

**FORMULATION AND EVALUATION OF GASTRORETENTIVE
DRUG DELIVERY SYSTEM OF ANTIDIABETIC DRUGS**

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Abstract

The current research was aimed to formulate, optimize and evaluate the gastroretentive formulation of antidiabetic drugs. The drugs chosen for the study were metformin, glipizide and mitiglinide (MTG), which are benefited by preparing stomach specific drug delivery systems in the form of floating matrix tablet and floating microsponges.

The floating matrix tablets for all the three drugs were prepared using HPMC K15M, as release retarding polymer along with other ionic and anionic polymeric substances. Final formulations were prepared using HPMC K15M and kappa carrageenan as the release retarding polymers. The optimization of metformin floating matrix tablet was done by simplex centroid design using HPMC K4 M (X_1), kappa-Carrageenan (X_2), gas-generating agent, sodium bicarbonate (X_3), as independent variable. The floating lag time (F_{lag}), drug released after 1 hour and time required for 90% drug release, were taken as dependent variables. Formulations were also evaluated for swelling, floating period and drug release. All the tablets showed acceptable physicochemical properties. Formulation prepared with 150mg of X_1 , 75mg of X_2 and 150mg of X_3 was found to be the optimum having good floating lag time and also matching the desirability criteria for drug release.

Floating matrix tablet of glipizide was optimization by applying Simplex lattice design (SLD) using kappa carrageenan, HPMC K15M and sodium bicarbonate as independent variable. The similarity factor (f_2), time to release 50% of drug and time to release 90% of drug were taken as dependent factors. The optimum values of selected variables was found to be 50.134mg of X_1 , 39.8654mg of X_2 and 10mg of X_3 , and this formulation showed highest desirability.

Floating matrix tablet of MTG was prepared using the combination of release controlling polymer HPMC K15M and sodium alginate. The final optimization of floating MTG formulation was done by applying 3^2 full factorial design using sodium alginate and HPMC K15M as independent variable. The floating lag time (F_{lag}), time to release 50% of drug (t_{50}) and time to release 90% of drug (t_{90}) were taken as dependent factors. All the formulations were evaluated for their physical properties, in vitro buoyancy studies, swelling studies and drug release study. Results showed that M-3 formulation containing maximum amount of both variables gave promising results, hence was considered as optimized batch.

Floating microsponges of MTG and glipizide were prepared by quasi emulsion technique. Glipizide microsponges were screened by Plackett–Burman design to find the potential risk factors and finally microsponges were optimized using Box–Behnken design. Optimization

of MTG microsponges was done by applying by applying 3^2 full factorial design. All the formulations were evaluated for micrometric properties, product yield, entrapment efficiency, buoyancy, and in vitro release. Further, characterization of formulations was done by FTIR, DSC and XRD and surface morphology of the optimized formulation was studied by SEM. Compatibility between the drug and excipient was proved by FTIR and DSC studies, XRD studies showed the molecular level distribution of the drug in polymeric matrix. SEM revealed the spherical and porous nature of microsponges.

Accelerated stability studies of optimized floating tablets and microsponges were performed as per the ICH guidelines and the results indicated no significant change in the release pattern of the drugs and other properties of the formulation on storage.

Radiological study was performed on healthy albino rabbits for checking the gastroretention for optimized floating tablets and microsponges of MTG and glipizide. The in vivo X-ray imaging study clearly indicated that the optimized formulations remained afloat in gastric fluid up to 12 h in the stomach of rabbit.

Pharmacodynamics studies of optimized microsponges of glipizide and MTG was performed on diabetic Albino wistar rats. Optimized microsponges of both drugs showed reduction in blood glucose level slowly but for extended period of time as compared to pure plain drug. Pharmacokinetic studies were performed on healthy Albino wistar rats. The study revealed the presence of both the drugs in the blood for more than 12 hrs which supports the pharmacodynamics effect of the drugs where the reduction of the blood glucose was observed for the period of 12hrs on comparison with pure drug.

The optimization of gastroretentive tablet and microsponges was successfully done by applying statistical design. It can be concluded that oral antidiabetic treatment may be achieved efficiently by preparing floating microspheres and floating tablets, which could results in increase in bioavailability along with extended duration of action resulting in possible reduction in dose and side effects of drug.

Brief description on the state of the art of the research topic

Despite remarkable innovations in the drug delivery system, oral route remains the chosen route for the administration of therapeutic agents. After oral administration, the drugs which are better absorbed from stomach suffer from mainly two adversities: the short gastric retention time and unpredictable short gastric emptying time, which can result in incomplete drug release from the dosage form in the absorption leading to diminished efficacy of administered dose¹. This demerit has led to the evolution of oral gastro-retentive dosage forms (GRDDS). Various gastroretentive drug delivery systems (GRDDS) have being designed and developed, including: high density sinking systems that is retained in the bottom of the stomach², low density floating systems that causes buoyancy in gastric fluid³⁻⁵, floating osmotic pump^{6,7}, floating pulsatile delivery system⁸, mucoadhesive systems that causes bioadhesion to stomach mucosa⁹, superporous hydrogel systems¹⁰, floating system by hot melt extrusion¹¹ etc. Multiparticulate gastroretentive formulations have also been explored, which avoids dose dumping, all or none effect and irritation at the site of release^{12,13}.

Drug absorption in the gastrointestinal tract is a highly variable process and prolonging gastric retention of the dosage form extends the time for drug absorption. GRDDS improves bioavailability of drugs those are locally active in the stomach, increases the duration of drug release, reduces drug waste, and improves the drug solubility that are less soluble in intestinal pH or colonic environment. Controlled release gastroretentive dosage forms enable prolonged and continuous input of the drug to the upper parts of the gastrointestinal tract and improve the bioavailability of medications that are characterized by a narrow absorption window^{14,15}. Due to all these reasons gastroretentive dosage forms (GRDF) are chosen for this research work.

Antidiabetic agents have been selected as a drug of choice for preparing GRDDS because Diabetes is the fourth leading cause of death by disease & a person dies from diabetes related causes in every 10seconds. GRDF provide us with new and important therapeutic options for delivery of anti-diabetic drug as they not only prolong dosing intervals, but also increase patient compliance¹⁶⁻²⁰. This research project was undertaken to prepare some of the GRDF for increasing the retention of anti-diabetic drugs in upper GIT. Modified formulations would stay in the stomach for longer period of time, releasing the drug slowly, thereby decreases the dosage frequency of drug and hence increase patient compliance.

Definition of the Problem

Type II diabetes mellitus is a chronic metabolic disorder and its occurrence has been increasing steadily all over the globe, particularly in poorly developed countries. The requirement of the antidiabetic drugs is to maintain the blood glucose level over the extended period of time to give better therapeutic efficacy and better patient compliance. Antidiabetic agents like biguanide derivatives, sulfonylurea and meglitinide analogs are having a strong rationale for preparing as gastroretentive dosage forms. Biguanide derivative, Metformin HCl has biological half-life is 1.5-1.6 h, and the main site of its absorption is proximal small intestine of the GIT. It has absolute bioavailability of 50-60 %, when administered orally due to its incomplete absorption²¹.

Glipizide is a second generation sulfonylurea and is one of the most widely used agents against Type II diabetes. It is a weak acid with 5.9 pKa value, and has a short biological half-life (3.4 ± 0.7 h) and requires 2–3 doses of 2.5–10 mg per day for treatment^{22,23}. Its site of the absorption is stomach which necessitates development of controlled-release dosage forms that are retained in the stomach, which would increase the absorption, improve drug efficiency, and decrease dose requirements.

Meglitinide analog, Mitiglinide is a mildly acidic drug with the pKa 4.45. It remains unionized in acidic environment, hence better absorbed from stomach. Recently, it was found that mitiglinide is better absorbed via the stomach and the gastric absorption was delayed when the gastric pH was higher than 5 pH²⁴.

Hence, these drugs were chosen for preparing the gastroretentive microsponges and tablet formulations for the better therapeutic efficacy, better patient compliance and efficient treatment of type II diabetes mellitus.

Objectives

This research project was undertaken to prepare GRDF of antidiabetic drugs for increasing their retention in upper GIT. Following are the objectives of the present research work:

1. To perform the preformulation studies of all the drugs used in the study.
2. To carry out the preliminary studies for selection of excipients and for preparing the floating matrix tablet of metformin, glipizide and mitiglinide.
3. To carry out the drug excipient compatibility studies.
4. To identify the key variable affecting the formulation of floating matrix tablet.
5. To apply an appropriate statistical design for the optimization of the floating matrix tablets.

6. To prepare and perform characterization and in vitro evaluation of the prepared floating matrix tablets.
7. To perform preliminary studies for preparing the microsponges of the selected drugs.
8. To carry out the drug excipient compatibility studies.
9. To find the key variables that affect the design of formulation and use of these variable in the optimization of floating microsponges.
10. To prepare, characterize and evaluate the prepared microsponges.
11. To perform the stability study of the optimized floating tablets and microsponges of the selected antidiabetic drugs as per ICH guidelines.
12. To perform radiological study of the formulations to determine gastric residence time of the optimized formulations, in-vivo.
13. To perform the in vivo pharmacodynamics and pharmacokinetic studies of the optimized microsponges of antidiabetic drugs on Albino wistar rats.

Scope of work

The antidiabetic drugs which are better absorbed from the upper part of GIT and have repeated frequency of administration, are the best candidates for preparing stomach specific formulations. Gastroretentive formulation of antidiabetic drugs would increase the residence time of the drugs in the stomach with sustained release pattern. This is particularly beneficial in enhancing the therapeutic effect with reduced side effects of drug. The sustained release of the drug is beneficial for decreasing the dosing frequency of the drug, which would lead to the increased patient compliance. Such formulations can be developed at the industrial level to get the maximum benefit and efficient treatment of type II diabetes mellitus.

Original contribution by the thesis

An important factor for the development of gastro retentive dosage form is the selection of suitable hydrophilic polymer, which provides acceptable flotation characteristics and release of the drug substance. The release mechanism of the drug from the polymeric matrix has been explained by many researchers, but in most of the studies, hydroxy propyl methyl cellulose (HPMC) is used as polymeric floating matrix system²⁵. But the combination of HPMC with other ionic and anionic polymeric substances and their effect on the release of the drug has not been explored much. In the present work, the floating matrix tablet of the selected antidiabetic drugs were prepared using the mixture of HPMC K15M and kappa carrageenan, which has not been prepared earlier. Carrageenans are reported to have

swelling property and thereby modifying the properties of polymeric matrices, to obtain tailor-made materials for drug delivery systems²⁶.

The drug MTG is a novel drug and has not been explored for the preparation of gastroretentive formulation despite of being better absorbed from the stomach. Moreover, it has short half-life and requires repetitive administration to maintain the therapeutic effect. It is a novel research to prepare the gastroretentive formulation of MTG.

Another type of dosage form prepared was microsponges. Microsponges offer an efficient drug delivery system for stomach specific delivery with high drug loading capacity i.e. upto 50 to 60%. The microsponges are expected to remain buoyant in the stomach due to lower density than that of the gastric fluids. Consequently dissolved drug will be released continuously in effective controlled manner from the floating system. Microsponges have stability over a pH range of 1 – 11. Stable up to temperature 130°C, free flowing and cost effective²⁷⁻²⁹. Microsponges were not explored for low density gastro retentive system until Priyanka Arya et. al., developed targeted floating curcumin microsponges for improved site specific absorption for gastric cancer³⁰. This study proved that microsponges have floating ability and can be used for the gastroretention of the drugs. Hence, floating microsponges are the novel way of preparing the gastroretentive formulations for antidiabetic drugs, which are required to be present in upper part of GIT for its better therapeutic action.

Methodology of Research and Results

a) Preformulation studies

The preformulation studies of metformin, glipizide and MTG was performed. Identification of the drugs was done by performing their physical evaluation and also by conducting the Fourier transform infrared spectroscopy (FTIR) study. The UV spectroscopy method was used for the analysis of the drugs in their dosage form. The wavelength at which the drugs showed the maximum absorbance was taken as λ_{max} and was further used for the preparation of calibration curve of drug in 0.1N HCl and methanol. UV analysis of MTG revealed that the absorptivity of the drug is very low. Only above the concentration of 200mcg/ml, the measureable amount of the radiations were being absorbed by the drug. As the dose of drug is very less, the method of analysis was not suitable for the analysis. Hence, high performance liquid chromatography (HPLC) method was developed for the analysis of drug. The mobile phase used for HPLC analysis of MTG was acetonitrile:HPLC water(55:45) and the pH was adjusted to 2.15 with phosphoric acid. The retention time of the drug was found to be 4.869 minutes and the R^2 value for the calibration curve was found

to be equal to 0.9982. This methods were used for in-vitro analysis of the formulations containing drug.

b) Floating matrix tablets

The floating matrix tablets of metformin were prepared by direct compression technique. The preliminary batches of metformin floating matrix tablets were prepared using HPMC K15M, as release retarding polymer along with other ionic and anionic polymeric substances. Formulations were prepared using sodium alginate, pullulan, kappa carrageenan, xanthan gum, poloxamer 68 in combination with HPMC K15M. Prepared formulations were evaluated for swelling, floating adhesive period and drug release. All the tablets showed acceptable physicochemical properties. Statistical analyses of data revealed that tablets prepared using HPMC K15M and kappa carrageenan, formulation F₂, is best in terms of showing excellent floating properties, extended adhesion periods and sustained drug release characteristics with similarity factor as 92 on comparison with the theoretical release of the drug. Hence, this combination of polymers was further evaluated by applying statistical design, to obtain the optimized gastro retentive formulation of metformin.

It was decided to develop floating matrix tablets containing metformin using simplex Centroid design as an optimization technique by changing the conc. of three factors simultaneously and keeping their total concentration constant. The amounts of matrixing agent, HPMC K4 M (X₁), release retarding polymer, kappa-Carrageenan (X₂), gas-generating agent, sodium bicarbonate (X₃), were selected as independent variable. The floating lag time (F_{lag}), drug released after 1 hour and time required for 90% drug release, were taken as dependent variables. The design was employed and evaluated using the Design-Expert® Software (version- 9.0.6, Stat-Ease) by running 14 experiments. The result of multiple regression analysis indicated that medium to high levels of X₁, medium level of X₂ and low level of X₃ should be used to manufacture the tablet formulation with desired in vitro floating time and release profile. Formulation M-SCD 7 with the quantities as X₁ 175mg, X₂ 75mg, X₃ 150mg, was found to be the optimum having good floating lag time and also matching the desirability criteria for drug release. The formulation also gave reasonably high adhesion retention period and swelling index desirable for ensuring the retention of formulation in the stomach. Hence, it was concluded that the mixture of kappa carrageenan and HPMC K 15 M increases the flexibility in the release pattern of the drug. However, increase amount of kappa carrageenan is not desirable as it hinders the controlled

release of the drug by increasing the hydration of the formulation and hence fastens the release of drug from the formulation.

Floating matrix tablet of glipizide was also prepared using the combination of hydrophilic polymer HPMC K15M with anionic and non-ionic polymers. The final optimization of floating glipizide formulation was done by applying Simplex lattice design (SLD) using kappa carrageenan, HPMC K15M and sodium bicarbonate as independent variable. The simplex lattice design for three-component system is represented by an equilateral triangle in two-dimensional space. The levels of the variables was decided from preliminary studies and the tablets were prepared by wet granulation technique using PVP K30. The similarity factor (f_2), time to release 50% (t_{50}) of drug and time to release 90% (t_{90}) of drug were taken as dependent factors. The design was employed and evaluated using the Design-Expert® Software (version- 9.0.6, Stat-Ease) by running 14 experiments. It was evident from the overlay plot that minimum amount of gas generating agent is sufficient to give the desired effect. Minimum concentration of HPMC K15M is required, whereas the amount of kappa carrageenan should be maximum. The optimum values of selected variables was found to be 50.134mg of X_1 , 39.8654mg of X_2 and 10mg of X_3 , and this formulation showed highest desirability.

Floating matrix tablet of MTG was also prepared using the combination release controlling polymer HPMC K15M and sodium alginate. The final optimization of floating MTG formulation was done by applying 3^2 full factorial design using sodium alginate and HPMC K15M as independent variable. The levels of the independent variables and the quantities of other excipients of the tablet was fixed from preliminary studies and the tablets were prepared by direct compression. The floating lag time (F_{lag}), time to release 50% of drug (t_{50}) and time to release 90% of drug (t_{90}) were taken as dependent factors. The design was employed and evaluated using the Design-Expert® Software (version- 9.0.6, Stat-Ease) by running 9 experiments. All the formulations were evaluated for their physical properties, in vitro buoyancy studies, swelling studies and drug release study. Results showed that M-3 formulation containing maximum amount of both variables gave promising results, hence was considered as optimized batch.

The floating matrix formulations of all the three drugs were prepared and optimized by applying appropriate design. For validating the model and checking out the reliability of the mathematical models, check point batches covering entire range of experimental domain

were prepared. The experimental and the predicted values of the check point batches had close agreement with each other, hence the models were successfully validated. There was no drug excipient interaction as reflected by Fourier transform infrared spectroscopy (FTIR) study. Accelerated stability studies were performed at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $75 \pm 5\%$ relative humidity (RH), according to the current International Conference on Harmonization (ICH) guidelines for six months. There was no significant change found in drug content and drug release profile on storage.

c) **Floating Microsponges of MTG**

Floating microsponges of MTG and glipizide were prepared and due to very high dose of metformin, its microsponges were not prepared as the formulation will become bulky. Floating microsponges of MTG were prepared by quasi emulsion method. The drug was dissolved in organic phase consisting of ethanol and dichloromethane, along with polymer (ethyl cellulose). Triethyl citrate was also added to this internal phase in order to facilitate the plasticity. The external aqueous phase was prepared of containing distilled water and polyvinyl alcohol (PVA). Then internal phase was added to external phase and the mixture was continuously stirred for 3-4 hrs. The mixture was immediately filtered to separate the microsponges product was washed and dried at room temperature for 24 h.

Preliminary batches were prepared for the screening of levels of excipient and process variables for the formulation of MTG microsponges. Observations of the study suggested that the solubility of the drug should be less in the external phase to enhance its entrapment in the polymeric matrix. Increased stirring speed decreased the particle size of the microsponges but speed beyond the optimum level caused the sticking of polymer to the walls of beaker. High temperature causes fast solidification of the polymeric matrix but particle size increased. The amount of triethyl citrate and the volume of organic and aqueous phase was fixed by the preliminary screening. The stirring speed of stirrer and temperature of the aqueous phase during the formulation of microsponges was optimized and was fixed for further studies. During the preliminary study, supported by literature survey, it was found that concentration of PVA affects the buoyancy and particle size of formulation. Also, the polymer concentration was found to affect entrapment efficiency and release pattern of the drug. Finally, the concentration of PVA (X_1) and ethyl cellulose (X_2) was considered to be the most important factors affecting the formulation of microsponges. Hence, the optimization of dosage form was done by taking these factors as independent variables and

by applying 3^2 full factorial design. Product yield (Y_1), % entrapment efficiency (Y_2), % buoyancy (Y_3) and % cumulative drug release (Y_4) were taken as dependent variables. Prepared formulations were evaluated for their physical properties, micrometric properties and drug release study. Using design expert software, optimized batch of MTG microsponges were obtained from the overlay plot, with the level of X_1 and X_2 as 0.47362 and -0.151682 respectively. The theoretical values of Y_1 , Y_2 , Y_3 and Y_4 were found to be 83.72%, 92.88%, 95.31%, 93.40%, respectively were found to be in close agreement with the practical values. The characterization of the optimized formulation was done by Differential Scanning Colorimetry (DSC), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD) study and FTIR. Compatibility between the drug and excipient was proved by FTIR and DSC studies. XRD studies showed the transformation of the drug from crystalline to amorphous state which indicated molecular level distribution of the drug in polymeric matrix. SEM revealed the spherical and porous nature of microsponges.

d) Floating Microsponges of Glipizide

Microsponges of Glipizide were prepared by quasi-emulsion solvent diffusion method. During the formulation of microsponges of MTG, it was found that there were various factors that affected the formulation. Screening of these formulation and process related factors by trial and error technique is time consuming and can be inaccurate at times. Hence, Plackett–Burman design was employed as the screening technique to determine the most significant factors that affected the formulation of microsponges using Design-Expert® software. This statistical tools helps in selecting the most important variables that can affect the formulation. The factor screened by the design were temperature (X_1), polymer concentration (X_2), stirring speed (X_3), amount of plasticizer (X_4), amount of PVA (X_5), volume of internal phase solvent (X_6), stirring time (X_7). The effect of these independent variables was checked on dependent variables (% yield and % entrapment efficiency). Pareto charts revealed that concentration of polymer i.e. ethyl cellulose, stirring speed and temperature significantly affected the formulation of microsponges. They were considered to be the most critical factors in the formulation of microsponges with positive sign indicating their positive influence on dependent variables.

Final optimization of glipizide microsponges was done using Box-Behnken design. A total of 15 batches were prepared as given by design expert. The effect of independent variables, selected from the screening design, was determined on the entrapment efficiency, buoyancy and cumulative drug release. The prepared microsponges were evaluated for various

micromeritic properties like angle of repose, bulk density, Carr's index and Hausner's ratio. The results indicated that the optimized formulation had entrapment efficiency of $90.81 \pm 1.2\%$, buoyancy of $92.3 \pm 2.25\%$ and drug release of $92.3 \pm 0.9\%$ in 12 hrs.

The optimized formulation was characterized by FTIR, DSC and XRD. The surface morphology of the optimized formulation was studied by SEM. FTIR and DSC studies showed no interaction between drug and polymer. Compatibility between the drug and excipient was proved by FTIR and DSC studies. XRD studies showed the molecular level distribution of the drug in polymeric matrix. SEM revealed the spherical and porous nature of microsponges. The optimized formulation of glipizide microsponges were subjected to stability same as that of MTG microsponges and there was no significant change observed in the glipizide microsponges on storage.

The stability studies of the optimized microsponges of MTG and Glipizide were performed at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $75 \pm 5\%$ relative humidity (RH), according to the current International Conference on Harmonization (ICH) guidelines for six months. The samples were analysed for physical changes, buoyancy, % drug content and % CDR. There was no significant change in observed in the microsponges on storage.

e) In-vivo radiographic studies³¹

The in-vivo radiographic studies were conducted on healthy albino rabbits weighing 2.0 kg to 2.2 kg. The protocol (BIP/IAEC/2015/05) for in vivo study was approved by the Institutional Animal Ethical Committee (IAEC) in accordance with guidance of committee for the purpose of control and supervision of experiments on animals (CPCSEA). In order to evaluate the in vivo residence time of the floating tablets, Floating tablet was prepared by incorporating the X-ray opaque material in the optimized formulation by replacing the drug with barium sulphate. The amount of the X-ray opaque material in the optimized tablets was kept sufficient to ensure visibility by X-ray, but at the same time the amount of barium sulphate was low enough to enable tablets to float.

For checking the gastroretention of optimized microsponges, the optimized microsponges incorporated with barium sulphate was put in capsule. These prepared tablets and capsules were given to albino rabbits for in vivo X-ray imaging study, to check the gastroretention of the formulations. The in vivo X-ray imaging study clearly indicated that the optimized formulation of floating tablet and microsponges remained afloat in gastric fluid up to 12 h in the stomach of rabbit. Hence, it could be concluded that the optimized formulations will show the good gastroretentive behaviour, in vivo when given to human beings.

f) In Vivo Studies

For conducting the in vivo studies of the optimized microsponges of glipizide and MTG, a protocol was approved by the Institutional Animal Ethical Committee (IAEC). Pharmacodynamic effect of optimized microsponges of glipizide and MTG was performed on diabetic Albino wistar rats. For inducing the diabetes in the rats, they were feeding with high fat diet, for the initial period of 2 weeks. Then, the rats were injected intraperitoneally (i.p.) with low dose of STZ (35 mg kg^{-1})³². The rats with the non-fasting PGL of $\geq 130 \text{ mg dl}^{-1}$ were considered diabetic and were selected for further pharmacological studies. The diabetic rats were divided into two groups with three rats each and the blood samples were taken on the regular intervals for up to 24hrs for checking the blood glucose levels, using Glucose Kit (Accu-Chek* Active Blood Glucose Monitor System). One group was given pure drug in suspension form and other group was given the optimized microsponges of glipizide in suspension form. The study demonstrated that pure glipizide showed the significant blood glucose reducing activity within one hour. Whereas, glipizide microsponges showed reduction in blood glucose level slowly but for extended period of time. Significant decrease in the blood glucose level was shown by the optimized glipizide microsponges for the period of 12hrs in diabetic rat. Similar effect was shown by the microsponges of MTG as compared with plain MTG drug, when given to the diabetic rats. The sustained hypoglycemic effect observed over a longer period of time in the case of microsponges is due to the slow release and absorption of glipizide and MTG over longer period of time.

Pharmacokinetic evaluation of the optimized glipizide and MTG microsponges was done using albino wistar rats. Six rats ($220 \pm 10 \text{ g}$) were kept in fasting condition for 24 h (but with free access to water) before being randomly assigned into two groups with three rats in each group.

Optimized gastroretentive microsponges of glipizide and plain drug was orally administered to the two groups of rats, respectively. Blood samples (0.5 ml) was collected from the fossa orbitalis vein at regular intervals of time till 24hrs. The blood samples were introduced into heparinized micro centrifuge tubes, and then separated by centrifugation. The samples were stored at -20°C till further analysis. 200 μl of plasma containing drug was taken in which an optimized quantity of internal standard was added. Then, 25 μl of trichloroacetic acid was added and the mixture was mixed on vortex mixer for 60sec. To this mixture, 1.5 ml of Acetonitrile was added and again mixed on vortex mixer. This mixture was centrifuged for 10min at 4,000 rpm at 4°C . Supernatant through 0.45 μm filter paper and then dried. The

residue was reconstituted with 100µl of mobile phase³³. 20µl of this sample is injected in HPLC for the analysis. Stationary phase was Agilent C 18 column (150 mm ×4.6 mm) with guard column. Mobile phase used for glipizide was Acetonitrile:HPLC water (55:45), pH adjusted to 3.5 with o-phosphoric acid. Glibenclamide was used as internal standard with detected wavelength was 240 nm.

For the pharmacokinetic study of MTG, same method as glipizide was used. But the bioanalytical method was slightly different. Mobile phase used for MTG was Acetonitrile:HPLC water (60:40), pH adjusted to 3.5 with o-phosphoric acid³⁴. Glipizide was used as internal standard with detected wavelength was 210 nm. Pharmacokinetic studies revealed the presence of both the drugs in the blood for more than 12 hrs which supports the pharmacodynamics effect of the drugs where the reduction of the blood glucose was observed for the period of 12hrs on comparison with pure drug.

Achievements with respect to objectives

1. Floating matrix tablets of all the three chosen drugs were prepared after performing the preformulation study of drugs and carried out extensive preliminary studies.
2. The key variable affecting the formulation of floating matrix tablet were identified and applied for the optimization of tablets using appropriate statistical design.
3. Drug excipient compatibility study was carried out and the characterized, in vitro evaluation of tablets was performed.
4. Floating microsponges were prepared for glipizide and MTG and after preliminary studies key variables were found for the optimization of microsponges using appropriate statistical design.
5. The characterization and in vitro evaluation of prepared microsponges was carried out successfully.
6. Stability study of the optimized floating tablets and microsponges was done as per ICH guidelines.
7. Radiological study of the optimized tablets and microsponges was performed on albino rabbits which confirmed their gastric residence in-vivo.
8. Pharmacodynamics and Pharmacokinetic studies of optimized microsponges of glipizide and MTG revealed the presence of both the drugs in the blood for extended time showing reduction of the blood glucose for the period of 12hrs.

Conclusion

An attempt was made to develop a gastroretentive drug delivery system of metformin, glipizide and MTG by preparing a floating matrix tablet and floating microsponges. The floating tablets prepared using HPMC K15M, and kappa carrageenan were found to be the best combination for preparing the gastroretentive formulation of metformin and glipizide. The formulations were optimized by applying appropriate statistical design. It was concluded that the mixture of kappa carrageenan and HPMC K 15M increases the flexibility in the release pattern of the drug. However, increase amount of kappa carrageenan is not desirable as it hinders the controlled release of the drug due to more hydration which results in increased release of drug from the formulation. Floating matrix tablet of MTG was prepared using HPMC K15M and sodium alginate, as this combination could sustain the release of the drug for the desired period as compared to other polymeric combinations. Floating microsponges were successfully formulated for glipizide and MTG with the sustained release pattern of the drug. The statistical designs proved to be the best tool for screening and optimization of microsponges. Radiological studies of optimized tablets and microsponges proved the ability of the formulations to retain in the stomach. Pharmacodynamic and pharmacokinetic studies proved the presence of the floating microsponges in the stomach for extended period. It was concluded that oral antidiabetic treatment can be achieved efficiently by preparing floating microspheres and floating tablets, which results in increase in bioavailability along with extended duration of action resulting in possible reduction in dose, less side effects, low overall cost of therapy and hence better patient compliance.

List of publications

1. A **research article** titled, “Controlled-release effervescent floating matrix tablets of metformin using combination of polymers”, has been published in, *International Journal of Pharmacy and Pharmaceutical Science*. 2016; 8(11):114-119.
2. A **research article** titled, “Application of simplex centroid design in formulation and optimization of floating matrix tablets of metformin”, has been accepted by, *Journal of Applied Pharmaceutics*.
3. A **review article** titled, “Gastroretentive Drug Delivery System – A Novel Approach for the Management of Diabetes Mellitus”, *Inventi Rapid: NDDS*. 2013; 2:1-7.
4. An **oral presentation** on, “Formulation and Evaluation of Floating Microsponges for Gastroretention of an Antidiabetic drug”, was given at *Pharma Submit & Expo by OMICS international*, on October 08-10, 2015.
5. Communicated a paper titled, “Microsponges of Glipizide: Formulation and Characterization”, for **poster presentation** at *Controlled Release Society Annual Meeting & Exposition 2017* in Boston.

References

1. Rouge N, Buri P, Doelker E. Drug absorption sites in the gastrointestinal tract and dosage form for site-specific delivery. *Int J Pharm*, 1996; 136:117-139.
2. Rouge N, Allemann E, Gex-Fabry M, Balant L, Cole ET, Buri P, Doelker E. Comparative pharmacokinetic study of a floating multiple-unit capsule, a high density multipleunit capsule and an immediate-release tablet containing 25 mg atenolol. *Pharm Acta Helvetiae*, 1998; 73: 81-7.
3. Hu L, Li Li, Yang X, Liu, W, Yang J, Jia Y, Shang C, Xu H. Floating matrix dosage form for dextromethorphan hydrobromide based on gas forming technique: In vitro and in vivo evaluation in healthy volunteers. *Eur. J. Pharm. Sci.*, 2011; 42: 99–105.
4. Patel B. Formulation and evaluation of Omeprazole gastroretentive floating tablets for the treatment of peptic ulcer. *Int J Scientific Res*. 2015; 4(6): 81- 82.
5. Shakya R, Thapa P, Saha RN. In vitro and in vivo evaluation of gastroretentive floating drug delivery system of ofloxacin. *Asian J Pharm Sci* 2013; 8: 191-98.
6. Khan AZ, Tripathi R, Mishra B. Floating elementary osmotic pump tablet for controlled delivery of diethylcarbamazine citrate: a water soluble drug. *AAPS Pharm Sci Tech*, 2011; 12(4):1312-23.

7. Mane SS, Kamble MS, Mane OR, Borwandkar VG, Aute PP, Chaudhari PD, Bhosale AV. Optimization of floating osmotic drug delivery system of diltiazem hydrochloride using 3^2 factorial design. *Indo Am J Pharm Res* 2013; 3(6): 4585-93.
8. Jagdale SC, Bari NA, Kuchekar BS, Chabukswar AR, Optimization studies on compression coated floating-pulsatile drug delivery of bisoprolol. *Biomed Res Int.* 2013; 1-11.
9. Pund S, Joshi A, Vasu K, Nivsarkar M, Shishoo C. Gastroretentive delivery of rifampicin: In vitro mucoadhesion and in vivo gamma scintigraphy. *Int. J. of Pharm.* 2011; 411, 106–112.
10. Gupta NV, Shivakumar HG. Development of a Gastroretentive Drug Delivery System based on Superporous Hydrogel. *Trop J Pharm Res.* 2010; 9(3): 257-264.
11. Vo AQ, Feng X, Morott JT, Pimparade MB, Tiwari RV, Zhang F and Repka MA. A novel floating controlled release drug delivery system prepared by hot-melt extrusion. *Eur. J. Pharm. Biopharm.* 2016; 98: 108-121.
12. Amrutkar PP, Chaudhari PD, Patil SB. Design and in vitro evaluation of multiparticulate floating drug delivery system of zolpidem tartarate. *Coll. Surf. B: Biointerfaces.* 2012; 89:182-187.
13. Dube TS, Ranpise NS, Ranade AN, Formulation and Evaluation of Gastroretentive Microballoons Containing Baclofen for a Floating Oral Controlled Drug Delivery System. *Current Drug Delivery.* 2014; 11(6):805 – 816.
14. Bharkatiya M, Kitawat S, Ojha A. Floating drug delivery systems- a review. *J Drug Delivery Ther.* 2014; 4(2):130-34.
15. Sandhan S, Singh R, Kakar S. Gastroretentive drug delivery systems- a review. *Afr J Pharm Pharm col.* 2015; 9(12):405-17.
16. Kuchekar BS, Boldhane SP. Gastroretentive Drug Delivery of Metformin Hydrochloride: Formulation and In Vitro Evaluation Using 3^2 Full Factorial Design. *Current Drug Delivery.* 2009; 6(5): 477 – 485.
17. Tack-Oon Oh, Ju-Young Kim, Jung-Myung Ha, Sang-Cheol Chi, Yun- Seok Rhee, et. al. Preparation and In Vivo Evaluation of Highly Porous Gastroretentive Metformin Tablets using a Sublimation method. *Eur J Pharm Biopharm.* 2013; 83(3): 460-7.
18. Priyadarshini R, Nandi G, Changder A, Chowdhury S, Chakraborty S, and Ghosh LK. Gastroretentive extended release of metformin from methacrylamide-g-gellan and tamarind seed gum composite matrix. *Carbohydrate Polymers.* 2015; 137:100-110.

19. Kalyankar, TM, Rangari NT, Khan M, Hosmani A and Sonwane A. Formulation and Evaluation of Mucoadhesive Pioglitazone HCl Microspheres. *Int. J. Pharma World Res.* 2010; 1(3): 1-14.
20. Dhakar RC, Maurya SD , Bhanu P, Sagar S ,Gupta AK ,Maurya G, Development and evaluation of mucoadhesive microspheres of pioglitazone maleate, *J Pharm Res.* 2011; 4(2): 456-458.
21. Pentikainen PJ, Neuvonen PJ & Penttila A. Pharmacokinetics of metformin after intravenous and oral administration to man. *European Journal of Clinical Pharmacology*, 1979; 16: 195 – 202.
22. Foster R, Plosker G. Glipizide: A Review of the Pharmacoeconomic Implications of the Extended-Release Formulation in Type 2 Diabetes Mellitus. *Pharmaco Economics.* 2000; 18(3): 289-306.
23. Chowdary KPR, Koteshwara RN, and Malathi K. Ethyl cellulose microspheres of gliplizide: characterization, in vitro and in vivo evaluation. *Indian Journal of pharmaceutical sciences* 2004; 66: 412- 416.
24. Urashima Y, Kayano E, KATO R, Effect of gastric pH on Mitiglinide absorption, *Clinical Pharmacy and Clinical Pharmacokinetics*, 2012; 29(2): 41-46.
25. Bettini R, Catellani PL, Santi P, Massimo G, Peppas NA, et al. Translocation of drug particles in HPMC matrix gel layer: effect of drug solubility and influence on release rate. *J Control Release* 2001; 70: 383–91.
26. Dorozynski P, Kulinowski P, Mendyk A, Jachowicz R. Gastroretentive drug delivery systems with l-dopa based on carrageenans and hydroxypropylmethylcellulose. *Int J Pharm.* 2011; 404 :169–75.
27. Nokhodchi A, Jelvehgari M, Siah MR, Mozafari M. Factors affecting themorphology of benzoyl peroxide microsponges. *Micron.* 2007; 38, 834–840.
28. Orlu M, Cevher E, Araman A. Design and evaluation of colon specific drug delivery system containing flurbiprofen microsponges. *Int. J. Pharm.* 2006; 318,103–117.
29. Kaity S, Maiti S, Ghosh AK, Pal D, Ghosh A, Banerjee S. Microsponges: a novel strategy for drug delivery system. *J. Adv. Pharm. Technol. Res.* 2010; 1: 283–290.
30. Arya P and Pathak K, Assessing the viability of microsponges as gastro retentive drug delivery system of curcumin: Optimization and pharmacokinetics, *Int. J. Pharm.* 2014; 460: 1– 12.

31. Sharma OP, Shah MV, Parikh DC & Mehta TA. Formulation optimization of gastroretentive drug delivery system for allopurinol using experimental design. *Expert Opin. Drug Deliv.* 2014;1-12.
32. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. *Pharmacological Research.* 2005; 52: 313–320.
33. Pandit V, Pai RS, Yadav V, Devi K, Surekha BB, Inamdar MN, and Sarasija S. Pharmacokinetic and pharmacodynamic evaluation of floating microspheres of metformin hydrochloride. *Drug Development and Industrial Pharmacy.* 2013; 39(1): 117–127.
34. Yu L, Su Z. Determination of Mitiglinide in rat plasma by high-performance liquid chromatography with UV detection. *Journal of Chromatography B.* 2006; 834 (1-2): 204–207.