LITERATURE SURVEY

Lipophilicity: The important role played by lipophilicity in governing the bioactivity of pharmaceuticals has been extensively reviewed [23-24]. The octanol-water partition coefficient (log P), a generally accepted physico-chemical parameter for characterization of lipophilicity, has been reported for a large variety of drugs. Examples include some antihypertensives [90], non-steroidal anti-inflammatory drugs [91], antibiotics [92] and others [93]. Lipophilicity has also been correlated to solubility [94], permeability [95], protein binding [96] and other pharmacokinetic properties [97] of drugs. Amongst antidiabetic drugs, lipophilic behaviour of repaglinide has been studied by Mandic and Gabelica [6]. The authors have shown that despite its zwitterionic nature, repaglinide has high lipophilicity (log P = 3.97). Enhanced partitioning of repaglinide into octanol phase is due to the fact that intramolecular electrostatic attraction between positively charged aromatic amino group and negatively charged carboxylate anion results in the formation of internal ion-pair. Log P data for glipizide, glibenclamide and some glitazones has also been reported [98-99, 7]. Various aspects of the lipophilic behaviour of five widely used thiazolidinediones; ciglitazone, troglitazone, netoglitazone, pioglitazone and rosiglitazone have been studied by Giaginis et al. [7]. All the studied compounds were found to be highly lipophilic. PPAR-γ activity was found to increase with decrease in lipophilicity.

Experimental measurement of partition coefficient is costly and time consuming and therefore, most of the data used in the literature is predicted. Various log P predictors such as milogP, IAlogP, ClogP, AlogP, Pallas 3.0, KowWin etc. are available. However, there is usually a wide variation in the log P values predicted by different software and therefore, the reliability of predicted data without any experimental verification is questionable [100]. Comparison of the octanol-water partition coefficients, calculated by different software, and the experimentally determined values for a large number of compounds has been reported by Machatha and Yalkowsky [25].

Ionization behaviour: Ionization pattern can profoundly affect the solubility, lipophilicity and biological activity of drugs. Degree of ionization, expressed as
pKa values, have been compiled by Avdeef [101] and Jambhekar [102] for a wide range of acidic and basic drugs. pKa values of some antihypertensives [90], sulfonamides [92] and non-steroidal anti-inflammatory drugs [91] have been reported. Amongst antidiabetic drugs, ionization of repaglinide has been studied by Mandic and Gabelica [6]. They have reported that repaglinide possesses two protonation sites and in aqueous solution exhibits amphoteric properties. The physico-chemical and pharmacological behaviour of repaglinide is determined mostly by the zwitterionic form. All sulfonylureas are weak acids due to the marked delocalization of the nitrogen lone electron pair by the sulfonyl group [103]. pKa values of gliclazide, glibenclamide and glipizide have been reported by different authors [98-99]. Dissociation constant of glimepiride, determined by spectrophotometric and solubility methods, has been reported by Grbic et al. [5].

**Solubility:** Several review articles [48 (a-d)] and research papers [104-109] are available on the solubilization of poorly soluble drugs. Solubility enhancement of some non-steroidal anti-inflammatory drugs [43(a), 43(b)], antibiotics [110-111], anti-HIV agent [112] and fungicide [113] has been reported. A wide variety of techniques based on physical modification [36] and/or chemical modification [35] of drug substances have been used. In the present context, use of co-solvents and surfactant systems is relevant and has been discussed. Co-solvent solubilization technique has been used for the solubility enhancement of nimesulide [32], valdecoxib [114], acetaminophen [115] and a wide variety of other drugs [116-118]. It has been reported [116] that greater the difference in polarity of the two solvents in a given mixed solvent system, the greater is the solubilization power. Micellar solubilization technique has been utilized for improving the solubilities of ibuprofen [118], cyclosporine A [33(a)] and fluasterone [33(b)]. The nature and micellar concentration of surfactant as well as ionic strength of the medium can influence significantly the solubilization of a drug in micellar solution.

Solubilities of some antidiabetic drugs have also been reported in the literature [119-127]. Amongst antidiabetic agents, gliclazide has been the most widely studied drug. Enhancement of aqueous solubility of gliclazide by
complexation with cyclodextrins [119-120], co-grinding with excipients [121], micronization [122] and surfactant solubilization [38(b)] techniques has been reported. Significant improvement in solubility could be achieved. Solubility enhancement of another sulfonlurea, glibenclamide has been studied using solid dispersions with polymeric excipient, polyethylene glycol 4000 [123] and co-precipitation with polyvinylpyrrolidone [124]. Combined effect of surfactant and pH [125] and formation of inclusion complex with cyclodextrin [126] have been employed in the case of glipizide. Complexation with cyclodextrins has also been used to enhance the solubility of nateglinide, repaglinide and glimepiride [127]. pH-solubility profile and thermodynamic parameters for the dissolution of repaglinide have been studied by Mandic and Gabelica [6].

**Drug-protein interaction:** The binding of drugs to plasma proteins can have important pharmacokinetic implications; especially when the drugs are highly bound with a small volume of distribution and narrow therapeutic index [71]. Serum albumin is the major drug binding protein in plasma and its interaction with a wide variety of drugs has been studied. A large number of reports, including some reviews, have been published in recent years on various aspects of the problem. Important reviews on the subject, arranged in chronological order include articles by Meyer and Guttman [77], Settle et al. [128], Vallner [129], Kragh-Hansen [58], Wood [130], Lin et al. [131], Kremer et al. [132] and Otagiri [133]. Some recent papers have reported studies on the molecular basis of the interaction of thiazide diuretic, bendroflumethiazide [134], anti-tumour drug, paclitaxel [135], antipsychotic drug, chlorpromazine [136], anti-HIV drugs [137], topoisomerase inhibitor, betulinic acid [138], central nervous system stimulant, caffeine [139], analgesic and antipyretic drug, paracetamol [140], N-alkyphenothiazines [141] and furosemide [142] with serum albumin. Various aspects such as binding parameters, binding sites, nature of interaction and the conformational changes involved have been studied. In some earlier papers, direct determination of binding parameters using equilibrium dialysis technique has been reported [76, 77, 129]. Chromatographic techniques have also been used in some cases [143-146]. In most of the recent studies, however, spectroscopic techniques have been
employed [134-135, 137, 142].

Such studies on antidiabetic drugs are few. Equilibrium dialysis technique has been used for some sulfonylurea drugs [147-152]. However these are very old references and only preliminary work has been reported.

Shibukawa et al. [153] have developed a novel chromatographic method for the binding of troglitazone to human serum albumin. Binding of a sulfonylurea antidiabetic (SU-118) with HSA has been studied using ultraviolet absorption and fluorescence spectroscopic techniques [82(d)]. It has been postulated that hydrophobic interactions are predominantly involved and drug can bind to both site I and site II on the HSA molecule. For the antidiabetic drugs used in the present work, detailed studies on various aspects of their complexation with serum albumin are not available. Few available reports include binding of glibenclamide [154] and glimepiride [155] with human serum albumin using mass spectrometry and liquid chromatography, respectively. Interaction with human plasma has been reported for repaglinide using ultrafiltration technique [156]. These reports do not provide any detailed information on the interaction mechanism involved. Pioglitazone hydrochloride is the only drug for which interaction with human serum albumin using fluorescence spectroscopic technique has been reported recently [157]. Results have indicated that pioglitazone hydrochloride has high affinity for human serum albumin, static quenching mechanism is operative and hydrogen bonding interaction is predominantly involved.

**Glycosylated albumin-drug interaction**

The effect of glycosylation of human serum albumin on the binding of furosemide, naproxen, procaine, phenylbutazone, salicylic acid, sulphamethoxazole, tolbutamide and warfarin has been reported by Koizumi et al. [69] using ultrafiltration technique. No alteration in the drug binding affinity was observed in the early stage when glycosylation occurred only at lys-525 site. On further glycosylation, binding affinity generally decreased due to a conformational change or steric hinderance. Bohney and Feldhoff [158] have studied the effect of bound fatty acids and non-enzymatic glycosylation
on the binding of tryptophan to human serum albumin. The influence of glycosylation on the binding affinity of some sulfonylureas and a biguanide, metformin hydrochloride has also been reported [159]. Tsuchiya et al. [87] have shown that compared to non-glycosylated albumin, the amount of bound sulfonylurea decreased by 44% in the case of tolazamide and acetoxyhexamide, 50% in the case of glibenclamide and 52% in the case of tolbutamide. Altered binding capacity is primarily due to the modification of the three-dimensional structure of albumin caused by the covalent binding of glucose. They concluded that free drug concentration can exceed the normal levels in diabetic patients with high concentration of glycosylated HSA. The binding properties of some hypoglycemic drugs to glycosylated HSA using fluorescence quenching method have also been investigated [159]. The authors have shown that the glycosylation not only decreases the ability of HSA to bind hypoglycemic drugs, it also changes the displacement pattern at site II. It is suggested that extensive glycosylation of plasma protein in diabetic patients complicates drug-drug interactions beyond those seen in normal people.

Drug-drug interaction: An important aspect of drug-drug interactions, relevant in the present context, is the competition between drugs for the binding sites on serum albumin. Such studies on a wide range of drug combinations have been reported [160-162]. For example, a fluoroquinolone, ciprofloxacin reduces the binding of antihypertensive drug, captopril resulting in large increase in the concentration of free captopril [163]. Similarly methotrexate toxicity has been observed when used in combination with aspirin [164]. Displacement of anticoagulants, warfarin and dicumarol from the binding sites in human serum albumin by various acidic drugs and free fatty acids have been studied by a number of authors [165-167]. Wilting et al. [168] have observed that the affinity of warfarin for albumin is decreased by chloride ions. Afifi [169] has investigated the influence of various monovalent and divalent cations and anions as well as various buffer systems on the binding of anti-inflammatory drug, tenoxicam to human serum albumin. Effect of some toxic ions and common ions on the binding of vitamin K3 to bovine serum
albumin has also been reported [170]. Effect of co-administered drugs on the oxaprozin binding to human serum albumin has been studied by Aubry et al. [171]. Some other recently reported drug-drug interactions include competitive binding of 3'-azido-3'-deoxythymidine and salicylic acid [172], propranolol and NSAIDs [173] and neomycin and ponceau S [174]. Drug-drug interactions are particularly important for highly protein bound drugs with a small margin of safety [175]. In general, displacement is clinically significant for low clearance drugs with a small volume of distribution and a low therapeutic index [94].

Amongst antidiabetic drugs used, competitive binding of sulfonylureas and anionic drugs [148 (b)], coumarin derivatives [148 (c)] and ethyl alcohol [149] using equilibrium dialysis technique has been reported. It has been recently reported [176] that the presence of gliclazide and metformin hydrochloride increase the free plasma concentration of caffeine. On the other hand, drug interactions with oral hypoglycemic agent, acarbose are uncommon. It has been reported that at normal therapeutic levels, acarbose does not change the pharmacokinetic profile of glibenclamide [178(a)] and digoxin [178(b)]. Acarbose, however, reduces the bioavailability of metformin hydrochloride [178(c)].

**Competitive binding of drugs and endogenous substances**

Three endogenous substances, bilirubin, hemin and chloride ions were used for the present studies.

**Bilirubin:** Bilirubin, the yellow breakdown product of heme catabolism, is excreted in bile and its levels are elevated in certain diseases. Bilirubin is a highly lipid-soluble substance; there is no blood-brain barrier for unbound bilirubin. Bilirubin is known to bind to human serum albumin at a specific high affinity site [179]. Free unconjugated bilirubin can enter the central nervous system, causing serious adverse effects [14] while albumin-bound bilirubin appears to be non-toxic [179]. Fluorescence quenching studies have been used by Levine [180] to study the binding affinity and binding capacity of albumin for bilirubin and the effect of a diuretic drug, furosemide on bilirubin-albumin interaction. It has also been reported [181] that the quenching of
tryptophan fluorescence by energy transfer from tryptophan to bilirubin, unmasks the tyrosine fluorescence in protein. A number of drugs have been shown to compete with bilirubin for its albumin-binding sites, resulting in bilirubin displacement and toxicity [182-184]. Cobinding of ceftriaxone sodium, a cephalosporin antibiotic and bilirubin to albumin is shown to be competitive [185]. Ceftriaxone is a strong bilirubin displacer. Competitive binding of bilirubin and twenty different drugs to HSA has been studied by Broderson [186]. Competitive binding mechanism has been suggested in most cases.

**Hemin**: Hemin is an important porphyrin residue of hemoglobin that binds to the hydrophobic region of albumin with high specificity [16]. Porphyrins and related compounds are also widely used as therapeutic agents. Hemin-bovine serum albumin interaction has been reported by Ponka et al. [16] and others [187]. Silva et al. [136], using fluorescence technique, have shown that hemin readily binds to bovine serum albumin and the binding causes conformational changes in albumin molecule. It has been suggested that the primary binding site for hemin on bovine serum albumin may be located asymmetrically between the two tryptophans along the sequence formed by subdomains IB and IIA, closer to tryptophan residue 212. From studies involving binding of a phenothiazine drug, chlorpromazine to BSA in the presence of hemin [136], it has been concluded that hemin makes tryptophan residues more accessible to the drug.

Hemin also binds to human serum albumin (HSA) with a high association constant [187]. Based on the magnitude of the binding constant and results of kinetic studies, Hrkal et al. [188] suggested that the primary binding site is located in a part of subdomain IB and a part of subdomain IIA. They proposed that the presence of C-terminal part of albumin was essential for the spatial configuration of the site. Adans and Berman [189] proposed that a chemical interaction of hemin with a group on protein (HSA) surface is followed by an entropy-controlled internalization of the hemin molecule.

**Chloride ions**: The salt (NaCl) concentration of blood is about 9 g/L which is equivalent to about 0.15 M. Sodium chloride solution of this concentration is
also referred to as 'normal saline'. HSA is known to bind a number of ions such as Cl', Br', I' etc. Chloride ion binding to bovine and human serum albumin has been studied by a variety of methods [190-193]. It has been suggested that there are two classes of chloride binding sites; strong sites and weak sites [17]. The effect of chloride ions on the binding of benoxaprofen [190], toлемитин [191], warfarin [167] and other drugs [192-193, 168] to HSA has been reported. In each case chloride ions were found to decrease the drug-HSA binding affinity.