INTRODUCTION

Every cell in the human body needs energy in order to function. The body’s primary energy source is glucose, a simple sugar resulting from the digestion of foods containing carbohydrates (sugars and starches). Glucose from the digested food is absorbed from the intestines into the blood stream. It circulates in the blood as a ready energy source for any cells that need it. However, cells are able to process glucose only with the help of insulin, a proteinaceous hormone, secreted by pancreas. When glucose enters bloodstream, the pancreas automatically produces the right amount of insulin to move glucose into the cells. Insulin binds to a receptor site on the outside of the cell and acts like a key to open doorway into the cell through which glucose can enter. Diabetes mellitus is a condition in which the pancreas no longer produces enough insulin or when cells stop responding to the insulin that is produced and the result is high blood glucose level, called hyperglycemia.

Diabetes mellitus can be classified as insulin-dependent diabetes mellitus (Type I) and non-insulin-dependent diabetes mellitus (Type II). Type I diabetes is characterized by a state of insulin deficiency resulting from autoimmune-mediated destruction of the pancreatic beta cells. Insulin, given subcutaneously, either by injection or insulin pump, is the primary treatment for patients with type I diabetes. Type II diabetes, on the other hand, is characterized mainly by insulin resistance; muscle, fat and liver cells do not respond properly to insulin and body needs more insulin to help glucose enter cells. When pancreas fails to keep up with the body’s need for insulin, high blood glucose levels build up (hyperglycemia). Type II diabetes is more common; over 90% of diabetic cases worldwide are type II. Moreover, its prevalence is increasing and has already reached epidemic proportions. Antidiabetic drugs, also called oral hypoglycemic agents, used alone or in combination provide effective therapy for type II diabetes mellitus [1-2].

Based on the chemical structures and mechanism of action, five major classes of hypoglycemic agents are available.
1. Sulfonylureas: All members of this class have an acidic functional group (sulfonylurea), a substituted aromatic ring and an alkyl group with 3-7 carbons on the urea nitrogen. Sulfonylureas stimulate beta cells in the pancreas to produce more insulin and also help the body use insulin. Sulfonylureas are further classified as first, second and third generation drugs based on their effectiveness. The first generation sulfonylureas are short acting due to the fast metabolic oxidation of the aromatic ring methyl groups into the inactive carboxylic acid metabolites. Second and third generation sulfonylureas are frequently used since they are generally longer acting, have fewer side effects and lesser drug interactions. Chemically, later generation drugs have more lipophilic groups than the previous generation. Classification scheme for sulfonylureas is given below and their basic structure is shown in Fig. 1. Substituents $R_1$ and $R_2$ for some representative drugs of this class are given in Table 1.
Table 1. Subsituents $R_1$ and $R_2$ for various sulfonylureas.

<table>
<thead>
<tr>
<th>Drug</th>
<th>$R_1$</th>
<th>$R_2$</th>
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<tbody>
<tr>
<td>Acetohexamide</td>
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<td><img src="image" alt="Acetohexamide" /></td>
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<tr>
<td>Chlorpropamide</td>
<td><img src="image" alt="Chlorpropamide" /></td>
<td>Cl</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td><img src="image" alt="Tolbutamide" /></td>
<td>H</td>
</tr>
<tr>
<td>Tolazamide</td>
<td><img src="image" alt="Tolazamide" /></td>
<td>H</td>
</tr>
<tr>
<td>Gliquidone</td>
<td><img src="image" alt="Gliquidone" /></td>
<td><img src="image" alt="Gliquidone" /></td>
</tr>
<tr>
<td>Glyclopyramide</td>
<td><img src="image" alt="Glyclopyramide" /></td>
<td>Cl</td>
</tr>
<tr>
<td>Gliclazide</td>
<td><img src="image" alt="Gliclazide" /></td>
<td>CH$_3$</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td><img src="image" alt="Glibenclamide" /></td>
<td><img src="image" alt="Glibenclamide" /></td>
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<tr>
<td>Glipizide</td>
<td><img src="image" alt="Glipizide" /></td>
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<td>Glimepiride</td>
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</table>
2. **Meglitinides**: The mechanism of action of meglitinides closely resembles that of the sulfonylureas. The meglitinides stimulate the release of insulin from pancreatic beta cells. However, this action is mediated through selective potassium channel blockers. Unlike the commonly used sulfonylureas, the meglitinides have a very short onset of action, shorter half-life and decreased risk of hypoglycemia. Repaglinide and nateglinide are two representative examples of this class. Chemical structures of the two drugs are shown in Fig. 2. The meglitinides are carboxylic (benzoic and phenylacetic) acid derivatives.

**Fig. 2.** Structures of the meglitinides.

![Chemical structure of Repaglinide](image)

**Repaglinide**

![Chemical structure of Nateglinide](image)

**Nateglinide**

3. **Biguanides**: Biguanides work by decreasing hepatic glucose production, decreasing absorption of glucose and improving body's ability to use insulin. As biguanides do not stimulate endogenous insulin secretion, hypoglycemia does not occur. Members of this class include phenformin,
buformin and metformin hydrochloride. However, phenformin and buformin are withdrawn from market since they are associated with high incidences of lactic acidosis. Metformin hydrochloride is the only biguanide which is commonly used. Basic structure of biguanides is shown in Fig.3. Due to the presence of biguanide group, formed as a condensation product of two guanidine groups, biguanides have basic and polar characteristics and hence poor bioavailability after oral administration. Subsituents $R_1$, $R_2$ and $R_3$ for various biguanides are given in Table 2.

![Basic structure of the biguanides.](image)

**Table 2.** $R_1$, $R_2$ and $R_3$ for various biguanides.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenformin</td>
<td>![Phenformin structure]</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Buformin</td>
<td>![Buformin structure]</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Metformin hydrochloride</td>
<td>![Metformin structure]</td>
<td>H</td>
<td>CH$_3$</td>
</tr>
</tbody>
</table>

**4. Thiazolidinediones:** Thiazolidinediones, also known as glitazones, are insulin sensitizers, useful in cases of insulin resistance. When administered alone, they do not have hypoglycemic or antihyperglycemic effects. However, when co-administered with insulin or other antidiabetic agents, notable improvement in insulin resistance is achieved. Pioglitazone, troglitazone, ciglitazone and rosiglitazone are some of the members of this class. Chemically they are derivatives of 2, 4- thiazolidinedione nucleus, substituted at position 5 with a benzyl group, which in turn is further substituted at position 4 with various lipophilic moieties (Fig.4). Subsituents $R_1$ for some representative glitazones are given in Table 3.
5. **Alfa-glucosidase Inhibitors**: Under normal conditions, the enzyme alfa-glucosidase generates glucose in the gastrointestinal tract by hydrolyzing the dietary complex carbohydrates. Inhibition of alfa-glucosidase prevents the hydrolysis of complex carbohydrates, thereby reducing the amount of glucose available for absorption from the gastrointestinal tract. Acarbose, voglibose and miglitol represent this class of drugs. Acarbose has a carbohydrate-like polymer structure. Voglibose and miglitol are polyhydroxylated cyclohexane derivatives (Fig. 5). The polar nature of these drugs precludes absorption after oral administration and ensures high concentrations in the gastrointestinal tract, where the drugs are designed to act.
Fig. 5. Structures of the alfa-glucosidase inhibitors.

Drugs used in the present work

I. Antidiabetic drugs

In the present work, five categories of antidiabetic drugs: sulfonylureas (gliclazide, glibenclamide, glipizide and glimepiride), meglitinide (repaglinide), biguanide (metformin hydrochloride), thiazolidinediones (pioglitazone hydrochloride and rosiglitazone maleate) and alfa-glucosidase inhibitor (acarbose) have been used. Some pharmacokinetic and physical properties of these drugs are described below.

1. Sulfonylureas: Most members of this class share similar kinetic properties for absorption, distribution, metabolism and excretion. Absorption after oral administration is generally rapid and complete (bioavailability~100%). Sulfonylureas are weak acids due to the marked delocalization of the nitrogen lone pair by the sulfonyl group. The pKa values are in the range 5.9-6.8. Sulfonylureas are extensively bound (98-99.5%) to plasma
proteins due to their lipophilic and acidic nature. Four sulfonylureas (gliclazide, glibenclamide, glipizide and glimepiride) were used in the present work. Gliclazide, \(\text{N-(hexahydrocyclopenta[c]pyrrol-2(1H)-ylcarbamoyl)-4-methylbenzenesulfonamide, C}_{16}\text{H}_{21}\text{N}_{3}\text{O}_{3}\text{S, mol. wt. 323.4}\), glibenclamide \(\text{N-(4-[N-(cyclohexylcarbamoyl)sulfamoyl]phenethyl)-2-methoxybenzamide, C}_{23}\text{H}_{28}\text{ClN}_{3}\text{O}_{5}\text{S, mol. wt. 494.0}\) and glipizide \(\text{N-(4-[N-(cyclohexylcarbamoyl)sulfamoyl]phenethyl)-5-methylpyrazine-2-carboxamide, C}_{21}\text{H}_{27}\text{N}_{5}\text{O}_{4}\text{S, mol. wt. 445.55}\) are white powders, while glimepiride \(\text{3-ethyl-4-methyl-N-(4-[N-((1r,4r)-4-methylcyclohexylcarbamoyl)sulfamoyl]phenethyl)-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide, C}_{24}\text{H}_{34}\text{N}_{4}\text{O}_{5}\text{S, mol. wt. 490.62}\) is a white to yellowish white crystalline powder.

2. **Meglitinides:** Repaglinide \{(S)-(+)\}-2-ethoxy-4-[2-(3-methyl-1-[2-(piperidin-1-yl)phenyl]butylamino)-2-oxoethyl]benzoic acid, C\(_{27}\)H\(_{36}\)N\(_2\)O\(_4\), mol. wt. 452.6\}, a commonly prescribed drug in this category, was used. It is a white powder, practically insoluble in water. Repaglinide is more rapid and short acting than sulfonylureas and produces fewer side effects. Its bioavailability is 56\%. In contrast to sulfonylureas, repaglinide possesses one weakly basic and one weakly acidic group resulting in amphoteric nature of the molecule. It is more than 98\% bound to plasma proteins.

3. **Biguanides:** Metformin hydrochloride \((\text{N,N-dimethylimidodicarbonimidic diamide hydrochloride, C}_{4}\text{H}_{11}\text{N}_{5}\text{.HCl, mol. wt. 165.63}\), the only guanide derivative in use, was studied. Metformin hydrochloride exists in white crystalline form and is freely soluble in water. Metformin hydrochloride is a basic drug, absorbed mainly from the small intestine with a bioavailability of about 60\%. Unlike sulfonylureas, it is not protein bound, is not metabolized and is rapidly eliminated by the kidney.

4. **Thiazolidinediones:** Pioglitazone hydrochloride \{(RS)-5-[4-(5-ethylpyridin-2-yl)ethoxy]benzyl\}thiazolidine-2,4-dione hydrochloride, C\(_{19}\)H\(_{20}\)N\(_2\)O\(_3\)S. HCl, mol. wt. 392.9\), a white crystalline solid and rosiglitazone maleate \{(RS)-5-[4-(2-[methyl(pyridine-2-y1)amino]ethoxy)benzyl\}thiazolidine-2,4-dione maleate, C\(_{18}\)H\(_{19}\)N\(_3\)O\(_3\)S. C\(_4\)H\(_4\)O\(_4\), mol. wt. 473.52\}, an off-white solid have been used in the present work. Thiazolidinediones are rapidly absorbed after oral administration with bioavailability in the range 80-99\%. These drugs
are metabolized in the liver by hydroxylation and demethylation. Glitazones contain both acidic and basic functionalities and are practically insoluble in water. They have high lipophilicity and are extensively bound to plasma proteins.

5. **Alfa-glucosidase Inhibitors:** Alfa-glucosidase Inhibitor used in the present work was acarbose \([(2R,3R,4R,5S,6R)-5-\{(2R,3R,4S,5S,6R)-3,4-dihydroxy-6-methyl-5-\{(1S,4R,5S,6S)-4,5,6-trihydoxy-3-(hydroxymethyl)cyclohex-2-en-1-yl\}amino]tetrahydro-2H-pyran-2-yloxy\}-3,4-dihydroxy-6-(hydroxymethyl]tetrahydro-2H-pyran-2-yl\}oxy\)-6-(hydroxymethyl)tetrahydro-2H-pyran-2,3,4-triol, \(C_{25}H_{43}NO_{18}\), mol. wt. 645.61), a white to yellowish white amorphous powder. It is freely soluble in water. Acarbose is not bound to plasma proteins. It is designed to act in the gastrointestinal tract, absorption after oral administration is negligible.

Pharmacokinetic and physical properties [3-7] of the antidiabetic drugs used in the present work are also summarized in Table 4.

Table 4. Pharmacokinetic and physical properties of the antidiabetic drugs used.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Physical property</th>
<th>Pharmacokinetic parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water solubility</td>
<td>Percent protein binding</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>Practically insoluble</td>
<td>98.0%</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>Practically insoluble</td>
<td>~99.0%</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>Practically insoluble</td>
<td>99.5%</td>
</tr>
<tr>
<td>Glipizide</td>
<td>Practically insoluble</td>
<td>98.5%</td>
</tr>
<tr>
<td>Pioglitazone hydrochloride</td>
<td>Practically insoluble</td>
<td>&gt;99.0%</td>
</tr>
<tr>
<td>Rosiglitazone maleate</td>
<td>Practically insoluble</td>
<td>99.8%</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>Practically insoluble</td>
<td>&gt;98.0%</td>
</tr>
<tr>
<td>Metformin hydrochloride</td>
<td>Freely soluble</td>
<td>Negligible</td>
</tr>
<tr>
<td>Acarbose</td>
<td>Freely soluble</td>
<td>Negligible</td>
</tr>
</tbody>
</table>

*Practically insoluble: <100\(\mu\)g/mL

**volume of distribution
II. Competing drugs and endogenous substances

a. Competing drugs

Four categories of competing drugs, a cephalosporin antibiotic (cefdinir), a fluoroquinolone antibiotic (sparfloxacin), a non-steroidal anti-inflammatory drug (parecoxib sodium) and analgesic and antipyretic drug (paracetamol) were used. Structures of the competing drugs are shown in Fig. 6. Some pharmacokinetic and physical properties [8-11] of various competing drugs used are described below.

1. **Cefdinir**: Cefdinir \(8\text{-}[2\text{-}(2\text{-amino}-1,3\text{-thiazol}-4\text{-yl})\text{-}1\text{-}hydroxy\text{-}2\text{-nitroso\text{-}ethenyln]amino\text{-}4\text{-}ethenyl\text{-}7\text{-}oxo\text{-}2\text{-thia}\text{-}6\text{-}azabicyclo[4.2.0]oct\text{-}4\text{-ene}\text{-}5\text{-carboxylic acid}, C_{14}H_{13}N_{5}O_{5}S_{2}, \text{mol. wt. 395.4} \) is white to slightly brownish yellow solid, slightly soluble in water. It is absorbed rapidly and well with half-life of 0.75 hr. Oral bioavailability is dose-dependent. Volume of distribution is 0.27 L/kg. Elimination is primarily via the kidneys.

2. **Sparfloxacin**: Sparfloxacin \(5\text{-amino\text{-}1\text{-cyclopropyl\text{-}7\text{-}[\{3R,5S\}3,5\text{-dimethylpiperazin\text{-}1\text{-yl}\}\text{-}6,8\text{-difluoro\text{-}4\text{-oxo\text{-}quinoline\text{-}3\text{-carboxylic acid, C}_{19}H_{22}F_{2}N_{4}O_{3}, \text{mol. wt. 392.4} \) is a yellowish crystalline powder and is practically insoluble in water. It is well absorbed following oral administration with bioavailability of 92%. Elimination half-life is 16-24 hours and volume of distribution is 1.7 L/kg. It is eliminated by both renal and non-renal routes and is widely distributed throughout the body.

3. **Parecoxib sodium**: Parecoxib sodium \(N\text{-}[4\text{-}(5\text{-methyl\text{-}3\text{-phenylisoxazol\text{-}4\text{-yl})phenylsulfonyl\text{-}propionamide, sodium salt, C}_{19}H_{17}N_{2}O_{3}Na, \text{mol. wt. 392.04} \) is a white to off-white solid that is very soluble in water. It is a pro drug of valdecoxib and is rapidly \(t_{1/2} =15\text{-}30 \text{ min} \) and completely converted via enzymatic hydrolysis to the pharmacologically active moiety, valdecoxib and propionic acid. The elimination half-life of valdecoxib is about 8 hours after the dosing of parecoxib sodium.

4. **Paracetamol**: Paracetamol \(N\text{-}[4\text{-}\text{(4\text{-hydroxyphenyl)ethanamide, C}_{9}H_{9}NO_{2}, \text{mol. wt. 151.17} \) is white to off-white powder that is freely soluble in water. It is well absorbed after oral administration with a bioavailability of about 100%. Its half life is 1-4 hours.
b. **Endogenous substances**

Endogenous substances are produced naturally in the body. Pharmacokinetics and toxicokinetics of endogenous substances has been reviewed by Marzo [12]. In the present work, bilirubin, hemin and chloride ions are used as endogenous substances. Some pharmacokinetic and physical properties of endogenous substances used in the present work are described below.

1. **Bilirubin**: Bilirubin is the yellow breakdown product of normal heme catabolism. Heme is found in hemoglobin, a principle component of red blood cells. Bilirubin is excreted in bile and its levels are elevated in certain diseases. The structure of bilirubin (Fig. 7) consists of an open chain of four pyrrole-like rings (tetrapyrrrole). Bilirubin \( \{2,7,13,17\text{-tetramethy}-1,19\text{-dioxo}-3,18\text{-divinyl}-1,10,19,22,23,24\text{-hexahydro}-21\text{H-bilene}-8,12\text{-dipropanoic acid}, \ C_{33}H_{36}N_4O_6, \ \text{mol. wt. 584.6}\} \) is apolar substance, practically insoluble in water. It is a dibasic acid and solubility increases with increase in pH [13]. Bilirubin is
very strongly but reversibly bound to human serum albumin. The binding forces are enthalpic and predominantly of electrostatic nature [14]. There is probably no blood-brain barrier for unbound bilirubin. Free bilirubin is highly toxic to cellular metabolism while albumin-bound bilirubin appears to be nontoxic.

Fig. 7. Structure of bilirubin

2. Hemin: Hemin, a reddish brown chloride of heme, is an iron-containing porphyrin produced from hemoglobin. Porphyrins are heterocyclic macrocycles characterized by the presence of four modified pyrrole subunits interconnected at their $\alpha$ carbon atoms via methine bridges (=CH-). The structure of hemin is shown in Fig. 8. Hemin { chloro[3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropanoato(2-)]iron(III), $C_{34}H_{32}N_4O_4FeCl$, mol. wt. 651.94} is practically insoluble in neutral water but is soluble under alkaline conditions [15]. Hemin is a highly lipophilic molecule and binds to the hydrophobic region of albumin with high specificity [16].

Fig. 8. Structure of hemin
3. **Chloride ions**: Chloride ions are the major extracellular anions, principally responsible for maintaining proper hydration, osmotic pressure and normal anion-cation balance in the vascular and intestinal fluid compartments. Chloride imbalance can lead to hypochloremia or hyperchloremia. The salt (NaCl) concentration of blood is about 9 g/L which is equivalent to about 0.15 M concentration of chloride ions. Sodium chloride solution of this concentration is also referred to as ‘normal saline’ and is commonly used in intravenous drips. Chloride ions are also reported [17] to bind to human serum albumin at some specific strong sites and weak sites.

**Gastrointestinal absorption of drugs**

The fate of the drug after oral administration is described by the LADMER system, according to which Liberation, Absorption, Distribution, Metabolism, Elimination and Response determine the overall therapeutic effect of the drug [18]. Drug absorption is the process of movement of drug from the site of administration to systemic circulation. For a drug to be absorbed, it needs to be in solution and therefore, for poorly-soluble drugs, drug dissolution is the rate-limiting step [19]. After entry into the systemic circulation, the drug is subjected to disposition processes such as distribution, metabolism and elimination. Distribution is the reversible transfer of drug between blood stream and other tissues and fluids. Drugs are eliminated from the body by biotransformation (metabolism) and excretion. The fraction of an administered dose of a drug that reaches the systemic circulation in unchanged form is known as the bioavailable dose. Bioavailability, defined as the rate and extent of drug absorption, is an important parameter in determining whether a therapeutically effective concentration will be achieved at the site of action.

**Biopharmaceutical properties**

Biopharmaceutical properties of drugs constitute major pharmaceutical factors, influencing the gastrointestinal absorption of drugs. Examples of such properties include lipophilicity, ionization behavior, drug solubility and dissolution rate, particle size and effective surface area, polymorphism and amorphism and drug stability. Some of these properties relevant in the present context are discussed below.
Lipophilicity: Lipophilicity represents the affinity of a drug molecule for lipophilic environment. It is usually measured by the ability of the drug to partition between 1-octanol (a model for biological membrane) and water or aqueous buffer [20]. The intrinsic lipophilicity (log P) of a compound refers to the equilibrium of the unionized drug between organic and aqueous phase. However, at physiological pH, many acidic and basic drugs are ionized and the overall ratio of drug, ionized and unionized, between the two phases is described as the distribution coefficient (log D). The distribution coefficient (log D) is widely used in recent years [21-22] to represent, the effective (or net) lipophilicity, taking into account both intrinsic lipophilicity and the degree of ionization. log P can be related to log D by the expression
\[ \log D = \log P + \log (1+10^{\text{pH} - \text{pK}_a}) \]
The magnitude and sign of log P/log D values can give an estimate of the ability of drug molecule to cross the biological membrane. For optimum bioavailability, the drug should be sufficiently hydrophilic to dissolve in the fluids at the absorption site and sufficiently lipophilic to cross the cellular barriers.

The partition/distribution coefficients can be measured experimentally by a variety of techniques, which are reviewed in a number of articles and books [23, 24(a)]. The simplest and most frequently used technique is the shake-flask technique. Leo et al. [23] have collected a vast amount of experimental data on the partition coefficient of drugs. Although experimental measurement of partition coefficient is reliable, it is costly and time consuming. Therefore, most of the data used in the literature is predicted. Various log P predictors such as milogP, lAlogP, ClogP, AlogP, Pallas 3.0 and KowWin are available. These programmes are based either on group contributions or molecular properties [24(b)]. Group contribution approach which includes atom-based methods and fragment-based methods is more commonly employed. Comparison of the calculated and experimentally determined data has been reported by Machatha and Yalkowsky [25].

Ionization Behaviour: Ionization behaviour of a drug constitutes an important biopharmaceutical property. The dissociation constant (pK_a) of the drug, the
lipid solubility of unionized drug and pH at the absorption site often influence the absorption characteristics of a drug throughout the gastrointestinal tract [26]. According to pH-partition hypothesis, the absorption of a weakly acidic or basic drug is determined chiefly by the extent to which the drug exists in its unionized form at the site of absorption. The extent of ionization of a drug at any pH can be calculated using appropriate form of Henderson-Hasselbatch equation

$$\log \frac{[A]}{[HA]} = pH - pK_a$$

for acidic drug, HA and

$$\log \frac{[BH]}{B} = pK_b - pH$$

for basic drug, B

Accordingly, a weakly acidic drug (pK_a 3) is more likely to be absorbed from the stomach (pH 1.2) where it is unionized and a weakly basic drug (pK_b 5) from the intestine (pH 6.8) where it is predominantly unionized. However, pH-partition theory has its limitations. The significantly larger surface area that is available for adsorption in the small intestine and larger small intestinal residence time, can compensate for the high degree of ionization of weakly acidic drugs at intestinal pH values [27]. Moreover, it has now been recognized that the gastrointestinal barrier is not completely impermeable to ionized drugs and unionized drugs must have sufficient lipid solubility to get absorbed [28]. Despite its limitations, the theory is still useful in basic understanding of the drug absorption process.

The extent of drug ionization under given conditions is also a useful quantity in relation to drug dissolution in the gastrointestinal tract, drug development process and various pharmacokinetic and pharmacodynamic drug interactions.

**Solubility and dissolution rate:** Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility of drug molecules. A drug is available for absorption only if it has sufficient solubility within the gastrointestinal tract. The poor aqueous solubility gives rise to
difficulties in the design of pharmaceutical formulations and leads to variable oral bioavailability [29]. Moreover, a solution of the drug is required for various drug development studies. In general, a drug is categorized as highly soluble if the highest dosage strength is soluble in 250 mL or less of aqueous media across the physiological pH range. Poorly-soluble drugs, on the other hand, can be defined as those with an aqueous solubility less than 100 μg/mL. Various approaches commonly used to enhance the solubility of poorly-soluble drugs include pH adjustment [30-31], use of co-solvents [32-33], selection of salt form [34-35], increase of specific surface area by reduction of particle size [36-37], use of surfactants for increased wettability [38-39], complex formation with excipients such as hydrophilic polymers and cyclodextrins [40-41], change of crystal form (polymorphism/ amorphism) [42], preparation of solid dispersions [43-44] and hydrotropy [45-46]. Reviews by Lipinski et al. [47], Panchagnule and Thomas [18] and Strickley [48 (a)] are also available on the subject.

**Drug interactions:** Drug interactions alter the biological activity, i.e. the amount and duration of drug's availability at receptor sites. Drug interactions may be classified as (i) pharmacokinetic (ii) pharmacodynamic and (iii) pharmaceutical interactions [49-50]. Pharmacokinetic interactions result in alteration of absorption, distribution, metabolism and excretion of the parent drug. Pharmacodynamic interactions, on the other hand, modify the activity of drug at the site of action. Pharmaceutical interaction occurs when drugs are mixed with excipients in formulations. However, it is also useful to categorize them as drug-protein, drug-drug and drug-excipient interactions. Drug-protein and drug-drug interactions, relevant in the present context, are discussed below.

**Drug-protein interactions:** An orally administered drug is absorbed through the gastrointestinal tract into the blood stream. In the blood stream drug interacts with plasma proteins, blood cells and body tissues. The main interaction of drug in the blood compartment is with plasma proteins. Serum albumin, α1-acid glycoprotein and lipoproteins are the three major proteins
responsible for drug binding in plasma. However, serum albumin which constitutes about 60% of total plasma protein concentration, has high binding capacity as well as binding affinity for a wide variety of exogenous and endogenous ligands [51]. The binding of drugs to serum albumin is reversible. The bound drug is in equilibrium with the free drug in plasma and is released as the free drug concentration falls below the therapeutic value. Thus the availability of drug at the site of action is maintained and the duration of action is prolonged. Complexation with plasma proteins also delays the metabolism and excretion of the drug [52]. An increased solubility, decreased toxicity and protection against degradation of the bound ligand are some other advantages of drug-albumin binding [53]. Human serum albumin (HSA) has been used in the present work due to its physiological significance.

A brief description of the structure and properties of human serum albumin is given below.

**Structure and properties of human serum albumin (HSA)**

Human serum albumin, the most abundant protein in blood plasma, is produced in the liver. It shows a typical blood concentration of 5 g/100 mL, has serum half-life of approximately 20 days and a molar mass of 66.5 kDa [51]. HSA has an isoelectric point of 4.7 and therefore, has a net negative charge at physiological pH [54]. Albumin plays a major role in numerous physiological activities. Albumin strongly contributes to osmotic pressure in the blood stream. It is a possible source of amino acids for various tissues and provides most of the acid/base buffering action of the plasma [55]. In addition, it is a well known carrier for endogenous and exogenous ligands such as fatty acids, bilirubin, hemin, thyroxine and a wide variety of drugs [56]. Its physiological and pharmacological properties have been extensively studied [57-60].

The first crystal structure of HSA at low resolution was reported by Carter and co-workers in 1989 [61] and its refined structure at 2.8 Å resolution was published by the same group in 1992 [56]. Later high resolution crystallographic analysis of HSA in 1999 by Curry et al. [62] and Sugio et al.
Introduction

[63] revealed not only the structural features of protein but also how protein binds a variety of ligands. The crystal structure of HSA is shown in Fig. 9. X-ray crystallographic analysis revealed that HSA is a heart shaped or equilateral triangular molecule; 80 Å on a side, with average thickness of 30 Å and a calculated molecular volume of about 88,249 Å³ [56]. The protein is a single polypeptide with 585 amino acid residues containing 17 disulfide bridges, one free thiol (34cys) and a single tryptophan (214trp). The three-dimensional structure of human serum albumin is influenced and stabilized to a great extent by the disulfide bridges. Albumin has a high percentage of ionic amino acids, glutamic acid and lysine, which confer a relatively high solubility to the protein. As far as secondary structure is concerned, albumin is a highly helical molecule with 67% α-helix content, 23% extended chain and 10% β-turn [63]. HSA is organized in three domains (I, II, III) and each of these is comprised of two subdomains (A and B).

Fig.9. X-ray crystallographic structure of fatty acid-free HSA.

(Subdomains and the two primary drug-binding sites of HSA are indicated. Dotted bar represents spatial dimension of the interdomain cleft, and asterisk indicates the position of Trp214).
Alb...
residues of polypeptide chains. Lysine 525 has been identified as the principle site of glycosylation [69]. Glycosylation also causes conformational changes in the HSA molecule; the environment of tryptophan residue becomes more hydrophobic. Shaklai et al. [70(a)] have shown that bilirubin-binding site is affected by glycosylation while hemin-binding site is unaffected. Structural changes in glycosylated albumin also lead to a reduction in the affinity for drugs and fatty acids [70(b)].

**Drug-HSA interaction**

Drug-HSA interactions are of considerable clinical and pharmaceutical interest, since the overall distribution, metabolism and efficacy of drugs in the body is governed by their affinities towards serum albumin. The bound drug is both pharmacokinetically and pharmacodynamically inactive; only the free drug has pharmacological activity. The effect is especially significant for highly protein bound drugs with a narrow therapeutic index and a small volume of distribution [71]. For these drugs, only a small alteration in bound fraction can produce a profound change in the pharmacodynamically active free drug concentration. The nature of forces involved in drug-protein interaction also play a significant role in drug action because the dissociation of drug-protein complex is required for release of free drug. Binding properties of drugs can be useful to create a reservoir from which the drug can be released slowly, for transport of poorly soluble drugs and to prevent or minimize drug degradation and drug toxicity [72]. It may be mentioned that albumin-binding is not always a desirable property. If a drug binds too tightly to HSA, it can get trapped in the blood stream, meaning higher doses are needed to ensure that the drug’s benefits are felt [73]. Working out which structural features of drugs are responsible for binding to HSA may also make it possible to design new compounds with appropriate HSA-binding properties. Structural basis of drug-HSA interaction has been reported by Ghuman et al. [74] and others [75].

The techniques which are commonly employed to study drug-HSA interaction include equilibrium dialysis [76-77], ultrafiltration [78], circular dichroism [55, 79], ultraviolet absorption spectroscopy [80] and fluorescence spectroscopy [81-82]. Variation in the HSA binding affinity in the diseased
state and other environmental factors such as pH, temperature, HSA concentration, presence of metal ions, chloride ions etc. provide useful information from clinical point of view [83-84]. Association constants for binding have also been correlated to physical properties such as hydrophobicity, pK_a values, steric factor and solubility parameter [85-86].

**Binding of drugs to glycosylated albumin**

Non-enzymatic glycosylation of albumin induces changes in the chemical, physical and ultimately functional and biological properties of albumin [87]. The concentration of glycosylated albumin is 2-3-fold higher in diabetic patients. Glycosylation of human serum albumin (HSA) alters its drug binding capacity thereby influencing the distribution and elimination of drugs and hence the duration and intensity of pharmacological effect [69, 49].

**Drug-drug interactions**

In-vivo drug-drug interactions may be pharmacokinetic or pharmacodynamic in nature. A pharmacokinetic interaction affects the absorption, distribution, metabolism and elimination of either drug while a pharmacodynamic interaction may increase/decrease the activity of the parent drug or cause unexpected side effects. An important aspect of drug-drug interaction, relevant in the present context involves the competition between drugs for the binding sites on human serum albumin (HSA). The binding of a drug is often influenced by the simultaneous binding of other drugs because serum albumin has a limited number of regions for high affinity binding and moreover, the protein is a flexible molecule [49, 57(a)]. Such information is important because alteration in the protein binding may alter the volume of distribution, clearance and elimination of a drug and may modulate its therapeutic effect. In general, plasma protein binding displacement is clinically significant for highly protein bound, low clearance drugs with a small volume of distribution and a narrow therapeutic index [88]. The mechanism involved is called competitive interference when two or more drugs compete with one another for the same high affinity binding site on serum albumin and non-competitive interference, when the drugs bind at different sites but cause conformational changes in the binding protein. In either case the biological
activity of drugs used in the combination is altered. Drug-drug interactions may also be manipulated to have desirable effects such as increased therapeutic efficiency, reduced toxicity, improved physicochemical characteristics of formulation etc. [89].