FLUORESCENCE AND DOCKING STUDIES ON THE BINDING OF COPPER AND COBALT COMPLEXES TO DNA AND RNA

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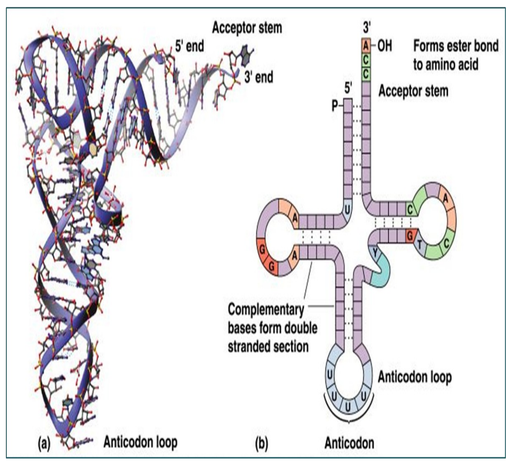
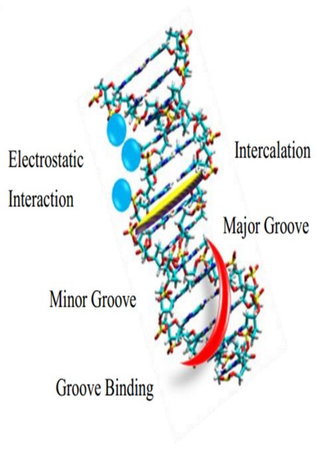
INTRODUCTION

- **DNA and RNA is highly structured, but generally considered to be an accessible target.**
- **DNA repair systems are available in the cell, whereas analogous enzymes for RNA repair are virtually unknown.**
- **DNA and RNA structures have unique binding pockets for small molecules, and its structural diversity could be exploited to design small molecules that can specifically be targeted to DNA/RNAs of particular interest.**



Secondary and tertiary structure of tRNA demonstrating



Structure of DNA

OBJECTIVE

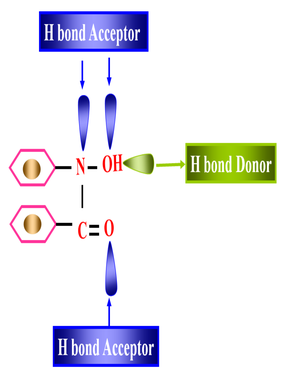
Investigation of interaction of Cu-NMBHA and Co-CBBHA to DNA/RNA by Fluorescence Spectroscopy, Viscosity measurement and Molecular Docking.

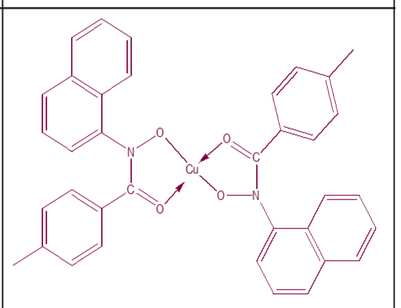
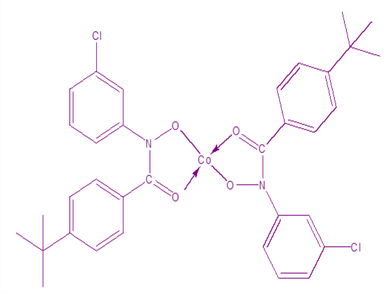
EXPERIMENTS

Hydroxamic acid molecules attract considerable attention because of their features namely-

DRUG LIKE molecule as it follows Lipinski's rule of five.

- **Neutral, polyfunctional molecules.**
- **Consists of pharmacological functionality.**
- **It shows both HBA and HBD capability.**



S.NO	HYDROXAMIC ACID	MOLECULAR WEIGHT	STRUCTURE
1	Cu-N-m-naphthyl-p-methyl-benzohydroxamic acid	616.16	
2	Co-N-m-chlorophenyl-p-tert-butylbenzohydroxamic acid	664.48	

SOLUTION OF HYDROXAMIC ACID

The stock solutions of hydroxamic acid, (0.01M) were prepared in DMSO and used further of various concentrations as obtained by mass dilution technique. The final concentration of hydroxamic acid was prepared in Citrate-phosphate buffer.

SOLUTION OF DNA/RNA

The stock solution of DNA was prepared in Tris-HCl buffer and RNA solution is prepared in citrate-phosphate buffer and used further of various concentration.

RESULT AND DISCUSSION

FLUORESCENCE METHOD

- Fluorescence emission spectra of hydroxamic acid carried out in Cary Varian Spectrofluoremeter.
- Fluorescence quenching is decrease in fluorescence intensity of luminescent species with interaction to other species.
- Binding constant Ksv were calculated by Stern—Volmer Equation.

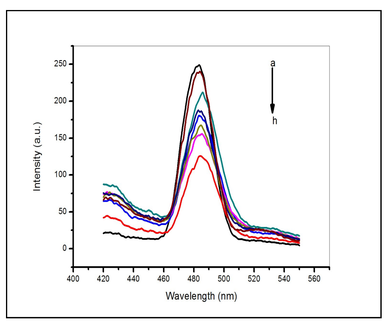
$$F_0/F = 1 + K_{SV}(Q)$$

where,

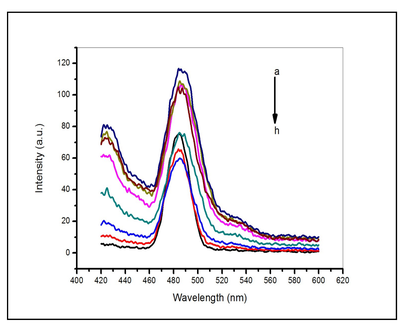
F_0 = the fluorescence intensities of the PBHA with t-RNA in the absence of quencher.

F = the fluorescence intensities of the PBHA with t-RNA in the presence of quencher.

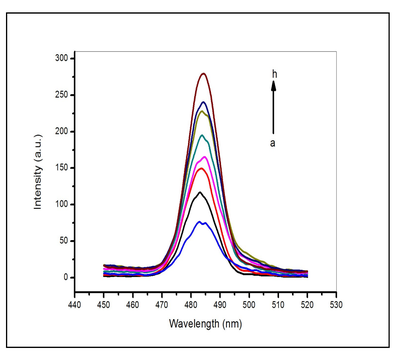
K_{sv} = the Stern-Volmer quenching constant, which is the measure of efficiency of quencher.



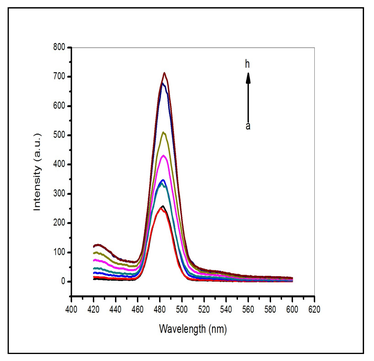
Fluorescence Emission Spectra and Stern-Volmer plot between Cu-NMBHA and DNA



Fluorescence Emission Spectra and Stern-Volmer plot between Cu-NMBHA and RNA



Fluorescence Emission Spectra and Stern-Volmer plot between Co-CBBHA and DNA



Fluorescence Emission Spectra and Stern-Volmer plot between Co-CBBHA and RNA

STERN-VOLMER CONSTANT	K_{sv} M^{-1}
Cu-NMBHA-DNA	$4.9\ 2 \pm 0.12 \times 10^3$
Cu-NMBHA-RNA	$4.61 \pm 0.07 \times 10^3$
Co-CBBHA-DNA	$3.57 \pm 0.40 \times 10^2$
Co-CBBHA-RNA	$3.36 \pm 0.06 \times 10^3$

COMPETITIVE BINDING BETWEEN ETHIDIUM BROMIDE - RNA WITH N-PHENYLBENZOHYDROXAMIC ACID

- Ethidium bromide is employed in the examination of the reaction, presumably binds initially to DNA/RNA through intercalation mode.
- This method is also used to elaborate binding mode.
- Like EtBr, if hydroxamic acid intercalate into the helix of DNA/RNA, it would compete with EtBr for the intercalation sites in DNA/RNA, and lead to a significant decrease in the fluorescence intensity of the DNA/RNA–EtBr complex.

VISCOMETRIC METHOD

In general, intercalation caused an increase in the viscosity of RNA solution due to lengthening of RNA helix as the base pair are pushed apart, and very little effect on the viscosity of RNA, if the electrostatic or groove surface binding occurs. The viscosity of PBHA-RNA complexes are obtained by the expression,

$$\eta'_{sp} / \eta_{sp} = [(t_{complex} - t_0)/ t_0] / [t_{control} - t_0 / t_0]$$

where,

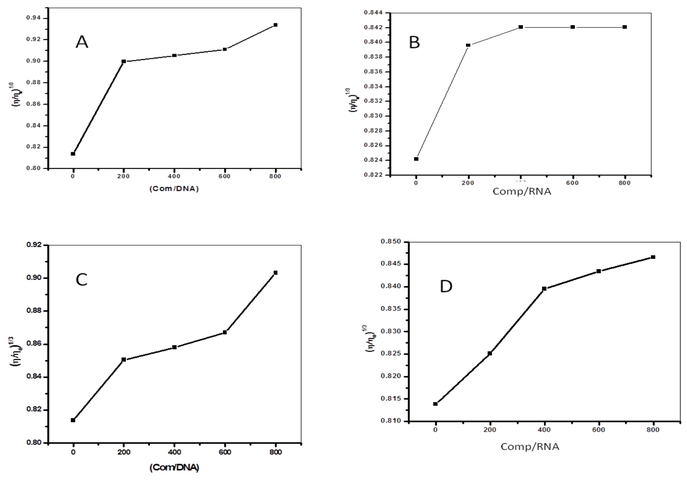
η'_{sp} = the specific viscosity of t-RNA in the presence of the ligand.

η_{sp} = the specific viscosity of t-RNA in the absence of the ligand .

$t_{complex}$ = the average efflux times of complex.

$t_{control}$ = the average efflux times of RNA.

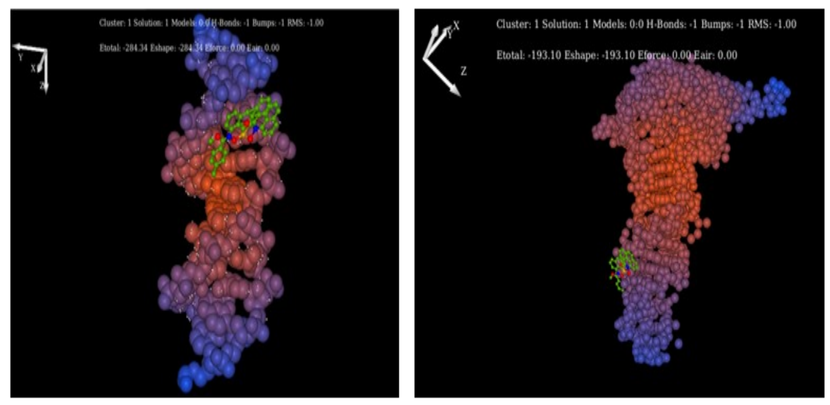
t_0 is the same for the buffer as described previously



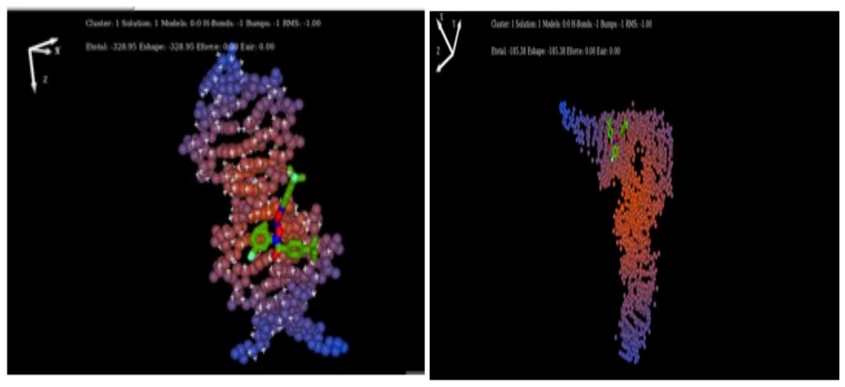
Graph plotted between $(\eta/\eta_0)^{1/3}$ versus (A)[Cu-NMBHA/DNA] (B) Cu-NMBHA/RNA (C) Co-CBBHA /DNA (D) Co-CBBHA /RNA

MOLECULAR DOCKING

Hex 8.0 cuda, was used to calculate DNA/RNA- hydroxamic acid docking assuming ligand as rigid.



Molecular docked structure of Cu-NMBHA with DNA & RNA



Molecular docked structure of Co-CBBHA with DNA & RNA

BINDING ENERGY	E value eV
Cu-NMBHA-DNA	-284.34
Cu-NMBHA-RNA	-193.10
Co-CBBHA-DNA	-328.95
Co-CBBHA-RNA	-385.35

CONCLUSION

Fluorescence quenching spectra revealed strong binding of Cu-NMBHA and Co-CBBHA to DNA/RNA.

EtBr displacement shows the decrease in emission intensity for both the hydroxamic acids. The relative viscosities of hydroxamic acid-DNA/RNA complexes have increased value as compared to DNA/RNA alone.

The docked posture of DNA/RNA with Cu-NMBHA and Co-CBBHA reveals the strong binding interactions as it has smaller value of binding energy. All the experimental evidences indicate that Cu-NMBHA and Co-CBBHA can strongly bind to DNA/RNA.

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