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Interaction between Human Holo-Transferrin and an Antibiotic Drug Ciprofloxacin

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Abstract: The mechanism of interaction between Human Holo-Transferrin (HHT) and an antibiotic drug ciprofloxacin (CIP) has been investigated using fluorescence, absorption spectroscopy.
The quenching mechanism of fluorescence of Human Holo-Transferrin by Ciprofloxacin was discussed. The binding sites number n and apparent binding constant K were measured by fluorescence quenching method. The results of Fluorescence spectra, Synchronous fluorescence spectra and UV/Vis absorption spectra of HHT in the presence of Ciprofloxacin were recorded and discussed.
Keywords: Human Holo-Transferrin, Ciprofloxacin, Fluorescence, UV/Vis absorption.

I. INTRODUCTION

Human Holo-Transferrin (HHT) is a member of the transferrin family that function as iron-binding and iron transport protein. HHT has been detected in various body fluids including plasma, bile, amniotic, celebrospinal and lymph fluids and also in breastmilk [1-4]. The fundamental biochemical function of transferrins is nothing but the transport of iron in the blood via reversible binding of metal iron. Transferrin may serve for specific targeting of molecules of interest for cancer cells and for the brain and several investigation on the ability of transferrins to target tumors were published [5-7]. In addition, transferrins act as iron transferrins act as iron transporters in the blood stream of vertebrates [8].
Ciprofloxacin is one of the most frequently prescribed antibiotics to treat infections of the respiratory or urinary tract, skin and soft skin [9]. Fluorescence Spectroscopy has been provide qualitative and quantitative information on the protein-ligand interactions.
In the present work, we made a special attempt to examine the binding mechanism of an antibiotic drug, Ciprofloxacin to Human Holo Transferrin (HHT) by means of fluorescence Spectroscopy, Synchronous fluorescence and UV-Visible absorption spectroscopy under simulated Physiological condition.

II. MATERIALS AND METHODS

Human Holo transferrin (HHT) and Ciprofloxacin were purchased from Sigma Aldrich Company, Bangalore. They were used without further purification.
Fluorescence spectra and intensities were recorded on a SHIMADZU RF 5301 PC SPECTROFLUOROPHOTOMETER equipped with a1cm quartz cell and a Xenon lamp.
UV Spectroscopy was performed on SHIMADZU 1800 PC UV-VISIBLE SPECTROPHOTOMETER linked with a personal computer with a quartz cuvette with a 1cm path length

III. RESULTS AND DISCUSSIONS

A. Fluorescence quenching mechanism:

The fluorescence technique is an important tool for the study of interaction between proteins and small probe molecules [10]. The degree of fluorescence quenching suggested that there are change in the protein structure and in the micro environment protein fluorescence is generally excited at the absorption maximum near 280 nm or at longer.
The fluorescence spectra of HHT with different concentrations of Ciprofloxacin at λ_{ex}=278 nm are shown in Fig.1. Ciprofloxacin exhibited native fluorescence at 290-400 nm.
The spectra indicate that, with increasing ciprofloxacin concentration the fluorescence intensity of HHT was decreased and the ciprofloxacin peak increased in a regular manner.

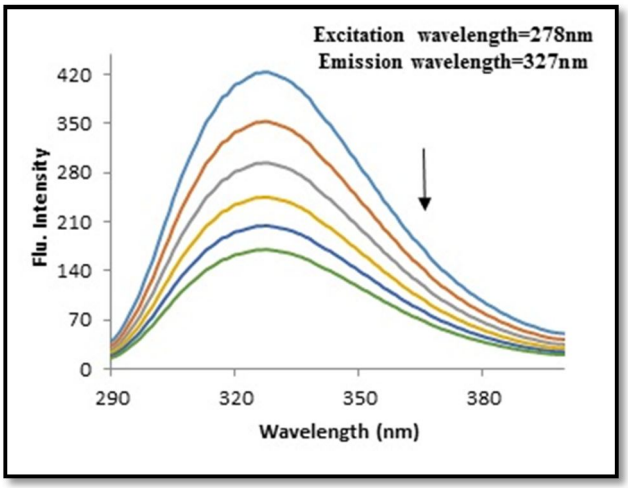

Fig:1 Steady-State fluorescence spectra HHT with Ciprofloxacin

B. Stern-Volmer Quenching Studies

The fluorescence quenching of a protein by small molecules can be static and/or dynamic in nature [11]. The Stern-Volmer equation is used for quenching,

$$\frac{F_0}{F} = 1 + K_q\tau_0[Q] = 1 + K_{SV}[Q] \qquad \text{----------(1)}$$

Where F_0 and F are the fluorescence intensity before and after addition of quencher, respectively. K_{sv} is the quenching constant. Fluorescence quenching behaviour of the protein and Stern-Volmer analysis of the relative fluorescence intensity (F_0/F) as a function of the quencher Concentration [Q] were applied to determine the quenching mechanism and rates Fig.2. Table 1 gives the K_{sv} and K_q values.

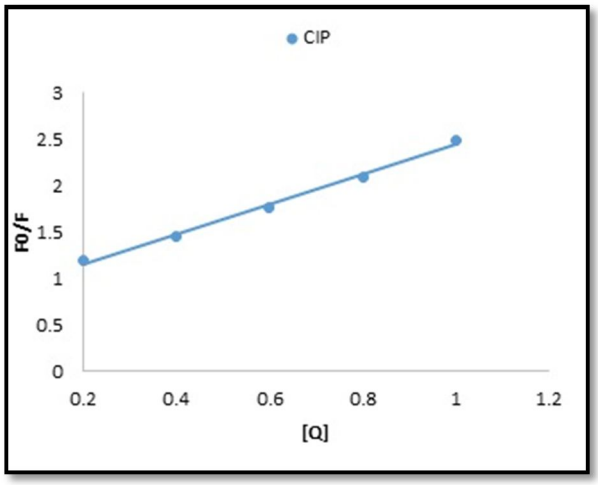

Fig.2 Stern-Volmer plot forfluorescence quenching of HHT

Table:1 Stern – Volmer (K_{SV}) and bimolecular quenching rate constant (K_q) of Holo Transferrin with Ciprofloxacin

Quenchers	K_{SV} x 10^5 (L mol^{-1})	K_q (L mol^1S^{-1})	R[a]	S.D[b]
Ciprofloxacin	1.6166	8.7181 x 10^8	0.995	0.7489

[a]→is the correlation coefficient

[b]→is the Standard Deviation

C. Analysis of Binding Constant

The values of Binding constant (K_a) and number of binding sites (n) are listed in Table.2.

Table:2 The binding constant (Ka), binding site (n) correlation coefficient (R) of HHT with Ciprofloxacin.

Quenchers	K_a x 10^6 (Lmol-1)	n	R	ΔGg (KJ mol-1)	ΔGe (KJ mol-1)
Ciprofloxacin	14.5077	1.2411	0.9976	259.72	14318.55

D. Characteristics Or Synchronous Fluorescence Spectra

Synchronous fluorescence spectroscopy provides information about the molecular environment and has severed advantages, such as sensitivity, Spectral simplification, spectral bandwidth reduction and the possibility to avoid various perturbing effects.

Figs 3 & 4 present the original peaks for the system when Δλ=20 & 40 nm. As we can see, an increase in ciprofloxacin concentration caused the fluorescence intensity to decrease in all system. These spectra do not show any shift in emission maxima.

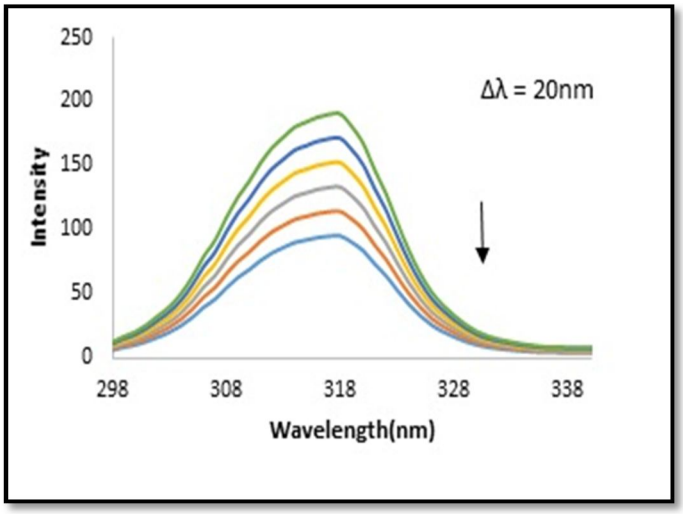

Fig :3 Synchronous Fluorescence Spectra for HHT with Ciprofloxacin having Δλ = 20nm

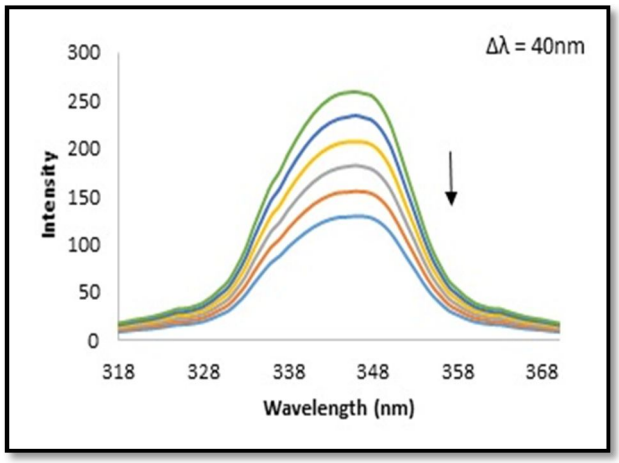

Fig :4 Synchronous Fluorescence Spectra for HHT with Ciprofloxacin having Δλ = 40nm

E. UV-Visible absorption spectra analysis

The UV-Visible absorption spectroscopy technique can be used to explore the structural change of protein and to investigate protein-ligand complex formation [12]. Fig.5 shows the UV/Visible absorption spectra of HHT without and with ciprofloxacin when the concentration of ciprofloxacin increases the absorption intensity also increases.

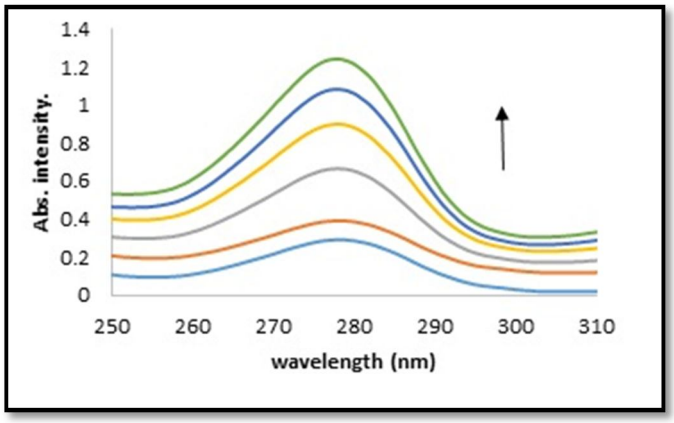

Fig:5 UV/Visible Absorption spectra of HHT with Ciprofloxacin.

IV. CONCLUSION

In this study, the effect of Ciprofloxacin on Human Holo Transferrin were characterized by fluorescence spectroscopy, Synchronous fluorescence Spectroscopy and UV/Visible absorption spectroscopy.

Binding parameters and number of binding sites were calculated. It can be proved that the binding affinity is higher here.

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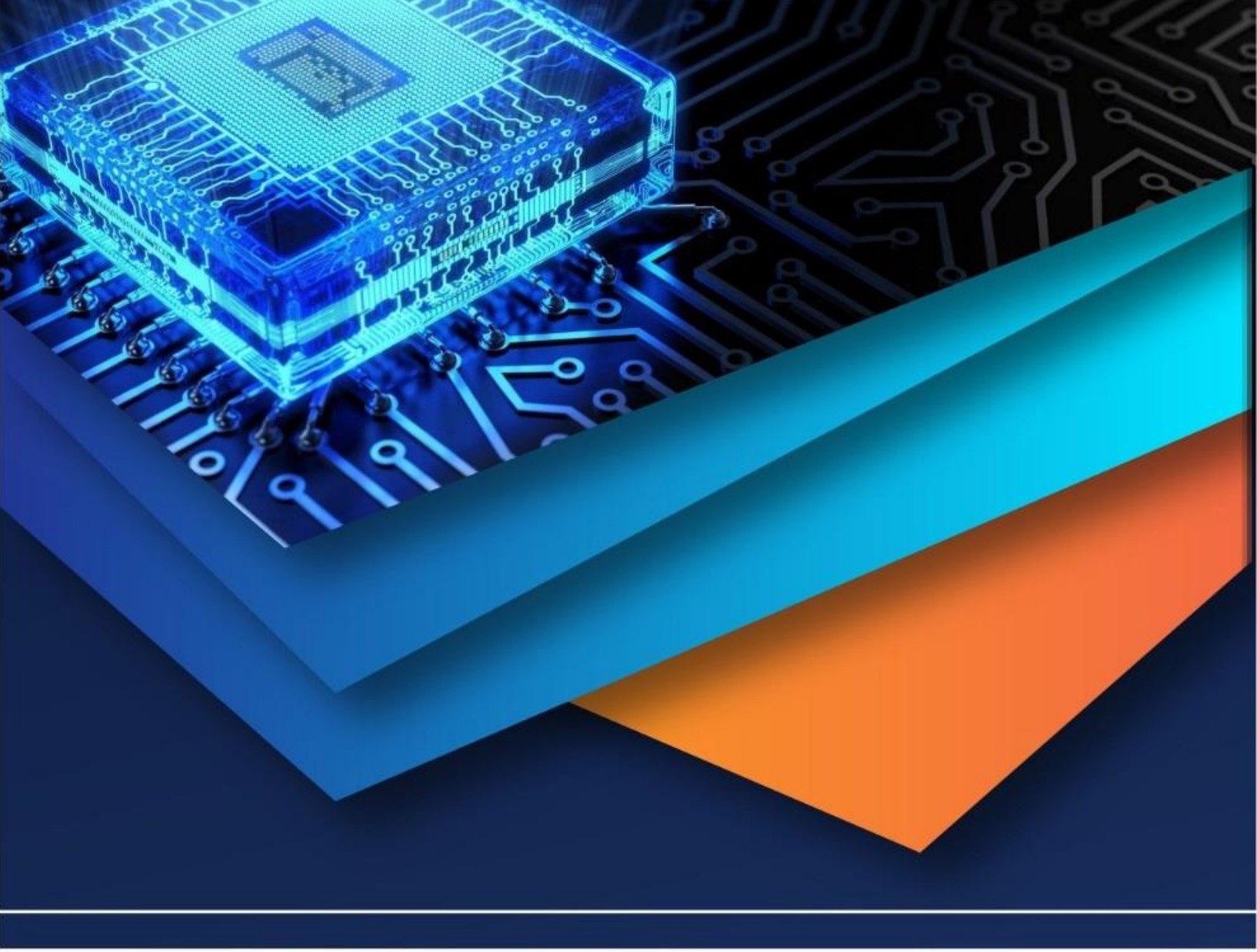

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