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A Study on the Interaction of 5-Fluorouracil with Human Serum Albumin using Fluorescence Quenching Method

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Abstract
5-Fluorouracil (5-FU) is extensively metabolized, and several metabolites have been detected in human serum albumin (HSA) with 5-fluorouracil at physiological conditions, using constant protein concentration and various drug contents. UV-Visible and fluorescence quenching method were used to analyze drug binding mode, the binding constant and the drug complexation on HSA. Static quenching was suggested by the fluorescence measurements. Thermodynamic parameters, different conditions including temperature to determine enthalpy change(ΔH) and entropy change(ΔS), indicating the hydrophobic force played a major role in the binding interaction of 5-Fluorouracil with HSA.
Keywords: 5-Fluorouracil, Human serum albumin, fluorescence quenching, Thermodynamic parameters.

1.INTRODUCTION

5-Fluorouracil (5-FU) (first introduced into medicine in 1957) is widely recognized today as effective treatment modalities, especially with tumors of the head, neck, and breast [1,2-4], and in combination drug regimens for cancer chemotherapy. It has been widely used in the treatment of cancer [5,6-8], and it exhibits also an antibacterial activity [9], and augments the bactericidal effect of antibiotics [10]. The other 5-XU have exhibited a weaker antiviral and antibacterial activity than that of 5-FU [11]. The structure 5-FU is shown in Fig. 1.

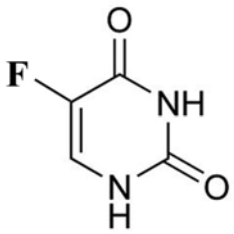

Fig. 1: Structure of 5Fluorouracil

Human serum albumin(HSA) is the most abundant protein constituent of blood plasma and serves as a protein storage component. It is synthesized in the liver, exported as a non-glycosylated protein, and present in the blood at around 40 mg mL^{-1} (~0.6 m Mol L^{-1}). The three-dimensional structure of human serum albumin has been determined through X-ray crystallographic measurement [12,13]. The globular protein consists of a single polypeptide chain of 585 amino acid residues and has many important physiological functions. HAS considerably contributes to colloid osmotic blood pressure and realizes transport and distribution of many molecules and metabolites, such as fatty acids, amino acids, hormones, cations and anions, and many divers drugs [14]. It is composed of three structurally similar domains (I, II and III), each containing two sub domains (A and B), stabilized by 17 disulfide bridges. Aromatic and heterocyclic ligands were found to bind within two hydrophobic pockets in subdomains IIA and IIIA, site I and site II [15]. HSA can interact with many endogenous and exogenous substances including many drugs. Drug interactions at protein binding level will in most case significantly affect the apparent distribution volume of the drugs and also affect the elimination rate of drugs. It is important to study the interaction of drug with the protein because protein-drug binding plays an important role in pharmacology and pharmacodynamics. The information on the interaction of serum albumin and drug can help us better understand the absorption and distribution of the drug.

In this paper, the interaction of 5-FU with HSA was studied under physiological conditions by fluorescence and UV-absorption spectroscopy.

2. MATERIALS AND METHODS

Human serum albumin and 5-fluorouracil were purchased from Sigma-Aldrich Chemical Company, Bangalore. Double distilled water was used throughout the experiments.

2.1 Absorption measurements

The absorption spectra were recorded on **Perkin elmer lamda 25 uv-visible spectrometer.**

2.2 Steady-state fluorescence measurements

The steady state fluorescence spectra were recorded **on Varian cary eclipse fluorescence spectrophotometer.** The concentration of HSA in all experiments were made as with 1.0 x 10^{-5} mol L^{-1}. The concentration of quencher (5-FU) were 2.0 x 10^{-4}mol L^{-1}.

3. RESULTS AND DISCUSSION

3.1 UV-Vis absorption spectroscopy

UV-Vis absorption measurements is a simple but effective method in detecting complex formation [16]. Complex formed of 5-FU with HSA was evident from the data of UV-Vis absorption spectra (Fig. 2). It is obvious that the intensity of UV absorption of HSA changes with the alteration of 5-FU concentration. Moreover, blue shift of maximum peak of HSA at 280 nm was also noticed probably due to complex formation between 5-FU and HSA [17].

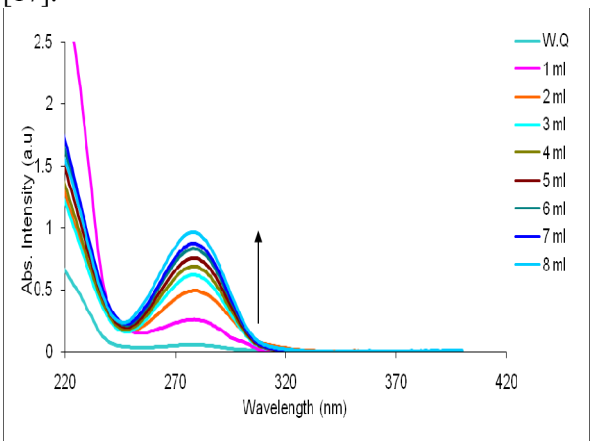

Fig. 2: Absorption spectra of HSA without and with different concentrations of 5FU

3.2 Fluorescence spectroscopy

The emission spectra of HSA in the presence of 5-FU with HSA at 288K and 293K are shown in Figs. 3 & 4. It is apparent that the emission spectra of HSA display a remarkable decrease by adding 5-FU, occurred at 335 nm with excitation at 280 nm. The results show that 5-FU can quench the fluorescence of HSA. The quenching efficiency increases greatly with a rise in the concentrations of 5-FU.

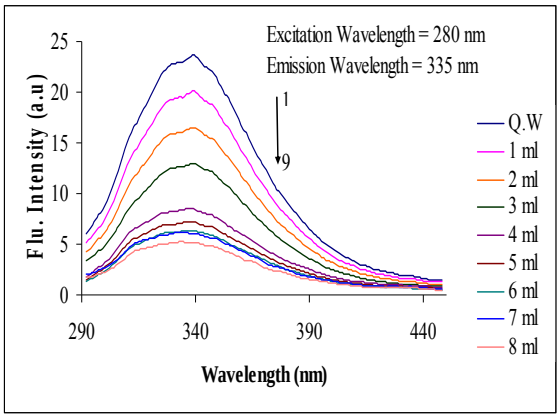

Fig. 3: Fluorescence spectra of HSA with 5FU at 288K

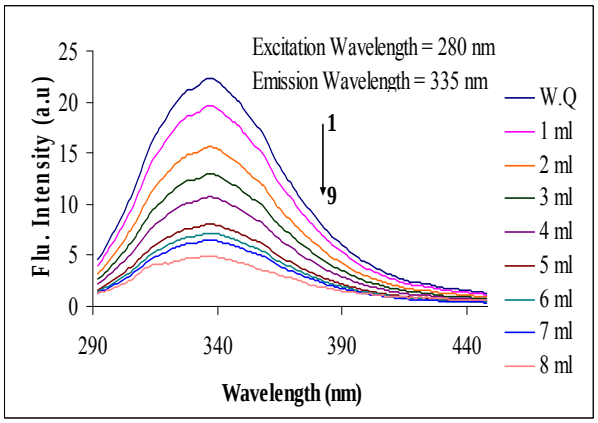

Fig. 4: Fluorescence spectra of HSA with 5FU at 293K

3.3 Quenching Mechanism

Quenching for the interaction of drug and protein can occur by different mechanisms, which are usually classified as dynamic quenching and static quenching. For other dynamic or static quenching, the Stern-Volmer relationship is suitable [18].

$F_0/F=1+K_{sv}$ [Q] -----(1)

Where F_0 and F are the fluorescence intensity in the absence and presence of the quencher, respectively; [Q] is the concentration of the quencher; and K_{sv} is the Stern-Volmer constant. In such an analysis, a plot of F_0/F *versus* [Q] will give a straight line with a slope of K_{sv} (Fig.5).

Table-1- Stern-Volmer (K_{sv}) quenching constants of the system 5-FU-HSA at different temperatures

Temperature	K_{sv} x 10^3 (L mol^{-1})	R^{2a}	S.D[b]
288K	4.8105	0.9600	1.0330
293K	3.4715	0.9727	1.0708

[a] ⟶ Regression co-efficient
[b] ⟶ Standard deviation

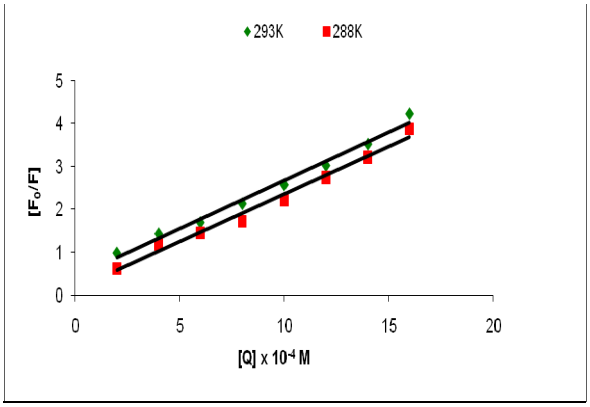

Fig. 5: Stern-Volmer plot for HSA with 5FU at 288K and 293K

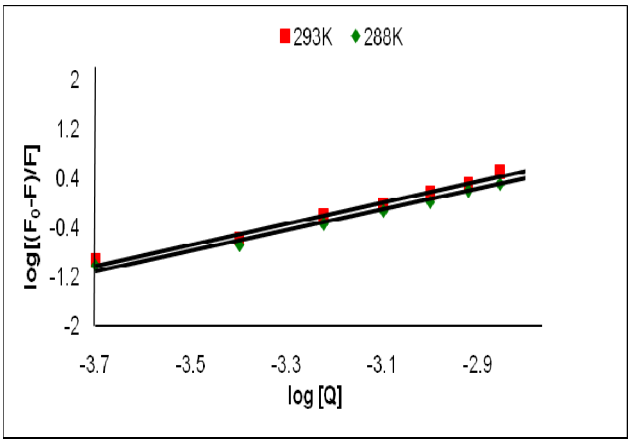

Fig. 6: Double log plot of HSA with 5FU at 288K and 293K

The corresponding quenching procedure constants at 288K and 293K are shown in Table 1. The result shows that the probable quenching mechanism of fluorescence of HSA by 5-FU is a static quenching procedure, because K_{sv} is increases the temperature should decreases.

3.4 The binding constant and the number of binding sites

When small molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules is given by the equation [19].

$\log(F_0/F) / F = \log K_a + n \log[Q]$ -----(2)

where K_a is the binding constant , and n is the number of binding sites per HSA.

Table 2 shows that the values of K_a for 5-FU and HSA decrease slightly with rise in temperature, which may be indicate that the compound of HSA and 5-FU is partly decomposed when the temperature rises. Whereas, the effect of temperature on the binding of HSA and 5-FU is relatively slight, which implies that hydrophobic force plays the major role in the reaction.

Table-2 Binding constant(K_a) and binding site(n) obtained by fitting Eqn. (2) at different temperature

Temperature	K_a x 10^3 (L mol^{-1})	n
288K	0.5011	1.0
293K	0.5623	1.0

3.5 Thermodynamic parameters and Nature of binding force

The interaction forces between drug and biomolecule may involve hydrophobic forces, electrostatic interactions, Van der Walls interactions, hydrogen bonds, etc. In order to elucidate the interaction of drug with HSA, we calculated the thermodynamic parameters from eqn.(3) – (5). If the temperature does not vary significantly, the enthalpy change (ΔH) can be regarded as a constant. The free energy change (ΔG) can be estimated from the following equation, based on the binding constant at different temperatures:

$\Delta G = -RT\ln K_a$ ------ (3)

Where R is the gas constant, T is the experimental temperature, and K is the binding constant at the corresponding T. Then the enthalpy change (ΔH) and entropy change (ΔS) can be calculated from the eqns. (4) and (5)

$\ln K_2/K_1 = [1/T_1 - 1/T_2]\, \Delta H/R$ ------(4)

where K_1 and K_2 are the binding constant at the experiment temperatures T_1 and T_2 respectively.

$\Delta G = \Delta H - T\Delta S$ ------(5)

The thermodynamic parameters for the interaction of drug with HSA is shown in Table 3.The negative sing for ΔG means that the interaction process is spontaneous. The positive ΔH and ΔS values indicate that the binding between drug and HSA is mainly ΔS-driven, with little contribution from the enthalpy factor, and the hydrophobic force may play a major role in the reaction [20].

Table-3 Thermodynamic parameters for the interaction of 5-FU with HSA at various temperatures

T(^{0}C)	ΔH (KJ mol^{-1})	ΔG (KJ mol^{-1})	ΔS (J $mol^{-1}K^{-1}$)
288K	16.17	-14.87	107.82
293K		-15.42	

4. CONCLUSION

The interaction between HSA and 5-FU has been studied by fluorescence and UV-Vis absorption spectra. The results indicate that the binding reaction of HSA and 5-FU is a single static quenching process. 5-FU can interact with HSA spontaneously through Van der Waals interaction and hydrogen bonds. The Thermodynamic parameter calculation shows that the acting force is mainly a hydrophobic one. The binding study of drugs to proteins is greatly important in pharmacy, pharmacology and biochemistry.

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