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Designed by Eng. Osama Al-Moaina
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Interaction Studies of Vinblastine Drug with Human Serum Albumin (HSA) by Spectroscopic and Molecular Modeling Studies

Waddhaah M. Al–Asbahy[1*], Manal Shamsi[2]

[1]*Department of Pharmacy, Al-Razi University, Sana'a, Yemen.*, [2]*Department of Chemistry, Taiz University, Taiz, Yemen.*

Abstract

Background: *The interactions of anticancer drugs with blood plasma constituents, particularly with human serum albumin (HSA) have a major influence on drug pharmacology and efficacy in overcoming the biological barriers to drug delivery and the targeting of active drugs to their specific site of action.* ***Aim:*** *to examine the interaction of vinblastine with human serum albumin (HSA) by means of various spectroscopic method (viz: UV/visible) in combination with molecular docking techniques.* ***Methods:*** *HSA was purchased from Sigma and used without further purification. Vinblastine, Tris(hydroxymethyl) aminomethane or Tris Buffer (Sigma), were used as received. Doubly distilled water was used as the solvent throughout the experiments. All reagents were of the best commercial grade and were used without further purification. Human serum albumin of* 1×10^{-3} *M was prepared by dissolving protein in Tris-HCl buffer solution at pH 7.4.* ***Results:*** *The results of fluorescence measurements indicate that Vinblastine has a strong ability to quench the intrinsic fluorescence of HSA through static quenching procedure. The binding constants (K) at different temperatures and thermodynamic parameters, enthalpy changes (ΔH) and entropy changes (ΔS) were calculated according to the fluorescence data. Furthermore, molecular docking studies revealed that the, Vinblastine was located to the entrance of site I by electrostatic and hydrophobic forces, which matched exactly with the corresponding experimental results.* ***Conclusion:*** *All the experimental results and theoretical data indicated that Vinblastine drug bound to HSA and was effectively transported and eliminated in the body. Such findings may provide useful guidelines for further drug design.*

Keywords: Vinblastine, human serum albumin, docking.

Introduction

Cancerous tumors are characterized by cell division, which is no longer controlled as it is in normal tissue [1-3]. "Normal" cells stop dividing when they come into contact with like cells, a mechanism known as contact inhibition. Cancerous cells lose this ability [4]. Cancer cells no longer have the normal checks and balances in place that control and limit cell division. The process of cell division, whether normal or cancerous cells, is through the cell cycle. The cell cycle goes from the resting phase, through active growing phases, and then to mitosis (division) [5]. The ability of chemotherapy to kill cancer cells depends on its ability to halt cell division. Usually, the drugs work by damaging the RNA or DNA that tells the cell how to copy itself in division.

If the cells are unable to divide, they die. The faster the cells are dividing, the more likely it is that chemotherapy will kill the cells, causing the tumor to shrink. They also induce cell suicide (self-death or apoptosis) [6].

Chemotherapy drugs that affect cells only when they are dividing are called cell-cycle specific. Chemotherapy drugs that affect cells when they are at rest are called cell-cycle non-specific. The scheduling of chemotherapy is set based on the type of cells, rate at which they divide, and the time at which a given drug is likely to be effective. This is why chemotherapy is typically given in cycles.

Vinblastine is an anti-cancer ("antineoplastic" or "cytotoxic") which belongs to a class of chemotherapy drugs called alkaloids. Plant alkaloids are made from plants. The vinca alkaloids are made from the periwinkle plant (catharanthus rosea). The plant alkaloids are cell-cycle specific. This means they attack the cells during various phases of division [7].

Albumin is emerging as a versatile protein carrier for drug targeting and for improving the pharmacokinetic profile of peptide or protein based drugs [8]. The specific delivery of a drug to cancer cells may be achieved by the use of targeting groups or by tuning the chemical and physical characteristics of the drug or drug carrier, such as hydrophobicity and molecular size [9]. Different types of macromolecules have been used as carrier molecules, such as liposomes, dendrimers, poly (ethylene glycol) polymers, nanoparticles, and protein biomolecules [10]. In particular, human serum albumin (HSA) is known to accumulate in tumours, being taken up by tumour cells at increased levels compared to normal cells because of enhanced tumour vascular permeability and prolonged retention time in the tumour interstitium due to the

*Corresponding author: Waddhaah M. Al–Asbahy , Department of Pharmacy, Al-Razi University, Sana'a, Yemen.

obstruction of lymphatic drainage, and has been exploited as the carrier conjugate of various anticancer drugs such as chlorambucil, doxorubicin, and paclitaxel [11,12]. The main role of HSA is to maintain the osmotic blood pressure and to scavenge free radicals as an antioxidant. It is an attractive macromolecular carrier, due to its lack of toxicity and immunogenicity make it an ideal candidate for drug delivery. In addition, HSA is the most multifunctional transport protein and play an important role in the transport and deposition of a variety of endogenous and exogenous substances in blood [13,14]. The interaction of drugs with protein result in the formation of a stable drug-protein complex, which can exert important effect on the distribution, free concentration and metabolism of the drug in the blood stream. Drugs distribution is mainly controlled by HSA, because most drugs circulate in plasma and reach the target tissues by binding to HSA [15]. Therefore, drug binding to proteins such as HSA has becomes an important determinant of pharmacokinetics, restricting the unbound concentration and affecting distribution and elimination [16].

Despite the numerous studies on Vinblastine and their interactions with bio-macromolecules (HSA), in this study, we have explored the interaction between new Vinblastine and one of the model human transporter proteins, HSA. The binding properties of this complex with HSA have been carried by means of spectroscopic method (UV spectra) and molecular docking techniques.

Aim of the study: the aim of the study was to examine the interaction of vinblastine with human serum albumin (HSA) by means of various spectroscopic method (viz: UV/visible) in combination with molecular docking techniques.

Subject and Methods

HSA (fatty acid free, 99%) was purchased from Sigma and used without further purification. Vinblastine (Loba chemie), Tris (hydroxymethyl) aminomethane or Tris Buffer (Sigma), were used as received. Doubly distilled water was used as the solvent throughout the experiments. All reagents were of the best commercial grade and were used without further purification.

Human serum albumin of 1×10^{-3} M was prepared by dissolving protein in Tris-HCl buffer solution at pH 7.4. The protein concentration was determined spectrophotometrically using an extinction coefficient of 35219 M^{-1} cm^{-1} at 280 nm [17]. Stock solution of complex (1×10^{-3} M) was prepared by dissolving complex in doubly distilled water. NaCl (analytical grade, 1M) solution was used to maintain the ionic strength of buffer at 0.1M. pH was adjusted to 7.4 by using HCl. Working standard solution was obtained by appropriate dilution of the stock solution.

HSA-binding experiments

UV-visible spectroscopy

Absorption spectra were recorded on a Janway UV-1700 pharmaspec UV/Vis spectrophotometer using cuvettes of 1-cm path length. HSA concentration was determined from absorption spectrum, taking the absorbance of a 1 mg/ml solution at 280 nm (λ_{max} Trp-214) as 1.80 x 10^{-6} M The value of binding constants can be calculated from the the method which described earlier by [18,19]. By assuming that there is only one type of interaction between Vinblastine and protein (HSA) in aqueous solution, the Eqs (1) and (2) can be established:

[HSA] + [Vinblastine Drug] ↔ HSA: Vinblastine

(1)

$$K = \frac{[HSA:Vinblastin]}{[HSA][Vinblastin]}$$

(2)

where K is binding constant for Vinblastine Drug

Assuming [HSA: Vinblastine Drug] = C_B,

$$K = \frac{C_B}{(C_{HSA} - C_B)(C_{Vinblastin} - C_B)},$$

(3)

where C_{HSA} and $C_{Vinblastine}$ are analytical concentration of HSA and drug in the solution, respectively.

According to Beer-Lambert law:

$$C_{HSA} = \frac{A_o}{\varepsilon_{HSA} \cdot l'}$$

(4)

$$C_B = \frac{A - A_o}{\varepsilon_{HSA} \cdot l},$$

(5)

Where A_o and A are the absorbance of HSA at 280 nm, in the absence and presence of Vinblastine Drug, respectively. ε_{HSA} and ε_B are the molar extinction coefficient of HSA and the bound complex, respectively and ℓ is the light path of the cuvette (1 cm).

By displacing ε_{HSA} and ε_B in equation (3) by equation (4) and (5), equation can be deduced, as follows:

$$\frac{A_o}{A - A_o} = \frac{\varepsilon_{HSA}}{\varepsilon_B} + \frac{\varepsilon_{HSA}}{\varepsilon_B K} \cdot \frac{1}{C_{Vinablasttin}}$$

(6)

Thus, the double reciprocal plot of $1/A\text{-}A_o$ vs. $1/ C_{Vinblastine\ Drug}$ is linear and binding constant can be estimated from the ratio of intercept to the slope.

Molecular docking

The rigid molecular docking studies were performed by using HEX 6.1 software [4], is an interactive molecular graphics program for calculating and displaying feasible docking modes of a pairs of protein and enzymes molecule. The Vinblastine drug was taken from its crystal structure as a CIF file and was converted to the PDB format using Mercury software. The crystal structure of the human serum albumin (PDB ID: 1h9z) was downloaded from the protein data bank

(http://www.rcsb.org./pdb). All calculations were carried out on an Intel Pentium 4, 2.4 GHz based machine running MS Windows XP SP2 as operating system. Visualization of the docked pose has been done by using PyMol (http://pymol.sourceforge.net/) molecular graphic program.

Results and discussion

In order to improve specific delivery of active drugs to their specific site of action, formulation of Vinblastine drug as chemotherapeutic agents were designed and their binding interaction with human serum albumin (HSA) was carried out by means various biophysical and molecular docking techniques. The good aqueous solubility of Vinblastine Drug **(Fig. 1)** facilitates its rapid cellular uptake, undergo selective localization and be able to induce programmed cell death of the cancer cells.

Serum albumin often increases the apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cell *in vivo* and *in vitro* **(Scheme. 1)**. Consequently, it is important, to understand the mechanism of interaction of a bioactive compound with protein. This works may provide salient information on the structural features that determine the therapeutic effectiveness of drugs and hence become an important rationale for designing new lead anticancer compounds in clinical medicine.

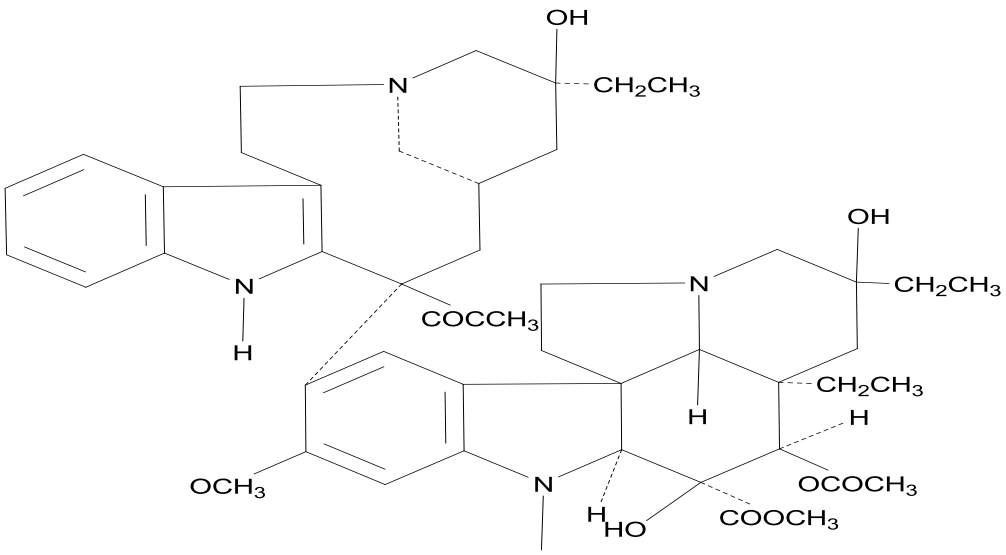
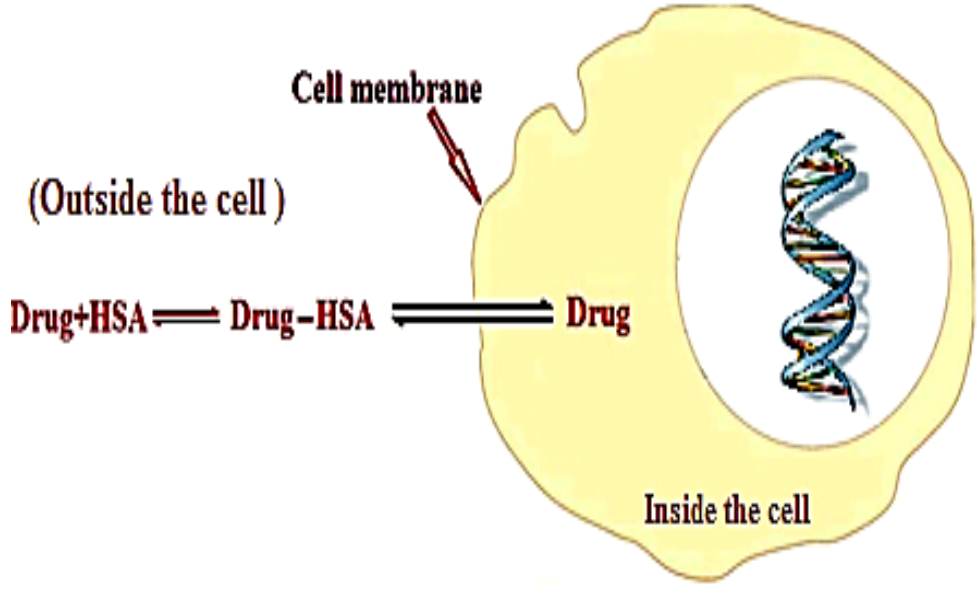


Figure 1: Line model structure of the Vinblastine Drug



Scheme. 1. Chemical equilibrium between the Vinblastine Drug–HSA system towards targeted delivery.

Electronic absorption studies

Electronic absorption spectroscopy is often employed to ascertain whether the changes in secondary structure of HSA was either simply due to collision between drug and protein electrostatistically or due to complex formation between them.

As shown in Fig. 1, upon addition of Vinblastine drug to HSA of fixed concentration (1.50 x 10^{-5} M), there is a sharp increase in absorption intensity *viz*. hyperchromism, which indicates that the drug interacts with HSA [20]. Upon binding to Vinblastine drug, the serum albumin undergoes a slow conformational transition from the original conformation to the final conformation (binding with drug). The conformational transitions probably enhance the complex formation between drug and protein [21]. As a result, continual increase in the absorbance value of intraligand band is observed. In order to assess the binding ability of the drug with HSA, the intrinsic binding constant K_b of the drug was calculated and found to be 6.42×10^{4} M^{-1}. The value of K_b suggests high affinity between Vinblastine Drug and HSA.

Binding mode

The interacting forces between drug and a biomacromolecule is composed of weak interactions such as electrostatic forces, hydrophobic interaction, hydrogen-bond formation, van der Waals forces and stereo-hindrance effect, etc. (**scheme 2**) [18].

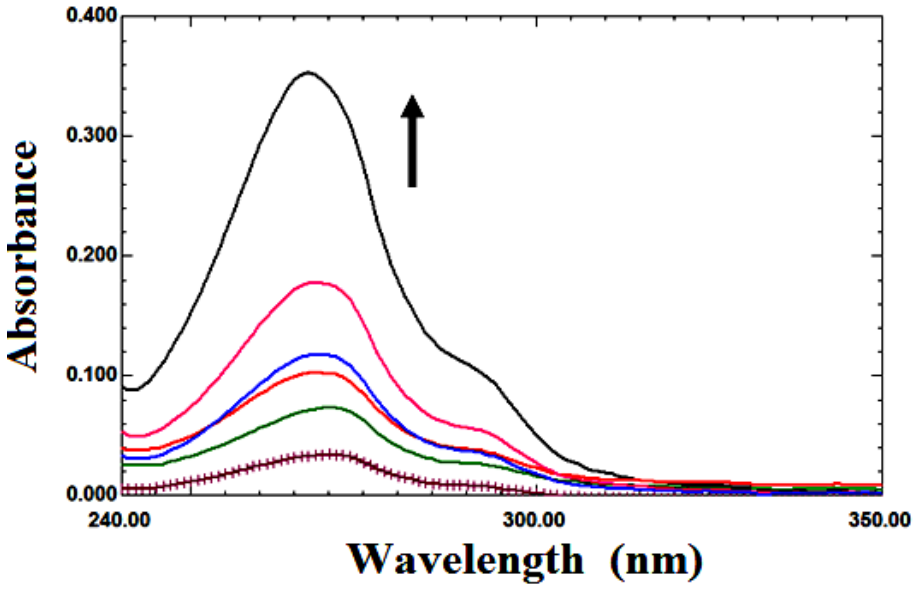
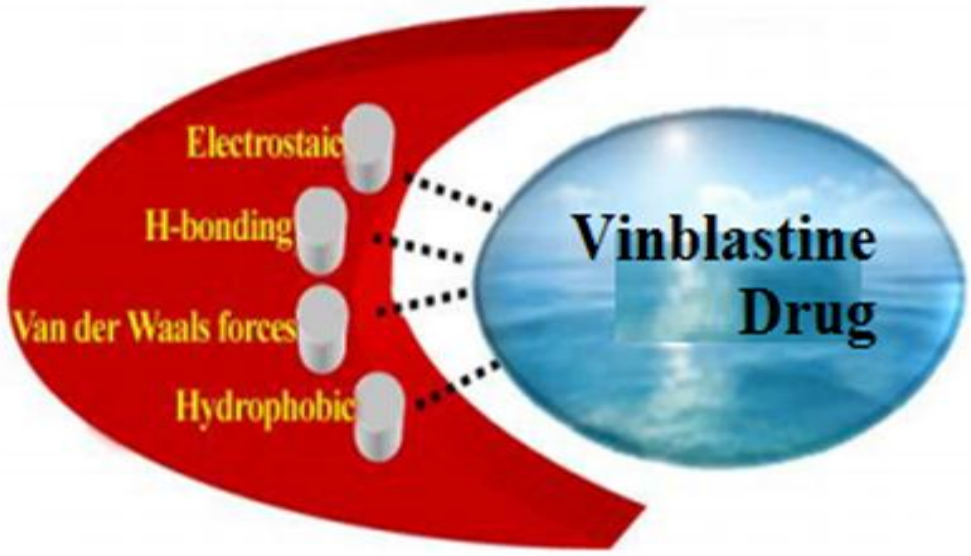


Figure 2. UV absorption spectra of the HSA– Vinblastine Drug system obtained in 5 mM Tris–HCl/50 mM NaCl buffer, pH 7.4, at room temperature: (a) Vinblastine, 0.60 x 10^{-5} M; (b) HSA, 1.50 x 10^{-5} M; (2–5) drug Vinblastine –HSA, the Vinblastine concentrations were 0.67, 1.0, 1.33, 1.67, 2.00 × 10^{-5} M, respectively. Arrows show the intensity changes upon increasing concentration of the Vinblastine. Inset: Plot of $1/A–A_o$ vs. 1/ [Vinblastine].



Scheme 2. Schematic illustration of non–covalent interactions between a protein and Vinblastine

The thermodynamic parameters, enthalpy change (ΔH), entropy change (ΔS) and free energy change (ΔG) are the main evidence for confirming binding modes. From the thermodynamic standpoint, ΔH> 0 and ΔS > 0 implies a hydrophobic interaction; ΔH< 0 and ΔS < 0 reflects the van der Waals force or hydrogen bond formation; and ΔH≈0 and ΔS > 0 suggests an electrostatic force [19]. The temperatures chosen for measurements were 299, 309 and 318 K so that HSA does not undergo any structural degradation. The thermodynamic parameters can be calculated from the van't Hoff equation:

$$\ln K = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} \quad (13)$$

$$\Delta G = \Delta H - T\Delta S = -RT \ln K \quad (14)$$

Where K is the Lineweaver–Burk static quenching constant at corresponding temperature and R is the gas constant, in which ΔH and ΔS of reaction could be determined from the linear relationship between ln K and the reciprocal absolute temperature (Fig. 3). The free energy change (ΔG) could be calculated by the Eq. (6). As shown in Table 1, ΔG and ΔH were negative, and ΔS was positive. Therefore, the formation of Vinblastine-HSA coordination complex was spontaneous and exothermic reaction. According to the views of Neméthy and Scheraga [22], Ross and Subramanian [23], the positive ΔS value is frequently taken as evidence for hydrophobic interaction.

Furthermore, specific electrostatic interactions between ionic species in aqueous solution are characterized by a positive value of ΔS and a negative ΔH value. Accordingly, it was not possible to account for the thermodynamic parameters of Vinblastine–HSA complex on the basis of a single interaction. It was more likely that both hydrophobic and electrostatic interactions were involved in the binding process.

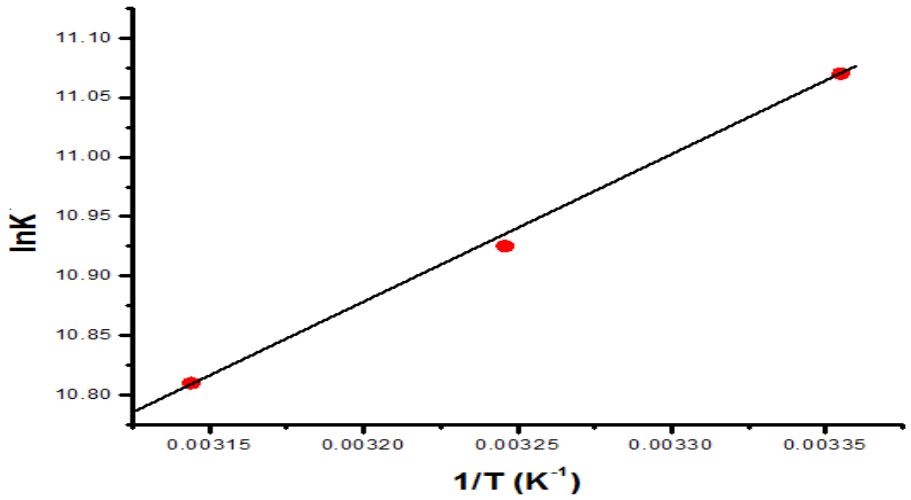


Figure 3. Van't Hoff plot for the interaction of Vinblastine and HSA.

Table 1: Thermodynamic parameters of Vinblastine - (HSA) interaction at pH 7.4

System	T (K)	K ×10^4 (M^{-1})	ΔG(J/mole)	ΔH(kJ/mole)	ΔS(J/moleK)
Vinblastine - (HSA)	299	6.42	-18.19	-1.238	+56.88
	309	5.55	-18.79		
	318	4.95	-19.41		

Molecular docking study

Molecular docking studies was further employed to analyze our understanding of the interaction between Vinblastine Drug and HSA. From the 3-D structure of crystalline albumin, it is known that HSA comprises three homologous domains (denoted I, II, and III): I (residues 1–195) II (196–383) and III (384–585) that assemble to form heart shaped molecule (Fig. 4). The principal region of drug binding sites of HSA are located in hydrophobic cavities in subdomain IIA and IIIA, which are corresponding to site I and site II, respectively and tryptophan residue (Trp-214) of HSA in subdomain IIA. There is a large hydrophobic cavity in subdomain IIA to accommodate the drug molecule. The resulting docking pattern is shown in Fig. 5. Which indicates that Vinblastine Drug is located within the subdomain IIA of HSA [24] suggesting the existence of hydrophobic interaction between them, which is consistent with the binding mode proposed in thermodynamic analysis and efficient fluorescence quenching of HSA emission in presence of Vinblastine

Drug. Furthermore, there are also a number of specific electrostatic interactions and hydrogen bonds,

because several ionic and polar residues in the proximity of the ligand play an important role in stabilizing the molecule via H-bonds and electrostatic interactions.

As shown in Fig. 6, there are hydrogen bond interactions between the main chain carbonyl oxygen of Arg-186 and terminal amino group of the complex and other between carbonyl groups of the drug and the residues Asp-173 Glu-184, Trp-214 and Lys-423 of HSA.

The results suggested that the formation of hydrogen bonds decreased the hydrophilicity and increased the hydrophobicity to keep stability in the Vinblastine Drug–HSA system. Therefore, the results of molecular docking indicated that the interaction between Vinblastine

Drug and HSA was dominated by hydrophobic forces, which was in accord with the experimental results.

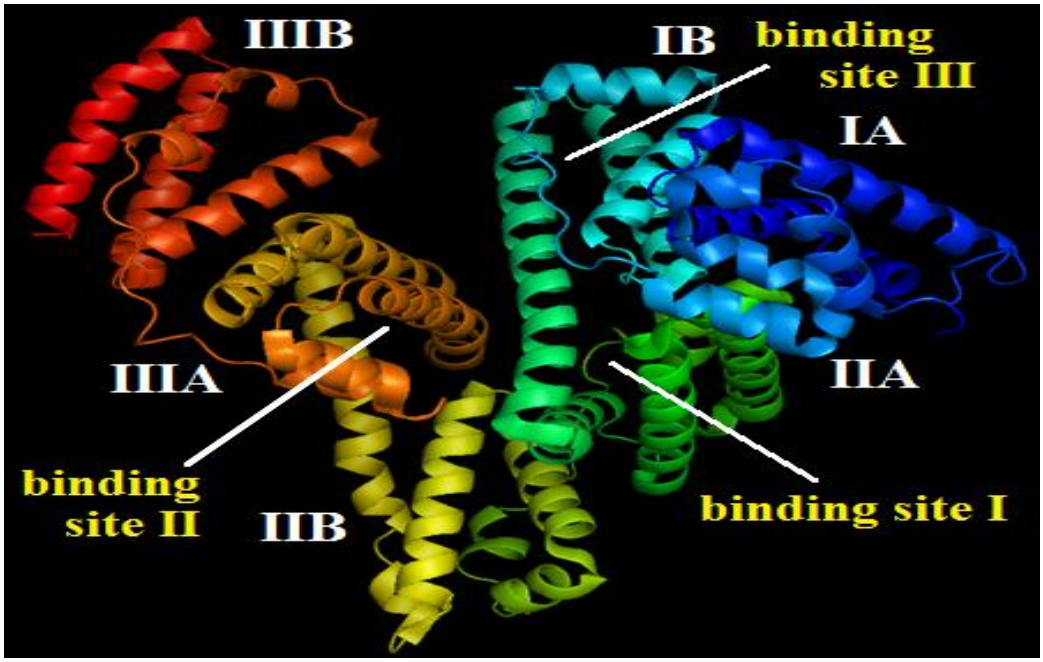


Figure 4. Modeling of X-ray crystallographic structure of HSA (PDB ID: 1h9z).

The domains and subdomains were displayed with different color, the every subdomain and classical binding site were marked in the corresponding location.

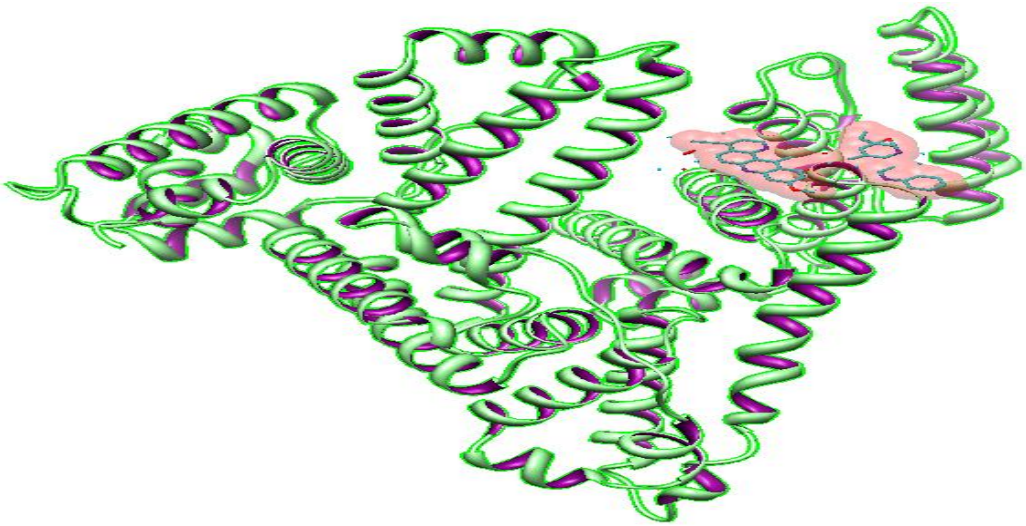


Figure 5. Molecular docked model of Vinblastine Drug (stick representation) located within the hydrophobic pocket in subdomain IIA of HSA.

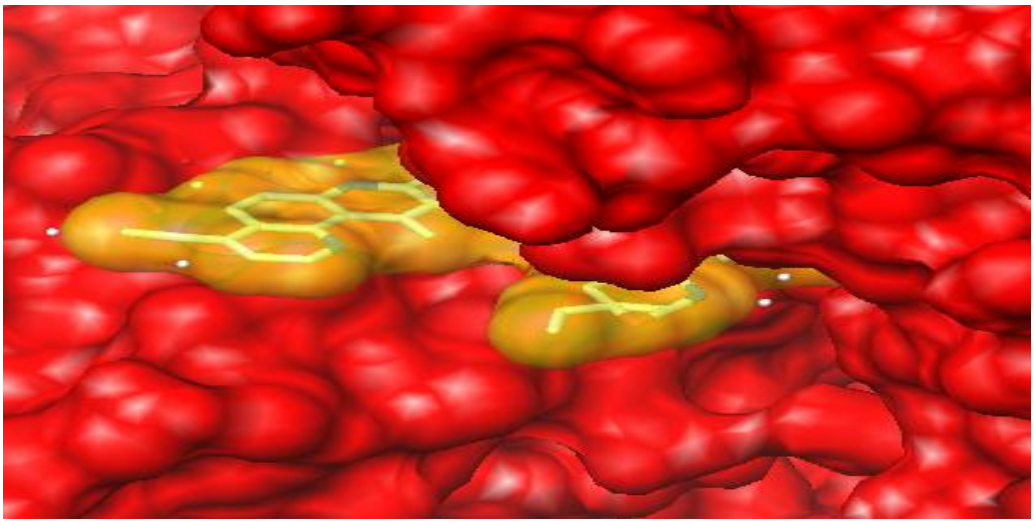


Figure 6. The interaction mode between Vinblastine Drug and HSA.

The Vinblastine Drug is showing in a central position (*yellow colour*) and HSA (*red colour*).

Conclusion

In this paper, the interaction between HSA and Vinblastine Drug was investigated by spectroscopic methods. The binding site was located at the subdomain IIA of HSA. The binding study of drugs with proteins is of great importance in pharmacy, pharmacology and biochemistry. This study is expected to provide some valuable information for the transportation and distribution of the physiologically important protein HSA with drugs.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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