th>
<th>UV$_{max}$ (nm)</th>
<th>Percentage change (at 260 nm)</th>
<th>Binding constant (Lmol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT-DNA</td>
<td>260</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-1</td>
<td>256</td>
<td>341</td>
<td>-</td>
</tr>
<tr>
<td>L-2</td>
<td>261</td>
<td>337</td>
<td>-</td>
</tr>
<tr>
<td>L-3</td>
<td>260</td>
<td>333</td>
<td>-</td>
</tr>
<tr>
<td>CT-DNA:L-1</td>
<td>257</td>
<td>341</td>
<td>77.41</td>
</tr>
<tr>
<td>CT-DNA:L-2</td>
<td>259</td>
<td>336</td>
<td>55.73</td>
</tr>
<tr>
<td>CT-DNA:L-3</td>
<td>261</td>
<td>338, 356</td>
<td>35.00</td>
</tr>
</tbody>
</table>

**4.4.2 Circular dichroism spectroscopy**

Circular dichroism spectral technique gives useful information on how the conformation of DNA is influenced by the binding of the drug. It detects changes in DNA morphology during drug-DNA interactions. This is because positive band due to base stacking (275 nm) and negative one due to right-handed helicity (246 nm) are quite sensitive to the mode of DNA interactions with small molecules. Circular dichroism technique is very profound method to analyse the structure of optically active materials such as proteins and DNA. The changes in CD signals of DNA observed on interaction with drugs may
often be assigned to the corresponding changes in DNA structure. The observed CD spectrum of calf thymus DNA consists of a positive band at 275 nm due to base stacking and a negative band at 245 nm due to helicity, which is characteristic of right-handed B form DNA. The effect of ligand-1, 2 & 3 on the conformation of the secondary structure of DNA was studied by keeping the concentration of CT-DNA at 15 µM while varying the concentration of drugs. (DNA:drug = 1.00:0, 1.00:0.25, 1.00:0.50, 1.00:1.00). There was clear change in CD spectra of CT-DNA on addition of ligand 1-3.

In titration experiments, successive addition of ligand-1 to CT-DNA showed change in intensity in both positive and negative side (figure-4.4). The ellipticity value of DNA increased in the range 270-290 nm and decreased at around 240 nm. These results suggested that change of the stacking style or orientation of the base pairs of DNA occurred. Meanwhile, change in the negative band of CT-DNA, suggesting that helical character of DNA was changing after binding. This type of change reflected the shift from B-like DNA structure toward one with some contributions from an A-like conformation. This phenomenon is due to groove binding that stabilise the right handed B-form of DNA. An increase in the positive band at 275 nm was due to the alterations induced in the DNA structure. Based on experimental results and previously published we concluded that L-1 was intercalating to the DNA without altering its basic nature.

For ligand-2 (figure-4.8), on gradual addition of L-2 into fix concentration of DNA, CD spectra exhibited clear change in both positive and negative region of DNA similar to L-1. This indicated that positive band increased slightly without any changes in their wave length. The enhancement of the CD band at 275 nm was due to the partial distortions induced in the DNA. Increase in CD signal at around 275 nm along with the increasing L-2 concentration is indicative of interaction for the intercalation of quinoline moiety with DNA base pairs, which concurs with the data reported. At the same time, decrease in the negative band at same wavelength is believed to be decrease in the helicity of CT-DNA without changing the basic nature. This change was mainly because of the interaction between quinoline ring and the base pairs of DNA and consistent with the earlier reports.
Figure-4.7: Circular dichroism spectra of CT-DNA in absence ($C_{CT-DNA}:C_L=1.0:0.0$) and presence ($C_{CT-DNA}:C_L=1.0:0.25-1.0$) of L-1 (288 K, TEN buffer, pH=8.0).

Figure-4.8: Circular dichroism spectra of CT-DNA in absence ($C_{CT-DNA}:C_L=1.0:0.0$) and presence ($C_{CT-DNA}:C_L=1.0:0.25-1.0$) of L-2 (288 K, TEN buffer, pH=8.0).
On stepwise addition of ligand-3 (figure-4.9), analogous changes in CD spectra observed as reported earlier in the case of L-1 and L-2. Gradual addition of ligand-3 into fix concentration of CT-DNA changed ellipticity both in positive and negative region. Circular dichroism studies indicated that positive band increased at around 275 nm on simultaneous increase of ligand concentration but this change is smaller as compared to L-1 and L-2. Further, change in negative band at around 245 nm is also visible which indicated that that L-3 intercalated with CT-DNA. Conformation changes in DNA due to the addition of ligands causes orientation of the DNA bases and results the interaction of ligands. These results are in agreement with the UV spectroscopic studies.

![Circular dichroism spectra of CT-DNA](image)

**Figure-4.9:** Circular dichroism spectra of CT-DNA in absence (C<sub>CT-DNA</sub>:C<sub>L</sub>=1.0:0.0) and presence (C<sub>CT-DNA</sub>:C<sub>L</sub>=1.0:0.25-1.0) of L-3 (288 K, TEN buffer, pH=8.0).

### 4.4.3 Thermal denaturation analyses

DNA melting or DNA denaturation is the process in which double stranded deoxyribonucleic acid (dsDNA) cooperatively unwinds and separates into single stranded deoxyribonucleic acid (ssDNA) through the breaking of hydrogen bonding between base pairs.\[^{46}\] It has been known since the 1950s, that heating a DNA solution
above room temperature results in the separation of strands. The temperature at which half of the DNA molecule is denatured, i.e. one half is in double helical form and the other half in a random coil state, is termed as the melting temperature (Tm) of the DNA.[47] The consequences of adduct formation on the stability of the double helix in CT-DNA is generally assayed by recording the DNA melting profiles. Cytosine-guanine base pairing is generally stronger than adenosine/thymine thus, higher Tm is associated with GC content.[48]

DNA melting occurs over a small range of temperature and alters its physical properties.[49] Generally, DNA absorbs maximum UV light at a wavelength of around 260 nm. When the temperature of solution containing dsDNA is raised, the absorbance at 260 nm remarkably increases. This is because double helical DNA absorbs lesser UV light by about 40% that the individual strand. The hypochromicity is due to the DNA base stacking which reduces the capacity of bases in DNA duplex to absorb UV light. The melting temperature of DNA depends on length of DNA,[50,51] the nucleotide sequence composition,[52,53] salt concentration (ionic strength of the added salt)[54] and generally lies between 50 °C-100 °C. Several other methods that can also be employed to denature DNA apart from heating are use of organic solvents such as formamide,[55] ligands,[56] pH of the solution, salt concentration[57] etc. Thermal response of DNA in presence of foreign molecules can give insight into their conformational changes when temperature is raised and offer information about the binding strength of drugs with DNA. Stabilization of calf thymus DNA through hydrogen bonding and electrostatic interactions are assayed by measuring the melting temperature. In the continuation of our work to determine the extent of binding mode of three ligands with CT-DNA we further measured the change in absorbance at around 260 nm as a function of temperature in absence and presence of L-1, L-2 & L-3 and compared their binding affinity with CT-DNA keeping DNA:ligand ratio 1:0 and 1:1 (figure-4.10). The values of Tm for DNA-ligand were determined by monitoring the absorbance of the system as a function of temperature ranged from 30-90 °C. For each monitored transition, the Tm of the assay solution was determined as the transition midpoint of the melting curves.
Figure 4.10: The melting curves of calf thymus DNA in absence (A) \([C_{\text{CT-DNA}}=15 \, \mu\text{M}]\) and presence (B, C & D) of ligands L-1, L-2 and L-3 \([C_L=15 \, \mu\text{M}]\) (TEN buffer, pH=8.0).

Figure 4.11: Fraction unfolded of calf thymus DNA in absence (A) \([C_{\text{CT-DNA}}=15 \, \mu\text{M}]\) and presence (B, C &D) of ligands L-1, L-2 and L-3 \([C_L=15 \, \mu\text{M}]\) (TEN buffer, pH=8.0).
The denaturation temperature of CT-DNA was found 66.2 °C in our experimental conditions. After addition of ligand-1, 2 and 3 Tm value increased from 66.2 °C to 67.9, 68.3 & 68.9 °C for CT-DNA: L-3, CT-DNA: L-2 & CT-DNA: L-1, respectively. It is well reported that, when temperature of DNA containing solution increases, the double stranded DNA gradually dissociates to single strands and generates a hyperchromic effect on the absorption spectra of DNA bases. This is quite noticeable from the Tm analysis that all three quinoline derivatives are intercalating with DNA on addition but minor groove binding is also an important factor under considerations. These values indicate that binding of L-1 is most profound in all three ligands. Moreover, all three ligands interact with double stranded CT-DNA and changes into ssDNA as can be seen by increase in the absorbance and Tm of CT-DNA. It is also clear from the figure-4.11 that the conversion of dsDNA to ssDNA is more profound in L-1 while least in L-3.

4.5 Conclusion

It can be concluded that binding of quinoline derivatives to DNA resulted in significant change in the structure and conformation of DNA. These ligands act as intercalator and results increase in the stability of DNA by raising Tm, increase in absorbance and alteration of DNA conformation. The absorption spectrum of DNA reveals higher degree of hyperchromism. Hyperchromism is a characteristic feature which is due to the interaction between aromatic chromophores of quinoline derivatives and base pairs of CT-DNA. CD spectrum of DNA in the presence of synthetic quinoline derivative show that both the negative and positive peaks change, but the change of the positive peak is bigger than that of the negative peak when small molecule is added into the DNA system.

These results suggest that quinoline derivatives can change the helicity of DNA and then induce the alteration of non-planar and tilted orientations of DNA bases, resulting in the changes of DNA base destacking and act as an intercalator. Binding of a small molecule to DNA is assumed to stabilize the helix against its thermal denaturation and the usual mark of stabilization is a rise in the transition temperature, Tm, for the double- to single-stranded form morphing of DNA. Experimentally, this is
accomplished by comparing $T_m$ for DNA in solution with and without the intercalator while monitoring some property dependent on the DNA helix. Due to the difference between the extinction coefficients of DNA bases in the double stranded form versus the single-stranded form at 260 nm, the absorbance increases sharply at $T_m$ as the DNA strands separate. DNA melting experiments reveals the helix stability as a result of intercalation of ligands into DNA. All the results exhibit the remarkable variation in the binding of ligands with CT-DNA.

Thus more ligands should be studied using CT-DNA which will give an idea how does these synthetic molecules interact in biological systems. This understanding may help to redesign new molecules for drug discovery.
4.6 References


