Serum albumins are the most abundant carrier protein in the circulatory system and are involved in the transportation and distribution of exogenous and endogenous materials in blood [1], including nutrients and drugs, mostly by the formation of noncovalent complexes at binding sites [2]. The absorption, distribution, metabolism, and excretion properties as well as the stability and toxicity of drugs can be significantly affected as a result of their binding to serum albumins [3]. Study of the interaction of different types of drugs to serum albumin is extremely important. As a kind of serum albumin, bovine serum albumin (BSA) has the advantages of medical importance, ready availability, low cost, and appreciable ligand/drug-binding properties. Bovine serum albumin (BSA) and human serum albumin (HSA) tertiary structures are also very similar, and the results of all studies are consistent with the fact that they have 76% sequence homology [4]. Several researchers are actively involved in the interaction of several synthesised or ready-made molecules with BSA and HSA to have an understanding of their behaviour. Both spectroscopic techniques and different types of biological assay experiments can be carried out to have a knowledge as to the behaviour of the chemical compounds [5].

Experimental methods generally used to study drug-protein binding interaction are briefly summarized below:

**FLUORESCENCE QUENCHING**

Fluorescence quenching can be described by the Stern-Volmer equation

$$\frac{F_0}{F} = 1 + k_q[Q] = 1 + KS_V[Q]$$

(1)

where $F_0$ and $F$ are the intensities of fluorescence before and after addition of any quencher, respectively, $k_q$ is known as the bimolecular quenching constant, $t_0$ is the lifetime of the fluorescent molecule in absence of the quencher, $[Q]$ being the concentration of the quencher, and $K_{SV}$ is known as the Stern-Volmer quenching constant. Hence, equation (1) was applied to determine $K_{SV}$ by linear regression of a plot of $F_0/F$ against $[Q]$. A linear Stern-Volmer plot indicates a single class of fluorophores, this also indicates that only one mechanism (dynamic or static) of quenching occurs. During static mechanism, complex formation occurs, and in such cases, $k_q$ can be calculated by the ratio between $K_{SV}$ and $t_0$. For BSA, the lifetime of the fluorophore tryptophan is approximately 5 ns [6]. The maximum possible value for diffusion-limited quenching (dynamic quenching) in water is approximately $10^{10} \text{ M}^{-1}\text{s}^{-1}$. When the value of the bimolecular quenching constant, $k_q$ is even higher, it could mean that there is a complex formation between protein and quencher, corresponding to a static mechanism.

**BINDING CONSTANTS**

Binding parameters are required in the study of pharmacokinetics. When small drug molecules bind independently to a set of equivalent sites on a protein/macromolecule and equilibrium between the free and the bound molecules has been attained, the fluorescence intensities obey the following equation [7,8]:

$$F = F_0 + (F_B - F_0) \frac{K_q}{1 + K_q[Q]}$$

(2)
log \([F_0-F/F]=n \log K_0+n \log 1/[[Q]-(F_0-F)][P]/F_0\)  \(\tag{2}\)

where, as usual, \(F_0\) and \(F\) are the fluorescence intensities before and after the addition of the quencher, respectively, \(K_b\) being the apparent binding constant to a set of sites, and \(n\) is the average number of binding sites per protein molecule, \([Q]\) and \([P]\) are the total quencher concentration and the total protein concentration, respectively. From the plot of \(\log (F_0-F)/F\) vs. \(\log (1/[[Q]-(F_0-F)][P]/F_0)\), the number of binding sites \(n\) and the binding constant \(K_b\) are calculated. Some authors determine the binding constants hence the binding affinity of molecules with protein using different technique by following modified Stern Volmer equation:

\[
\log (F_0-F)/F=\log K_0+n \log [Q] \tag{3}
\]

Plot of \(\log (F_0- F)/F\) against \(\log [Q]\) are used to determine the values of \(K_b\) and \(n\) from the intercept and the slope respectively.

**CIRCULAR DICHROISM (CD)**

CD spectrum of HSA exhibits two negative bands at 208 and 222 nm, which are typical of an \(\alpha\)-helix structure of the protein. When the binding of a molecule to HSA result in a decrease in negative ellipticity at all wavelengths of the far-UV CD without any significant shift of the peaks, it is indicative of changes in the protein secondary structure, and decrease of the \(\alpha\)-helix content in protein. The CD spectrum for BSA in aqueous solution is characteristic of macromolecules with high alpha-helical content, with two well-defined ellipticity values at 208 and 222 nm \([9,10]\].

**ISOTHERMAL TITRATION CALORIMETRY**

The interaction between a drug and protein can involve hydrophobic forces, electrostatic interactions, van der Waals interactions, hydrogen bonds, etc. in order to interpret the binding mode values of some thermodynamic parameters like enthalpy changes (\(\Delta H\)), free energy changes (\(\Delta G\)), and entropy changes (\(\Delta S\)) of interactions are required. In order to comprehend the interaction of a small drug or model drug molecule with BSA, the thermodynamic parameters can be calculated from the following equations. If the temperature does not vary much, the enthalpy change (\(\Delta H\)) can be regarded as a constant. The free energy change (\(\Delta G\)) can be estimated from the following equation:

\[
\Delta G=RT \ln K_0 \tag{4}
\]

where \(T\) is the experimental temperature, \(R\) the gas constant and \(K_0\) the binding constant at corresponding \(T\). Then the enthalpy change (\(\Delta H\)) and entropy change (\(\Delta S\)) can be calculated from the following equation:

\[
\ln K_0/K_1=\left[1/T-1/T_2\right] \Delta H/R \tag{5}
\]

where \(K_1\) and \(K_2\) are the binding constants at the experiment temperatures \(T_1\) and \(T_2\), respectively.

The sign and magnitude of the thermodynamic parameter associated with various individual kinds of interaction which may take place in the protein association processes were judicially characterized by Ross et al. \([11]\). The negative sign for \(\Delta G\) is indicative of a spontaneous interaction. The positive \(\Delta H\) and positive \(\Delta S\) indicate that hydrophobic forces may play a major role in the binding.

**FT-IR**

Hydrogen bonding and the coupling between transition dipoles are the primary factors that play a crucial role in understanding the conformational sensitivity of the amide bands of proteins. The protein amide I and II bands at 1640–1650 cm\(^{-1}\) (mainly C=O stretching) and 1548–1560 cm\(^{-1}\) (C–N stretching coupled with N–H bending), are then correlated with the structural changes in proteins. The difference spectra [(protein solution+polyphenol solution)-(protein solution)] provide information about the conformational changes that develops upon interaction between the protein and drug molecule \([5,12,13]\).

**REFERENCES**