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10. SUMMARY AND CONCLUSION

10.1 Summary of investigation

The drug absorption is unsatisfactory and highly variable among and between individuals, despite excellent in vitro release patterns of controlled or sustained release formulations. The reasons for this are essentially physiological and usually affected by the gastrointestinal (GI) transit of the form, especially its gastric residence time (GRT), which appears to be one of the major causes of the overall transit time variability. Over the past three decades, the pursuit and exploration of devices designed to be retained in the upper part of the GI tract has advanced consistently in terms of technology and diversity, encompassing a variety of systems and devices including floating (buoyancy) systems or low-density systems. Based on the mechanism of buoyancy, two distinctly different technologies, i.e., noneffervescent and effervescent systems have been utilized in the development of floating systems in the form of tablets. However, the gastric emptying of a multiparticulate floating system (floating microspheres) would occur in a consistent manner with small variations in individual. Stomach specific (gastric retention) will provide advantages such as the delivery of drugs with narrow absorption windows in the small intestinal region. Furthermore, improved bioavailability is expected for drugs that are absorbed readily upon release in the GI tract. These drugs can be delivered ideally by slow release from the stomach. Many drugs categorized as once-a-day delivery have been demonstrated to have suboptimal absorption due to dependence on the transit time of the dosage form, making traditional extended release development challenging.
Therefore, a system designed for longer gastric retention will extend the time within which drug absorption can occur in the small intestine.

Glipizide is a second-generation sulfonylurea that can acutely lower the blood glucose level in humans by stimulating the release of insulin from the pancreas and is typically prescribed to treat type II diabetes (non-insulin-dependent diabetes mellitus). Its short biological half-life (3.4 ± 0.7 hr) necessitates that it be administered in 2 or 3 doses of 2.5 to 10 mg per day. Moreover, the site of absorption of weak acidic drug glipizide (pKa = 5.9) is in stomach.

The aim of this thesis was to improve the oral bioavailability of glipizide by the development and optimization of controlled release floating formulations including non effervescent floating tablets (NEFT), effervescent floating tablets and microspheres. To reach at the objective of the current research, whole experimental work was divided into following five major parts. i) Preformulation study of drug including drug-excipient interaction testing., ii) Bioanalytical method development and validation of glipizide in rabbit plasma for in vivo pharmacokinetic study, iii) Design, development; optimization and pharmacokinetic study of non effervescent floating tablet of glipizide, iv) Design, development, optimization and pharmacokinetic study of effervescent floating tablet of glipizide, v) Design, development, optimization and pharmacokinetic study of floating microspheres of glipizide. The thesis, being the documentation of the entire research work, comprises of ten chapters placed accordance with the chronological sequence of the work plan.

The Preformulation study of GPZ was carried to characterize the drug, to access the physiochemical and pharmaceutical properties of drug, and to construct the standard
calibration curves in various mediums. The study was also important to know the stability of the drug in simulated gastrointestinal fluids and compatibility testing between drug and excipients used in the development of formulations. The linear regression coefficient ($r^2$) value of all standard calibration plot of GPZ in various mediums was equivalent to one. Hence, the calculated linear equations of standard calibration plot were used for the determination of unknown quantity of drug in various mediums. The drug was stable in simulated gastric as well as in simulated intestinal fluid up to 96 hrs. The solubility of the drug increases when the pH of the medium increases from 1.2 to 7.4. The flow property of the drug powder was found to be poor, which had been improved during formulation development. From the results of isothermal stress testing studies, the drug was found to be compatible with proposed excipients of the formulations.

To quantify the small quantity of drug present in rabbit plasma, HPLC method was adopted. A simple and sensitive reversed phase HPLC isocratic method has been developed and validated for the estimation of GPZ in rabbit plasma using UV detector. A good resolution was obtained between GPZ and internal standard (gliclazide) with retention times of 7.32 min and 9.02 min respectively. The method was found to be linear ($r^2=0.999$) within the analytical range of 25.38 to 2046.45 ng/ml. The results obtained proved that the method development was accurate and reproducible, and drug was stable in rabbit plasma.

The design, development, optimization and in vivo pharmacokinetic study of non-effervescent floating tablet of GPZ were carried out. The $3^2$ factorial design-based optimization was employed to investigate the effect of two independent process variables (factors), i.e., amount of HPMC K4M (55, 60 and 65 %) and Carbopol 934 (12.5, 15 and
17.5 %) on the dependent variables like time required to release 50 % of drug (t50), 80 % of drug (t80) and similarity factor (f2). The tablets were formulated at all nine possible combinations of variables by keeping the concentration of other excipients constant. The optimized model equations relating Y1(t50), Y2(t80) and Y3(f2) as responses are given in Eq. 10.1, Eq.10.2 and Eq.10.3 respectively.

\[
Y_1 (t_{50}) = 9.66 + 1.63 X_1 + 3.21 X_2 + 0.78 X_1 X_2 \quad (10.1)
\]

\[
Y_2 (t_{80}) = 15.92 + 2.18 X_1 + 3.83 X_2 + 1.34 X_1 X_2 \quad (10.2)
\]

\[
Y_3 (f_2) = 68.87 + 2.19 X_1 - 2.72 X_2 - 5.87 X_1 X_2 - 22.15 (X_2)^2 \quad (10.3)
\]

In the case of \(Y_1\) (t50) and \(Y_2\) (t80), coefficients \(b_1\) and \(b_2\) were found to be significant, with an interaction of \(b_{12}\). It is observed from Eq. 10.1 and 10.2 that all the coefficients are positive. It signifies dependent variable, t50 (Y1) increases on increasing the value of independent variables, amount of HPMC K4M (X1) or/and Carbopol 934. In Eq. 10.3, the coefficient of \(X_1\) is positive and the coefficient of \(X_2, X_1X_2\) and \((X_2)^2\) are negative. It indicates that dependent variable, \(Y_3\) (f2) is directly proportional with amount of HPMC K4M (X1) and inversely proportional to the amount of Carbopol 934. The optimized formulation was obtained by applying constraints (t50, 6 - 10 h; t80, 12 - 24 h and f2, 50-100) on dependent variable responses and independent variables. The recommended concentrations of the independent variables were calculated by the Design Expert software with highest desirability near to 1.0. The optimum values of selected variables obtained using Design Expert software were 124.33 mg of \(X_1\) and 25.76 mg of \(X_2\) which was considered as optimum formulation (NEFT-O) comprised 10 mg GPZ, 124.33 mg HPMC K4M, 25.76 mg carbopol 934, 33.91 mg mannitol, 2 mg Aerosil and 3 mg talc. The in vivo pharmacokinetic study of NEFT-O resulted that \(C_{\text{max}}, \text{AUC}_{0-\infty}\) and \(t_{1/2}\) value...
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were found to be 12.52 ± 1.23 µg/mL 114.08 ± 1.23 µg.h/mL, and 12.24 ± 0.46 h respectively. The biological half-life of glipizide in NEFT-O (12.24 ± 0.46 h) was increased from its reported value i.e. 3.4 ± 0.7 hr signifies the controlled release of drug in formulation.

The design, development, optimization and \textit{in vivo} pharmacokinetic study of effervescent floating tablet of GPZ have been performed. The $3^2$ factorial design-based optimization was employed to investigate the effect of two independent process variables (factors), \textit{i.e.}, HPMC K4M (70, 80 and 90; $X_1$) and NaHCO$_3$ (14, 24 and 34 %; $X_2$) on the dependent variables like floating lag time (FLT), time required to release 50 % of drug ($t_{50}$) and 80 % of drug ($t_{80}$). The tablets were formulated at all nine possible combinations of variables by keeping the concentration of other excipients constant. The optimized equation of responses $Y_1$ (FLT), $Y_2$ ($t_{50}$) and $Y_3$ ($t_{80}$) are given in Eq. 10.4, Eq.10.5 and Eq.10.6 respectively.

\begin{align*}
Y_1 (\text{FLT}) &= 37.33 +13.50 X_1 -12.00 X_2 - 4.25 X_1 X_2 - 3.50 X_1^2 \quad (10.4) \\
Y_2 (t_{50}) &= 5.27 + 1.80 X_1 -2.42 X_2 + 0.30 X_1 X_2 -1.18 X_1^2 \quad (10.5) \\
Y_3 (t_{80}) &= 11.40 + 1.11 X_1 -1.89 X_2 - 0.90 X_1^2 \quad (10.6)
\end{align*}

In all cases ($Y_1, Y_2$ and $Y_3$), the coefficients $b_1$, $b_2$ and $b_{11}$ were significant where as coefficient of interaction, $b_{12}$ was insignificant in $Y_3$. In all equations, $b_1$ is positive and $b_2$ is negative. It signifies dependent variable, FLT, $t_{50}$ and $t_{80}$ are directly proportional with amount of HPMC K4M and inversely proportional with NaCO$_3$. The optimized formulation was obtained by applying constraints on dependent variable responses and independent variables. The constraints were: FLT, 13 to 60 sec; $t_{50}$, 6 to 10 h; $t_{80}$ and 12...
to 24 h. The optimum values of selected variables obtained using Design Expert software were 87.52 mg of $X_1$ (HPMC K4M) and 16.35 mg of $X_2$ (NaHCO$_3$). The final composition of optimized formulation (EFT-O) comprised 10 mg GPZ, 87.52 mg HPMC K4M, 16.35 mg NaHCO$_3$, 71.13 mg MCC, 8 mg citric acid, 12 mg povidone, 2 mg Aerosil and 3 mg talc. The \textit{in vivo} pharmacokinetic study of EFT-O resulted that $C_{\text{max}}$ and $AUC_{0-\infty}$ value were found to be 11.74 ± 1.07 μg/m and 105.40 ± 6.51 μg⋅h/mL, and 12.24 ± 0.46 h respectively. The biological half-life ($t_{1/2}$) of glipizide in EFT-O (11.16 ± 0.44 hr) is increased from its reported value (3.4 ± 0.7 hr) signifies the controlled release of drug.

The design, development, optimization and \textit{in vivo} pharmacokinetic study of floating chitosan microspheres of GPZ have been performed. The $3^2$ factorial design-based optimization was employed to investigate the effect of two independent process variables (factors), \textit{i.e.}, drug polymer ratio (1:1, 1:2 and 1:3; $X_1$), percentage of sodium TPP (3, 4 and 5; $X_2$) on the dependent variables like drug entrapment efficiency (DEE), particle size (PS) and time require for 80 % of drug release ($t_{80}$). The floating microspheres were formulated at all nine possible combinations of variables by keeping other formulation parameters constant. The optimized equation of responses $Y_1$ (DEE), $Y_2$ (PS and $Y_3$ ($t_{80}$) are given in Eq. 10.7, Eq.8. and Eq.10.9 respectively.

\begin{equation}
Y_1 (\text{DEE, %}) = 64.14 + 16.63X_1 + 8.21X_2 + 3.40X_1X_2 + 0.83X_1^2 - 1.53X_2^2 \quad (10.7)
\end{equation}

\begin{equation}
Y_2 (\text{PS, μm}) = 667.44 + 14.83X_1 + 9.83X_2 + 2.50X_1X_2 \quad (10.8)
\end{equation}

\begin{equation}
Y_3 (t_{80}, \text{hr}) = 10.98 + 0.68X_1 + 1.40X_2 - 0.36X_1X_2 \quad (10.6)
\end{equation}
In all cases ($Y_1$, $Y_2$ and $Y_3$), the coefficients $b_1$, $b_2$ and $b_{11}$ were significant where as coefficient of interaction, $b_{12}$ was insignificant in $Y_3$. In all equations, $b_1$ and $b_2$ are positive. It signifies dependent variable, DEE, PS and $t_{90}$ are directly proportional with amount of chitosan and Sodium TPP. The optimized formulation was obtained by applying constraints (DEE, 70 to 95%; Particle size, 643 to 698 μm (entire range) and $t_{90}$ and 10 to 12 h) on dependent variable responses.

The optimum values of selected variables ($X_1 = 1.247; X_2 = 4.03$ %) and their responses (72.55 % of DEE, 675 μm of PS and 11.35 hr of $t_{90}$.) were predicted using Design Expert software and these predicted values were found good agreement with experimental results (DEE, 73.96 %; PS, 682 μm and $t_{90}$, 11.58 hr). The in vivo pharmacokinetic study of optimized floating microspheres (FM-O) equivalent to 2.5 mg of glipizide resulted that $C_{\text{max}}$ and AUC$_{0-\infty}$ value were found to be $2.88 \pm 0.29$ μg/m and $38.46 \pm 2.26$ μg.h/mL, and $12.24 \pm 0.46$ h respectively. The biological half-life ($t_{1/2}$) of glipizide in EFT-O (13.55 ± 1.36 hr) is increased from its reported value (3.4 ± 0.7 hr) signifies the controlled release of drug.

10.2 Conclusion of investigation

Glipizide is a second-generation sulfonylurea for the treatment of type II diabetes having short biological half-life (3.4 ± 0.7 hrs) and beings to BCS class II category drug. To meet the challenges in the oral bioavailability and short half-life of drug, floating drug delivery systems including non-effervescent tablets, effervescent tablets and floating microspheres of glipizide were designed, developed and optimized through $3^2$ factorial designs. The in vivo Pharmacokinetic study of optimum formulations i.e. non-effervescent floating tablet (NEFT-O), effervescent floating tablet (EFT-O) and
floating microspheres (FM-O) were carried out in rabbit. A highly sensitive analytical method was developed and validated using RP-HPLC for the estimation of drug in rabbit plasma. The floating lag time of NEFT and FM formulations were Zero and floating lag time of EFT was less than one minutes. The biological half life of NEFT-O (12.24 ± 0.46 hrs), EFT-O (11.16 ± 0.44 hrs) and FM-O (13.55 ± 1.36 hrs) were found much greater in compare with glipizide molecule (3.4 ± 0.7 hrs). It concludes that all the prepared formulations were floating controlled release formulations with desired characteristics.