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Probing the Binding of Two Antibiotics to Human – Holo Transferrin: A Combined Spectroscopic and Docking study.

S. Bakkialakshmi*, T.Anupriya
Department of Physics, Annamalai University, Annamalai Nagar, Tamilnadu, India-608 002

ABSTRACT

Ciprofloxacin (CPFX) and Tetracycline (TC) are two of the most widely used antibiotics in human and veterinary medicines. Their existence in the environment has experienced much attention because of the potential adverse effects on humans and ecosystem functions. In this paper, the interaction mechanism between the two antibiotics and Human holo transferrin by spectroscopic and molecular docking methods. The distance r, between the antibiotics and the protein was obtained according to FRET which pointed at a successful formation of a drug – protein complex. Molecular docking study provided possible binding sites of antibiotics within the proteins. The obtained data can be convenient for determining usage drug doses in drug delivery.

Keywords: Human holo transferrin, ciprofloxacin, Tetracycline, FRET, Molecular Docking.

*Corresponding Author Email: bakkialakshmis@rocketmail.com
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INTRODUCTION

The binding of drugs to human serum proteins is especially important as this may affect both the drug activities and their disposition [1]. Consequently, knowledge of drug binding to plasma proteins (such as human serum albumin and transferrin) is essential in order to understand the pharmacodynamics and pharmacokinetics of drugs [2].

Transferrins are a family of bilobal iron – binding proteins that share a closely related three – dimensional fold and exist in the blood and other bodily secretions as well as in avian egg white that controls the levels of iron in the bodies of vertebrates [3]. Human Holo transferrin (HHT) (HHT) is the main Fe^{3+} transport protein in human serum. It is capable of a compact reversible binding with two equivalents of Fe^{3+} has been shown to bind a wide variety of other metal ions [4]. The HHT protein contains 679 amino acid residues and has a molecular weight of ~ 79 KD. It is stabilized by 19 intra – chain disulfide bonds [5]. HHT Composed of two homologous lobes, termed the N – and C - lobe. Each lobe, in turn, comprises two domains (N_1 and N_2 and C_1 and C_2) that are connected by a flexible hinge, and each lobe can independently bind to a Fe^{3+} ion. The highly specific binding site for Fe^{3+} is created when the domains of a lobe are closed around iron (holo transferrin), whereas iron release requires the two domains to open up [6].

The introduction of antibiotics more than 20 years ago given clinicians with a range of antibacterial agents that have broad spectrum of activity to act against both Gram – negative and Gram – positive bacteria [7]. Ciprofloxacin (CPFX) and Tetracycline (TC) (structures shown in Fig 1) which belong to the group of antibiotics, are frequently used in many human and veterinary applications [8,9]. However, due to the deficient metabolism and the relative ineffectiveness of conventional water treatment technologies in removing them [10], they have recently been observed in waste waters, surface and ground water and in drinking water as well [11-13]. Treatment of antibiotics in animals could also induce some adverse effects like fatal liver failure, renal dysfunction and embryo toxicity to humans [14 -16].

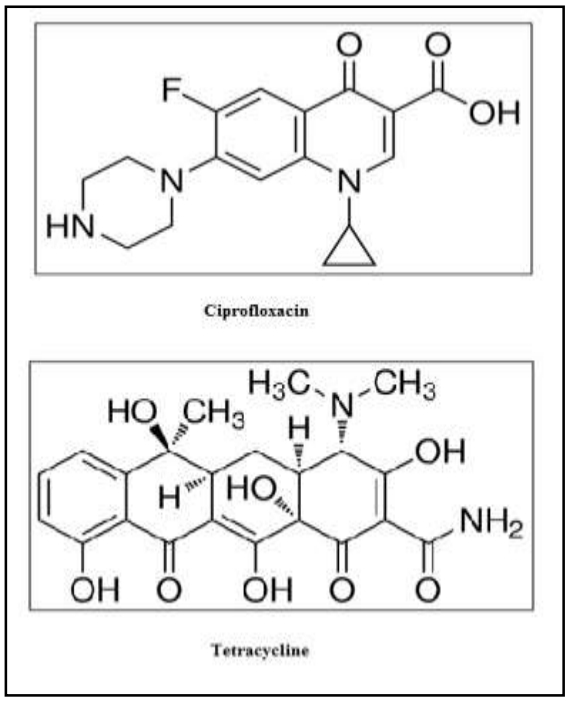

Figure 1: **Molecular structure of Ciprofloxacin and Tetracycline**

MATERIALS AND METHOD

Human holo Transferrin and the two antibiotics (Ciprofloxacin and Tetracycline) were purchased from Sigma-Aldrich. Absorption spectra were recorded on SHIMADZU 1800 PC UV-VISIBLE SPECTROPHOTOMETER at the temperature 25^0 C (a slit 2 nm). Fluorescence emission spectra were registered with the use of SHIMADZU RF 5301 PCSPECTROFLUORO PHOTOMETER at the temperature 25^0 C (excitation and emission slits at 5 nm)

The docking calculations of the association of fluoroquinolones with HHT, were undertaken using the Auto dock 4 programme. The crystal structure of HHT, HHT-CPFX and HHT-TC complex were retrieved from the RCSB Protein Data Bank (1suv). The best docking results were applied to Weblab-Viewer lite.

RESULTS AND DISCUSSION:

Effect of solvents on UV/vis absorption and fluorescence spectra of HHT.

Fig. 2 shows the absorption spectra of HHT recorded in eleven different solvents. The absorption has good extension of the visible region in all the different solvents under study. From Fig. 2, it could be noted that HHT is in all the investigated solvents a strong and intended absorption band in the 225 nm – 280 nm wavelength region. The spectra in hexan-lol and cyclohexane are structured; a loss of the vibration free structure was observed in more polar solvents. Appreciable change was detected in energy transition in various solvents which suggests that

solvent stabilization of the ground state species is significant. the absorption spectrum is very broad for all the solvents and there is presence of more than one isomeric form in the ground state [17,18].

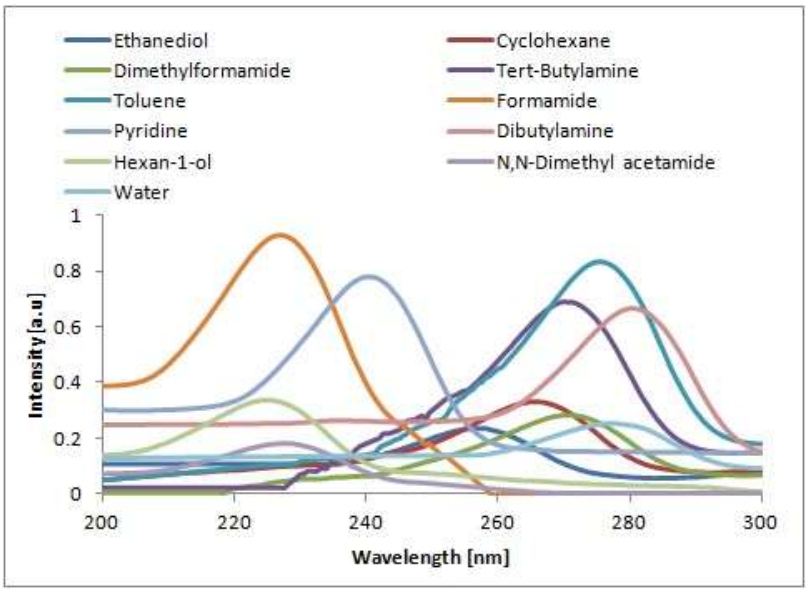

Figure 2: UV/Vis absorption spectra of HHT with Different Solvents.

HHT is practically insoluble in water at neutral and medium acidic pH, while soluble in both polar and non-polar organic solvents. From Table 1, it could be noted that the absorbance of HHT is strongly dependent on solvent polarity.

Fig.3 demonstrates the significant solvent dependent shifts in emission maxima; HHT depicts structured emission in non-polar solvents, but in other solvents of moderate and high polarity it shows broad and structural emission. Absorption and emission wavelengths are presented in Table 1 along with stokes shift values, Energy (E), Ionization Potential (ID), Electron Affinity (EA), Molar Extinction coefficient (ε) and the solvent parameter (z). the calculated values are presented in Table 1. There are remarkable changes in stokes shift values in different solvents.

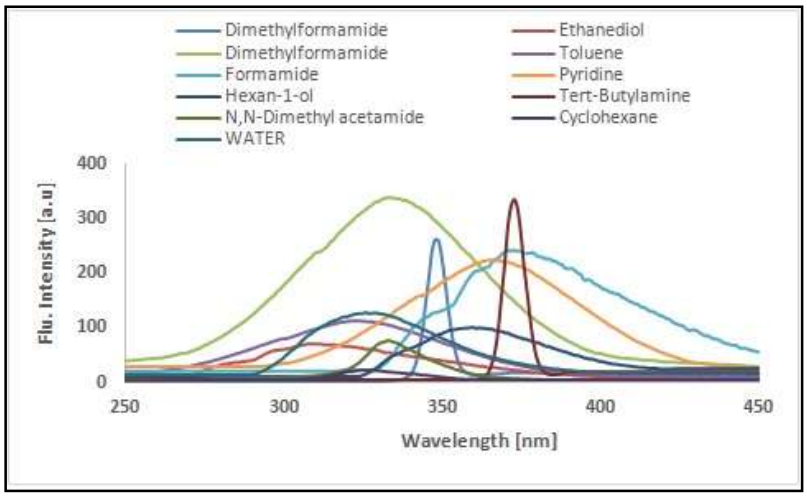

Figure 3 Fluorescence emission spectra of HHT With Different Solvents

Table 1: Absorption and emission wavelength, Ionization potential, Electron Affinities of charge transfer complex, molar extinction co-efficient [log ε], Solvent parameter [Z] and Stoke's shift [cm^{-1}] of Human Holo Transferrin in different solvents.

Solvents	λ_{abs} [nm]	λ_{ems} [nm]	Energy[E]	Ionization potential [eV]	Electron Affinity [eV]	log ε [$M^{-1}cm^{-1}$]	Z X 10^{-6} [nm]	Dielectric constant [ε]	Refractive index [n]	Orientation polarizability [Δf]	Stoke's shift [cm^{-1}]
Ethanediol	257	308	4.8340	11.2925	-0.7438	-3.5900	1.1124	41.4	1.4318	0.1551	6442.97
Dimethyl formamide	269.5	333	4.6096	11.012	-0.5139	-3.5694	1.0608	36.7	1.4305	0.1547	7075.72
Toluene	275	324	4.5176	10.897	-0.4196	-3.5606	1.0396	2.38	1.4967	0.0106	5499.43
Formamide	227	371	5.4726	12.0907	-1.3981	-3.6439	1.2594	111	1.4475	0.1544	17098.68
Pyridine	240	363	5.1765	11.7206	-1.0947	-3.6197	1.1912	12.4	1.5102	0.1193	14118.43
Dibutylamine	280	349	4.4369	10.7961	-0.3369	-3.5528	1.0210	3.20	1.415	0.0738	7060.99
Hexan-1-ol	225	360	5.5215	10.7715	-1.4482	-3.6478	1.2706	13.06	1.4178	0.1456	16666.66
Tert-Butylamine	270	373	11.7794	19.9742	-7.8600	-3.5686	1.0588	4.31	1.377	0.1134	10227.38
N,N-Dimethyl acetamide	227	333	5.4726	12.0907	-1.3981	-3.6439	1.2594	37.8	1.4384	0.1526	14022.83
Cyclohexane	266	325	4.6705	11.0881	-0.5763	-3.5751	1.0748	1.9	1.426	0.0155	6824.75
Water	278	327	4.4688	10.836	-0.3696	-3.559	1.0284	80.1	1.33	0.1938	5390.18

Fig. 4. depicts the plot of stoke's shift versus fluorescence intensity of HHT in different solvents. To comprehend the polarity effect of HHT in various solvents, solvent dependent spectral shifts were investigated. The lippert-Mataga equation [19-22] shows that solvent dependence of stoke's shift for a compound depends on change in the dipole moment of the fluorescence moiety upon excitation, the dielectric constant, and the refractive index of the solvents being used [19-25].

$$\overline{\nu_A}-\overline{\nu_F}= \frac{2}{hc}\left(\frac{\varepsilon-1}{2\varepsilon-1}-\frac{n^2-1}{2n^2+1}\right)\left(\frac{\mu_E-\mu_a}{a^3}\right)^2+\text{constant} \quad \text{-- (1)}$$

where ν_A and ν_F are the wave numbers (cm-1) of the absorbance and fluorescence emission respectively, h the Planck's constant, c the speed of light in a vacuum, 'a' the radius of the cavity in which the fluorosphore resides μ_E and μ_a, the dipole moment in the excited and ground states, respectively, ε and n the dielectric constant and the index of refraction of the solvents, respectively.

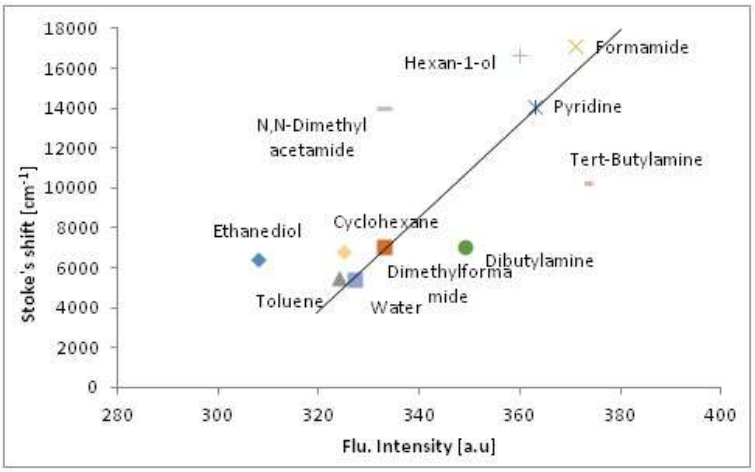

Figure 4: Plot of Stoke's shift versus fluorescence intensity of HHT

The Lippert-Mataga plot can be obtained by plotting stoke's shift versus the term in the brackets in the above equation referred to, as the orientation polarizability (Δf) of the solvent, which is the result of both the mobility of the electrons in the solvent and the dipole moment of the solvent.

$$\Delta f = \frac{\varepsilon-1}{2\varepsilon-1}-\frac{n^2-1}{2n^2+1} \quad \text{--------- (2)}$$

Fig. 5 represents the Lippert-Mataga plot of HHT in different solvents. Calculated values are tabulated in Table 1.

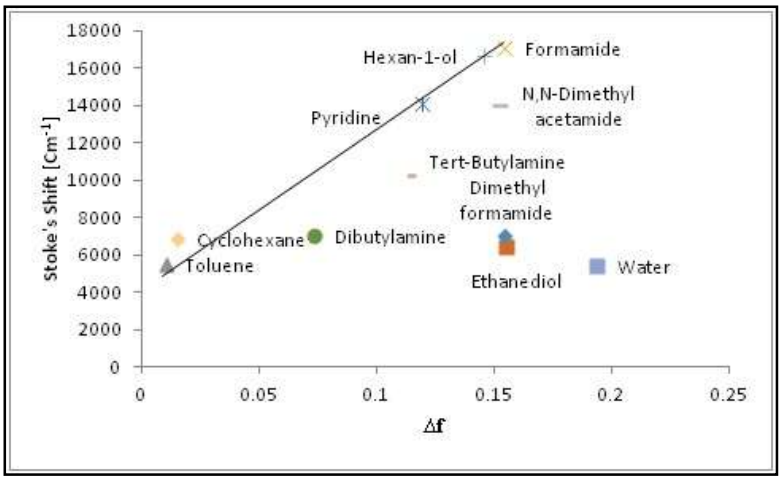

Figure 5: Lippert-Mataga plot of HHT in different solvents

Energy Transfer from HHT to Antibiotics

According to the Forster resonance energy transfer theory, energy transfer occurs under the following conditions [26]. (i) when the donor can produce fluorescence light; (ii) when the fluorescence emission spectrum of the donor and UV absorbance spectrum of the acceptor overlap; and (iii) when the distance of approach between donor and acceptor is lower than 7 nm. According to the Forster's theory, the energy efficiency E is defined according to the following equation:

$$E = 1- (F_0 /F) = R_0^6 /R_0^6 + r^6 \qquad \text{----- (3)}$$

Where F and F_0 are the fluorescence intensities of HHT in the presence and absence of antibiotics, respectively, r is the distance from the ligand to the tryptophan residue of the protein, and R_0 the Forster critical distance, at which 50% of the excitation energy is transferred to the acceptor. It can be calculated from the donor emission and acceptor absorption spectra using the Forster equation:

$$R_0^6 = (8.79 \times 10^{-25}) k^2 n^{-4} \phi J \qquad \text{---------(4)}$$

Where k^2 is the orientation factor related to the geometry of the donor and acceptor of dipoles, N is the average refractive index of the medium in the wavelength range, where spectral overlap is significant, ϕ is the fluorescence quantum yield of the donor, J is the effect of the spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor [26] which can be calculated as follows:

$$J = \frac{F(\lambda)\,\Sigma(\lambda)\,\Delta(\lambda)}{F(\lambda)\;\Delta(\lambda)} \qquad \text{----------(5)}$$

Here, F (λ) is the fluorescence intensity of the donor in the absence of the acceptor at wavelength λ and Σ (λ) is the uv molar absorption coefficient of the acceptor at λ [26]. The parameters regarding the Forster resonance energy transfer are presented in Table 2.

The spectral overlap between the fluorescence emission spectrum of HHT and the UV – Vis absorbance spectrum of CPEX (and TC) are shown in Fig 6. A and B. According to equations 3-4 for HHT- CPFX, Calculation gave J = 1.43 J/cm^3 L mol^{-1}, E = 59.85 , R_0 = 1.813 nm and r = 1.696 nm. The estimated values are listen in Table.2.

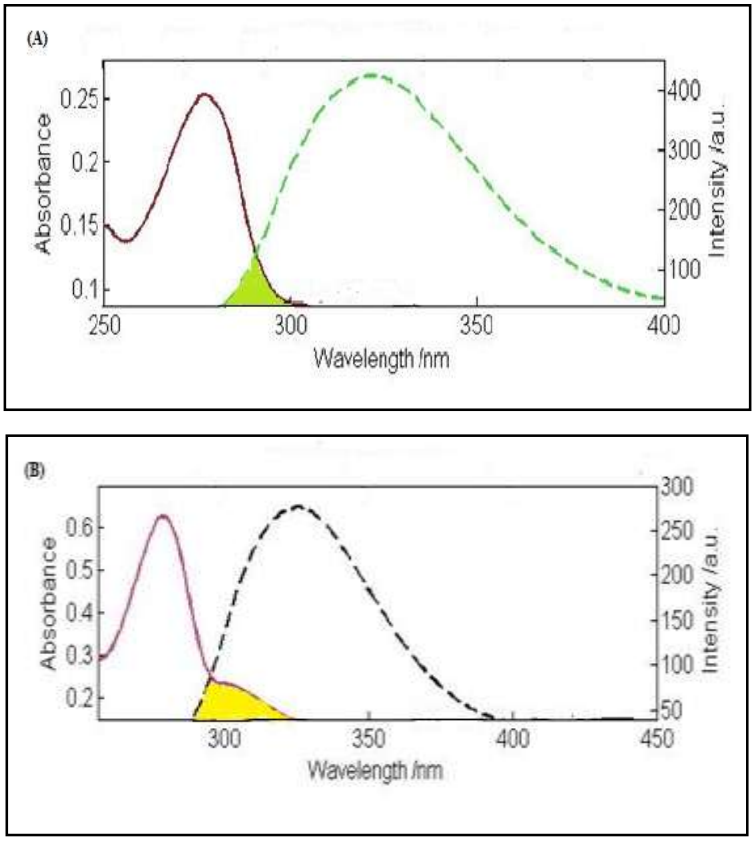

Figure 6 The overlap of UV absorption spectra of HHT (solid line) with the fluorescence emission spectra of Ciprofloxacin (dotted line) (A) and Tetracycline (B).

Table:2 Efficiency transfer energy [E], Critical energy transfer distance [R_o] of Human Holo Transferrin with Antibiotic drugs

Quenchers	Energy [E]	R_o [nm]	J [cm^{3}M^{-1}] [10^{-15}]	R[nm]
Ciprofloxacin	59.85	1.813	1.43	1.696
Tetracycline	68.28	1.733	1.09	1.528

Molecular Dockings:

To further realize the interaction of antibiotics with HHT interaction models were produced using molecular docking techniques. Autodock 4 was used to determine the best docking result. It was found that the inhibiting constant for (HHT + CPFX) is 2.92 μM and for (HHT + TC) is 24.76 Mm. The best docking results for the (HHT+CPFX) and (HHT +TC) are shown in Figs 7 & 8 and the distance was determined from this model. The estimated results were given in Table .3.

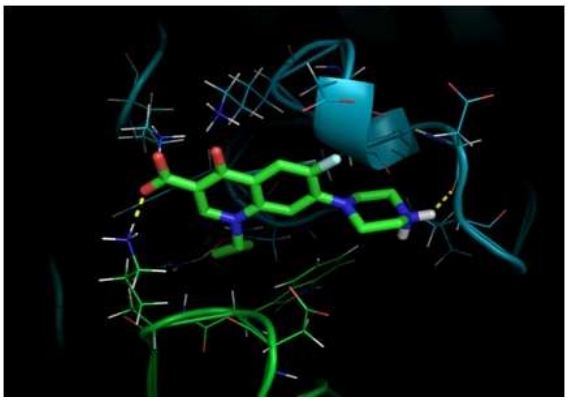

Figure 7 3D view of docking interaction of HHT with Ciprofloxacin

Table 3: Human Holo Transferrin with Ciprofloxacin and Tetracycline

Ligand	Target protein ID	Binding Energy Δ*E* [kcal/mol]	Bonded Residues	Inhibition Constant *Ki* [μM]	RMSD [Å]	H- Bond
Ciprofloxacin Tetracycline	1suv	-7.45 -6.28	ASP245, LYS358 ASN 608, LEU 609, LEU 609 & LEU 607	2.92 24.76	53.843 16.711	2.1, 1.7 2.0,2.0, 2.1 & 2.3

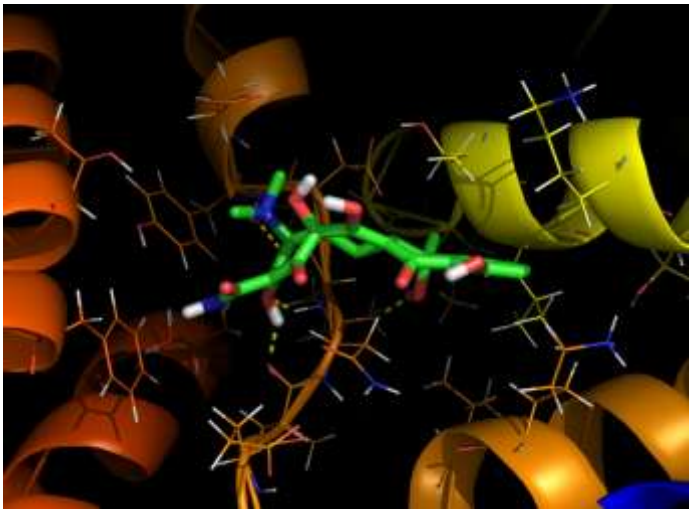

Figure 8 3D view of docking interaction of HHT with Tetracycline

CONCLUSION:

In this study, spectroscopic and molecular docking techniques have been used in order to investigate the binding properties of antibiotics with HHT. The complex formation between HHT with antibiotics could result in an increase in binding affinity of Antibiotics to HHT. In addition, it was shown that there existed one set of independent binding sites for Antibiotics on HHT. In summary, the present study provides useful data pertaining to pharmacology and pharmacodynamics of antibiotics.

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