Chapter-7

Summary and Conclusion
7. SUMMARY AND CONCLUSION

7.1. SUMMARY

Glipizide is an oral hypoglycemic agent belonging to the second-generation sulfonylurea used for the treatment of type II diabetes mellitus. Glipizide lowers blood glucose concentration by stimulating the release of insulin from the pancreas. It is a BCS class II drug, which has low solubility and high permeability. It has a short biological half-life (3.4±0.7 hours). The treatment with some conventional dosage forms like capsules and tablets of oral hypoglycemic agents is associated with short half-life, low bioavailability, uncontrolled drug release, high dosage requirement, poor absorption and shorter duration of action. Therefore, development of a sustained release formulation which remains at the absorption site for extended period of time is an important approach. Several approaches have been developed to prolong the gastric residence time of the dosage form at the absorption time. One of the most attractive researches in drug delivery is the design and formulation of sustained release nano-systems that are able to deliver drugs at appropriate times and at the right dosage with reduced dosing frequency. The objective of the study was to formulate Glipizide nanoparticles that may enhance solubility and dissolution rate. The polymeric nanoparticles of Glipizide were made using biodegradable and biocompatible polymers: PLA, Eudragit RL100 and Eudragit RS100 to attain a sustained release effect.

The nanoparticles were prepared by solvent evaporation method by varying concentration of polymers such as PLA, Eudragit RL100, and Eudragit RS100 and surfactant (PVA). The nanoparticles were characterized by particle size, entrapment efficiency, drug loading and in-vitro drug release studies. The effect of variables like polymer concentration (PLA, Eudragit RL100, and Eudragit RS100) and surfactant concentration (PVA) on particle size, entrapment efficiency and drug loading were investigated.

Design Expert Software was used for the optimization of polymeric nanoparticles.

- **Glipizide Loaded PLA Nanoparticle Formulations:** the particle size was found to be in the range of 295.5 – 720.4 nm. The particle size of nanoparticles increased with increase in amount of polymer but decreased with increase in the concentration of surfactant (PVA). The entrapment efficiency and drug loading...
were found to be in the range of 50.67 - 79.16% and 14.1 - 36.78% respectively. The entrapment efficiency and drug loading decreased with increase in the amount of PLA polymer but increased with increase in PVA concentration. The in-vitro drug release studies were carried out in phosphate buffer pH 6.8. The percentage cumulative drug release from the nanoparticles was found to be in the range of 73.72% to 78.12% in 10 hrs at pH 6.8. From the study it was observed that drug release decreased with increase in polymer concentration due to the high viscosity of PLA polymer this could be attributed to the fact that on contact with the dissolution medium, surface of nanoparticles becomes wet and forms viscous gel layers. As the concentration of PLA increase the viscosity of the gel layer also increases while the diffusion coefficient of drug decreases.

- **Glipizide Loaded Eudragit RL100 Nanoparticle Formulations**

  The particle size was found to be in the range of 201.1 to 427.5 nm. The particle size of nanoparticles increased with increase in amount of polymer but decreased with increase in the concentration of surfactant (PVA). The entrapment efficiency and drug loading were found to be in the range of 59.78% - 79.53% and 23.13% - 37.11% respectively. As the concentration of polymer increased, entrapment efficiency and drug loading increased but entrapment efficiency and drug loading were found to decrease with an increase in surfactant concentration. The in-vitro drug release studies were carried out in phosphate buffer pH 6.8. The release profile of Glipizide loaded nanoparticles showed the cumulative drug release range of 64.31 - 78.56 % in 10 hrs at pH 6.8. The results showed that the release was dependent on the concentration of polymer. An increase in the polymer concentration caused a decrease in the release rate because it increases the density of the molecules in the given space; as a consequence of which release was reduced. However, the percent cumulative drug release increased with increase in surfactant concentration which could be attributed to the decrease in particle size and increase in surface area available for the dissolution.

- **Glipizide Loaded Eudragit RS100 Nanoparticle Formulations**

  The particle size was found to be in the range of 243 - 864.5 nm. The particle sizes of nanoparticles decreased with increase the concentration of surfactant (PVA) but increased with increase in amount of polymer. The entrapment efficiency and drug loading decreased with increase in polymer concentration but increased with increase in surfactant concentration.
loading were found to be in the range of 58.43% to 81.23% and 15.43% to 40.12% respectively. Entrapment efficiency and drug loading increased with increase in the polymer concentration but after some time entrapment efficiency and drug loading start decreased with increase in polymer concentration due to formation of more compact polymeric coat. However the entrapment efficiency and drug loading were found to increase with increase in PVA concentration. The \textit{in-vitro} drug release studies were carried out in phosphate buffer pH 6.8. The release profile of Glipizide loaded nanoparticles showed the cumulative drug release from 69.52 - 81.44 % in 10 hrs at pH 6.8. The results showed that the release was dependent on concentration of polymer. An increase in the polymer concentration caused a decrease in the release rate because polymer increases the density of the molecules in the given space as a consequence of which release is reduced. However the percent cumulative drug release increased with increase in surfactant concentration which could be attributed to the decrease in particle size and increase in surface area available for the dissolution.

The optimized formulation was made using appropriate amount of polymer and surfactant. The \textit{in-vitro} drug release studies of optimized batches of PLA, Eudragit RL100 and Eudragit RS100 nanoparticles were carried out in phosphate buffer pH 6.8 for 10 hrs. The kinetics of drug release data of PLA, Eudragit RL100 and Eudragit RS100 polymeric nanoparticles was detected by fitting to zero-order kinetics, first-order kinetics, Higuchi model and Korsmeyer-Peppas kinetic models. The kinetic model with the highest value of the coefficient of correlation was considered to be the best fit model. The release mechanism of drug was evaluated. The highest correlation coefficient for PLA and Eudragit RL100 loaded nanoparticles was obtained for Higuchi model. Higuchi kinetic model indicated that drug release followed the diffusion mechanism. In case of Eudragit RS100 loaded nanoparticles, the highest correlation coefficient was found in the order Korsmeyer-Peppas kinetic model. This indicated that drug release followed a combination of diffusion as well as erosion mechanisms. Optimized batches were further evaluated for Fourier Transform Infrared Spectroscopy, Differential Scanning Calorimetry and Transmission Electron Microscopy. FTIR studies showed that there was no interaction between drug, polymer and excipients used in drug-loaded nanoparticles and DSC studies showed that drug was efficiently entrapped inside the polymer in the polymeric nanoparticles.
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Transmission Electron Microscopy showed the particles were found to be almost spherical in shape with smooth morphology.

*In-vivo* studies were carried out to check the efficacy of Glipizide loaded polymeric nanoparticles which were compared with pure drug for antidiabetic activity. Out of the above three optimized formulations, Eudragit RS100 loaded nanoparticles formulation was selected for animal study. The antidiabetic activity was studied on albino wistar rats using Streptozotocin induced Diabetic Mellitus Type II method. Overnight fasted animals were induced with Diabetes Mellitus in by a single injection of streptozotocin (60 mg/kg body weight i.p. in 0.1M citrate buffer pH 4.5). An incubation of 7 days was given to the infected rats for developed and stabilized diabetes and was confirmed by elevated blood glucose levels determined at 72h and on 7th day after injection. Only rats with blood glucose level > considered diabetic and used in the antidiabetic study. Treatment was given orally once daily for 28days. The blood glucose levels were estimated on 0th, 7th, 14th, 21th and 28th days in normal and STZ-induced diabetic rats. A significant increase was observed in STZ-induced diabetic rats in blood glucose level when compared with normal rats. The treatment with nanoparticle formulation (50mg/kg body weight) and standard drug Glipizide (10 mg/kg) showed a significant reduction in plasma glucose level compared to diabetic rats.

When compared to normal rats Estimation of biochemical parameters: Insulin, total cholesterol, triglycerides, HDL, SGOT, SGPT, HDL cholesterol levels found to be significantly decreased and serum total cholesterol, triglyceride levels were significantly increased in streptozotocin induced diabetic rats. The administration of nanoparticle formulation and standard drug Glipizide significantly increased the HDL-C levels and reduced the elevated serum total cholesterol, triglyceride levels when compared to diabetic control rats. In case of SGOT, SGPT, glycosylated hemoglobin and serum insulin, diabetic rats showed a significant increase in the SGOT, SGPT, glycosylated hemoglobin levels and a decrease in serum insulin levels compared with normal rats. In diabetic rats, administration of nanoparticle formulation and standard drug Glipizide significantly reduced the serum SGOT, SGPT, glycosylated hemoglobin levels and significantly increased the serum insulin level when compared to diabetic control rats.
The rat liver was weighed and 10% liver homogenate was prepared with 0.1 M phosphate buffer (pH 7.0). The liver homogenate was used to measure Lipid peroxidation, reduced glutathione and catalase activity. On estimation of antioxidant parameters the lipid peroxide level was found to be significantly higher in streptozotocin-diabetic group compared to normal group. Administration of formulation and Glipizide significantly decreased the level of lipid peroxide when compared with diabetic group. In addition the streptozotocin-diabetic group showed a decrease in glutathione and catalase activity compared with normal group. Administration of formulation and Glipizide reduce the levels of glutathione and catalase during diabetes.

The rat pancreas was washed with saline and fixed in 10% formaldehyde solution for histopathological studies.

### 7.2 CONCLUSION

- The investigation indicated that solvent evaporation method can be successfully used for preparation of polymeric nanoparticles.
- **As the concentration of the polymer PLA increased, particle size also increased** but entrapment efficiency and drug loading decreased. However as the concentration of surfactant (PVA) increased, particle size decreased but entrapment efficiency and drug loading increased.
- As the Eudragit RL100 concentration increased, particle size, entrapment efficiency and drug loading increased but all the parameters decreased with an increase in the concentration of surfactant (PVA).
- As the Eudragit RS100 concentration increased, particle size increased. Entrapment efficiency and drug loading increased with increase in the polymer concentration, however the two parameters decreased with increase in polymer concentration beyond a point. As the concentration of surfactant (PVA) increased, particle size decreased but entrapment efficiency and drug loading were found to increase.
- **In-vitro** drug release studies on Glipizide loaded polymeric nanoparticle showed an initial burst release which could be due to adsorbed drug on the surface of nanoparticles. This was followed by a slower and sustained release rate from the nanoparticles.
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- The release of the drug from optimized batches of Glipizide loaded PLA and Eudragit RL100 nanoparticles was found to follow the Higuchi model and in case of Eudragit RS100 nanoparticles showed Korsmeyer-Peppas release kinetics.

- Out of the three optimized formulations, Eudragit RS100 nanoparticle formulation was selected for animal studies. *In-vivo* study showed that oral administration of the nanoparticle formulation of Glipizide had a greater potential for antidiabetic and antioxidant effect as evidenced by the studies performed in streptozotocin induced diabetic rats.

- In conclusion, the potential of Glipizide nanoparticle formulations can be further explored following long term pharmacokinetic and pharmacodynamic studies in human volunteers.