CHAPTER 7

Summary and conclusion
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The study of drug plasma protein interactions is a very important phenomenon in the design and clinical phases of drug discovery. They play a significant and important role affecting the distribution, clearance, metabolism, and pharmacological performance of drugs. Consequently, control of the extent of protein binding may be associated with the ability to modify the pharmacokinetics of a drug substance. Since blood contains hundreds of proteins, there is a high probability that most small molecules will exhibit some level of binding. Depending upon whether the drug is a weak or strong acid or base, or is neutral, it can bind to a single blood protein, and to multiple proteins (e.g. serum albumin, acid-glycoprotein (AAG) or lipoproteins) as well.

Keeping in view the above-mentioned facts the present work illustrated in this thesis was undertaken to investigate in detail the binding of selective drug compounds under different physico-chemical conditions (e.g. pH, temperatures, ionic strength and dielectric constant). Bovine serum albumin (BSA) was selected as the plasma protein in the present instances except in case of lamotrigine where horse serum was used.

The binding of nimesulide, a cox-2 inhibitor, to BSA was investigated by equilibrium dialysis method at different temperatures, ionic strengths and pH conditions. The Scatchard plots as well as non-linear regression were performed based on these drug-protein binding data. The number of binding sites (n), the value of association constant (K) at different conditions and different thermodynamic parameters (i.e., standard free energy change ($\Delta G^0$), standard enthalpy change ($\Delta H^0$) and standard entropy change ($\Delta S^0$) of nimesulide-BSA binding were determined. The result shows that number of binding sites is around 2.0 and the value of association constant is decreasing with increasing temperature, pH and ionic strength. It also reveals that the value
of $\Delta G^0$ and $\Delta H^0$ were highly negative and $\Delta S^0$ has very small positive value. The result indicates that the interaction between nimesulide and BSA is exothermic and spontaneous in nature. It is postulated that nimesulide–BSA interaction may occur due to ionic interaction and the hydrogen bonding between drug and protein.

The equilibrium dialysis data of diclofenac sodium binding with BSA at different temperatures (20°C, 30°C and 40°C), pH (6.4, 7.4 and 8.4) and ionic strength ($\mu = 0.1, 0.2$ and 0.3) were interpreted by nonlinear regression method using Graphpad prism software. The analysis showed that the interaction between diclofenac sodium with BSA results in two-sites saturable binding. A decrease in association constant was observed with increasing temperature. The average standard free energy change ($\Delta G^0$), the standard enthalpy change ($\Delta H^0$) and the standard entropy change ($\Delta S^0$) were found to be negative for both site-I and site-II binding. The negative enthalpy change suggests the binding between diclofenac sodium and the binding sites of BSA were spontaneous and exothermic. The negative value of $\Delta H^0$ and $\Delta S^0$ indicates hydrogen bonding and van der Waal's forces are the major mechanism for diclofenac sodium and BSA interaction. Increase in pH and ionic strength also causes decrease in association constant of diclofenac sodium and BSA binding.

Lamivudin, a dideoxynucleoside analogue used in combination with other agents in the treatment of human Immunodeficiency virus type 1 (HIV – 1) infection, was utilized as third agent for plasma-protein binding studies. The binding of this drug with BSA was studied at different temperatures, pH's and ionic strength by equilibrium dialysis method. The data obtained were interpreted by nonlinear regression method using Graph-pad prism software. The analysis showed that the interaction between lamivudine with BSA appears to be a saturable binding with no depletion. A decrease in association constant was observed with increasing temperature. The negative enthalpy change suggests the binding between lamivudine and the binding sites of BSA were spontaneous and exothermic. The negative value of $\Delta H^0$ and positive value of $\Delta S^0$ indicates
hydrogen bonding and hydrophobic interaction together are the major mechanism for protein binding. Increase in pH and ionic strength also causes decrease in association constant of lamivudine and BSA binding. The binding studies lamivudine with serum from different species (horse, rat, rabbit and goat) were carried out by equilibrium dialysis method at constant temperature, pH and ionic strength and no significant variation in total serum protein binding of lamivudine was observed with the exception of the goat plasma where some insignificant variations were recorded.

Lamotrigine, a phenyltriazine derivative and an antiseizure drug, binding with horse serum were studied by equilibrium dialysis method at different temperatures and dielectric constants. Result suggests that a decrease in association constant was observed with increasing temperature. The negative enthalpy change suggests the binding between lamotrigine and the binding sites of proteins in horse serum were spontaneous and exothermic. The negative value of $\Delta H^0$ and positive value of $\Delta S^0$ indicates that the binding process was due to hydrophobic interaction. This was again established by the fact that is the decreasing binding constant with decreasing dielectric constant. Binding study of lamotrigine with serum from different species (horse, rat, rabbit and goat) showed that there were very little significant changes in plasma protein binding in the above-mentioned species and lowest binding was only recorded in the case of goat serum.

In conclusion, the investigation results suggest that drug plasma protein interactions are mostly reversible, non-covalent and mostly enthalpy driven. In our study we have explained that plasma-protein binding of drugs is clearly influenced by concentration of drug molecules, temperature, pH, dielectric constant and ionic strength of the medium. A little significant changes was observed in in-vitro plasma protein binding of lamivudine and lamotrigine with species variation and the in-vitro study resulted lowest binding with goat plasma.