SUMMARY AND CONCLUSIONS

In the present thesis some physico-chemical characteristics and pharmacologically relevant drug interactions for five categories of antidiabetic drugs have been studied. The selected drugs included four sulfonylureas (gliclazide, glibenclamide, glimepiride and glipizide), a meglitinide (repaglinide), a biguanide (metformin hydrochloride), two thiazolidinediones (pioglitazone hydrochloride and rosiglitazone maleate) and alpha-glucosidase inhibitor (acarbose). Following aspects have been studied: i) Drug estimation, ii) Ionization behavior and lipophilicity, iii) Solubility enhancement using co-solvent solubilization, micellar solubilization and drug combination techniques, iv) Mechanism of interaction with non-glycosylated and glycosylated human serum albumin, v) Competitive drug-drug and drug-endogenous substances binding on human serum albumin.

1. Drug Estimation

Ultra-violet absorption spectrophotometric methods have been standardized for the estimation of eight drug samples. A range of solvents, based on the solubility of drugs, stability of drug solutions and other specific requirements for the experiments carried out subsequently, were used. The accuracy, sensitivity and reproducibility of drug estimation methods was determined from various optical parameters such as wavelength for maximum absorption ($\lambda_{\text{max}}$), Beer’s law limits, molar extinction coefficient ($\varepsilon$) and sensitivity and statistical parameters such as coefficient of determination ($R^2$), 95% confidence interval and standard error of estimate (SEE), obtained from the data.

2. Drug Ionization and Lipophilicity

Drug ionization data (pK$_a$ values) were used to calculate the percentage ionization of drugs at different pH values in some physiologically relevant locations in the gastrointestinal tract such as stomach, intestine and blood. It was concluded that sulfonylureas exist as neutral species at pH 1.2 and predominantly anionic species at pH 6.8 and 7.4. Glitazones and repaglinide exist as cationic species at pH 1.2 and predominantly anionic species in the pH range 6.8-7.4 while metformin hydrochloride exists as
cationic species at all pH values. Acarbose exists as cationic species in the acidic conditions of the stomach (pH 1.2) and neutral species in the intestine (pH 6.8) and blood (pH 7.4).

1-octanol-buffer (pH 7.4) distribution coefficients (logD_{app}) of various drug samples were determined by shake flask method. The values were also corrected for drug ionization to determine the intrinsic lipophilicity. It was also concluded that predicted data, calculated using commercially available software is not very reliable and for a meaningful correlation only experimental data should be used. The information about drug ionization and lipophilicity can be useful in understanding the drug absorption process and various drug interactions.

3. Solubility Enhancement: Following four techniques were used to enhance the solubility of seven poorly-soluble antidiabetic drugs. (i) pH modification, (ii) Co-solvent solubilization, (iii) Micellar solubilization and (iv) Drug combination approach.

3.1 pH modification

pH-solubility profiles of various drugs were determined. For sulfonylureas, solubility in alkaline range was much higher than in the acidic range. All sulfonylureas except glipizide exhibited a small amphoteric behavior. Repaglinide and pioglitazone hydrochloride had very pronounced amphoteric character. Rosiglitazone maleate was also amphoteric but the effect of pH on solubility was not very pronounced. Noticeable enhancement in solubility was observed only at extremes of pH scale and maximum solubility was much less than 1mg/mL in most cases.

3.2 Co-solvent solubilization

For co-solvent solubilization technique, polyethylene glycol 400, polyethylene glycol 8000, propylene glycol, glycerol, ethanol and propylene glycol + ethanol (1:1 by volume) were used as co-solvents at concentrations of 20 and 40%. Results have been expressed as solubility (μg/mL) and solubilization efficiency. In the case of sulfonylureas and repaglinide, at 20% concentration of co-solvent in water, 3-4.5 times enhancement in aqueous solubility was observed. On increasing the concentration of co-solvent to 40%, the increase was very large (146 times) for glimepiride while in other cases it
varied from 7-29 times. The combination of co-solvent and buffer, however, produced synergistic increase in solubility of drugs. At 20% concentration of co-solvent, 12-209 times increase was observed as compared to 3-4.5 times for co-solvent solutions in water. The corresponding increase at 40% concentration of co-solvent was enormous; 153-763 times as compared to 7-146 times in aqueous medium.

In the case of glitazones, the solubilization efficiency of co-solvents was much higher in aqueous medium as compared to the corresponding values for sulfonylureas and repaglinide. The maximum increase was 42-53 times and 513-792 times at 20 and 40% co-solvent concentrations, respectively. However, the corresponding increase for the combined effect of co-solvent and buffer was much less. This may be due to the basic nature of glitazones with buffer solubilities lower than aqueous solubilities. Solubility greater than 10 mg/mL in the case of gliclazide, repaglinide and glitazones and about 1 – 5 mg/mL in the case of glipizide, glimepiride and glibenclamide could be attained. The significance of solubility data in relation to the development of drug formulation has also been discussed.

### 3.3 Micellar Solubilization

Micellar solubilization of poorly-soluble antidiabetic drugs was also studied using anionic (SDS), cationic (CTAB) and non-ionic (Tween-80) surfactants as well as ionic + non-ionic surfactant mixtures at 50 mM micellar concentration in water, salt (0.15 M NaCl) and buffer (PB, pH 7.4) media. A very large enhancement in aqueous solubility was observed in each case. In general, non-ionic surfactant was found to be a better solvent as compared to ionic surfactants in aqueous and salt solutions while cationic surfactant (CTAB) was a better solvent in buffer medium. Amongst ionic surfactants, all sulfonylureas except glipizide had higher solubility in cationic surfactant (CTAB) while glipizide and pioglitazone hydrochloride had higher solubility in anionic surfactant (SDS). For rosiglitazone maleate and repaglinide, the solubility was not much affected by the nature of ionic surfactant. The increase in aqueous solubility obtained from solubilization efficiency data was about 2-91 times, 20-25 times and 6-30 times for sulfonylureas, repaglinide and glitazones, respectively.
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Solubility in surfactants was also determined in the presence of 0.15 M NaCl. In the case of anionic surfactant (SDS), the presence of salt increased solubilization in all cases while for cationic (CTAB) and non-ionic (Tween-80) surfactants, the effect of salt was, in general, much smaller. Moreover, it also varied with the nature of drug sample. Significant decrease in solubility has also been observed for some sulfonylureas in Tween-80. The solubilization efficiency was about 7-112 times, 20-26 times and 8-33 times for sulfonylureas, repaglinide and glitazones, respectively. Increase/decrease in aqueous solubility in the presence of salt has also been used to get an estimate of the possible locus of solubilization of drugs in the micellar medium. It was concluded that in anionic surfactant (SDS), all the drugs except glipizide are solubilized in the inner core of the micelle. In cationic surfactant (CTAB), glipizide, pioglitazone hydrochloride and rosiglitazone maleate are solubilized in the inner core while gliclazide and glimepiride are solubilized in the outer palisade layer. Solubility of glibenclamide and repaglinide was not much affected by the presence of salt. In non-ionic surfactant (Tween-80) most of the drugs were solubilized in the outer palisade layer.

The combined effect of surfactant and buffer was synergistic in most cases; an enormous increase in solubility was observed. For sulfonylureas, repaglinide and glitazones, respectively the enhancement of aqueous solubility by the combined effect of surfactant and buffer was about 9-357, 62 and 4-35 times in SDS; about 37-1333, 366 and 73-102 times in CTAB and about 19-100, 153 and 2-12 times in Tween-80. Thus the synergistic effect of surfactant and buffer is relatively more pronounced for ionic surfactants, especially cationic surfactant (CTAB) as compared to non-ionic surfactant (Tween-80). In the case of glitazones, however, in some cases the presence of surfactant and buffer had negative effect on solubilization.

In general, surfactant mixtures were found to be much better solubilizing agents than single surfactants. The observed solubility was found to be much larger than the value calculated for the equimolar mixture of the two surfactants. The solubility enhancement was specially significant for gliclazide and glimepiride where 241-388 and 405-431 times, respectively increase in aqueous solubility was observed. The synergistic effect of mixed

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surfactant systems varied with the dissolution medium used. In most cases, 0.15 M NaCl was most effective while buffer was least effective. Thus combined effect of surfactant mixtures and buffer was not synergistic. In NaCl medium, surfactant mixtures produced enormous enhancement in aqueous solubility in most cases. For gliclazide, glibenclamide, glimepiride, pioglitazone hydrochloride and rosiglitazone maleate, the enhancement of aqueous solubility was about 124-131, 824-878, 589-667, 173-503 and 239-269 times, respectively.

Surfactant mixtures were found to be particularly good solvents for some very poorly-soluble antidiabetic drugs. Glibenclamide, glimepiride, pioglitazone hydrochloride and rosiglitazone maleate, with aqueous solubilities in the range 0.006 - 0.030 mg/mL could be dissolved up to about 5.24, 4.27, 7.06 and 8.25 mg/mL, respectively in mixed surfactant systems at 25°C. A very high solubility (>14mg/mL) could also be attained for gliclazide. Surfactant solubilization parameters; molar solubilization capacity ($\chi$), micelle-water partition coefficient ($\kappa$) and standard free energy change of solubilization ($\Delta G^\circ$), gave quantitative estimate of the solubilization efficiency of the surfactant system.

### 3.4 Drug Combination Approach

Since combination therapy is common in type II diabetes, drug combination approach has also been used to enhance the solubility of poorly-soluble antidiabetic drugs. Such an approach has been used for the first time. Antidiabetic-antidiabetic binary and ternary drug combinations have been studied and enhancement of solubility has been expressed in terms of the solubilization efficiency data. In binary systems, the solubility of very poorly-soluble drugs, glibenclamide and glimepiride increased by about 64 and 73 times, respectively in the presence of rosiglitazone maleate and that of rosiglitazone maleate increased by about 41 times in the presence of repaglinide. About 11-24 times increase has been observed for other combinations. Solubility of repaglinide in the presence of sulfonylureas and glibenclamide in the presence of repaglinide, however, decreased by about 2-5 times. In general, the combined effect of combination drug and buffer produced synergistic enhancement of aqueous solubility in the case of
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Sulfonylureas and repaglinide. About 11-68 times increase in aqueous solubility was observed for some drug combinations. The solubility of glitazones in the presence of other drugs, however, decreased in buffer medium. The decrease was quite large (12-33 times) for some drug combinations.

Ternary drug combinations consisted of the binary drug combinations containing fixed concentration (100 μM) of freely-soluble drug, acarbose. In the ternary systems, in general, the enhancement of solubility was much larger as compared to the binary systems. More than 100 times enhancement in aqueous solubility was observed only in ternary systems. The effect was especially significant in combinations involving sulfonylureas and rosiglitazone maleate. Gliclazide, glibenclamide and glimepiride could be dissolved up to 18.95, 3.76 and 7.68 mg/mL in the presence of rosiglitazone maleate + acarbose while rosiglitazone maleate could be dissolved up to 9.49 mg/mL in the presence of sulfonylureas + acarbose. In terms of solubilization efficiency, an enormous increase (507-1200 times) in solubility of all sulfonylureas except glipizide and 47-309 times increase in solubility of rosiglitazone maleate was observed. About 17 and 11-15 times increase in the solubility of glimepiride and pioglitazone hydrochloride was also observed. A 631 and 1200 times increase in the solubility of practically insoluble drugs, glibenclamide and glimepiride, respectively is particularly noticeable. Combined effect of ternary drug combination and buffer produced much larger enhancement in solubility as compared to aqueous ternary systems for some drug combinations. In general, the solubility enhancement was enormous for sulfonylureas. The presence of buffer, however, retarded the dissolution process in the case of glitazones. Both increase as well as decrease in drug solubility due to the presence of combination drug has lot of significance in the design of multi-drug formulations for combination therapy in the treatment of type II diabetes.

The results have been discussed considering that the second drug acts as a co-solvent for the parent drug. The extent of enhancement of aqueous solubility has been discussed in terms of the relative magnitudes of the solute-
solute, solute-solvent and various inter- and intramolecular solvent-solvent interactions.

4. DRUG INTERACTIONS

4.1 Mechanism of interaction with human serum albumin (HSA)

Mechanism of interaction and detailed physico-chemical characterization of the binding of four antidiabetic drugs, gliclazide, glimepiride, glipizide and repaglinide with human serum albumin has been studied using fluorescence spectroscopic technique. Results have been discussed in terms of the stoichiometry of interaction, binding parameters, thermodynamics of the binding process, the nature of the forces involved in the binding, identification of the drug binding sites and the fluorescence quenching mechanism involved.

Quenching of intrinsic HSA fluorescence was monitored. Small red shift (4-8 nm) in emission wavelength showed that the bound drugs decreased the hydrophobicity in the microenvironment of tryptophan. The stoichiometry of interaction was determined by the method of continuous variation. There was only one class of binding sites with association constants of the order of $10^5$. At low drug: protein ratios about 46-74% of the added antidiabetic drugs were bound at physiological temperature. Thus only about 26-54% of drug is free for physiological effect. Thermodynamic parameters, calculated from the temperature-dependence of association constants, were used to predict the nature of interaction involved. In the case of glimepiride and glipizide, due to temperature-dependent conformational changes, binding behavior at temperatures greater than 25°C was different and therefore, the nature of interaction in the temperature range 15-25°C was also different from that in the range 25-37°C. High negative $\Delta G^\circ$ values in each case showed spontaneity of the binding process. Large positive enthalpy change ($\Delta H^\circ$) and entropy change ($\Delta S^\circ$) in all cases, except glimepiride in the lower temperature range and glipizide in the higher temperature range, indicated that in general, the interaction is entropy driven and hydrophobic interactions are primarily involved in the binding of these drugs to HSA. For glimepiride in the lower temperature range, hydrogen bonding and Van der Waal's interactions were involved while for glipizide in the higher temperature range, hydrogen
bonding, electrostatic and hydrophobic interactions were involved. A comparison of percentage quenching at excitation wavelengths of 295 and 280 nm showed that tyrosine residues are involved in the binding of all drugs except glimepiride.

In order to further understand the nature of interaction involved, binding studies were also carried out in the presence of hydrophobic probe (1-anilino-naphthalene – 8 – sulfonate, ANS), site I-specific probe (Dansylamide, DA) and site II-specific probe (Dansylsarcosine, DSS). In the case of ANS, quenching of HSA by drugs and ANS under identical conditions and displacement of ANS from HSA-ANS complex by drugs was studied. Both studies showed that although hydrophobic interactions are involved, primary drug binding sites do not coincide with the ANS binding sites. From the fluorescence probe displacement studies, carried out in the presence of site-specific probe, it was concluded that gliclazide and repaglinide bind to site II on the HSA molecule. Although glimepiride and glipizide bind to both sites I and II, they have greater affinity for site II than site I. In most cases, the aromatic ring of 411Tyr appears to be involved in the binding within site II; the guanidino moiety of 410Arg is not involved. Glimepiride, however, binds at a different region within site II since tyrosine residues are not involved in the binding of this drug. For binding of glipizide in the higher temperature range, the contribution of electrostatic interactions in binding indicates that due to temperature-induced conformational changes, arginine residues are also involved.

Stern-Volmer analysis of data was useful in understanding the quenching mechanism involved. Downward curvature in simple Stern-Volmer plots showed that the tryptophan residues of HSA are not fully accessible to the drugs. Quenching constants, determined from modified Stern-Volmer equation were of the order of $10^4$. From the downward curvature in stern-volmer plots and the temperature-dependence of quenching constants, it was concluded that dynamic quenching mechanism dominates over the static process. High magnitude of the rate constant for quenching ($10^{13}$ M$^{-1}$s$^{-1}$) showed that the process is not entirely diffusion controlled; specific drug-protein interactions are also involved.
4.2 Complexation of antidiabetic drugs with glycosylated human serum albumin (G-HSA)

Since concentration of glycosylated albumin is 2-3 fold higher in diabetic patients and glycosylation alters the drug binding affinity of albumin, binding of four antidiabetic drugs (gliclazide, glimepiride, glipizide and repaglinide) with glycosylated albumin has also been studied. The results have been compared with those for non-glycosylated albumin. Glycosylation decreased the binding affinity of HSA for the drugs used. The order of association constants was $10^4$ for glycosylated HSA as compared to $10^5$ for non-glycosylated HSA. The percentage drug bound was only about 21-38% as compared to 46-74% for non-glycosylated HSA at physiological temperature. Thus the percentage of free drug available for antihyperglycemic effect was about double (62-79%) compared to the values for non-glycosylated HSA. Much higher free drug concentrations available for pharmacological effect can lead to the risk of hypoglycemia (low blood glucose levels). The nature of interaction was predicted from the thermodynamic parameters for the binding, determined from temperature-dependence of association constants. Large positive $\Delta H^0$ as well as $\Delta S^0$ values for all the drugs except gliclazide showed that hydrophobic interactions are predominantly involved in the binding. Large negative $\Delta H^0$ values and small positive $\Delta S^0$ values in the case of gliclazide showed the involvement of hydrogen bonding and electrostatic interactions.

Site specificity for G-HSA, determined from fluorescence probe displacement studies, was same as that for non-glycosylated HSA; gliclazide and repaglinide bind only at site II whereas glimepiride and glipizide bind at both site I and II. However, much larger percentage displacement of site II-specific probe (DSS) in G-HSA indicated that glycosylation causes conformational changes in albumin and the binding region within site II is different for glycosylated and non-glycosylated albumin. Simple Stern-Volmer plots were linear in all cases showing thereby that the tryptophan residues are fully accessible to the drugs. Stern-Volmer analysis, therefore, also indicated conformational changes in albumin as a result of glycosylation since tryptophan residues which were buried in HSA are fully accessible to drugs in G-HSA. Increase in quenching constant with increase in temperature in most
cases showed that dynamic quenching mechanism is involved for both glycosylated and non-glycosylated HSA.

4.3 Drug-drug interaction: Competitive drug-protein binding

Competitive binding of four antidiabetic drugs: gliclazide, glimepiride, glipizide and repaglinide in the presence of four categories of competing drugs; one non-steroidal anti-inflammatory drug (parecoxib sodium), two antibiotics (sparfloxacin and cefdinir), analgesic and antipyretic drug (paracetamol) and two antidiabetic drugs (metformin hydrochloride and repaglinide) has been studied. Competitive binding of competing drugs in the presence of antidiabetic drugs was also studied. Data has been expressed in terms of association constant (K), percentage of drug bound (β) and the percentage of free drug in the absence and presence of competing drugs. Results have been interpreted in terms of change in the percentage of free drug due to competitive binding (Δα) and the competitive binding mechanism has been proposed.

4.3.1 Binding of antidiabetic drugs in the presence of competing drugs

In most cases, the presence of competing drug decreased the association constant of the parent antidiabetic drug. However, increase as well as practically no change in association constants were also observed in some cases. Percentage binding of antidiabetic drugs in the presence of various competing drugs was also calculated. Due to the physiological significance of low drug:protein ([D]/[P]) ratios, all data has been analyzed at the lowest [D]/[P] ratio studied (0.6). Large decrease, up to 32%, in the percentage binding of antidiabetic drugs was observed in most cases. Percentage of bound gliclazide, however, increased by about 15% in the presence of repaglinide. Decreased binding affinity resulted in increase in the percentage of free antidiabetic agent available for antihyperglycemic effect.

To get a quantitative idea about the effect of competing drugs on the binding of antidiabetic drugs, change in the percentage of free antidiabetic drug due to competitive binding (Δα) was calculated for each drug combination. For this purpose less than 1% change was considered as no change, 1-5%, 5-10%, 10-20% and >20% change was considered as very
small change, small change, large change and very large change, respectively. A very large increase (>20%) was observed in the percentage of free gliclazide in the presence of sparfloxacin, parecoxib sodium and metformin hydrochloride and free repaglinide in the presence of cefdinir, sparfloxacin and parecoxib sodium. A large increase (10-20%) was observed in the percentage of free gliclazide in the presence of paracetamol, free glimepiride in the presence of repaglinide and metformin hydrochloride and free repaglinide in the presence of gliclazide and glimepiride. The percentage of free gliclazide, however, decreased by 15% in the presence of repaglinide. On comparing the competitive binding results for various antidiabetic drugs, it was concluded that a very large change (>20%) was observed in the binding of gliclazide and repaglinide while a large change (10-20%) was observed for gliclazide, glimepiride and repaglinide. For glipizide, the change was relatively small (< 10%).

4.3.2 Binding of competing drugs in the presence of antidiabetic drugs

Association constants for the binding of four competing drugs (cefdinir, sparfloxacin, parecoxib sodium and paracetamol) in the absence and presence of the antidiabetic drugs were also determined. The association constants of competing drugs increased in the presence of antidiabetic drugs in most cases. Maximum increase was observed for the binding of antibiotics (cefdinir and sparfloxacin) in the presence of antidiabetic drugs and paracetamol in the presence of glimepiride. Decreased binding affinity was observed only for parecoxib sodium in the presence of gliclazide and glimepiride.

Thus in most cases, competitive binding resulted in large decrease in the percentage of free drug. The criterion adopted for quantitative estimate of the competitive binding in terms of the change in percentage of free drug ($\Delta \alpha$) was same as described previously (sec. 4.3.1). A very large decrease (>20%) was observed in the percentage of free cefdinir in the presence of glipizide, repaglinide, free sparfloxacin in the presence of glimepiride and glipizide and free paracetamol in the presence of glimepiride. A large decrease (10-20%) was observed in the percentage of free cefdinir in the presence of gliclazide.
and free sparfloxacin in the presence of gliclazide and repaglinide. The percentage of free parecoxib sodium, however, increased by about 17 and 10%, respectively in the presence of gliclazide and glimepiride. Such combinations may produce serious fluctuations in the blood glucose level of diabetic patients and should be avoided. Other cases, where the change was small can be considered safe.

4.3.3 Competitive binding mechanism: For the binding of antidiabetic drugs in the presence of competing drug as well as the binding of competing drugs in the presence of antidiabetic drugs, the relative magnitudes of the association constants of drugs used in the combination could not explain the binding behavior. Site-specificity of the binding and competing drugs and the possible conformational changes in the HSA molecule as a result of competitive binding could explain the competitive binding mechanism involved.

For the binding of antidiabetic drugs, high affinity site II- specific antidiabetic drugs, gliclazide and repaglinide could not displace low affinity site II – specific competing drugs resulting in decreased binding and consequent increase in the percentage of free antidiabetic drugs. Since glimepiride and glipizide bind at both site I and Site II, it appears that in the presence of site II – specific competing drugs, these drugs mainly occupy site I and hence change in the binding affinity is very small. Thus combinations of studied competing drugs with repaglinide and gliclazide increase the risk of hypoglycemia while the corresponding combinations with glimepiride and glipizide are safe. Increased binding of the competing drugs in the presence of antidiabetic drugs could be explained on the basis of conformational changes in HSA molecule caused by simultaneous binding of competing and antidiabetic drugs. Thus efficacy of the competing drugs decreases in the presence of antidiabetic drugs.

4.4 Mechanism of interaction of endogenous substances with human serum albumin and their effect on antidiabetic drug–albumin interactions

4.4.1 Mechanism of interaction of bilirubin and hemin with human serum albumin: The association constants for the binding were of the order
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of $10^5$ for both ligands. However, hemin had higher affinity for HSA as compared to bilirubin. At low ligand:protein ratios, about 82-86% of ligands were bound. Studies carried out in the presence of site-specific probes showed that both hemin and bilirubin bind at site I as well as site II in HSA; bilirubin, however, had more affinity for site II than site I. Stern-Volmer analysis of fluorescence quenching data showed that the tryptophan residues of HSA are fully accessible to these ligands and static quenching mechanism is predominantly involved. Quenching constants were of the order of $10^4$ and $10^5$, respectively in the case of bilirubin and hemin.

4.4.2 Competitive binding of antidiabetic drugs and endogenous substances: Bilirubin, hemin and chloride ions were used as endogenous substances for competitive binding studies. Association constants for the binding of all the antidiabetic drugs decreased in the presence of bilirubin. In the presence of hemin, association constants of gliclazide and glimepiride increased significantly while those of glipizide and repaglinide decreased. The presence of chloride ions decreased the association constants of all drugs except glimepiride where a small increase was observed. Among various antidiabetic drugs the presence of endogenous substances produced a large change in the percentage binding for gliclazide and repaglinide. The corresponding change for glimepiride and glipizide was smaller. Change in the percentage free drug due to competitive binding ($\Delta\alpha$), calculated from the data, was used to get quantitative estimate of the effect of competing endogenous substances on the binding of antidiabetic drugs. The criterion used for subsequent discussion was same as described earlier (sec. 4.3.1). A very large increase (>20%) was observed in the percentage of free gliclazide in the presence of bilirubin and free repaglinide in the presence of bilirubin and hemin. A large increase (10-20%) was observed in the percentage of free gliclazide in the presence of chloride ions and free glipizide in the presence of hemin. However, the percentage of free gliclazide in the presence of hemin decreased by about 18% and free glimepiride in the presence of hemin and chloride ions decreased by 14.4 and 8.5%, respectively.

Competitive binding mechanism involved could be explained on the basis of the relative magnitudes of the association constants and site-
specificity of the drugs and competing endogenous substances. The results also indicated that in some cases, the presence of two ligands simultaneously caused conformational changes in the HSA molecule. The change in the percentage of free hypoglycemic agent due to competitive binding can result in serious fluctuations in the blood glucose levels of diabetic patients.

**Bilirubin:** Relatively lower affinity antidiabetic drugs could not dissociate strong HSA-bilirubin complex resulting in decreased binding of antidiabetic drugs. In the case of repaglinide, bound bilirubin probably blocks the accessibility of drug to the binding region within site II.

**Hemin:** Glipizide and repaglinide could not displace strongly bound hemin from HSA and hence decrease in the binding affinity of these drugs. Increase in the binding affinity of gliclazide and glimepiride; on the other hand, can be due to the fact that in the presence of these drugs, hemin probably occupies site I. Simultaneous binding of two strongly bound ligands causes conformational changes in HSA resulting in increased binding.

**Chloride ion:** Antidiabetic drugs in spite of their higher binding affinity could not displace chloride ions from HSA resulting in decreased binding of antidiabetic drugs. In the presence of Cl' ions, glimpiride probably occupies site I and unfolding of protein due to simultaneous binding of two ligands may be responsible for increase in the binding of this drug.