FORMULATION AND EVALUATION OF GASTRORETENTIVE DRUG DELIVERY SYSTEM OF ANTIDIABETIC DRUGS

A Thesis Submitted to Gujarat Technological University

for the Award of

Doctor of Philosophy

in

Pharmacy

By

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Under the Supervision of

Dr. Naazneen Surti

GUJARAT TECHNOLOGICAL UNIVERSITY AHMEDABAD

March - 2018

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ABSTRACT

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

Identification of drugs was done by physical characterization and FTIR scan. The analytical methods on UV spectrophotometer and HPLC were developed for the *in vitro* analysis of drug. The bioanalytical methods were developed for MTG and GLP for evaluating pharmacokinetic parameter of the drug *in vivo* wistar rats.

The floating matrix tablet was prepared for MH 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. 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.

Another antidiabetic drug, for which the gastroretentive formulations were developed, was MTG. The floating matrix tablet of MTG was prepared using the combination of release controlling polymer HPMC K15M and sodium alginate. The final optimization was done by applying 3^2 full factorial design, taking floating lag time (F_{lag}), time to release 50% of drug (t_{50}) and time to release 90% of drug (t_{90}) as dependent factors. All the formulations were evaluated and results showed that M-3 formulation containing maximum amount of both variables gave promising results, hence was considered as optimized batch.

A Gastroretentive multiparticulate system of MTG was developed as floating microsponges by quasi-emulsion solvent diffusion method. The primary screening of formulation related and process related variables was done by trial and error technique. The final optimization of dosage form was done by applying 3^2 full factorial design by taking concentrations of PVA (X_1) and ethyl cellulose (X_2) as independent factors and product yield (Y_1), % entrapment efficiency (Y_2), % buoyancy (Y_3) and % cumulative drug release (Y_4) of

microsponges as dependent responses. Using design expert software, optimized batch of MTG microsponges, (F-0) was 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 responses were found to be in close agreement with the practical values. The characterization of the optimized formulation showed the compatibility between the drug and excipient and spherical, porous nature of microsponges.

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 microsponges of glipizide were prepared by quasi emulsion technique. Primary screening of variables was done by Plackett–Burman design to find the potential risk factors and finally microsponges were optimized using Box–Behnken design. All the formulations were evaluated for product yield, entrapment efficiency, buoyancy, and in vitro release. The desirability function and overlay plot indicated GBB-8 (with X_1 at 0-level and X_2, X_3 at 1-level), as optimized formulation. The physicochemical characterization of optimized formulation showed no interaction between the drug and polymer and the complete dispersion of the drug in polymeric matrix and also the porous, spherical nature of the formulation.

Radiological study was performed on healthy albino rabbits for checking the gastroretention for optimized floating tablets and microsponges of MTG and GLP. 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. Pharmacokinetic studies of optimized microsponges of glipizide and MTG 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.

Dedication

My memory goes deep down the lane of life when we left everything and moved out of Kashmir. Leaving behind all we could call ours. Facing many hardships in life and doing that happily, my father ensured not to let our dreams die. He was always the encouraging force for me to start my doctorate. Although my work never really struck a chord in him, he was an unwavering supporter through the seemingly endless years. He was immensely proud that his daughter was working towards a PhD. Unfortunately, he did not stay with us to witness this proud day in my life, but I still feel his existence every day.

So, with pride and affection, I dedicate this thesis to my father, Late Raj Nath Raina.

LOVE YOU PAPA

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"When you start achieving your dream, there will always be obstacles, doubters, mistakes and setbacks along the way. But with hard work, perseverance and support of your dear ones there is no limit to what you can achieve"

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Meenakshi Bhavesh Patel

List of Contents

List of Abbreviation

ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
AUC	Area Under Plasma Concentration Curve
AUMC	Area Under the First Moment of the Concentration
CDR	Cumulative Drug Release
C_{max}	Maximum Plasma Concentration
CP	Carbopol p 934
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
CR	Controlled Release
CS	Calcium Silicate
DAD	Diode-array Detector
DCM	Dichloromethane
DM	Diabetes Mellitus
DoE	Design of Experiment
DSC	Differential Scanning Calorimetry
ES	Eudragit S
F	Floating Force
FT-IR	Fourier-Transform Infrared
GDM	Gestational Diabetes Mellitus
GIT	Gastrointestinal Tract
GLB	Glibenclamide
GLP	Glipizide
GRDDS	Gastro-Retentive Dosage Forms
GRDF	Gastroretentive Dosage Forms
GRT	Gastric Retention Time
HBS	Hydrodynamically Balanced Systems
HCl	Hydrochloric Acid

HEC	Hyrdoxy Ethyl Cellulose
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxy Propyl Methyl Cellulose
HQC	High Quality Control
IAEC	Institutional Animal Ethical Committee
ICH	International Conference on Harmonization
IP	Indian Pharmacopoeia
IS	Internal Standard
K_{el}	Elimination Rate Constant
LLE	Liquid-Liquid Extraction
LLOQ	Lower Limit of Quantification
LOD	Limit of Detection
LOQ	Limit of Quantification
LQC	Low Quality Control
MDS	Microsponge Delivery System
MH	Metformin Hydrochloride
MQC	Medium Quality Control
MRT	Mean Residence Time
MTG	Mitiglinide Calcium Dihydrate
$NaHCO_3$	Sodium Bicarbonate
NIDDM	Non-Insulin-Dependent Diabetes Mellitus
PABA	Paraaminobenzoic Acid
PD	Pharmacodynamic
PK	Pharmacokinetic
PMA	Polymethacrylic Acid
PVA	Polyvinyl Alcohol
PVP	Poly Vinyl Pyrrolidone
QC	Quality Control
RH	Relative Humidity
RP-HPLC	Reversed Phase - High Performance Liquid Chromatography
RSM	Response Surface Methodology
SCD	Simplex Centroid Design
SEM	Scanning Electron Microscopy

SLD	Simplex Lattice Design
SPE	Solid Phase Extraction
SUPAC	Scale Up and Post Approval Changes
$t_{1/2}$	Elimination Half-Life
TEC	Triethylcitrate
t_{max}	Time for Maximum Plasma Concentration
USFDA	United State Food and Drug Administration
USP	United State Pharmacopoeia
UV	Ultraviolet
WHO	World Health Organization
XRD	Powder X-Ray Diffraction

List of Symbols

Symbol	Name
%	Percentage
±	Positive or Negative
°C	Degree Celsius
µg	Micrograms
cm	Centimeter
conc.	Concentration
gm	Gram
mg	Milligram
min	Minute
hr	Hour
s	Second
λ	Lambda
mMol	Millimole
rpm	Rotations per minute
mg	Milligram

List of Figures

List of Tables

CHAPTER 1

Introduction

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 faces the problem of the short gastric retention period. This results in the incomplete absorption of the drug, as the dosage form doesn't stay at the site of absorption for a longer period of time, which lead to decreased efficacy of the drug[1]. This shortcoming has led to the evolution of oral gastro-retentive dosage forms (GRDDS). Various gastroretentive dosage forms have being designed and developed, including: high density sinking systems that is retained at the bottom of the stomach[2], low density floating systems that causes buoyancy in gastric fluid[3-5], floating osmotic pump[6,7], floating pulsatile delivery system[8], mucoadhesive systems that causes bioadhesion to stomach mucosa[9], superporous hydrogel systems[10], floating system by hot melt extrusion[11] 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].

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) was selected for the present research work.

Antidiabetic agents have been selected as a drug of choice for preparing GRDDS because Diabetes is a fatal disease & a person dies from diabetes related causes in every 10seconds. The treatment of type II diabetes involves the lifelong intake of antidiabetic drugs to control the blood glucose levels. There are many antidiabetic drugs which need to be in stomach for getting absorbed. Gastroretentive dosage forms provides an efficient approach to deliver such anti-diabetic drugs to the upper part of gastrointestinal tract (GIT), thereby increase patient compliance and offers better treatment of disease[16-20]. This research project was undertaken to prepare GRDDS of anti-diabetic drugs for increasing their retention in upper GIT, thereby maintaining the therapeutic level of drug.

1.1 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. World Health Organization (WHO) reports refer India as the potential diabetic capital of the world, with the number of patients of the disease expected to increase from three to six crores by 2030[21].

For the proper management of the disorder, the medicament has to be taken at regular intervals of time, lifelong. Conventional antidiabetic oral dosage forms offer no control over drug delivery, leading to fluctuations in plasma drug concentration and causes irregular glucose level in the patient's body.

This shows that there is utmost requirement of the antidiabetic drugs to maintain the blood glucose level over the extended period of time for better therapeutic efficacy of drug. Antidiabetic agents like biguanide derivatives, sulfonylurea and meglitinide analogs are having a strong rationale for preparing gastroretentive dosage forms as they are absorbed from the upper part of gastrointestinal tract. Development of GRDDS of such drugs keep the dosage form in the upper part of gastrointestinal tract and releases the drug in the sustained manner for the desired period. Literature supports the improved delivery of antidiabetic drugs, when gastric retention period is increased, leading to better control of the disease condition[22-25]. Although many researchers have worked on the topic, still there is scope to make the treatment of diabetes better by preparing other gastroretentive dosage forms.

Hence, the present research was focused on preparing the gastroretentive multiparticulate system and tablet formulations, of antidiabetic drugs to achieve better patient compliance and efficient treatment of type II diabetes mellitus.

1.2 Aim of the Research Work

The aim of the present work was to formulate and evaluate the gastroretentive drug delivery system of antidiabetic drugs in order to increase their retention in stomach, which ultimately results in the increase of 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.

1.3 Objectives of Present Work

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 gastroretentive floating matrix tablet.
5. To apply an appropriate statistical design for the optimization of the floating matrix tablets.
6. To prepare, optimize and evaluate 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 in Albino wistar rats.

1.4 Scope of Research 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.

1.5 Rationale of the Present Research Work

1.5.1 Rationale for Selection of Antidiabetic Drugs

Many antidiabetic drugs like, metformin, repaglinide, glipizide mitiglinide etc., are better absorbed from the upper part of gastrointestinal tract, but they suffer from the drawback of short residence time in stomach. The conventional dosage forms of these drugs can't hold the formulation in the upper part of GIT for a long time, which causes incomplete absorption of the drug. Hence, can lead to fluctuations of the drug concentration in the blood, which is not desirable for the treatment of Diabetes. Moreover, the drugs have a short half-life which demands the repeated administration of the dosage form to maintain the therapeutic level of the drug in the body. The gastroretentive dosage forms help in overcoming all these drawbacks of antidiabetic drugs which leads to increase in bioavailability of such drugs and hence helps in better management of diabetes.

1.5.2 Rationale for Preparing Gastroretentive Formulations

Gastroretentive drug delivery system retains the dosage form in the stomach and enables the sustained release of the drug[26]. This is beneficial for the drug which have their target site of absorption, in upper part of gastrointestinal tract. As the dosage form offers sustained release, it avoids the repeated administration of the drug, which is advantageous for the drug with short half-life[27]. The continuous release of the drug at appropriate site ensures the maintenance of the drug level in the blood of patient, which is highly essential for the proper treatment of diabetes. Hence, GRDDS provides a new and important therapeutic option for delivery of anti-diabetic drug as they not only prolong dosing intervals, but also increase patient compliance as compared to existing conventional and other controlled release dosage forms.

1.5.2.1 Selection of Gastroretentive Floating Matrix Tablet

In the present research work, two types of gastroretentive dosage forms are prepared. The gastroretentive floating matrix tablet and floating microsponges of antidiabetic drugs. Floating matrix tablets are the unit dosage form that are convenient to formulate and provides the benefit of releasing the drug in the stomach for long period of time[28]. This dosage form would give better therapeutic activity of antidiabetic drugs thereby would maintain the blood glucose level efficiently.

1.5.2.2 Selection of Gastroretentive Floating Microsponges

Multiparticulate systems have potential for targeting drug molecule to its target site, but have low drug loading capacity[29, 30]. 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 on the surface of the stomach liquid contents 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 effective. As microsponges offer an efficient drug delivery system, hence, in present research, gastroretentive floating microsponges were explored for antidiabetic drugs [31,32].

1.6 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 system[33]. 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 systems[34].

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 floating microsponges, which 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[35-37]. Microsponges were not explored for low density gastro retentive system until Arya et. al., developed targeted floating curcumin microsponges for improved site specific absorption for gastric cancer[38]. 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.

1.7 Outline of Thesis

The description on the present research work has been divided into seven major chapters of the thesis. The first chapter of introduction includes brief background, definition of the problem, aim, objectives, rationale of the present research work and Original contribution by the thesis. Chapter 2 presents literature review on various phases related to the work including the description about diabetes, literature related to the drugs used in the present work, gastroretentive floating matrix tablets, details of floating microsponges, profile of the drug and polymers used in the final preparation of gastroretentive formulations. Chapter 3 includes the material and method used in the present research work, preformulation studies of drugs including physical characterization, analytical and bioanalytical methods for the analysis of drugs. Chapter 4 includes the details of the matrix floating tablets of metformin hydrochloride (MH). It includes the development, evaluation and optimization of formulation by simplex centroid design, it further includes the results, discussion and conclusion of the work. Chapter 5 is divided in two parts: Chapter 5A deals with the formulation of gastroretentive floating matrix tablet of mitiglinide calcium dihydrate (MTG). The chapter includes evaluation, optimization (by two factor three level full factorial design), results, discussion and conclusion of the work. The second part of the chapter 5B, includes the formulation of floating microsponges of MTG. It includes the experimentation,

optimization (by two factor three level full factorial design) and evaluation of the formulations. It further contains the results obtained with the experiments performed and detailed discussion on it with conclusion. It also includes the *in vivo* studies of the optimized gastroretentive floating microsponges of MTG. Chapter 6 comprises of two sections: chapter 6A deals with the formulation of gastroretentive floating matrix tablet of glipizide (GLP). The chapter includes evaluation, optimization (by simplex lattice design), results, discussion and conclusion of the work. Chapter 6B deals with floating microsponges of GLP. It involves the experimentation part, including the screening of critical factors by Plackett and Burman design and final optimization of formulation by Box-Behnken design. It also includes the *in vivo* studies of the optimized gastroretentive floating microsponges of GLP. Chapter 7 includes the final conclusions of the research work.

1.8 References

1. Rouge, N., Buri, P. and Doelker, E., 1996. Drug absorption sites in the gastrointestinal tract and dosage forms for site-specific delivery. *International Journal of Pharmaceutics*, *136*(1-2), pp.117-139.

2. Rouge, N., Allémann, E., Gex-Fabry, M., Balant, L., Cole, E.T., Buri, P. and Doelker, E., 1998. Comparative pharmacokinetic study of a floating multiple-unit capsule, a high-density multiple-unit capsule and an immediate-release tablet containing 25 mg atenolol. *Pharmaceutica Acta Helvetiae*, *73*(2), pp.81-87.

3. Hu, L., Li, L., Yang, X., Liu, W., Yang, J., Jia, Y., Shang, C. and Xu, H., 2011. Floating matrix dosage form for dextromethorphan hydrobromide based on gas forming technique: *in vitro* and *in vivo* evaluation in healthy volunteers. *European Journal of Pharmaceutical Sciences*, *42*(1), pp.99-105.

4. Garg, T., Kumar, A., Rath, G. and Goyal, A.K., 2014. Gastroretentive drug delivery systems for therapeutic management of peptic ulcer. *Critical Reviews™ in Therapeutic Drug Carrier Systems*, *31*(6), pp. 531-557.

5. Shakya, R., Thapa, P. and Saha, R.N., 2013. *In vitro* and *in vivo* evaluation of gastroretentive floating drug delivery system of ofloxacin. *Asian Journal of Pharmaceutical Sciences*, *8*(3), pp.191-198.

6. Khan, Z.A., Tripathi, R. and Mishra, B., 2011. Floating elementary osmotic pump tablet (FEOPT) for controlled delivery of diethylcarbamazine citrate: a water-soluble drug. *AAPS PharmSciTech*, *12*(4), pp.1312-1323.

7. Mane, S.S., Kamble, M.G., Mane, O.R., Borwandkar, V.G., Aute, P.P., Chaudhari, P.D. and Bhosale, A.V., 2013. Optimization of Floating Osmotic Drug Delivery System of Diltiazem Hydrochloride Using 3^2 Factorial Design. *Indo American Journal of Pharmaceutical Research*, *3*(6), pp.4585-4593.

8. Jagdale, S.C., Bari, N.A., Kuchekar, B.S. and Chabukswar, A.R., 2013. Optimization studies on compression coated floating-pulsatile drug delivery of bisoprolol. *BioMed Research International*, *2013*, pp.1-11.

9. Pund, S., Joshi, A., Vasu, K., Nivsarkar, M. and Shishoo, C., 2011. Gastroretentive delivery of rifampicin: *In vitro* mucoadhesion and *in vivo* gamma scintigraphy. *International Journal of Pharmaceutics*, *411*(1), pp.106-112.

10. Gupta, N.V. and Shivakumar, H.G., 2010. Development of a gastroretentive drug delivery system based on superporous hydrogel. *Tropical Journal of Pharmaceutical Research*, *9*(3), pp.257-264.

11. Vo, A.Q., Feng, X., Morott, J.T., Pimparade, M.B., Tiwari, R.V., Zhang, F. and Repka, M.A., 2016. A novel floating controlled release drug delivery system prepared by hot-melt extrusion. *European Journal of Pharmaceutics and Biopharmaceutics*, *98*, pp.108-121.

12. Amrutkar, P.P., Chaudhari, P.D. and Patil, S.B., 2012. Design and *in vitro* evaluation of multiparticulate floating drug delivery system of zolpidem tartarate. *Colloids and Surfaces B: Biointerfaces*, *89*, pp.182-187.

13. Dube, T.S., Ranpise, N.S. and Ranade, A.N., 2014. Formulation and evaluation of gastroretentive microballoons containing baclofen for a floating oral controlled drug delivery system. *Current Drug Delivery*, 11(6), pp.805-816.

14. Bharkatiya, M., Kitawat, S., Ojha, A., 2014. Floating drug delivery systems- a review. *Journal of Drug Delivery and Therapeutics*. 4(2), pp.130-34.

15. Darandale, S.S. and Vavia, P.R., 2012. Design of a gastroretentive mucoadhesive dosage form of furosemide for controlled release. *Acta Pharmaceutica Sinica B*, *2*(5), pp.509-517.

16. Boldhane, S.P. and Kuchekar, B.S., 2009. Gastroretentive drug delivery of metformin hydrochloride: formulation and *in vitro* evaluation using 32 full factorial design. *Current Drug Delivery*, *6*(5), pp.477-485.

17. Oh, T.O., Kim, J.Y., Ha, J.M., Chi, S.C., Rhee, Y.S., Park, C.W. and Park, E.S., 2013. Preparation of highly porous gastroretentive metformin tablets using a sublimation method. *European Journal of Pharmaceutics and Biopharmaceutics*, *83*(3), pp.460-467.

18. Priyadarshini, R., Nandi, G., Changder, A., Chowdhury, S., Chakraborty, S. and Ghosh, L.K., 2016. Gastroretentive extended release of metformin from methacrylamide-g-gellan and tamarind seed gum composite matrix. *Carbohydrate Polymers*, *137*, pp.100-110.

19. Kalyankar, T.M., Nalanda, T., Mubeena, K., Avinash, H. and Arvind, S., 2010. Formulation and evaluation of mucoadhesive pioglitazone HCl microspheres. *International Journal of Pharma World Research*, *1*(3), pp.1-14.

20. Dhakar, R.C., Maurya, S.D., Sagar, B.P., Gupta, A.K. and Maurya, G., 2011. Development and evaluation of mucoadhesive microspheres of pioglitazone maleate. *Journal of Pharmaceutical Research*, *4*(2), pp.456-458.

21. Shaw, J.E., Sicree, R.A. and Zimmet, P.Z., 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*, *87*(1), pp.4-14.

22. Parveen, R., Singh, A.P., Bhargav, R.K., Verma, A. and Shrivastav, P., 2016. Formulation and evaluation of floating tablet of Metformin HCL. *World Journal of Pharmacy and Pharmaceutical Science*, *5*(7), pp.1317-1326.

23. Meka, V. S., Pillai, S., Dharmalingham, S. R., Sheshala, R., Gorajana, A., 2015. Preparation and *in vitro* Characterization of a Non-Effervescent Floating Drug Delivery System for Poorly Soluble Drug, Glipizide. *Acta Poloniae Pharmaceutica - Drug Research*, *72*, pp.193-204.

24. Sharma, M., Kohli, S. and Dinda, A., 2015. *In vitro* and *in vivo* evaluation of repaglinide loaded floating microspheres prepared from different viscosity grades of HPMC polymer. *Saudi Pharmaceutical Journal*, *23*(6), pp.675-682.

25. Kamila, M.M., Mondal, N., Ghosh, L.K. and Gupta, B.K., 2009. Multiunit floating drug delivery system of rosiglitazone maleate: development, characterization, statistical optimization of drug release and *in vivo* evaluation. *AAPS PharmSciTech*, *10*(3), pp. 887–899.

26. Chavanpatil, M., Jain, P., Chaudhari, S., Shear, R. and Vavia, P., 2005. Development of sustained release gastroretentive drug delivery system for ofloxacin: *in vitro and in vivo* evaluation. *International Journal of Pharmaceutics*, *304*(1), pp.178-184.

27. Nama, M., Gonugunta, C.S.R. and Veerareddy, P.R., 2008. Formulation and evaluation of gastroretentive dosage forms of clarithromycin. *AAPS PharmSciTech*, *9*(1), pp. 231-237.

28. Dey, S., Dutta, S. and Mazumder, B., 2012. Formulation and evaluation of floating matrix tablet of atenolol for gastro-retentive drug delivery. *International Journal of Pharmacy and Pharmaceutical Sci*ence. *4*, pp.433-7.

29. Streubel, A., Siepmann, J. and Bodmeier, R., 2003. Multiple unit gastroretentive drug delivery systems: a new preparation method for low density microparticles. *Journal of Microencapsulation*, *20*(3), pp.329-347.

30. Nappinnai, M. and Sivaneswari, S., 2013. Formulation optimization and characterization of gastroretentive cefpodoxime proxetil mucoadhesive microspheres using 3^2 factorial design. *Journal of Pharmacy Research*, *7*(4), pp.304-309.

31. Embil, K. and Nacht, S., 1996. The microsponge® delivery system (MDS): a topical delivery system with reduced irritancy incorporating multiple triggering mechanisms for the release of actives. *Journal of Microencapsulation*, *13*(5), pp.575-588.

32. Nacht, S. and Katz, M., 1990. The microsponge: a novel topical programmable delivery system. *Drugs and the Pharmaceutical Sciences*, *42*, pp.299-325.

33. Bettini, R., Catellani, P.L., Santi, P., Massimo, G., Peppas, N.A. and Colombo, P., 2001. Translocation of drug particles in HPMC matrix gel layer: effect of drug solubility and influence on release rate. *Journal of Controlled Release*, *70*(3), pp.383-391.

34. Dorożyński, P., Kulinowski, P., Mendyk, A. and Jachowicz, R., 2011. Gastroretentive drug delivery systems with l-dopa based on carrageenans and hydroxypropylmethylcellulose. *International Journal of Pharmaceutics*, *404*(1), pp.169-175.

35. Nokhodchi, A., Jelvehgari, M., Siahi, M.R. and Mozafari, M.R., 2007. Factors affecting the morphology of benzoyl peroxide microsponges. *Micron*, *38*(8), pp.834-840.

36. Orlu, M., Cevher, E. and Araman, A., 2006. Design and evaluation of colon specific drug delivery system containing flurbiprofen microsponges. *International Journal of Pharmaceutics*, *318*(1), pp.103-117.

37. Kaity, S., Maiti, S., Ghosh, A.K., Pal, D., Ghosh, A. and Banerjee, S., 2010. Microsponges: A novel strategy for drug delivery system. *Journal of Advanced Pharmaceutical Technology & Research*, *1*(3), pp.283–290.

38. Arya, P. and Pathak, K., 2014. Assessing the viability of microsponges as gastro retentive drug delivery system of curcumin: Optimization and pharmacokinetics. *International Journal of Pharmaceutics*, *460*(1), pp.1-12.

CHAPTER 2

Literature Review

2.1 Overview

GRDDS is an approach to prolong the gastric residence time, thereby aiming site-specific drug release in the upper GIT for local or systemic effects. Gastroretentive dosage forms can remain in the gastric region for long periods and hence significantly prolong the gastric retention time (GRT) of drugs. Prolonged GRT enables the controlled delivery of drugs in stomach which can evade the repeated administration of dosage form of the drugs with short half-life. Literature suggests that GRDDS has gained huge popularity in the field of oral drug delivery recently, as it can release the drug slowly that can combat many shortcomings allied with conventional oral delivery, including poor bioavailability. Studies demonstrate that the drugs which has to be in the upper part of the GIT, have been prepared as gastroretentive dosage forms using various approaches. Such formulations improves the therapeutic efficacy of the drug and enhances the patient compliance.

GRDFs have been researched for many antidiabetic drugs and studies revealed that the gastroretentive form of drug has led to better management of the disease status. Although, a lot of work has been done, but still there is tremendous scope to develop such dosage form of antidiabetic drugs. Moreover, there are certain antidiabetic drugs which have a strong rationale for developing GRDDS, but no research has been reported to be performed on them.

2.2 Diabetes Mellitus

Diabetes mellitus (DM) is chronic and complex metabolic diseases characterized by hyperglycemia arising due to deficiency of Insulin secretion or resistance to insulin function, or both. Insulin hormone, produced in the pancreas, within the β-cells of the islets of Langerhans, regulates the glucose level in the body[1]. Diabetes mellitus is considered to be the highest growing public health problem throughout the world and there is an imperative need for improvement in diabetes care as the occurrence of diabetes is increasing at an alarming rate. Uncontrolled Diabetic in the patients affect their key organs like, heart and

blood vessels, eyes, kidneys, nerves, GIT and gums and teeth and cause many complications[2].

Mostly, type II diabetes is increasing in the people living in urban areas[3]. Although, the exact cause of DM is uncertain still the reason may be genetic and but in most of the cases, occurrence of DM is attributed to low physical activity and unhealthy diet. The genetic reason cannot be avoided, but a person can control the diet, rich in fat and starch, which on digestion gets converted to glucose. The increased level of glucose, caused due to starch, can be regulated by the inhibition of a-amylase and a-glucosidase, digestive enzymes of starch[4,5].

The basis treatment of diabetes involves lifestyle modification through increased physical activity and attention to food intake[6,7]. The drugs used for treatment of diabetes mellitus include insulin and insulin analogues, sulfonylureas, Meglitinides, Biguanides, Thiazolidinediones and α glucosidase inhibitors. Now-a-days combination products are being used for the better management of the diabetes. Except insulin all other antidiabetic drugs are given by oral route, which is considered as preferable route for the administration of therapeutic agents.

2.2.1 Types of Diabetes

There are three types of Diabetes:

Type I: This type of diabetes is also called as insulin-dependent diabetes or juvenile diabetes. Approximately 10% of all diabetes cases are of type I. This results because of β-cell destruction which leads to insulin insufficiency. In such type of DM the patients' needs to take insulin daily, to normalize the glucose level in the blood. If insulin is not taken, it can be life threatening.

Type II diabetes: This type of diabetes is also called as non-insulin dependent diabetes. About 90% of all diabetes cases are of DM type II, which is caused because of insulin resistance on account of the inefficient use of insulin by the body. The high level of glucose in the body is because of the insulin resistance or its deficiency. As the symptoms are very common and less noticeable, the disease remains undiagnosed for years, which complicate the condition. The reasons for type II diabetes may be family history of DM, past history of

gestational diabetes (diabetes in pregnancy), diet and advancing age[8,9]. This type of diabetes can be treated by different hypoglycemic agents, which will be discussed later in the chapter.

Gestational diabetes mellitus (GDM): This type of DM is a provisional disorder that happens in the second or third trimester of pregnancy. According to a 2014 analysis by the Centers for Disease Control and Prevention, the prevalence of gestational diabetes is as high as 9.2%.

2.2.2 Complications of Diabetes Mellitus[10-12]

DM is a condition caused due to the hormonal imbalance which can lead to lot of complications in long term. The complications due to diabetes develop gradually, if there is less control on the blood sugar levels. Eventually, diabetes complications may be disabling or even life-threatening. Some of the possible complications are discussed below:

Cardiovascular disease. Diabetes dramatically increases the risk of various cardiovascular problems, including coronary artery disease with chest pain (angina), heart attack, stroke and narrowing of arteries (atherosclerosis). If you have diabetes, you are more likely to have heart disease or stroke.

Nerve damage (neuropathy). Excess sugar can injure the walls of the tiny blood vessels (capillaries) that nourish your nerves, especially in your legs. This can cause tingling, numbness, burning or pain that usually begins at the tips of the toes or fingers and gradually spreads upward. Left untreated, you could lose all sense of feeling in the affected limbs. Damage to the nerves related to digestion can cause problems with nausea, vomiting, diarrhea or constipation. For men, it may lead to erectile dysfunction.

Kidney damage (nephropathy). The kidneys contain millions of tiny blood vessel clusters (glomeruli) that filter waste from your blood. Diabetes can damage this delicate filtering system. Severe damage can lead to kidney failure or irreversible end-stage kidney disease, which may require dialysis or a kidney transplant.

Eye damage (retinopathy). Diabetes can damage the blood vessels of the retina (diabetic retinopathy), potentially leading to blindness. Diabetes also increases the risk of other serious vision conditions, such as cataracts and glaucoma.

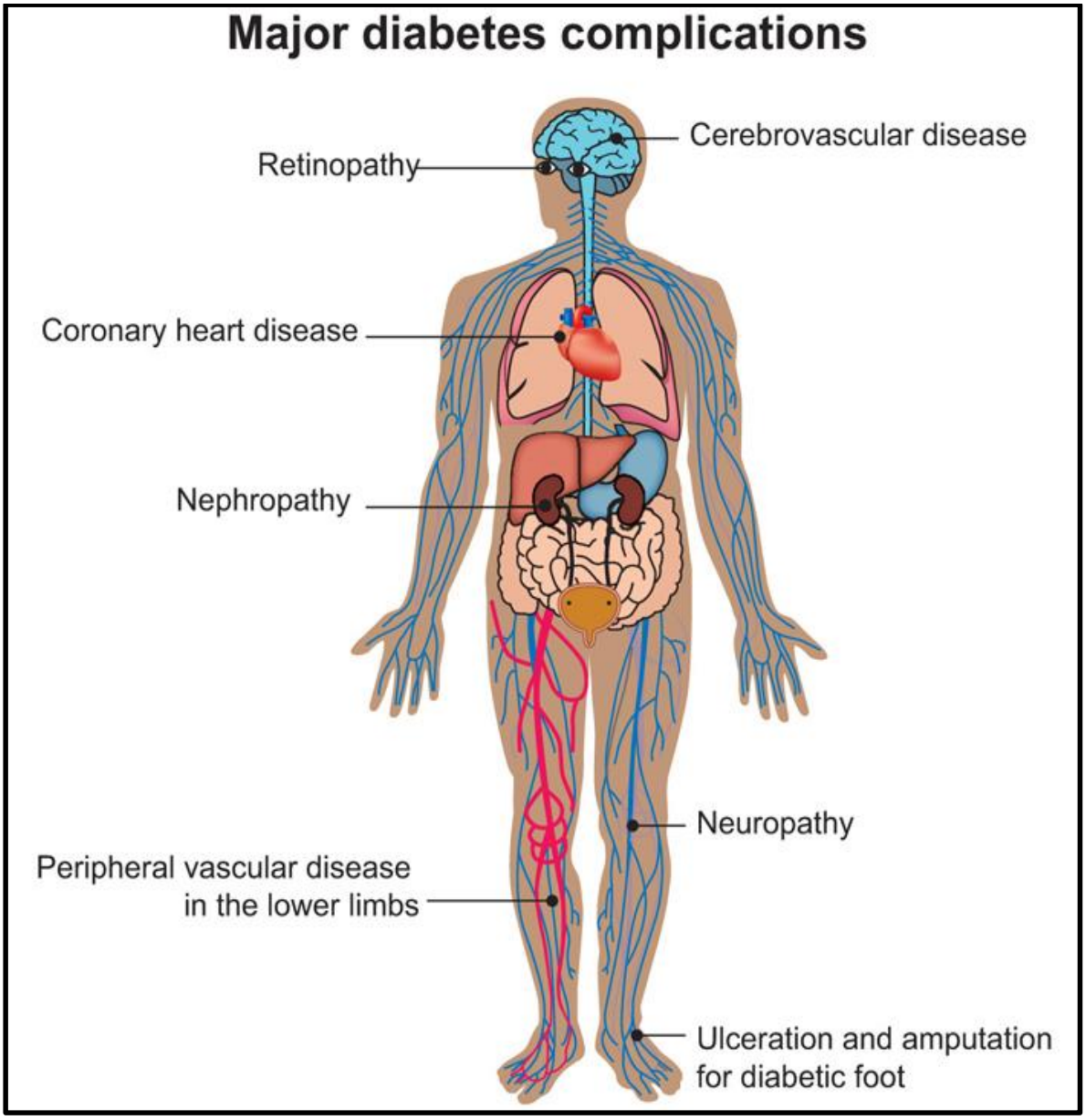


FIGURE 2.1 Complication of Diabetes Mellitus

Source: http://www.psdcfoundation.org/our-work-2/complications/

Foot damage. Nerve damage in the feet or poor blood flow to the feet increases the risk of various foot complications. Left untreated, cuts and blisters can develop serious infections, which often heal poorly. These infections may ultimately require toe, foot or leg amputation.

Skin conditions. Diabetes may leave you more susceptible to skin problems, including bacterial and fungal infections.

Hearing impairment. Hearing problems are more common in people with diabetes.

Alzheimer's disease. Type 2 diabetes may increase the risk of Alzheimer's disease. The poorer your blood sugar control, the greater the risk appears to be. Although there are theories as to how these disorders might be connected, none has yet been proved.

2.2.3 Treatment of Diabetes Mellitus

DM can be managed by changing the lifestyle through diet control and exercise and by checking the blood sugar level regularly[13]. However, if this doesn't work, the only way is to take antidiabetic drugs and in worst case insulin therapy is required. The list of antidiabetic drug used in the treatment of DM is given in table 2.1

TABLE 2.1 List of antidiabetic drugs used in the treatment of DM[14]

Sr. No	Category/ Class	Drugs with brandnames
1	Biguanides:	Metformin (Glucophage, Glucophage XR, Glumetza) phenformin
2	Sulfonylureas:	
2a	First-Generation Sulfonylurea	Tolazamide (Tolinase) Tolbutamide Acetohexamide (Dymelor)
2b	Second-Generation Sulfonylurea	Glibenclamide (Daonil) Glipizide (Glucotrol) Gliquidone (Glurenorm) Glyclopyramide (Deamelin-S) Glimepiride (Amaryl) Gliclazide (Diamicron)
3	Alpha-Glucoside Inhibitors:	Acarbose (Precose) Miglitol (Glyset)
4	Meglitinides derivatives:	Repaglinide (Prandin) Nateglinide (Starlix) mitiglinide

5	Glitazones (Thiazolidinediones):	Rosiglitazone (Avandia) Pioglitazone (Actos) troglitazone
6	Glucagonlike peptide–1 (GLP-1) agonists	Dulaglutide (Trulicity) Exenatide (Byetta) Liraglutide (Victoza) Lixisenatide (Lyxumia)
7	Dipeptidyl peptidase IV (DPP-4) Inhibitors	Linagliptin (Tradjenta) Saxagliptin (Onglyza) Sitagliptin (Januvia) Vildagliptin (Galvus)

All the antidiabetic drugs act on hyperglycemic condition caused due DM by different mechanisms. Various mode of action of antidiabetic drugs is shown in figure 1.2.

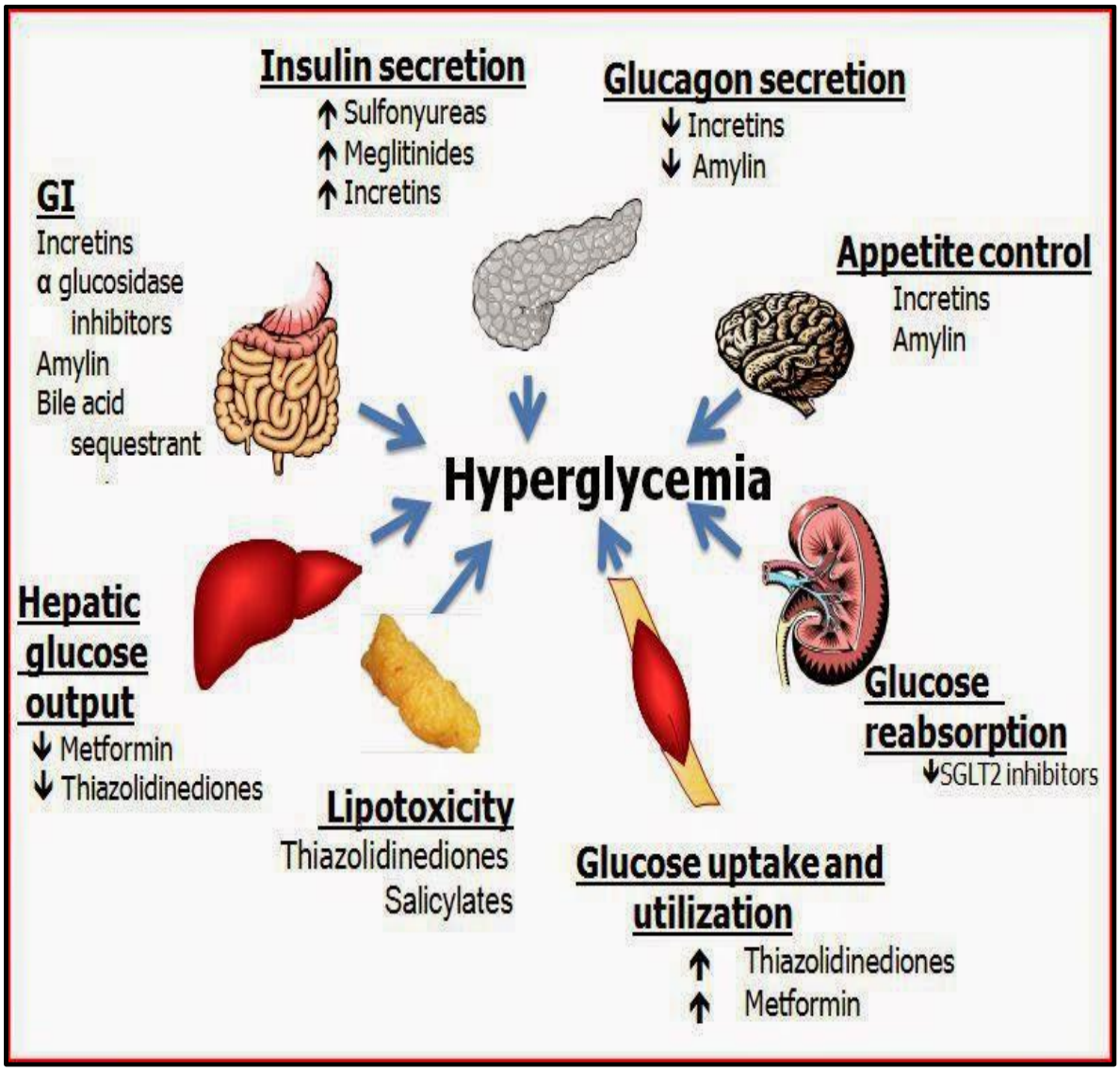


FIGURE 2.2 Mode of action of antidiabetic drug
Source: http://forumofdiabetes.blogspot.in/2013/05/diabetes complications.html

2.3 Gastroretentive Drug Delivery System

GRDDS is the system which retains in the stomach for long period of time, which is not possible to achieve with the conventional dosage form of the drug. It can provide the controlled release of the drug in the upper part of GIT, thereby enhance its absorption. Control release formulation provides the continuous release of the drug hence is beneficial for the drugs with short half-life as the frequency of the dosing decreases[15,16]. Literature suggests that sustained release GRDDS is highly beneficial for the drugs with special characteristics.

2.3.1 Potential drug candidates for Gastroretentive drug delivery systems

- Drugs that are absorbed in the proximal part of the gastrointestinal tract[17].
- Drugs those are locally active in the stomach e.g. misroprostol, antacids etc. Drugs that have narrow absorption window in gastrointestinal tract (GIT) e.g. l-dopa, furosemide, paraaminobenzoic acid (PABA), riboflavin etc[18].
- Drugs those are getting degraded in the intestinal or colonic pH or which give the local effect e.g. ranitidine HCl, captopril, metronidazole[19, 20].
- Drugs that disturb normal colonic microbes e.g. antibiotics and the antibacterial agents' used for the treatment of Helicobacter pylori related peptic ulcers[21].
- Drugs that less soluble at high pH e.g. captopril, diazepam, verapamil HCl[22].

2.3.2 Drugs those are unsuitable for gastro drug delivery systems[23]

- Drugs that have partial acid solubility doesn't make any sense to keep in the stomach for extended time, e.g. phenytoin etc.
- Drugs that are not stability in the gastric environment e.g. erythromycin etc.
- Drugs intended for site specific release in the colon as the target site is colon, e.g. corticosteroids and 5- amino salicylic acid etc.

2.3.3 Approaches to Gastric Retentive Delivery of Drug

To formulate a successful stomach specific or gastroretentive drug several techniques are currently used such as

***Hydrodynamically balanced systems (HBS)*[24]**

This system has buoyant materials incorporated which enable the device to float. The gelatinous polymer barrier formation results from the swelling of hydrophilic polymer. Drug is released from the dosage form by diffusion and erosion of the gel barrier.

***Raft systems incorporating alginate gels*[25]**

This system is especially advantageous when a patient can't take the solid dosage form orally and still there is a need to developing sustained released gastroretentive formulation. These systems are liquid formulations with gelling agent and carbonated component, which upon reaction with gastric acid forms gel with entrapped gas bubbles, hence enables floating.

***Bioadhesive or Mucoadhesive systems*[26]**

These systems are used to restrict a delivery device within the stomach to increase the drug absorption process in a site-specific manner. This approaches involves the use of bioadhesive polymers which remain adhered to the epithelial surface of stomach, thereby retaining the drug in upper part of GIT.

***High density systems*[27]**

This approach involves formulation of dosage forms with the density higher than the normal stomach content. These formulations are prepared by incorporating heavy core such as iron powder, zinc oxide and titanium oxide etc, along with the drug in the formulation. This formulation of high density pellet is based on assumption that heavy pellets might remain longer in the stomach, since they are positioned in the lower part of the antrum. But, practical applicability of this system in human beings has not been observed and no system has been marketed.

***Expandable, unfolding and Swelling system*[28]**

This type of gastroretentive formulation is prepared based on the fact that a dosage form should be bigger than pyloric sphincter to withstand gastric transit. However, the dosage

form must be small enough to be swallowed, and must not block the pylorus either by single dose or by accumulation.

Swelling system swell to an extent that prevents the exit of the dosage form, from the stomach, through the pylorus. This dosage form retaines in the stomach for a longer period of time. These systems may be referred as a "Plug type system," since they exhibit tendency to remain logged in the pyloric sphincters.

Unfoldable systems are made of biodegradable polymers. They are available in different geometric forms like tetrahedron, ring or planner membrane of bioerodible polymer compressed within a capsule which extends in the stomach.

Magnetic systems[29]

These are the systems which includes external stimuli as magnetic field for site specific drug delivery. Some magnetically active compounds can be incorporated in the dosage form to achieve site specificity in stomach by external stimuli.

Floating drug delivery system[30-32]

Floating systems, first described by Davis in 1968, have bulk density lower than that of the gastric fluid and thus remain buoyant in stomach for a prolonged period. Swelling delivery systems are capable of swelling to a size that prevents their passage through the pylorus. Upon coming in contact with gastric fluid, the polymer imbibes water and swells; as a result the dosage form is retained in the stomach for a longer period of time.

The literature survey suggests that there are several techniques to prepare GRDDS. However, all the techniques have their own advantages and disadvantages. This is the reason that the marketed gastroretentive formulations are not formulated by applying all the approached available. Table 2.2 displays the list of marketed gastroretentive formulations of different drugs, with the brand name, manufacturer and the technology involved for the preparation.

TABLE 2.2 List of marketed gastroretentive formulations[33,34]

Sr. No.	Active pharmaceutical ingredient	Product	Manufacturer	Type of formulation
1	Rifaximin	Xifaxan	Lupin, India	Bioadhesive tablets
2	Ofloxacin	Zanocin OD	Ranbaxy, India	Effervescent floating system
3	Metformin hydrochloride	Riomet OD	Ranbaxy, India	Effervescent floating system
		Glumetza	Depomed, Inc., USA	Polymer-based swelling technology: AcuForm
4	Ciprofloxacin	Cifran OD	Ranbaxy, India	Effervescent floating system
		ProQuin_XR	Depomed, Inc., USA	Polymer-based swelling technology: AcuForm
		Cipro XR	Bayer, USA	Erodible matrix-based system
5	Levodopa(100 mg), Benserazide(25 mg)	Madopar®	Roche products, USA	Floating, CR capsule.
6	Diazepam (15 mg)	Valrelease®	Hoffmann-LaRoche, USA.	Floating capsule
7	Al-hydroxide(95 mg), Mg carbonate(358 mg)	Liquid Gaviscon®	GlaxoSmithKline, India.	Raft system
8	Carvedilol	Coreg CR	GlaxoSmithKline	Gastroretention with osmotic system
9	Misoprostol	Cytotec	Pharmacia Ltd, UK	Bilayer floating capsule

2.3.4 Literature Review on the Gastroretentive Dosage Forms of Antidiabetic Drugs

2.3.4.1 Glipizide

In the category of Sulfonylureas, Glipizide is the drug which is an ideal candidate for preparing GRDDS because it has short biological half-life (3.4 ± 0.7 h) which necessitates it be administered in two or three doses of 2.5 to 10 mg per day.

Effervescent tablets of glipizide were prepared by ***Patel et al.,***[35] using chitosan (CH), hydroxypropyl methylcellulose (HPMC), carbopol P 934 (CP), polymethacrylic acid (PMA), citric acid, and sodium bicarbonate. Tablets with 5% effervescent base had longer lag time than 10%. This work described, a matrix floating-bioadhesive tablet incorporating an insoluble active ingredient. The tablets with the least lag time of buoyancy were those which were prepared with 10% of effervescent base but changing the polymer type of mixture ratio did not change the duration of buoyancy. Tablets containing 20% of HPMC and 80% CH or 80% of CP and 20% of PMA were optimum from both the bioadhesion and prolonged drug release rate point of view.

Also Controlled-release Floating microspheres were prepared by ***Pandya et al***.[36] to increase residence time of Glipizide in the stomach using calcium silicate (CS) as porous carrier and Eudragit® S as polymer. The prepared microspheres exhibited prolonged drug release (~8 h) and remained buoyant for >10 h. In vitro studies demonstrated diffusion-controlled drug release from the microspheres. The release pattern of glipizide in simulated gastric fluid from all floating microspheres followed the Higuchi matrix model and the Peppas-Korsmeyer model. The developed formulation overcomes and alleviates the drawbacks and limitations of sustained-release preparations in the drug-delivery through the introduction of CS-based floating microspheres suitable for controlled release of drug after oral administration. The prepared microspheres could be compressed into tablets or filled into capsules.

In another attempt Hydrodynamically balanced drug delivery systems for Glipizide was prepared by ***Seshagiri et al.,***[37] using HPMC K4M and HPMC K15M polymers by solvent casting sintering technique. The study revealed that the formulations of HBS of Glipizide formulated has exhibited a floating lag time of less than 5 minutes and floating time of more than 22 hrs. The results indicate that gas powered Hydrodynamically Balanced Tablets of Glipizide containing HPMC K15M provides a better option for controlled release action and improved bioavailability.

2.3.4.2 Repaglinide

Research on the Antidiabetic drugs of Meglitinide category proved that GRDDS could possibly be beneficial in terms of increasing bioavailability of drugs. ***Jain et al.,***[38] prepared floating microspheres of repaglinide by the emulsion solvent diffusion technique using calcium silicate (FLR) as porous carrier and Eudragit S as polymer. The microparticles were found to be regular in shape and highly porous and formulation demonstrated favorable in

vitro floating and release characteristics. It was concluded that prepared system, could possibly be beneficial in terms of increased bioavailability of repaglinide. ***Jain et al***.,[39] evaluated gastro-retentive performance and pharmacokinetic parameters of optimized floating microspheres (RgFMCS4) consisting of calcium silicate (CS) as porous carrier; repaglinide (Rg) and Eudragit S (ES) as polymer. The optimized formulation gave satisfactory in-vitro floating and drug release characteristics. The relative bioavailability of repaglinide loaded floating microspheres was found to be increased almost 3.17 times in comparison to that of the marketed tablet. Developed formulation was found to be safer and more effective which is the need of day in pharmaceutical industry as an alternative DDS for a highly prevalent and chronic disease like type II diabetes mellitus

2.3.4.3 Metformin

Metformin is the drug which is most benefited if prepared in the form of gastroretentive dosage form because its absolute oral bioavailability is 50-60% due to its site-specific absorption limitations. The pharmacokinetic (PK) and pharmacodynamic (PD) rationale for development of metformin Controlled Release gastroretentive formulations (CR-GRDF) was established by ***Stepensky, D., et al.,***[40] It was concluded from the results that metformin has good PK-PD rationale for developing CR-GRDDS and such formulation could prove to be clinically advantageous.

A single unit controlled-release gas-generating gastroretentive metformin tablet was formulated by ***Tutunji Lara***.[41] The gas-generation helped the tablets to float on top of the dissolution vessel contents. Hydroxyethylcellulose (HEC) was considered as a good polymer base that was able to float in a short period of time in the designed formulation and control the release of metformin over the period of 10 hours.

Ali et al.,[24] formulated and optimized hydrodynamically balanced system for metformin as a single unit floating capsule. It was concluded on the basis of buoyancy and in vitro release kinetics that optimized formulation containing 500 mg of metformin granulated with 5% of ethyl cellulose, and 150 mg of HPMC K4M (extragranular) gave the best in vitro release of 97% in 12 h in simulated gastric fluid at pH 3. The release of metformin from the matrix formulation followed zero order release kinetics. Gamma Scintigraphic studies revealed that the optimized HBS capsule was retained in the gastric region (stomach) for a prolonged period and pharmacokinetic studies showed an increase in AUC as compared to immediate release capsules of metformin.

Floating tablets of metformin were prepared by ***Boldhane et al.,***[42] using sodium alginate as a gelling agent; sodium carboxymethylcellulose as release modifier, and Eudragit NE 30 D as sustained release polymer to control the initial burst release. The optimization study using a 3^2 full factorial design revealed that the amount of sodium alginate and sodium carboxymethylcellulose had a significant effect. This study showed that the Metformin GR tablets prepared using sodium alginate and sodium carboxymethylcellulose can successfully be employed as a once-a-day oral controlled release gastroretentive drug delivery system.

Jain and Gupta [43] prepared and characterized beads of Gelucire 43/01 for floating delivery of metformin hydrochloride (MH). The in vitro data obtained for floating beads of metformin HCl showed exceptional buoyancy. Prepared formulation showed better controlled release behavior when compared with its conventional dosage form and comparable release profile with marketed sustained release product of metformin HCl. It was concluded that the beads of Gelucire 43/01 can be considered as an effective carrier for the design of a gastroretentive multiparticulate drug delivery system of highly water-soluble antihyperglycemic drugs like metformin HCl for the effective management of type 2 diabetes mellitus.

2.3.4.4 Rosiglitazone

Due to its short biological half-life (3.5 ± 0.5 hrs) and instability at higher pH rosiglitazone maleate requires controlled release and was used as the drug in this study.

A multiunit floating drug delivery system of rosiglitazone maleate was developed by ***Kamila et al.,***[44] by encapsulating the drug into Eudragit® RS100 through non aqueous emulsification/solvent evaporation method. In vitro release was optimized by a {3, 3} simplex lattice mixture design to achieve predetermined target release. In vivo evaluation of the optimized formulation was done on streptozotocin-induced diabetic rats, which suggested that floating microspheres of rosiglitazone could be a promising approach for better glycemic control.

Rosiglitazone maleate is an anti-diabetic drug which is highly unstable at basic pH and is extensively absorbed from the stomach. Hence ***Gupta et al.,***[45] prepared Chitosan/poly(vinyl alcohol) interpenetrating polymer network type superporous hydrogels using a gas blowing method, employing glyoxal as the crosslinking agent for Rosiglitazone maleate. It was found that the introduction of a small amount of Poly(Vinyl Alcohol) enhanced the mechanical strength but reduce the swelling ratio. The drug release from superporous hydrogels was sustained for 6hrs. So, it was concluded that chitosan-based superporous hydrogels could be

used as a gastroretentive drug delivery system for rosiglitazone maleate in view of their swelling and prolonged drug release characteristics in acidic pH.

Broadly, the gastroretentive formulations can be classifies as unit drug delivery system and multiparticulate drug delivery system. For the present research work, both type of GRDDS were developed. The formulations developed were gastroretentive floating matrix system, based in effervescent technology and gastroretntive floating microsponges of the selected drugs.

2.4 Gastroretentive Floating Matrix Tablets

Floating matrix tablets low-density controlled release systems that have sufficient buoyancy to float over the gastric contents and remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from the stomach. This results in an increased gastric retention time and a better control of the fluctuations in plasma drug concentration. However, besides a minimal gastric content needed to allow the proper achievement of the buoyancy retention principle, a minimal level of floating force (F) is also required to keep the dosage form reliably buoyant on the surface of the meal.

In present work, the gastroretentive floating matrix tablets of metformin, glipizide and mitiglinide were developed by effervescent technology. The sodium bicarbonate was used as gas generating agent. Hydroxypropyl methyl cellulose (HPMC K15M) was used as release retarding polymer along with other ionic and anionic polymers. The effect of the combination of polymers was explored on the release and floatation behavior of the drug.

2.4.1 Literature Review on the Gastroretentive Floating Matrix Tablets

Gastroretentive Floating matrix tablet has been researched by many researchers. ***Dorozynski et al.,*** used carrageenans and their mixtures with HPMC for preparing gastro retentive drug delivery systems of l-dopa. The formulations showed linear increase in the releasing rate constantly. In such formulations, carrageenans can modify the properties of polymeric matrices, to obtain tailormade materials for drug delivery systems[46]. In another attempt, the

effect of different viscosity grade HPMC polymers was checked on the Gastro retentive dosage form of metformin HCl[47].

Gambhire et.al., developed an oral floating matrix tablet formulation of diltiazem hydrochloride with the aim of prolonging gastric residence time and increase its bioavailability[48]. The tablets were prepared by direct compression technique, using polymers such as hydroxypropylmethylcellulose (HPMC, Methocel K100M CR), Compritol 888 ATO, alone or in combination and other standard excipients and the optimization was done by applying factorial design. Results reveled the comparable release profiles between the commercial product and the developed dosage form.

Basak et. al., developed floating matrix tablet for ciprofloxacin with HPMC K100M as release retarding polymer by effervescent technology[49]. Prepared formulation could keep remain in the stomach form more than 8hrs. Hence, this gas powered floating matrix tablet was considered to be promising delivery system for ciprofloxacin with sustained release action and improved drug availability.

In another study, ***Patel et.al.,*** investigated the influence of Xanthan Gum and Guar Gum blends on dipyridamole release from floating Matrix Tablets[50]. It was concluded that the ratio of xanthan to guar gum had equal or dominant role as controlling factor on kinetics of drug release compared to content of polymer blends.

Asnaashari et. al., prepared floating matrix tablet of metronidazole to retain the drug in the stomach for a long time for better eradication of Helicobacter Pylori in peptic ulcer diseases[51]. HPMC, psyllium and carbopol in different concentrations were used as floating agents, and sodium bicarbonate was added as a gas-forming agent. These systems could float in the gastric condition and control the drug release from the tablets.

2.5 Gastroretentive Floating Microsponges

The Microsponge Delivery System (MDS) is a patented polymeric system consisting of porous microspheres[52]. They are tiny sponge like spherical particles that consist of a numerous interlocking cavities within a non-collapsible structure with a large porous surface through which active ingredient are released in a controlled manner. Recently their use is also being investigated for oral drug delivery. The size of the microsponge's ranges from 5-300μm in diameter with upto 250000 pores. This results in a large reservoir within each microsponge, which can be loaded with up to its own weight of active agent[53,54].

Microsponges are the dosage forms which are porous, highly cross-linked, polymeric microspheres. They are tiny sponge like spherical particles with a large porous surface area and having ability to entrap wide range of drug. It also enhances stability of drug, reduce side effects of drug, modified drug release favorably. Microsponges are being investigated for their application in effective oral drug delivery system. Recently, the curcumin is formulated as floating microsponge with high loading capacity.

Microsponges having the size ranges from 5-300 μm in diameter. A typical microsponges having 25 μm sphere can have up to 250000 pores and an internal pore structure equivalent to 10 feet in length, providing a total pore volume of about 1 mL/g for extensive drug retention. For the microsponges the surface can be varied from 20 to 500 m^2/g and the pore volume range from 0.1 to 0.3 cm^3/g. Because of this reason microsponges results in a large reservoir within each system and therefore, microsponges having ability to load the drugs with up to its equivalent weight.

In oral applications, the microsponges system has been shown to increase the rate of solubilization of poorly water soluble drugs by entrapping such drugs in the pores of microsponges. As the pores of microsponges are very small and the drug particles during the formulation entrapped within the pores of microsponges and due to size reduction of drug particles showing the significant increase in the surface area of drugs and thereby greatly increase the rate of solubilization.

The microsponges are prepared by many techniques. Free Radical Suspension Polymerization: (Bottom up approach), where drug is dispersed in the monomeric system and then the polymerization is carried out. Other technique is Quasi-emulsion solvent diffusion method: (Top down approach), which is a biphasic system. The hydrophobic drug is solubilized in organic phase with solubilized polymer. This organic phase is then added to the aqueous phase containing emulsifier. The microsponges are formed by complete removal of organic phase facilitated by continuous stirring. This technique can be modified by adding a porogen to increase the porosity of the microsponges.

2.5.1 Advantages of Floating Microsponge Drug Delivery System

- Microsponges exhibit site specific drug delivery system. i.e., Stomach specific.
- Microsponges having an efficient drug delivery system for stomach specific delivery along with high drug loading capacity. i.e., up to 50-60%.

- Due to lower density than the gastric contents, microsponges are being expected to remain buoyant on the surface of gastric contents.
- Consequently dissolved drug will be released continuously in effective controlled manner from the floating system.
- Microsponges have the ability to entrap wide range of active due to its numerous interconnected pores, and can adsorb high quantity of active on its surface and/or load into the bulk of particles.
- Microsponges favourably modifies drug release and provides maximum efficacy.
- Microsponges offers extended product stability over a pH range of 1 to 11.
- They are free flowing, cost effective and stable up to temperature 130°C.

2.5.2 Characteristics of Drugs to be Entrapped in the Microsponges

- Drug should exhibit complete miscibility in polymer.
- It must be inert to polymer and do not increase the viscosity of the resulting preparation during formulation.
- It should be water immiscible or almost slightly soluble.
- It should be stable in polymerization conditions.
- To obtain the desired release rate for a given period of time, the polymer design of the microsponge for active must be adjusted and payload.

2.5.3 Literature Review on Microsponges

Osmani, et.al. Worked on microsponge based drug delivery system for augumented gastroparesis therapy and also carried out their evaluations[55]. In this study, the authors found that based on drug polymer ratio, the drug released from the microsponges was shown extended release pattern. They also observed that the in-vitro drug release from capsules which are loaded with microsponges was superior to conventional marketed formulation.

Arya, et.al., Assessed the viability of microsponges as gastro retentive drug delivery system of curcumin for improved site specific absorption for gastric cancer[56]. Modified Quasi emulsion solvent diffusion method was used to formulate microsponges using 3^2 full factorial design. The effect of different levels of ethyl cellulose and polyvinyl alcohol concentration, selected as independent variables was determined on the % entrapment efficiency, % buoyancy and % cumulative drug release. Scanned Electron Microscopy

revealed spherical and porous microsponges. DSC confirmed molecular dispersion of the drug in the microsponges polymeric matrix. DRIFT revealed no chemical interaction between the drug and polymer used. The pharmacokinetic evaluation of optimized batch revealed 10-fold increase in bioavailability as compared to native curcumin which proved the superiority of microsponges as gastro retentive drug delivery system.

Nokhodchi, et.al., prepared encapsulated form of benzoyl peroxide using microsponge technology and explored the factors affecting the morphology and other characteristics of the resultant products[57]. The microsponges were prepared using an emulsion solvent diffusion method by adding an organic internal phase containing benzoyl peroxide, ethyl cellulose and dichloromethane into a stirred aqueous phase containing polyvinyl alcohol (PVA). Results indicated that the morphology and particle size of microsponges were affected by drug:polymer ratio, stirring rate and the amount of emulsifier PVA, used.

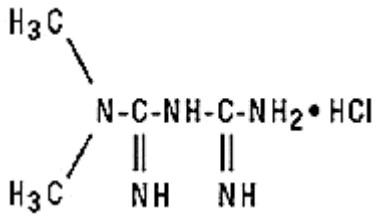
Sonali, et.al., prepared prednisolone loaded microsponges for colon drug delivery system. In-vitro and pharmacokinetic study were conducted[58]. In-vitro dissolution study of microsponges showed that drug release in colon could be controlled by Eudragit RS-100 and drug release followed zero order kinetic. Microsponges released the drug for over 8 hrs.

Karthika, et.al., prepared the tablet of lornoxicam microsponge, for the treatment of arthritis. Authors noted that the production yield and mean particle size were depended upon the amount of emulsifying agent[59]. The prepared microsponges give the prolonged drug release characteristic and it was proved to be an ideal drug delivery system which can be used as new alternative for mechanically strong tablet.

Jain, et.al. Described in their study that they had designed microsponge based on colon specific drug delivery system containing paracetamol[60]. For the preparation of microsponge they utilized Eudragit S-100 which contain paracetamol in varying amounts and quasi-emulsion solvent diffusion method was used to prepare. The microsponges were prepared by optimizing various process parameters. The *in vitro* release data showed a bi-phasic pattern with an initial burst effect. They observed that in the first hour drug release from microsponges was found to be between 18-30% and cumulative percent release at the end of 12th hour was noted to be between 74-98%. The colon specific tablets were prepared by compressing the microsponges followed by coating with pectin: HPMC mixture. *In vitro* release studies exhibited that compression coated colon specific tablet formulations started releasing the drug at 6th hour corresponding to the arrival time at proximal colon. The study presents a new approach for colon specific drug delivery.

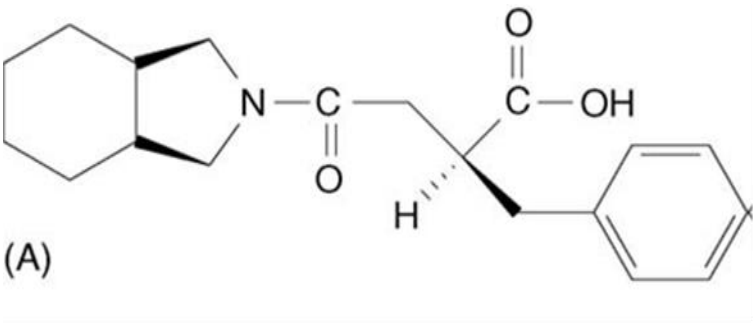
2.6 Drug Profile

2.6.1 Metformin Hydrochloride[61-63]

Name of drug	**Metformin Hydrochloride**
IUPAC Name	3-(diaminomethylidene)-1,1-dimethylguanidine;hydrochloride
Description	Metformin hydrochloride is a biguanide antihyperglycemic agent used in the treatment of non-insulin-dependent diabetes mellitus not responding to dietary modification. Metformin improves glycemic control by improving insulin sensitivity and decreasing intestinal absorption of glucose.
Molecular formula	$C_4H_{12}ClN_5$
Molecular Weight	165.625 g/mol
Structural formula	
CAS no.	1115-70-4
Log P	-2.64
Pka	12.4
Water Solubility	1.38 mg/mL
Physical form	Solid
Half life	6.2 hours. Duration of action is 8-12 hours.
Bioavailability	50 to 60% under fasting conditions
Protein binding	Metformin is negligibly bound to plasma proteins.
Elimination	Metformin does not undergo hepatic metabolism and it is excreted unchanged in the urine.
Volume of distribution	654 L for metformin 850 mg administered as a single dose

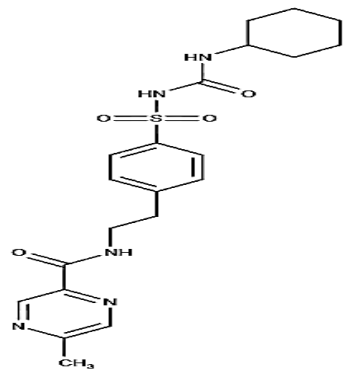
Indication	It is used for the control of hyperglycemia and its associated symptomatology in patients with non-insulin-dependent diabetes mellitus (NIDDM; type II).
Pharmacological class	Biguanide derivatives
Mechanism of action	Metformin decreases intestinal absorption of glucose, increases distribution of glucose from the blood into the tissues, decreases the production of glucose in liver and decreased insulin requirements for glucose disposal. Metformin is not effective in absence of insulin and it has no effect on pancreatic insulin secretion.
BCS Class	It belongs to BCS class III
Pharmacodynamics	Metformin is an oral antihyperglycemic agent that improves glucose tolerance in patients with NIDDM, lowering both basal and postprandial plasma glucose.
Dosage form	Tablets immediate release and extended release
Dose	Tablets of 500 mg or 850 mg immediate release and 500 mg and 1000 mg extended release tablets. Dose depends upon the condition of patient but the maximum recommended daily dose of extended release tablet in adult is 2500 mg
Melting point	223-226 °C
Therapeutic range	The therapeutic range was found to be up to 40 mg in divided doses 30 minutes before a meal of adequate caloric content. Dose may be increased in intervals of 2.5 to 5 mg a day according to a glucose response.
Marketed product	Riomet, Actoplus Met, Actoplus Met XR, Glucophage XR, Avandamet, Fortamet, Glucophage, Glucovance, Glumetza, Janumet, etc.

2.6.2 Mitiglinide Calcium Dihydrate[64-68]

Name of the drug	**Mitiglinide Calcium Dihydrate**
Structure	 (A)
Category	Antidiabetic
IUPAC Name	calcium bis((2S)-4-[(3aR,7aS)-octahydro-1H-isoindol-2-yl]-2-benzyl-4-oxobutanoate) dihydrate
Molecular formula	$C_{38}H_{52}CaN_2O_8$
Molecular Weight	Average: 704.918
Trade Name	Glufast
Route of administration	oral
pKa	4.62
Log P	2.9
Half Life[69]	1.5 hrs
Solubility	It is freely soluble in methanol and in ethanol (99.5), and slightly soluble in water.
t_{max}	0.25hrs
Melting point	179 -185ºC
Absorption	Better absorption when pH is below 5

Mechanism of action[70-72]	Mitiglinide is thought to stimulate insulin secretion by binding to and blocking ATP-sensitive K(+) (K(ATP)) channels (Kir6.2/SUR1 complex, KATP channels) in pancreatic beta-cells. Closure of potassium channels causes depolarization which stimulates calcium influx through voltage-gated calcium channels. High intracellular calcium subsequently triggers the exocytosis of insulin granules.
Dosage	***Tablets:*** GLUFAST 5 mg: Mitiglinide Calcium Hydrate 5 mg\ tablet three times daily just before the meals. GLUFAST 10 mg: Mitiglinide Calcium Hydrate 10 mg\tablet three times daily just before the meals.
Side Effects	hypoglycemia symptoms, constipation, diarrhea , feeling hungry and headache, Hypoglycemia, Hepatic dysfunction:
Storage	Store at room temperature (1-30ºC) in a well-closed container.

2.6.3 Glipizide[73,74]

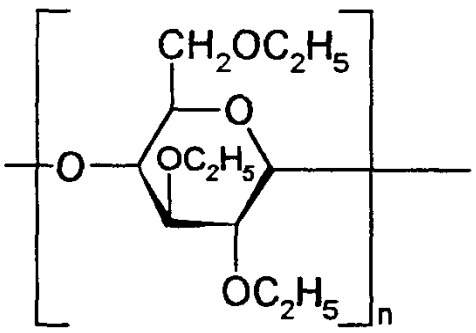
Name of drug	**Glipizide**
IUPAC Name	N-[2-(4-{[(cyclohexylcarbamoyl)amino]sulfonyl}phenyl)ethyl]-5-methylpyrazine-2-carboxamide
Discription	An oral hypoglycemic agent which is rapidly absorbed and completely metabolized
Molecular formula	$C_{21}H_{27}N_5O_4S$
Molecular Weight	445.535
Structural formula	

CAS no.	29094-61-9
Log P	1.91
Pka	5.9
Water Solubility	37.2 mg/L
Physical form	Solid
Half life	2 to 5 hours
Bioavailability	100%
Protein binding	98 to 99%
Elimination	The primary metabolites are excreted mainly in the urine.
Clearance	The total plasma clearance of glipizide was 42.2+/-5.4 ml/min.
Volume of distribution	11 L
BCS class	Class II
Indication	It is used for the control of hyperglycemia and its associated symptomatology in patients with non-insulin-dependent diabetes mellitus (NIDDM; type II).
Pharmacological class	Sulfonylurea
Storage	Store it at room temperature and away from excess heat and moisture. Keep in an airtight container and keep away from children.
Mechanism of action	Sulfonylureas likely bind to ATP-sensitive potassium-channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Depolarization stimulates calcium ion influx through voltage-sensitive calcium channels, raising intracellular concentrations of calcium ions, which induces the secretion, or exocytosis, of insulin.
Absorption	Gastrointestinal absorption is uniform and rapid.
Pharmacodynamics	Glipizide, a second-generation sulfonylurea, is used with diet to decrese blood glucose in patients with diabetes mellitus type II. The primary mode of action of glipizide appears to be the

	stimulation of insulin secretion from the beta cells of pancreatic islet tissue. In humans glipizide lower the blood glucose by stimulating the release of insulin from the pancreas, an effect dependent upon functioning beta cells in the pancreatic islets. Fasting insulin levels are not elevated even on long-term glipizide administration, but the postprandial insulin response continues to be enhanced after at least 6 months of treatment. Some patients fail to respond initially, or gradually lose their responsiveness to sulfonylurea drugs, including glipizide
Dosage form	Tablet, film coated, extended release
Dose	Initial-2.5 to 5 mg/day as a single dose may increase slowly. Doses greater than 15 mg may be given in 2 divided doses. Max: 40 mg/day.
Melting point	200-203°C
Therapeutic range	The therapeutic range was found to be up to 40 mg in divided doses 30 minutes before a meal of adequate caloric content. Dose may be increased in intervals of 2.5 to 5 mg a day according to a glucose response.
Marketed product	glipizide, Glucotrol XL, glipizide Tablets ER

2.7 Polymer Profile

2.7.1 Ethyl Cellulose[75,76]

Name	**Ethyl cellulose**
Structure	 Where, "n" can differ to provide a varied variety of molecular weights.

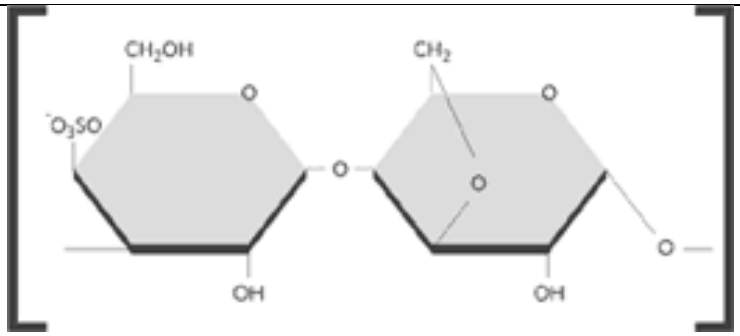
Synonyms	Aquacoat ECD, Aqualon E462, Ethocel, Sure lease
Molecular weight	454.513 g/mol
Empirical Formula	$C_{12}H_{23}O_6(C_{12}H_{22}O_5)_nC_{12}H_{23}O_5$
Solubility	Practically insoluble in glycerin, propylene glycol, and water. Ethylcellulose that contains less than 46.5% of ethoxyl groups is freely soluble in chloroform, methyl acetate, and tetrahydrofuran, and in mixtures of aromatic hydrocarbons with ethanol(95%)
pH	5.5 to 8.0
Viscosity	46 cP, 5 % in toluene/ethanol 80:20(lit.)
Melting Point	240 -255 ºC
Density	0.4 g/cm^3
Description	▪ Ethyl Cellulose is soluble in a wide range of solvents such as aliphatic alcohols, chlorinated solvents, and natural oils. It is insoluble in water, glycerin, and prolylene glycol.
Storage	Store in tightly packed container in cool and dry place
Regulatory Status	Approved as a direct food additive in the US. Code of Federal Regulations, Title 21CFR 172.870 as an emulsifier, film former, protective colloid stabilizer, suspending agent, or thickener
Health and Safety	Acceptable daily intake of not specified (No quantitative limit)
Functional Category	▪ Binder ▪ Filler ▪ Coating agent ▪ Viscosity increasing agent
Application	Microencapsulation, Sustained-release tablet, Tablet coating, Tablet granulation,

2.7.2 Hydroxy Propyl Methyl Cellulose (HPMC K15M)[77,78]

Name	**HPMC K15M**
Synonym/brand name	Benecel MHPC; E464; HPMC; Methocel; Hydroxypropyl methylcellulose; methyl hydroxypropylcellulose; Tylopur;.Metolose.
CAS no.	[9004-65-3]
Solubility	Soluble in cold water, forming a viscous colloidal solution; practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane, and mixtures of water and alcohol. Certain grades of hypromellose are soluble in aqueous acetone solutions, mixtures of dichloromethane and propan-2-ol, and other organic solvents.
Viscosity	A wide range of viscosity types are commercially available. Aqueous solutions are most commonly prepared, although hypromellose may also be dissolved in aqueous alcohols such as ethanol and propan-2-ol provided the alcohol content is less than 50% w/w. Dichloromethane and ethanol mixtures may also be used to prepare viscous hypromellose solutions. Solutions prepared using organic solvents tend to be more viscous; increasing concentration also produces more viscous solutions.
Acidity	pH = 5.5–8.0 for a 1% w/w aqueous solution.
Stability	HPMC powder is hygroscopic after drying but still is stable material. Solutions of HPMC are stable at pH 3–11. As the temperature increases, the viscosity of solutions decreases. Upon heating and cooling, it undergoes a reversible sol–gel transformation, respectively. The gel point is 50–90°C, depending upon the grade and concentration of material.

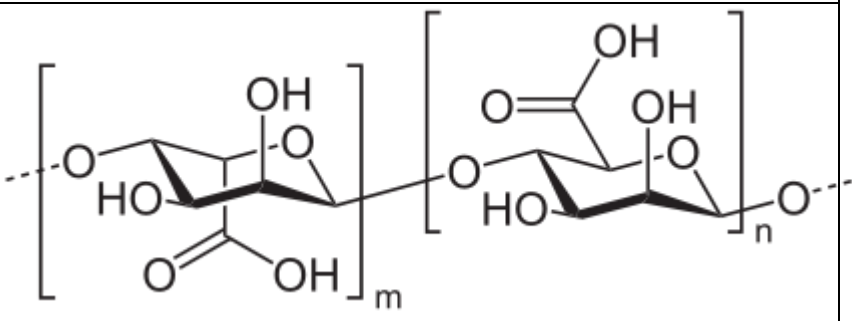
Description	HPMC is widely used in oral products, hypromellose is primarily used as a tablet binder, in film-coating, and as a matrix for use in extended-release tablet formulations.
Application	HPMC is widely used as an excipient in oral and topical pharmaceutical formulations. It is generally regarded as a nontoxic and nonirritant material, although excessive oral consumption may have a laxative effect.
Storage	It should be stored in a well-closed container, in a cool, dry place.

2.7.3 kappa-Carrageenan[46,79,80]

Name	**Kappa carrageenan**
Structure	
Types of carrageenan	Kappa carrageenan Iota carrageenan Lambda carrageenan
Description	Carrageenan is positioned in the cell wall of the seaweed plant tissue. It is a high molecular weight polysaccharide with 15% to 40% of ester-sulfate content. It is formed by alternate units of D-galactose and 3.6 anhydro-galactose (3.6-AG) joined by α-1,3 and β-1,4 -glycosidic linkage. Higher levels of ester sulfate indicates lower solubility temperature and lower gel strength. Kappa carrageenan has an ester sulfate content of about 25 to 30% and a 3, 6-AG content of about 28 to 35%.

Solubility	Carrageenans are soluble in hot water at temperatures above its gel melting temperature. The normal solubility temperature is between 40º and 70º C, depending on the concentration of solution and the presence of cations.
Viscosity	Commercial carrageenans are generally available in viscosities ranging from about 5 to 800 cps when measured in 1.5% solutions at 75ºC.
Melting point	50ºC to 70ºC
Stability	Carrageenan solutions are stable at neutral or alkaline pHs
Gelling mechanism	Due to the formation of a double helix structure by the carrageenan polymers, hot aqueous solution of kappa carrageenans have the ability to form thermo-reversible gels upon its cooling. At temperatures above the melting point of the gel, carrageenan polymers exist in solution as random coils. On cooling of the solution, a three-dimensional polymer network builds up in which double helices form the junction points of the polymer chains. Further cooling leads to aggregation of these junction points to build a three-dimensional gel structure. The presence of kinks in the chain, as well as the quantity, type and position of ester sulfate groups have important effects on gelling properties.
Gel Texture	Kappa carrageenan gels in the presence of certain cations. It produces firm and stiff gels in aqueous solutions with potassium salts. The gel strength is directly proportional to the concentration of carrageenan and salts. The increase of potassium or calcium salts concentration in aqueous carrageenan solutions will result in an increase in the gelling temperature.
Storage	It should be stored in a well-closed container, in a cool, dry place.

2.7.4 Sodium Alginate[81,82]

Name	**Sodium alginate**
Structure	
Synonym/Brand name	Alginato sodico; algin; alginic acid, sodium salt; E401; Kelcosol; Keltone; natrii alginas; Protanal; sodium polymannuronate
CAS no	9005-38-3
Solubility	Slowly soluble in water forming colloidal solution, Insoluble in organic solvent
Acidity	pH=7.2 (1% w/v aqueous solution)
Viscosity	1% w/v aqueous solution at 20 ○c around 20 - 400 cps. Above pH 10 viscosity decreases.
Stability	Stable at pH 4-10. Below pH 3 alginic acid is precipitated
Description	Odorless and tasteless, white to pale yellowish-brown colored powder. Sodium alginate consists chiefly of the sodium salt of alginic acid,which is a mixture of polyuronic acids composed of residues of D-mannuronic acid and L-guluronic acid.
Application	Stabilizing agent, suspending agent, tablet and capsule disintegrant, tablet binder, viscolizing agent.
Storage	Store in an airtight container in cool and dry place

2.8 Rationale for Selection of Drugs

2.8.1 Rationale for Selecting Metformin for Preparing GRDDS

Metformin HCl is an oral hypoglycemic agent, which belongs to the class of biguanide derivatives. Its 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 absorption[83]. To increase the bioavailability of metformin, controlled release gastro-retentive dosage forms would be beneficial.

2.8.2 Rationale for Selecting Mitiglinide Calcium Dihydrate for Preparing GRDDS

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 pH[84].

2.8.3 Rationale for selecting Glipizide for preparing GRDDS

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[85,86]. 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.

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.

2.9 References

1. American Diabetes Association, 2014. Diagnosis and classification of diabetes mellitus. *Diabetes Care*, *37*(1), pp.S81-S90.

2. Hod, M., Merlob, P., Friedman, S., Schoenfeld, A. and Ovadia, J., 1991. Gestational diabetes mellitus: a survey of perinatal complications in the 1980s. *Diabetes*, *40*(2), pp.74-78.

3. Sobngwi, E., Mbanya, J.N., Unwin, N.C., Kengne, A.P., Fezeu, L., Minkoulou, E.M., Aspray, T.J. and Alberti, K.G.M.M., 2002. Physical activity and its relationship with obesity, hypertension and diabetes in urban and rural Cameroon. *International Journal of Obesity*, *26*(7), pp.1009-1016.

4. Tan, Y., Chang, S.K. and Zhang, Y., 2017. Comparison of α-amylase, α-glucosidase and lipase inhibitory activity of the phenolic substances in two black legumes of different genera. *Food Chemistry*, *214*, pp.259-268.

5. Rossi, E.J., Sim, L., Kuntz, D.A., Hahn, D., Johnston, B.D., Ghavami, A., Szczepina, M.G., Kumar, N.S., Sterchi, E.E., Nichols, B.L. and Pinto, B.M., 2006. Inhibition of recombinant human maltase glucoamylase by salacinol and derivatives. *The FEBS Journal*, *273*(12), pp.2673-2683.

6. Lindström, J., Ilanne-Parikka, P., Peltonen, M., Aunola, S., Eriksson, J.G., Hemiö, K., Hämäläinen, H., Härkönen, P., Keinänen-Kiukaanniemi, S., Laakso, M. and Louheranta, A., 2006. Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. *The Lancet*, *368*(9548), pp.1673-1679.

7. Chandalia, M., Garg, A., Lutjohann, D., von Bergmann, K., Grundy, S.M. and Brinkley, L.J., 2000. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *New England Journal of Medicine*, *342*(19), pp.1392-1398.

8. American Diabetes Association, 2014. Standards of Medical Care in Diabetes—2014. Diabetes Care 2014; 37 (Suppl. 1): S14–S80Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2014; 37 (Suppl. 1): S81–S90. *Diabetes Care*, *37*(3), pp.887-887.

9. American Diabetes Association, 2015. Standards of medical care in diabetes—2015 abridged for primary care providers. *Clinical Diabetes*, *33*(2), pp.97-111.

10. Alberti, K.G.M.M. and Zimmet, P.F., 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic Medicine*, *15*(7), pp.539-553.

11. Nathan, D.M., 1993. Long-term complications of diabetes mellitus. *New England Journal of Medicine*, *328*(23), pp.1676-1685.

12. Diabetes Control and Complications Trial Research Group, 1993. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *New England Journal of Medicine*, *1993*(329), pp.977-986.

13. Tuomilehto, J., Lindström, J., Eriksson, J.G., Valle, T.T., Hämäläinen, H., Ilanne-Parikka, P., Keinänen-Kiukaanniemi, S., Laakso, M., Louheranta, A., Rastas, M. and Salminen, V., 2001. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *New England Journal of Medicine*, *344*(18), pp.1343-1350.

14. http://www.diabetes.co.uk/Diabetes-drugs.html.

15. Mathiowitz, E., 1999. *Encyclopedia of Controlled Drug Delivery*. Wiley, New York, vol-I, pp. 9-11.

16. Shivkumar, H.G., Gwda, D.V. and Pramod Kumar, T.M., 2004. Floating controlled drug delivery systems for prolong gastric residence. *Indian Journal of Pharmaceutical Education*, *38*(4), pp.172-179.

17. Rouge, N., Buri, P. and Doelker, E., 1996. Drug absorption sites in the gastrointestinal tract and dosage forms for site-specific delivery. *International Journal of Pharmaceutics*, *136*(1-2), pp.117-139.

18. Goole, J., Vanderbist, F. and Amighi, K., 2007. Development and evaluation of new multiple-unit levodopa sustained-release floating dosage forms. *International Journal of Pharmaceutics*, *334*(1), pp.35-41.

19. Fell, J.T., Whitehead, L. and Collett, J.H., 2000. Prolonged gastric retention: using floating dosage forms. *Pharmaceutical Technology*, *24*(3), pp.82-90.

20. Dave, B.S., Amin, A.F. and Patel, M.M., 2004. Gastroretentive drug delivery system of ranitidine hydrochloride: formulation and in vitro evaluation. *Aaps PharmSciTech*, *5*(2), pp.77-82.

21. Rajinikanth, P.S., Balasubramaniam, J. and Mishra, B., 2007. Development and evaluation of a novel floating in situ gelling system of amoxicillin for eradication of Helicobacter pylori. *International Journal of Pharmaceutics*, *335*(1), pp.114-122.

22. Matharu, R.S. and Sanghavi, N.M., 1992. Novel drug delivery system for captopril. *Drug Development and Industrial Pharmacy*, *18*(14), pp.1567-1574.

23. Streubel, A., Siepmann, J. and Bodmeier, R., 2006. Gastroretentive drug delivery systems. *Expert Opinion on Drug Delivery*, *3*(2), pp.217-233.

24. Ali, J., Arora, S., Ahuja, A., Babbar, A.K., Sharma, R.K., Khar, R.K. and Baboota, S., 2007. Formulation and development of hydrodynamically balanced system for metformin: in vitro and in vivo evaluation. *European Journal of Pharmaceutics and Biopharmaceutics*, *67*(1), pp.196-201.

25. Prajapati, V.D., Jani, G.K., Khutliwala, T.A. and Zala, B.S., 2013. Raft forming system—An upcoming approach of gastroretentive drug delivery system. *Journal of Controlled Release*, *168*(2), pp.151-165.

26. Gite, S.M., Bhusari, S.S., Sav, A. and Morade, V., 2012. Formulation and evaluation of gastro retentive mucoadhesive sustained release pellets of Acyclovir. *International Journal of Drug Delivery*, *4*(3), pp.386.

27. Rouge, N., Allémann, E., Gex-Fabry, M., Balant, L., Cole, E.T., Buri, P. and Doelker, E., 1998. Comparative pharmacokinetic study of a floating multiple-unit capsule, a high-density multiple-unit capsule and an immediate-release tablet containing 25 mg atenolol. *Pharmaceutica Acta Helvetiae*, *73*(2), pp.81-87.

28. Klausner, E.A., Lavy, E., Friedman, M. and Hoffman, A., 2003. Expandable gastroretentive dosage forms. *Journal of Controlled Release*, *90*(2), pp.143-162.

29. Kakar, S., Batra, D., Singh, R. and Nautiyal, U., 2013. Magnetic microspheres as magical novel drug delivery system: A review. *Journal of Acute Disease*, *2*(1), pp.1-12.

30. Choi, B.Y., Park, H.J., Hwang, S.J. and Park, J.B., 2002. Preparation of alginate beads for floating drug delivery system: effects of CO 2 gas-forming agents. *International Journal of Pharmaceutics*, *239*(1), pp.81-91.

31. Li, S., Lin, S., Daggy, B.P., Mirchandani, H.L. and Chien, Y.W., 2003. Effect of HPMC and carbopol on the release and floating properties of gastric floating drug delivery system using factorial design. *International Journal of Pharmaceutics*, *253*(1), pp.13-22.

32. Kawashima, Y., Niwa, T., Takeuchi, H., Hino, T. and Itoh, Y., 1992. Hollow microspheres for use as a floating controlled drug delivery system in the stomach. *Journal of Pharmaceutical Sciences*, *81*(2), pp.135-140.

33. Simoni, P., Cerrè, C., Cipolla, A., Polimeni, C., Pistillo, A., Ceschel, G., Roda, E. and Roda, A., 1995. Bioavailability study of a new, sinking, enteric-coated ursodeoxycholic acid formulation. *Pharmacological Research*, *31*(2), pp.115-119.

34. Chordiya, M., Gangurde, H., Borkar, V. and Chandwad, N., 2017. Technologies, optimization and analytical parameters in gastroretentive drug delivery systems. *Current Science*, *112*(5), pp.946-953.

35. Patel, J.K. and Chavda, J.R., 2010. Formulation and evaluation of glipizide floating-bioadhesive tablets. *Brazilian Archives of Biology and Technology*, *53*(5), pp.1073-1085.

36. Pandya, N., Pandya, M. and Bhaskar, V.H., 2011. Preparation and in vitro characterization of porous carrier-based glipizide floating microspheres for gastric delivery. *Journal of Young Pharmacists*, *3*(2), pp.97-104.

37. Seshagiri, B., Chowdary, D. and Priya, Y.D., 2011. Formulation and evaluation of sintered gastro retentive tablets of glipizide. *International journal of pharmacy and pharmaceutical sciences*, *3*(4), pp.128-135.

38. Jain, S.K., Awasthi, A.M., Jain, N.K. and Agrawal, G.P., 2005. Calcium silicate based microspheres of repaglinide for gastroretentive floating drug delivery: Preparation and in vitro characterization. *Journal of Controlled Release*, *107*(2), pp.300-309.

39. Jain, S.K., Agrawal, G.P. and Jain, N.K., 2006. A novel calcium silicate based microspheres of repaglinide: in vivo investigations. *Journal of Controlled Release*, *113*(2), pp.111-116.

40. Stepensky, D., Friedman, M., Srour, W., Raz, I. and Hoffman, A., 2001. Preclinical evaluation of pharmacokinetic–pharmacodynamic rationale for oral CR metformin formulation. *Journal of Controlled Release*, *71*(1), pp.107-115.

41. Tutunji, L., 2005. Development of a gastroretentive controlled release metformin delivery system. PhD thesis, Temple University.

42. Boldhane, S.P. and Kuchekar, B.S., 2009. Gastroretentive drug delivery of metformin hydrochloride: formulation and in vitro evaluation using 32 full factorial design. *Current Drug Delivery*, *6*(5), pp.477-485.

43. Jain, S.K. and Gupta, A., 2009. Development of Gelucire 43/01 beads of metformin hydrochloride for floating delivery. *AAPS PharmSciTech*, *10*(4), pp.1128.

44. Kamila, M.M., Mondal, N., Ghosh, L.K. and Gupta, B.K., 2009. Multiunit floating drug delivery system of rosiglitazone maleate: development, characterization, statistical optimization of drug release and in vivo evaluation. *AAPS PharmSciTech*, *10*(3), pp887.

45. Gupta, N.V. and Shivakumar, H.G., 2010. Preparation and characterization of superporous hydrogels as gastroretentive drug delivery system for rosiglitazone maleate. *DARU Journal of Pharmaceutical Sciences*, *18*(3), p.200.

46. Dorożyński, P., Kulinowski, P., Mendyk, A. and Jachowicz, R., 2011. Gastroretentive drug delivery systems with l-dopa based on carrageenans and hydroxypropylmethylcellulose. *International Journal of Pharmaceutics*, *404*(1), pp.169-175.

47. Kshirsagar, R.V., Jain, V. and Wattamwar, S., 2009. Effect of different viscocity grade HPMC polymers on gastroretantive drug delivery of Metformin. *International Journal of Applied Pharmaceutics*, 1(1), pp. 44-50.

48. Gambhire, M.N., Ambade, K.W., Kurmi, S.D., Kadam, V.J. and Jadhav, K.R., 2007. Development and in vitro evaluation of an oral floating matrix tablet formulation of diltiazem hydrochloride. *AAPS PharmSciTech*, 8(3), pp.E166-E174.

49. Basak, S.C., Rao, K.N., Manavalan, R. and Rao, P.R., 2004. Development and in vitro evaluation of an oral floating matrix tablet formulation of ciprofloxacin. *Indian Journal of Pharmaceutical Sciences*, *66*(3), p.313.

50. Patel, V.F. and Patel, N.M., 2007. Statistical evaluation of influence of xanthan gum and guar gum blends on dipyridamole release from floating matrix tablets. *Drug Development and Industrial Pharmacy*, *33*(3), pp.327-334.

51. Asnaashari, S., Khoei, N.S., Zarrintan, M.H., Adibkia, K. and Javadzadeh, Y., 2011. Preparation and evaluation of novel metronidazole sustained release and floating matrix tablets. *Pharmaceutical Development and Technology*, *16*(4), pp.400-407.

52. Won R: Method for delivering an active ingredient by controlled time release utilizing a novel delivery vehicle which can be prepared by a process utilizing the active ingredients as a Porogen. 1987; US Patent No. 4690825.

53. Osmani, A. M., H Aloorkar, N., S Kulkarni, A., K Kulkarni, P., Hani, U., Thirumaleshwar, S., & R Bhosale, R., 2015. Novel cream containing microsponges of anti-acne agent: Formulation development and evaluation. *Current Drug Delivery*, 12(5), pp.504-516.

54. Embil, K. and Nacht, S., 1996. The microsponge® delivery system (MDS): a topical delivery system with reduced irritancy incorporating multiple triggering mechanisms for the release of actives. *Journal of Microencapsulation*, 13(5), pp.575-588.

55. Osmani, R.A.M., Aloorkar, N.H., Thaware, B.U., Kulkarni, P.K., Moin, A., Hani, U., Srivastava, A. and Bhosale, R.R., 2015. Microsponge based drug delivery system for augmented gastroparesis therapy: Formulation development and evaluation. *Asian journal of Pharmaceutical Sciences*, *10*(5), pp.442-451.

56. Arya, P. and Pathak, K., 2014. Assessing the viability of microsponges as gastro retentive drug delivery system of curcumin: Optimization and pharmacokinetics. *International Journal of Pharmaceutics*, *460*(1), pp.1-12.

57. Nokhodchi, A., Jelvehgari, M., Siahi, M.R., Mozafari, M., 2007. Factors affecting themorphology of benzoyl peroxide microsponges. *Micron*, 38, pp.834–840.

58. Singh, R.P. and Prajapati, S.K., 2014. Formulation and Evaluation of Prednisolone loaded Microsponges for Colon Drug Delivery: in-vitro and Pharmacokinetic study. *International Journal of Pharmaceutical Sciences and Research*, 5(5), p.1994.

59. Karthika, R., Elango, K., Ramesh Kumar, K. and Rahul, K., 2013. Formulation and evaluation of lornoxicam microsponges tablets for the treatment of arthritis. *International Journal of Pharmaceutical Innovations*, 3(2), p.29.

60. Jain, V. and Singh, R., 2011. Design and characterization of colon-specific drug delivery system containing paracetamol microsponges. *Archives of Pharmaceutical Research*, 34(5), pp.733-740.

61. https://www.drugs.com/monograph/metformin-hydrochloride.html

62. https://pubchem.ncbi.nlm.nih.gov/compound/Metformin_hydrochloride#section=Top

63. https://www.fda.gov/ohrms/dockets/dailys/02/may02/053102/800471e6.pdf

64. Japanese Pharmacopoeia 2016.Society of Japanese Pharmacopoeia. *Seventeenth Edition (JP XVII) pp.* 1258-1259.

65. Jin, L. and Cao, Q., 2012. Effect of gemfibrozil on the pharmacokinetics of mitiglinide in rats. *Arzneimittelforschung*, *62*(01), pp.40-45.

66. https://pubchem.ncbi.nlm.nih.gov/compound/6918235

67. https://www.drugbank.ca/salts/DBSALT001868

68. Mitiglinide Calcium Hydrate. Drugs of the Future, 25(10), pp.1034-1042.

69. Mikoshiba, I., Suzuki, H. and Kiyono, Y., 2003. *Drug composition for Blood Sugar Control*. U.S. Patent Application 10/519,102.

70. Reimann, F., Proks, P. and Ashcroft, F.M., 2001. Effects of mitiglinide (S 21403) on Kir6. 2/SUR1, Kir6. 2/SUR2A and Kir6. 2/SUR2B types of ATP-sensitive potassium channel. *British Journal of Pharmacology*, *132*(7), pp.1542-1548.

71. Liang, Y., Sun, J., Xie, L., Kang, A., Xie, Y., Chen, W.D., Lv, H. and Wang, G.J., 2007. Simple, Sensitive, and Rapid LC–ESI-MS Method for Quantification of Mitiglinide in Human Urine. *Chromatographia*, *66*(3-4), pp.165-170.

72. Ojima, K., Kiyono, Y. and Kojima, M., 2004. Pharmacological and clinical profile of mitiglinide calcium hydrate (Glufast), a new insulinotropic agent with rapid onset. *Folia pharmacologica Japonica*, 124(4), pp.245-255.

73. https://pubchem.ncbi.nlm.nih.gov/compound/glipizide

74. Pentikäinen, P.J., Neuvonen, P.J. and Penttilä, A., 1983. Pharmacokinetics and pharmacodynamics of glipizide in healthy volunteers. *International Journal of Clinical Pharmacology, Therapy, and Toxicology*, *21*(2), pp.98-107.

75. https://pubchem.ncbi.nlm.nih.gov/compound/24832091

76. https://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@rn+@rel+9004-57-3

77. Phadtare, D., Ganesh Phadtare, N.B. and Asawat, M., 2014. Hypromellose—a choice of polymer in extended release tablet formulation. *World Journal of Pharmacy and Pharmaceutical Science*, 3, pp.551-566.

78. https://pubchem.ncbi.nlm.nih.gov/compound/57503849.

79. http://www.agargel.com.br/carragena.html

80. Hermansson, A.M., Eriksson, E. and Jordansson, E., 1991. Effects of potassium, sodium and calcium on the microstructure and rheological behaviour of kappa-carrageenan gels. *Carbohydrate Polymers*, *16*(3), pp.297-320.

81. http://www.fao.org/docrep/w6355e/w6355e0x.htm.

82. https://pubchem.ncbi.nlm.nih.gov/compound/5102882.

83. Pentikainen, P.J., Neuvonen, P.J. & Penttila, A., 1979. Pharmacokinetics of metformin after intravenous and oral administration to man. *European Journal of Clinical Pharmacology*, 16, pp.195 – 202.

84. Urashima, Y., Kayano, E., KATO, R., 2012. Effect of gastric pH on Mitiglinide absorption, *Clinical Pharmacy and Clinical Pharmacokinetics*. 29(2), pp.41-46.

85. Foster, R., Plosker, G., 2000. Glipizide: A Review of the Pharmacoeconomic Implications of the Extended-Release Formulation in Type 2 Diabetes Mellitus. *Pharmaco Economics*, 18(3), pp.289-306.

86. Chowdary, K.P.R., Koteshwara, R.N., and Malathi, K., 2004. Ethyl cellulose microspheres of gliplizide: characterization, in vitro and in vivo evaluation. *Indian Journal of Pharmaceutical Sciences*, 66, pp. 412- 416.

CHAPTER 3

Preformulation Studies

3.1 Materials and Equipments

TABLE 3.1 List of Materials used during research work

Sr. no.	Name	Supplier
1	Metformin Hydrochloride	Sanofi-Aventis Ltd., Ankleshwar
2	Glipizide	MicroLabs Mumbai
3	Mitiglinide Calcium Dihydrate	Cadila healthcare
4	Glibenclamide	Sanofi-Aventis Ltd., Ankleshwar
5	Sodium bicarbonate	Sulab Reagents, Suvidhinath laboratories, Baroda
6	Hydroxy propyl methyl cellulose (HPMC K15M)	Astron Chemicals, Ahmedabad
7	Pullulan	DKSH India Pvt. Ltd., Mumbai, India
8	Sodium Alginate	Sulab Reagents
9	Kappa-carrageenan	Chemdyes corporation, Gujarat
10	Xanthan gum	Sulab Reagents
11	Poloxamer 188	Signet Chemical Co. Pvt. Ltd., Mumbai
12	Microcrystalline cellulose	Thrien Enterprise, Ahmedabad
13	Magnesium stearate	Sulab Reagents
14	Ethyl cellulose	Colorcon, Mumbai
15	Polyvinyl Alcohol	Sulab, Suvidhinath laboratories
16	Ethanol	Balaji drugs, Gujarat

17	Dichloro methane	Qualikems
18	Triethylcitrate	S D Fine Ltd.
19	Water for HPLC	Sisco research lab.
20	Acetonitrile (HPLC grade)	Gujarat Chemicals, Gujarat
21	O-Phosphoric acid	Gujarat Chemicals, Gujarat
22	Methanol (HPLC grade)	Gujarat Chemicals, Gujarat

TABLE 3.2 List of Equipments used during research work

Sr. No.	Instrument name	Company and model
1	Digital Balance	Shimadzu Corporation, Japan (AX200)
2	Sonicator	D-compact, EIE Instrument, Mumbai
3	Rotary tablet compression machine	Cronimach Instrument
4	Monsanto hardness tester	M. Shah and corp., India
5	Roche friabilator	DBK Friability apparatus, Electro quip Inst., Ahmadabad
6	Digital Vernier Caliper	Aerospace, India
7	Digital pH meter	Equip-Tronics (EQ-610)
8	UV Spectrophotometer	Shimadzu Corporation, Japan(UV-1800)
9	Fourier transform infrared spectrophotometer	Alpha -1, Bruker Optics, Germany
10	Differential Scanning Calorimeter	Perkin Elmer (Pyris 1 DSC)
11	Gas Chromatography	Auto System XL, Perkin Elmer
12	Scanning Electron Microscope	Model ESEM EDAX XL-30, Philips, Netherlands
13	Trinocular Microscope	Carl Zeiss
14	X-Ray diffractrometer	Model No. Xpert MPD, Philips, Holland

15	Mechanical stirrer	REMI Equipments Pvt. Ltd.
16	Magnetic Stirrer	REMI Equipments Pvt. Ltd.
17	Dissolution Test Apparatus	Electrolab, Mumbai, India
18	Cooling Centrifuge	Remi Electrotechnic Ltd.(CM-112 PLUS)
19	High performance liquid chromatography	Agilent Technologies.1220 LC Infinity with DAD and autosampler, C18 column (100 mm × 4.6 mm, 3.5 μ)
20	Humidity control oven	Sun instrument Pvt. Ltd.

3.2 Experimental Work

3.2.1 Identification of Drugs

Prior to formulation development, the identification of procured drug is one of the preliminary tests to be performed to verify and ensure the purity of procured drug sample. Identification test is also included as a compendia test to provide an aid in verifying the identity of articles as they are purpoted[1]. In the present research work identification of drug was performed by its appearance, solubility, melting point and Fourier-transform infrared (FT-IR) spectroscopy.

3.2.1.1 Description of Drugs[2, 3]

Physicochemical properties of drugs such as state, colour, odour and taste was physically examined and compared with the reported description of drugs.

3.2.1.2 Melting Point[2, 3]

Melting point is one of the identification test for organic compounds. The melting point of the drug was determined using capillary melting point method. The drug was filled in a thin walled capillary tube, with sealed one end. The capillary was then put in melting point

apparatus and the temperature of the apparatus was gradually increased. The temperature range over which the drug melts was observed visually.

3.2.1.3 Solubility

Solubility tests were performed as a part of test for purity[1]. Solubility of drug was measured by taking 10 mg of drug in a test tube followed by stepwise addition of 0.1 ml of solvent. Addition of solvent was continued till the sample was dissolved completely[4]. Solubility was recorded in form of the solvent required for solubilisation of the drug powder and was compared with reported values.

3.2.1.4 Identification of Drug by FTIR

Fourier transform Infra-red (FT-IR) is the tool for solid state characterization of pharmaceutical solids[5]. The identification of the drug was done by (FT-IR) spectroscopic method using Alpha Bruker FTIR spectrophotometer. The drug was mixed with suitable amount of KBr and converted into pellets using KBr press at 20 psi for 10 min. The disc thus prepared was placed in a sample compartment and scanned at transmission mode in the region of 4000 to 400 cm^{-1}. The IR spectrum of the drug thus obtained was compared with standard spectra of the drug.

3.2.2 Analytical Methods

Analytical methods were required at different stages of research for the estimation of drug content. Suitable Analytical techniques were developed for accurate, precise and convenient analysis of drug during preformulation, optimization, *in vitro* and *in vivo* measurements.

3.2.2.1 Development of UV Spectrophotometric Method for Estimation of Metformin Hydrochloride

UV spectrophotometric method for estimation of Metformin Hydrochloride (MH) in Methanol and in 0.1 N HCl is reported in literature[6]. For estimation of MH in tablet dosage form, already established assay method was used. Calibration curve for MH was prepared in 0.1 N HCl at 230 nm.

3.2.2.1.1 Calibration Curve of MH in 0.1 N HCl as a Solvent

The calibration curve of MH in 0.1 N HCl was used for determination of drug release during *in vitro* release measurements.

Preparation of stock solution:

Accurately weighed 100 mg of MH was transferred in 100 ml volumetric flask. The drug was dissolved and diluted upto the mark with 0.1 N HCl to give a solution with concentration of 1000 µg/ml. An aliquot of 10 ml from the above solution was withdrawn and diluted upto 100 ml with 0.1 N HCl to obtain a stock solution having concentration of 100µg/ml.

Preparation of solutions to obtain calibration curve

Appropriate aliquots from stock solution of MH (0.5, 1, 1.5, 2, 2.5, 3, 3.5,4, 4.5 and 5 ml) were accurately withdrawn in 10 ml volumetric flask and diluted upto the mark with 0.1 N HCl to obtain the final concentration of solution in range of 5-50 µg/ml. For spectroscopic measurement drug free solvent was used as a blank. To measure the λ_{max}, solution of 10 µg/ml was scanned in the range of 200-400 nm using double beam spectrophotometer. Absorbance of prepared solutions of calibration plot was measured at λ_{max} of MH. The procedure for measurement of absorbance was performed in triplicate. Mean value of the absorbance (n=3) was plotted against concentration to obtain a calibration curve.

3.2.2.2 Development of UV Spectrophotometric Method for Estimation of Mitiglinide Calcium Dihydrate (MTG)

UV spectrophotometric method for estimation of Mitiglinide Calcium Dihydrate (MTG) in Methanol is reported in literature[7]. Calibration curves for MTG were plotted in Methanol at 259 nm.

3.2.2.2.1 Calibration curve of MTG in Methanol as a solvent

The calibration curve of MTG in Methanol was used for determination of content, entrapment efficiency and stability of the developed formulation.

Preparation of stock solution:

Accurately weighed 100 mg of MTG was transferred in 100 ml volumetric flask. The drug was dissolved and diluted upto the mark with methanol to give a solution with concentration of 1000 µg/ml.

Preparation of solutions to obtain calibration curve

Stock solution was appropriately diluted with methanol to get series of solution with a concentration range of 100-1000 µg/ml. For spectroscopic measurement drug free solvent was used as a blank. To measure the λ_{max}, solution of 400µg/ml was scanned in the range of 200-400 nm using double beam spectrophotometer. Absorbance of prepared solutions of calibration plot was measured at λ_{max} of MTG. The procedure for measurement of absorbance was performed in triplicate. Mean value of the absorbance (n=3) was plotted against concentration to obtain a calibration curve.

3.2.2.3 Development of High Performance Liquid Chromatography Method for Estimation of MTG in Dissolution Samples

RP-HPLC method was developed and validated using HPLC with DAD detector infinity LC for estimation of MTG in dissolution sample. The need to develop separate method for analysis of dissolution arises from the fact that MTG has very low absorptivity in UV range to detect the drug release during dissolution.

3.2.2.3.1 Analytical Method Development

The amount of MTG from the dissolution media was analysed by RP-HPLC (Agilent Technologies 1120 series, Germany) using C18 Column (4.6 X 100mm, 3.5 µ). Acetonitrile and water in a ratio of 55:45 (pH adjusted to 2.15 with o-phosphoric acid) was used as the mobile phase at a flow rate of 1ml/min. Detection of eluent was done at 210 nm. To prepare a calibration curve, standard solutions of MTG was prepared in 0.1 N HCL in the concentration range of 5-100 µg/ml[8].

3.2.2.3.2 Analytical Method Validation

The Method developed was validation as per the ICH guidelines.

a) Linearity and Range

The linearity and range was determined by analyzing seven independent levels of calibration curve in the range of 5-100 µg/ml of MTG. The calibration curve of area under curve (AUC) of chromatographic peak vs. concentration was plotted and a correlation coefficient and regression line equation for MTG was calculated.

b) Precision

Repeatability

Solutions of MTG (40 µg/ml) were analyzed six times using developed HPLC method and % RSD was calculated.

- **Intraday precision**

Solutions containing 20, 60 and 100 µg/ml of MTG were analyzed three times on the same day using developed RP-HPLC method and % RSD was calculated.

- **Interday precision**

Solutions containing 20, 60 and 100 µg/ml of MTG were analyzed three times on three different day using developed RP-HPLC method and % RSD was calculated.

c) Accuracy

The developed HPLC method was checked for the accuracy. It was determined by calculating the recovery of MTG from Tablet formulation by standard addition method. The target concentration of the sample was taken 40 µg/ml of MTG. The spiking was done at three levels 80 %, 100 % and 120 %. The amount of MTG was calculated at each level and % recoveries were computed.

d) Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined by the standard deviation of the response and the slope using the equations.

$$\mathrm{LOD} = \frac{3.3 \text{ X } \sigma}{\mathrm{s}}$$

$$LOQ = \frac{10 \text{ X } \sigma}{S}$$

Where, σ is the SD of intercept of regression line and S is the slope of the corresponding calibration curve.

e) Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity was assessed by analyzing the analyte in the presence of placebo. The results were based on three replicate analyses.

f) Robustness of method

For robustness evaluation of HPLC method a few parameters like composition of mobile phase, flow rate and pH of mobile phase were deliberately changed. One factor was changed at one time to estimate the effect. Each factor selected was changed at three levels (-1, 0, +1) with respect to optimized parameters.

Robustness study was performed in following altered chromatographic conditions:

- Variation in mobile phase (± 2%)
- Variations in the flow rate (±0.1 ml/min)
- Variation in pH of mobile phase (± 0.2)

Three replicate solutions of MTG (40 µg/ml) were analyzed as per the altered chromatographic conditions and chromatograms were recorded.

g) System suitability parameters

As per USP-24, system suitability tests were carried out on freshly prepared standard stock solution of MTG. 20 µl solution was injected under optimized chromatographic condition and parameters such as retention time, theoretical plates and peak asymmetry were studied to evaluate the suitability of the system.

3.2.2.4 Bioanalytical Method Development and Validation of MTG in Rat Plasma

The process of pharmaceutical product development requires a suitable bioanalytical method to quantify drugs and/or their metabolites in the biological matrices like blood, serum,

plasma and /or urine. A well-developed bioanalytical method is an integral part of *in vivo* studies of the drugs[9-11].

The development of bioanalytical method for the drug is dependent on chemical structure, molecular weight, solubility, polarity and pKa of the drug. Along with chemical properties, sample preparation technique for bioanalytical method plays an important role in the development process. Biological samples such as serum, plasma, blood and urine contain many endogenous components and the challenge for method development is the extraction of analyte from extremely complex matrices. Also the separation of endogenous interferences in such a way that it does not overlap with the retention time of analyte is an important requirement for the bioanalytical method.

Various sample preparation techniques such as protein precipitation, solid phase extraction (SPE) and liquid-liquid extraction (LLE) methods are generally employed for bioanalytical method development. LLE is a method used for the separation of a mixture using two immiscible solvents. In most LLEs, one of the phases is aqueous and the other is an immiscible organic solvent[12-13]. Protein precipitation is a technique in which a miscible organic solvent such as acetonitrile, acetone or methanol is added to biological sample to precipitate proteins. Other agents that can be used for protein precipitation are metal ion such as zinc sulphate, salt such as aluminium chloride or acids such as trichloroacetic acid, perchloric acid, metaphosphoric acid and tungstic acid to alter pH of biological samples. Protein precipitation method can provide a fast and simple extraction procedure that can be applied to both hydrophilic and hydrophobic compounds[13].SPE involves use of two phases one a solvent with analyte and second the solid phase of sorbent. In this method, the sample is loaded onto a solid phase, from which first the undesired components are removed by washing them with suitable solvent followed by using an appropriate organic solvent to recover desired analyte[14].

Another important factor for bioanalytical method development is to select proper internal standard (IS) during analysis. One aspect to be considered for selection of IS is the structural similarity of IS with that of analyte. During bioanalytical method IS is added to both calibration standards and samples at fixed known concentration which can facilitate quantification of the analyte.

Existing literature reveals that few bioanalytical methods for estimation of MTG has been developed and validated. Xiao et al has reported liquid chromatography with electrospray ionization method for estimation of MTG in human plasma[15]. Lushan et al has developed

bioanalytical HPLC method for estimation of MTG in rat plasma in isocratic mode[16].In present study an HPLC method for estimation of Mitiglinide in rat plasma was used with slight modification with that of the reported method.

3.2.2.4.1 Optimization of Chromatographic Conditions

Selection of internal standard

Metformin hydrochloride was tried as an internal standard but its peak was not obtained at detection wavelength of MTG. Glipizide was selected as an internal standard as it can be resolved well from MTG with acceptable peak symmetry.

Procedure for extraction of drug from plasma

Based on the method developed by Lushan et al., for extraction of MTG from rat plasma, liquid-liquid extraction procedure was selected [16,17]. A 200µl of plasma containing drug was taken to which 20µl of internal std. (2mg/ml) was added. To this 25µl of trichloroacetic acid was added to increase the intensity of peak. Resulting solution was mixed on vortex mixer for 60sec and 1.5 ml of Acetonitrile was added to it. Obtained mixture was again mixed on vortex mixer for 60sec followed by centrifugation at 4,000 rpm for 10min at 4ºC. Supernatant was carefully separated, filtered through 0.45 µm filter paper and collected in a glass tube. The organic layer was evaporated to dryness. Obtained residues were reconstituted with 100µl of mobile phase and 20µl of this sample was injected in HPLC.

Bioanalysis of Mitiglinide by RP-HPLC method in plasma[18]

The chromatographic separation was achieved on Agilent C 18 column (150 mm ×4.6 mm) equipped with guard column. The mobile phase consisted of Acetonitrile and water (60:40) (pH adjusted to 3.5 with o-phosphoric acid) in a ratio of 60:40. The HPLC system was operated at a flow rate of 1.2ml/min in the isocratic mode and 210 nm was used as a detection wavelength.

3.2.2.4.2 Bioanalytical Method Validation

Developed bioanalytical method was validated as per USFDA guidelines.

Preparation of stock and standard solutions

Primary stock solutions of MTG were prepared in acetonitrile and further dilutions were done using HPLC grade acetonitrile:water (50:50). The Glipizide solution of 10µg/ml was prepared in acetonitrile.

a) Linearity

Linearity of developed method was established in the range of 200-2000 ng/ml (200, 500, 1000, 2000, 5000, 10000 and 20000 ng/ml) of MTG. The concentration of internal standard (GLP) was 2000 ng/ml. All the measurements were done in triplicates. The regression analysis of the area ratios (analyte/internal standard) vs. concentration curve was carried out. The linearity was confirmed by correlation coefficient.

b) Precision and accuracy

Precision and accuracy were determined using replicate analysis (n=6) of quality control samples at three concentrations. The analysis was performed on same day and on three consecutive days. SD and %RSD were calculated for the results obtained.

c) Extraction Recovery

Extraction recovery was analysed by comparing mean peak areas of six extracted low quality control (LQC) samples to mean peak areas of six un-extracted reference solutions. Internal standard recovery was calculated at concentrations of 2000 ng/ml.

d) Selectivity

Selectivity for the method was analysed to confirm the ability of method to discriminate the MTG in the presence of other components in sample matrix and to quantify it. The selectivity was determined by injecting six blank rat plasma samples extracted with the same method as that of the sample but without addition of IS. Selectivity was carried out at the lower limit of quantification (LLOQ) of 200ng/ml.

e) Stability

Stability of MTG and IS in rat plasma were examined by spiking plasma samples at bench top stability and freeze-thaw stability. For freeze thaw stability, QC samples were processed

for three freeze-thaw cycles and at last the sample was analysed as per the process of analysis and amount of MTG was assayed. For bench top stability, samples were stored at ambient temperature in replicates and analysed for content of MTG.

3.2.2.5 Development of UV Spectrophotometric Method for Estimation of Glipizide (GLP)

UV spectrophotometric method for estimation of Glipizide (GLP) in Methanol is reported in literature[19]. Method with slight modification was used for analysis of GLP for content formulation, for entrapment efficiency, for stability study and for *in vitro* dissolution.

3.2.2.5.1 Calibration Curve of GLP in Methanol as a Solvent

The calibration curve of GLP in Methanol was used for determination of content, entrapment efficiency and stability of the developed formulation.

Preparation of stock solution:

Accurately weighed 10 mg of GLP was transferred in 100 ml volumetric flask. The drug was dissolved and diluted upto the mark with methanol to give a solution with concentration of 100µg/ml.

Preparation of solutions to obtain calibration curve

Stock solution was appropriately diluted with methanol to get series of solution with a concentration range of 5-25 µg/ml. For spectroscopic measurement drug free solvent was used as a blank. To measure the λ_{max}, solution of 10µg/ml was scanned in the range of 200-400 nm using double beam spectrophotometer. Absorbance of prepared solutions of calibration plot was measured at λ_{max} of GLP. The procedure for measurement of absorbance was performed in triplicate. Mean value of the absorbance (n=6) was plotted against concentration to obtain a calibration curve.

3.2.2.5.2 Calibration Curve of GLP in 0.1 N HCl as a Solvent

The calibration curve of GLP in 0.1 N HCl was used for determination of dissolution samples to obtain *in vitro* drug release.

Preparation of stock solution:

Accurately weighed 10 mg of GLP was transferred in 100 ml volumetric flask. The drug was dissolved and diluted upto the mark with methanol to give a solution with concentration of 100µg/ml.

Preparation of solutions to obtain calibration curve

Stock solution was appropriately diluted with 0.1 N HCl to get series of solution with a concentration range of 5-40 µg/ml. For spectroscopic measurement drug free solvent was used as a blank. To measure the λ_{max}, solution of 10µg/ml was scanned in the range of 200-400 nm using double beam spectrophotometer. Absorbance of prepared solutions of calibration plot was measured at λ_{max} of GLP. The procedure for measurement of absorbance was performed in triplicate. Mean value of the absorbance (n=6) was plotted against concentration to obtain a calibration curve.

3.2.2.6 Bioanalytical Method Development and Validation of GLP in Rat Plasma

Existing literature reveals that few bioanalytical methods for estimation of GLP has been developed and validated. Yin et al has reported UPLC with tandem mass spectrometry method for estimation of GLP in human plasma[20]. Atif et al has developed bioanalytical HPLC method for estimation of GLP in human plasma in isocratic mode [21].In present study an HPLC method for estimation of Glipizide in rat plasma was developed and validated.

3.2.2.6.1 Optimization of Chromatographic Conditions

Selection of internal standard

Based on the method used by researcher in thesis Glibenclamide (GLB) was selected as an internal standard as it can be resolved well from GLP with acceptable peak symmetry.

Procedure for extraction of drug from plasma

Based on the literature, liquid-liquid extraction procedure was selected for extraction of GLP from rat plasma. 200µl of plasma containing drug was taken to which 20µl of internal std.

(200 ng/ml) was added. To this 25µl of trichloroacetic acid was added to increase the intensity of peak. Resulting solution was mixed on vortex mixer for 60sec and 1.5 ml of Acetonitrile was added to it. Obtained mixture was again mixed on vortex mixer for 60sec followed by centrifugation at 4,000 rpm for 10min at 4ºC. Supernatant was carefully separated, filtered through 0.45 µm filter paper and collected in a glass tube. The organic layer was evaporated to dryness. Obtained residues were reconstituted with 100µl of mobile phase and 20µl of this sample was injected in HPLC.

Bioanalysis of Glipizide by RP-HPLC method in rat plasma[16]

The chromatographic separation was achieved on Agilent C 18 column (150 mm ×4.6 mm) equipped with guard column. The mobile phase consisted of Acetonitrile and water (pH adjusted to 3.5 with o-phosphoric acid) in a ratio of 55:45. The HPLC system was operated at a flow rate of 1.2ml/min in the isocratic mode and 240 nm was used as a detection wavelength.

3.2.2.6.2 Bioanalytical Method Validation

Developed bioanalytical method was validated as per USFDA guidelines.

Preparation of stock and standard solutions

Primary stock solutions of GLP were prepared in acetonitrile and further dilutions were done using acetonitrile:water (50:50). The Glibenclamide solution of 10µg/ml was prepared in acetonitrile.

a) Linearity

Linearity of developed method was established in the range of 100-3200 ng/ml (100, 200, 400, 800, 1600 and 3200 ng/ml) of GLP. The concentration of internal standard (GLB) was 200ng/ml. All the measurements were done in triplicates. The regression analysis of the area ratios (analyte/internal standard) vs. concentration curve was carried out. The linearity was confirmed by correlation coefficient.

b) Precision and accuracy

Precision and accuracy were determined using replicate analysis (n=6) of quality control samples at three concentrations. The analysis was performed on same day and on three consecutive days. SD and %RSD were calculated for the results obtained.

c) Extraction Recovery

Extraction recovery was analysed by comparing mean peak areas of six extracted low quality control (LQC) samples to mean peak areas of six un-extracted reference solutions. Internal standard recovery was calculated at concentrations of 200 ng/ml.

d) Selectivity

Selectivity for the method was analysed to confirm the ability of method to discriminate the MTG in the presence of other components in sample matrix and to quantify it. The selectivity was determined by injecting six blank rat plasma samples extracted with the same method as that of the sample but without addition of IS. Selectivity was carried out at the lower limit of quantification (LLOQ) of 100ng/ml.

e) Stability

Stability of GLP and IS in rat plasma were examined by spiking plasma samples at bench top stability and freeze-thaw stability. For freeze thaw stability, QC samples were processed for three freeze-thaw cycles and at last the sample was analysed as per the process of analysis and amount of GLP was assayed. For bench top stability, samples were stored at ambient temperature in replicates and analysed for content of GLP.

3.3 Results and Discussion

3.3.1 Identification of Drugs

3.3.1.1 Identification of Drugs by Description, Solubility and Melting Point

An identification of metformin hydrochloride (MH), Mitiglinide Calcium Dihydrate (MTG) and Glipizide (GLP) based on physical examination and melting point results are mentioned in following table 3.3(a), (b) and (c).

TABLE 3.3 (a) Identification of MH

Sr. No.	Test	Specification	Observation	Inference
1	State	Solid crystalline	Solid crystalline	Complies
2	Colour	White	White	Complies
3	Taste	Bitter	Not Performed	Complies
4	Melting Point	222 -226 ° C	224– 226°C	Complies
5	Solubility	Freely soluble in water Slightly soluble in ethanol	Freely soluble in water Slightly soluble in ethanol	Complies

TABLE 3.3 (b) Identification of MTG

Sr. No.	Test	Specification	Observation	Inference
1	State	Solid crystalline	Solid crystalline	Complies
2	Colour	White	White	Complies
3	Taste	Bitter	Not Performed	Complies
4	Melting Point	179-185°C	180-184°C	Complies
5	Solubility	Freely soluble in methanol and slightly soluble in water	Freely soluble in methanol and slightly soluble in water	Complies

TABLE 3.3 (c) Identification of GLP

Sr. No.	Test	Specification	Observation	Inference
1	State	Solid crystalline	Solid crystalline	Complies
2	Colour	White	White	Complies
3	Taste	Bitter	Not Performed	Complies
4	Melting Point	200-203°C	200° – 202°C	Complies
5	Solubility	Practically insoluble in water Soluble in 0.1 N NaOH	Practically insoluble in water Soluble in 0.1 N NaOH	Complies

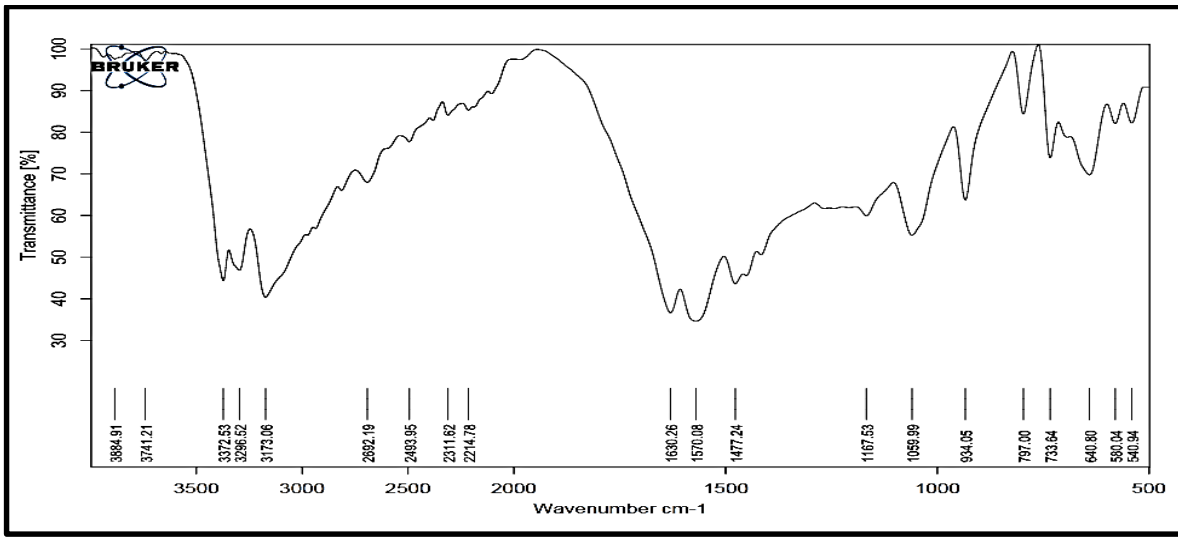
From the tables 3.3 (a), (b) and (c), it was observed that the obtained value of melting point of all the drugs was found to be similar to the reported value which proved that the received

drug samples meet the reported properties. Any impurity, if present, will cause variation in the melting point of a given drug substance. From the above test it was found that the sample drug complies with the standard test of MH, MTG and GLP.

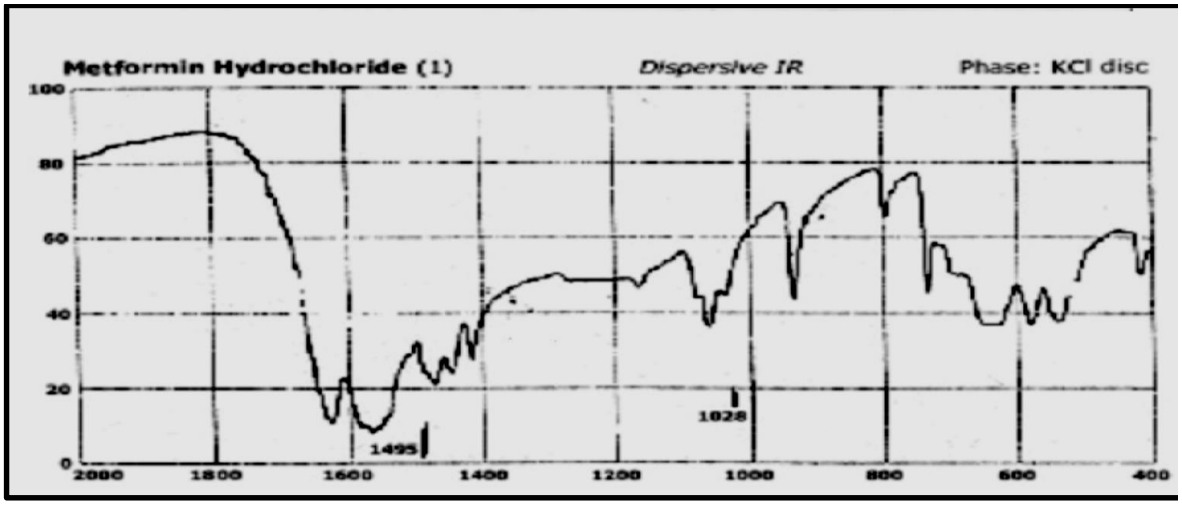
3.3.1.2 Identification of drugs by FTIR

FTIR spectra of MH, MTG and GLP was obtained and compared with reference IR spectra for identification and confirmation of various functional groups. The observed and reported spectra of MH is depicted in Fig. 3.1.

3.3.1.2.1 FTIR Study of Metformin Hydrochloride



(a)



(b)

FIGURE 3.1 (a) Observed FT-IT Spectra of MH (b)Reference FT-IR Spectra of MH

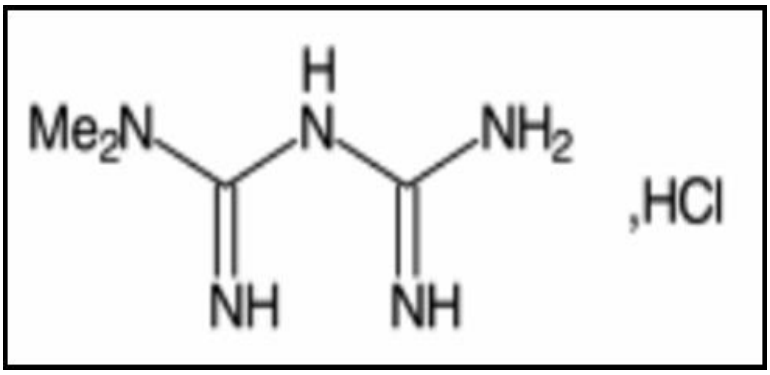
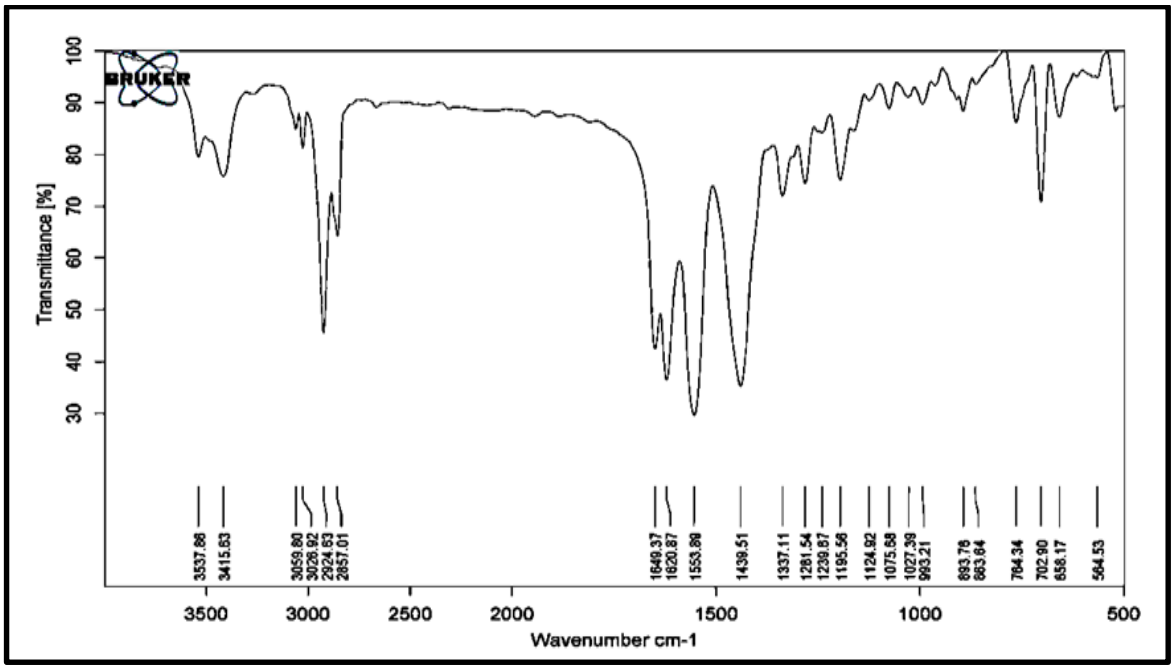


FIGURE 3.2 Structure of Metformin Hydrochloride

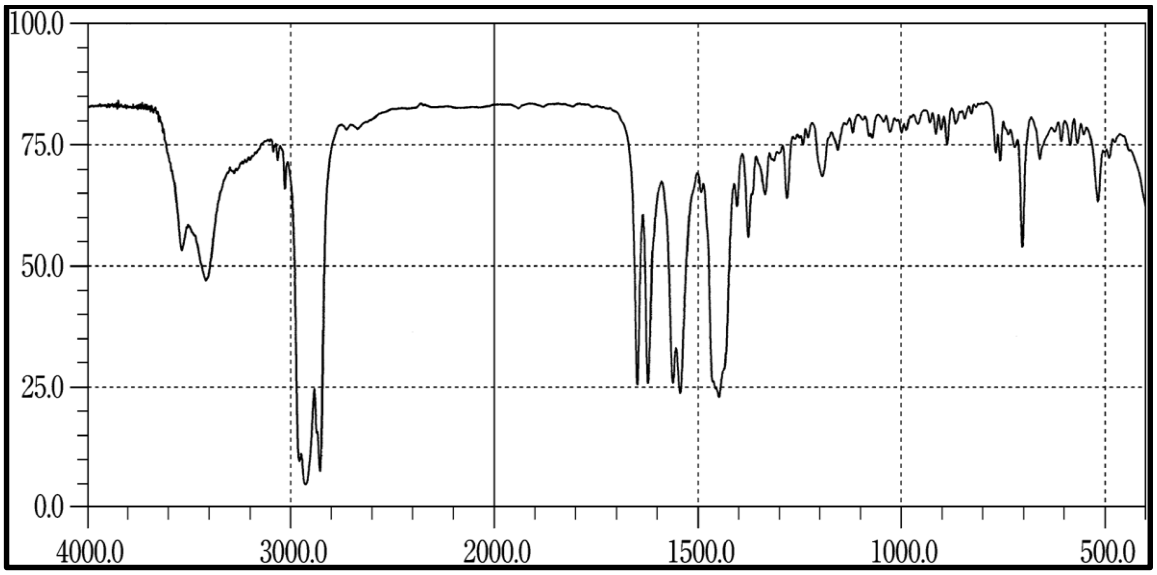
TABLE 3.4 (a) Interpretation of FT-IR spectra of MH

Peak Assignment	Wave Number (cm^{-1})	
	Reported Data	Observed Data
N-H stretching	3372	3372.53
Asymmetric N-H stretching	3300	3296.52
Symmetric N-H stretching	3176	3173.06
Asymmetric N-H deformation	1566	1570.08
N-H deformation	1626	1630.26

3.3.1.2.2 FTIR Study of Mitiglinide Calcium Dihydrate



(a)



(b)

FIGURE 3.3 (a)Observed FT-IR Spectra of MTG (b)Reference FT-IR Spectra of MTG

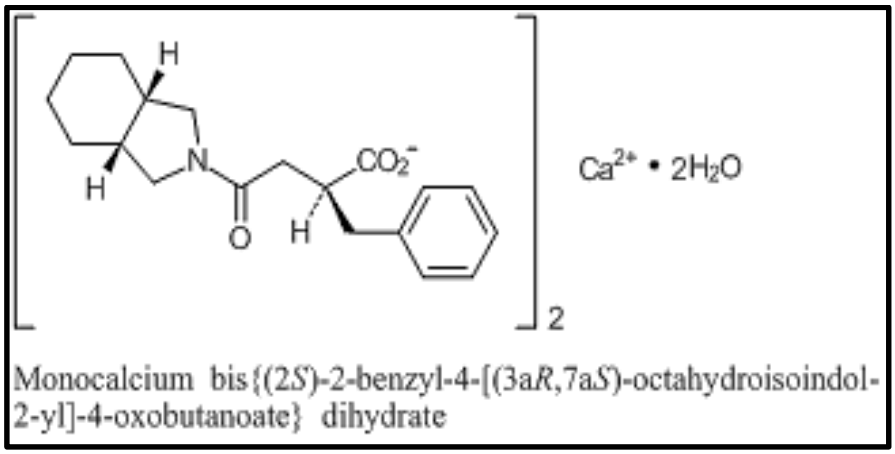


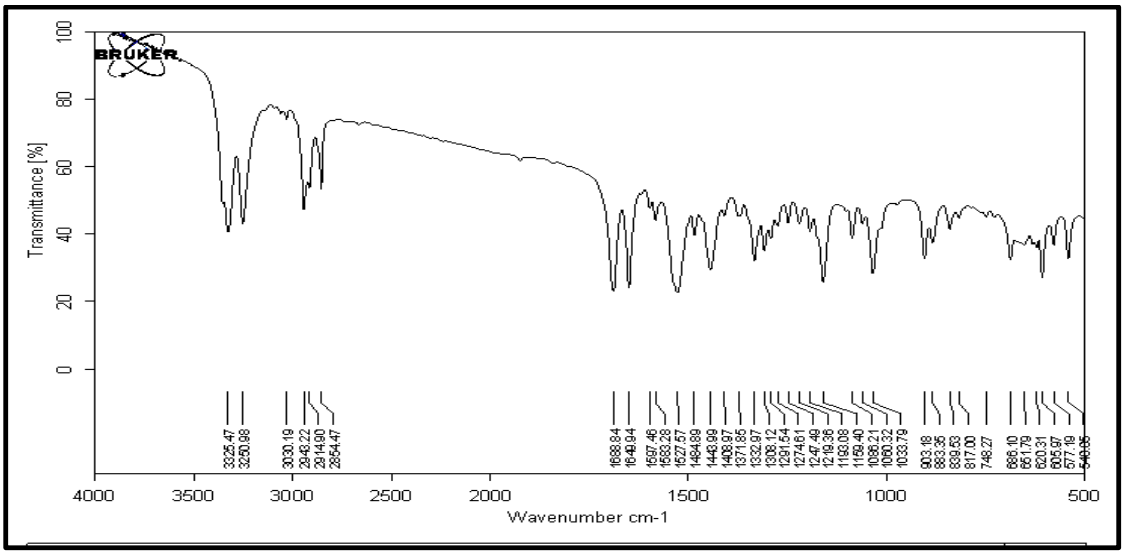
FIGURE 3.4 Structure of MTG

TABLE 3.4 (b) Interpretation of FT-IR spectra of MTG

Peak Assignment	Wave Number (cm^{-1})	
	Reported Data	Observed Data
N-H stretch	3424	3416.55
C-H stretch	2854 ~2926	2924.24, 2869.21, 2850.93
N-H bend	1618	1622.60
Aromatic C=C stretch	1495 ~1562	1544.75

3.3.1.2.3 FTIR Study of Glipizide

(a)



(b)

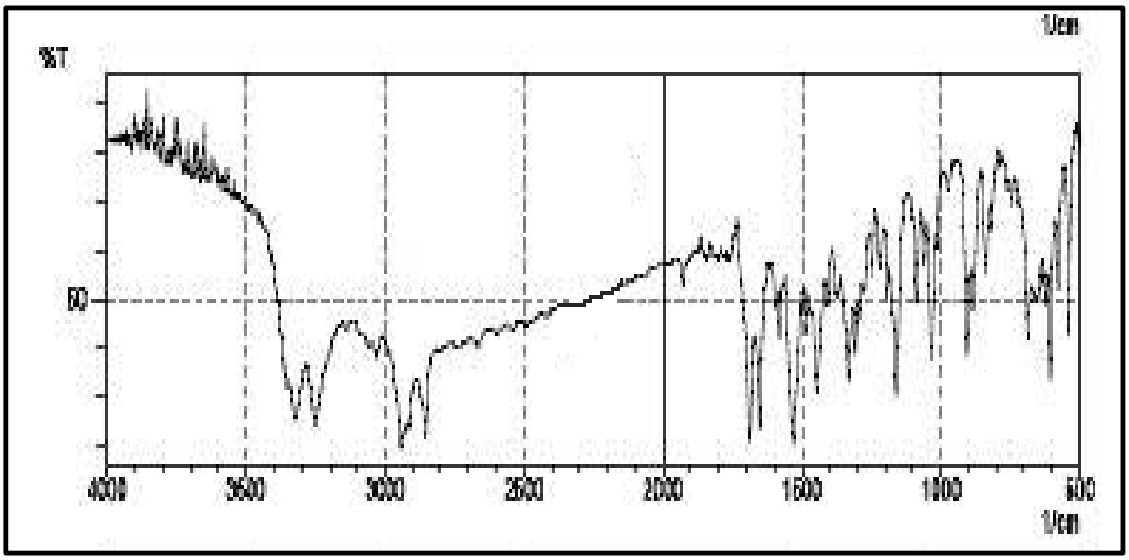


FIGURE 3.5 (a)Observed FT-IR Spectra of GLP (b)Reference FT-IR Spectra of GLP

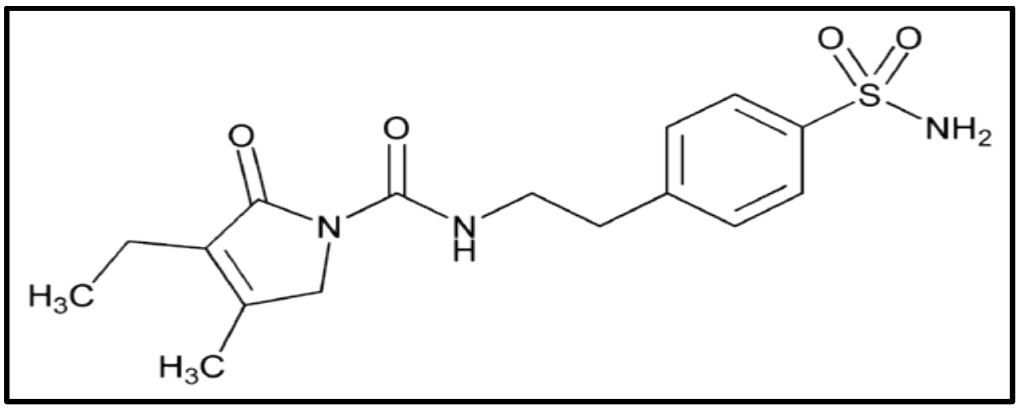


FIGURE 3.6 Structure of GLP

TABLE 3.4 (c) Interpretation of FT-IR spectra of GLP

Peak Assignment	Wave Number (cm^{-1})	
	Reported Data	Observed Data
Aromatic –CH stretching	3030	3030.19
Aliphatic –CH stretching	2940	2943.22
Aromatic C=C stretching	1700-1500	1597.48,1649.49, 1688.84

Interpretation of FTIR spectra of MH, MTG and GLP suggests that the observed peak list meets with that of the reference peak list. The observation confirms that the drugs obtained are pure.

3.3.2 Analytical Methods

Analytical methods that are accurate, simple and rapid are needed for estimation of drug content to determine content, drug release, entrapment efficiency (if applicable) and *in vivo* release of drug during formulation and optimization stage.

3.3.2.1 Development of UV Spectrophotometric Method for Estimation of MH

Simple, accurate, precise and rapid ultraviolet (UV) spectrophotometric methods are reported in literature for estimation of MH in bulk, dosage form and stability samples. Hence, calibration curves of MH were prepared in methanol and 0.1 N HCl to determine content and *in vitro* release.

3.3.2.1.1 Standard Calibration Curve of MH in 0.1N HCl by UV Spectrophotometer

Calibration curve of MH was prepared in 0.1 N HCl at 230 nm in the concentration range of 5-50 µg/ml. The overlay spectra and calibration curve are depicted in Fig. 3.7 (a) and (b). Data obtained for the calibration is shown in Table 3.5. The regression analysis was performed and correlation coefficient of 0.999 was obtained for the calibration curve.

TABLE 3.5 Calibration Data for MH in 0.1 N HCl

S.N.	Conc. (µg/ml)	Abs at 230 nm * (n=3)
1.	5	0.170±0.002
2.	10	0.302±0.005
3.	15	0.411±0.004
4.	20	0.518±0.004
5.	25	0.652±0.006
6.	30	0.758±0.005
7.	35	0.893±0.005
8.	40	1.038±0.005
9.	45	1.134±0.006
10.	50	1.247±0.004

*** Results are expressed as Mean ± SD**

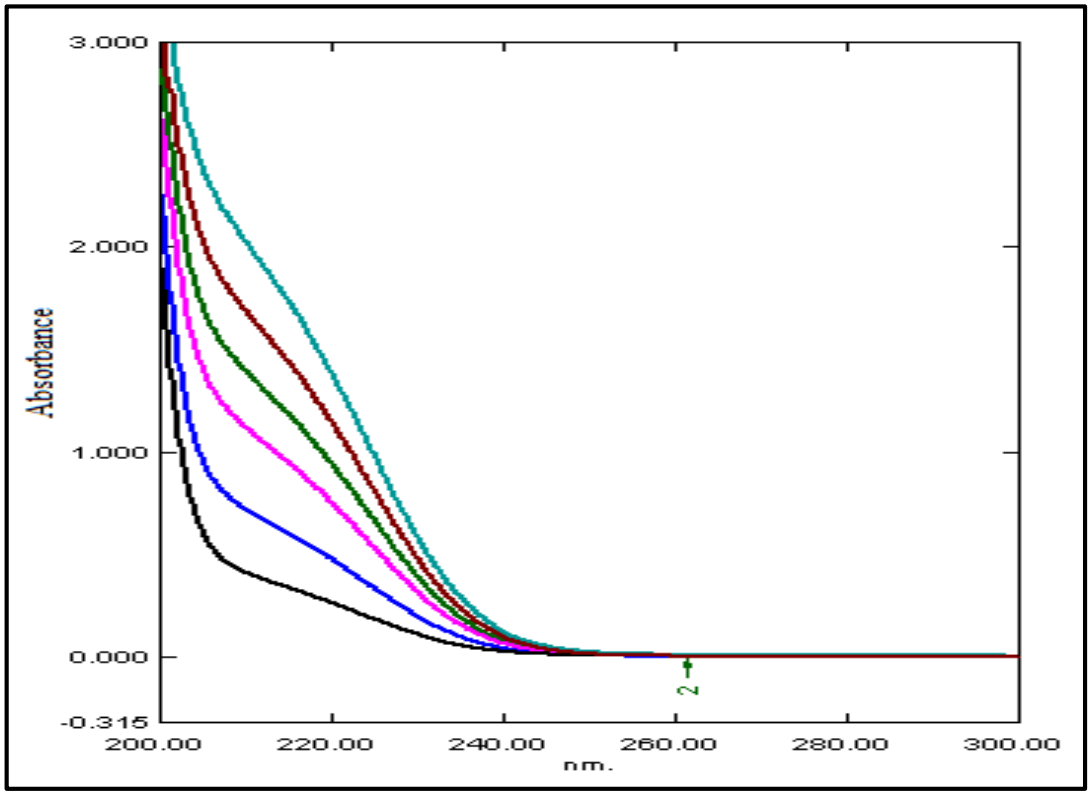


FIGURE 3.7 (a) Overlay UV spectra of MH in 0.1 N HCl

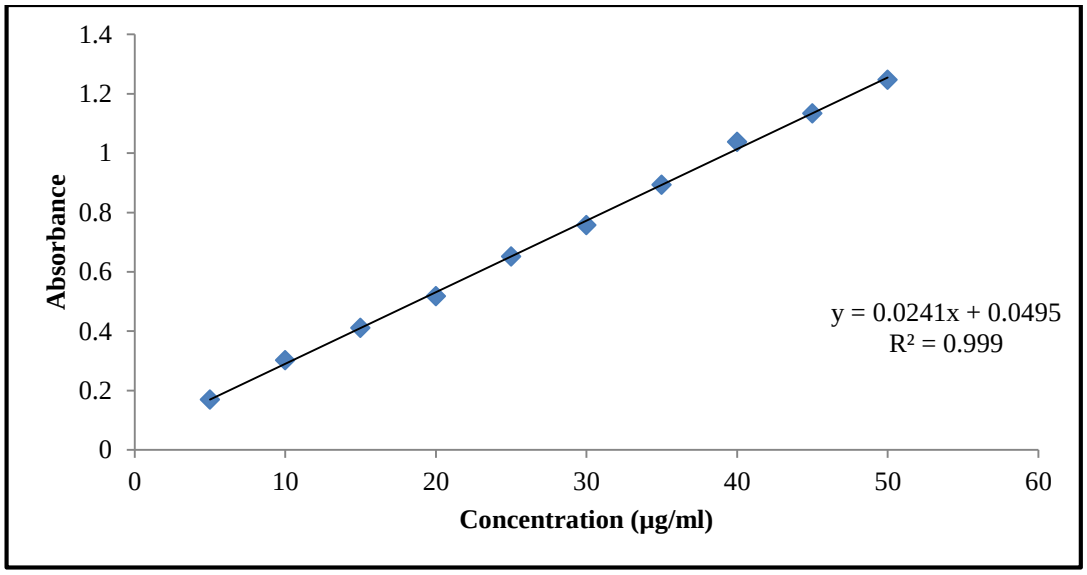


FIGURE 3.7 (b) Calibration curve of MH in 0.1 N HCl

3.3.2.2 UV Spectrophotometric Method for Estimation of MTG

3.3.2.2.1 Standard Calibration Curve of MTG in Methanol

Calibration curve of MTG was prepared in methanol at 259 nm in the concentration range of 100-1000 µg/ml.

TABLE 3.6 Calibration Data for MTG in Methanol

S.N.	Conc. (µg/ml)	Abs at 230 nm * (n=3)
1.	100	0.075±0.001
2.	200	0.156±0.002
3.	300	0.235±0.002
4.	400	0.293±0.003
5.	500	0.379±0.004
6.	600	0.446±0.002
7.	700	0.543±0.006
8.	800	0.603±0.005
9.	900	0.699±0.008
10.	1000	0.751±0.007

*** Results are expressed as Mean ± SD**

The calibration curve is depicted in Fig. 3.8. Data obtained for the calibration is shown in Table 3.6. The regression analysis was performed and correlation coefficient of 0.9984 was obtained for the calibration curve.

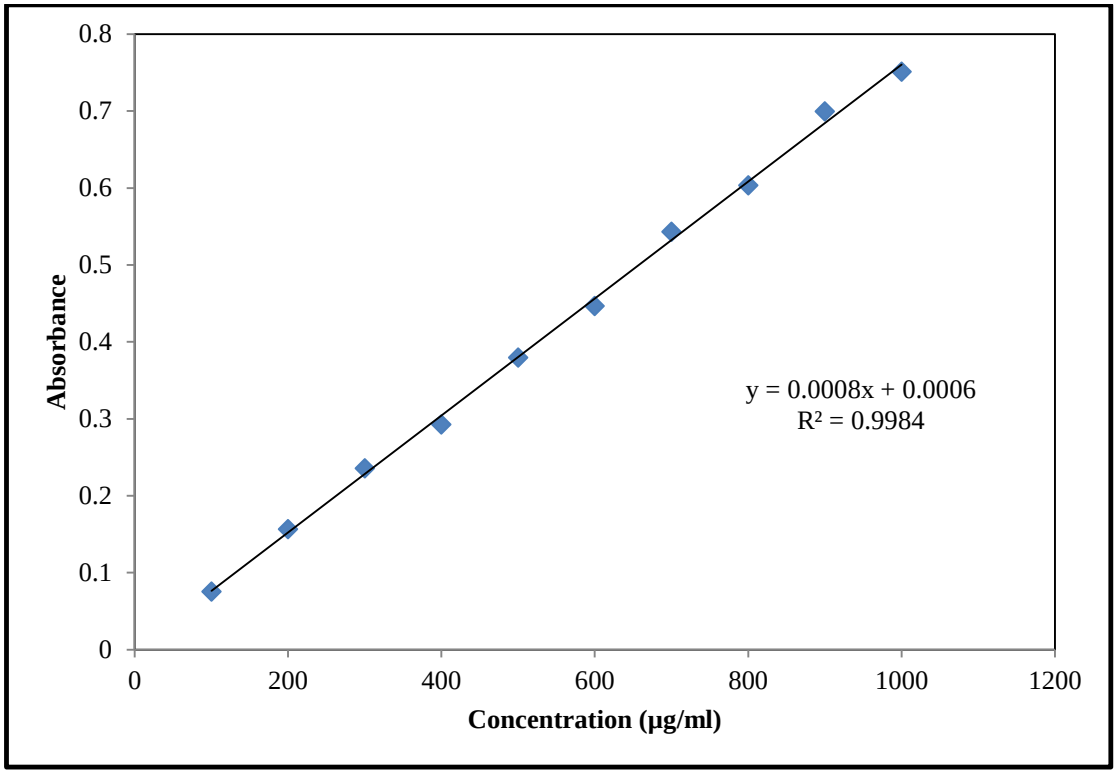


FIGURE 3.8 Calibration curve of MTG in Methanol

3.3.2.3 High Performance Liquid Chromatography Method for Estimation of MTG in Dissolution Samples.

An RP-HPLC method was developed and validated for estimation of MTG in *in vitro* release samples.

3.3.2.3.1 Analytical Method Development

To select the optimum chromatographic conditions several trials were performed and following chromatographic condition was selected as given in Table 3.7 which gave good characteristics of peak.

TABLE 3.7 Optimized chromatographic conditions for estimation of MTG for in vitro drug release

Equipment	Agilent 1220 Infinity LC equipped with DAD detector
Mobile phase	Acetonitrile: water(55:45) (pH adjusted to 2.15 with phosphoric acid)
Column	C_{18} (150X4.6mm,3.5 µ)
Column temperature	Ambient
Injection volume	20 µl
Flow rate	1ml/min
Wavelength	210 nm
Diluent	Acetonitrile: water(55:45)

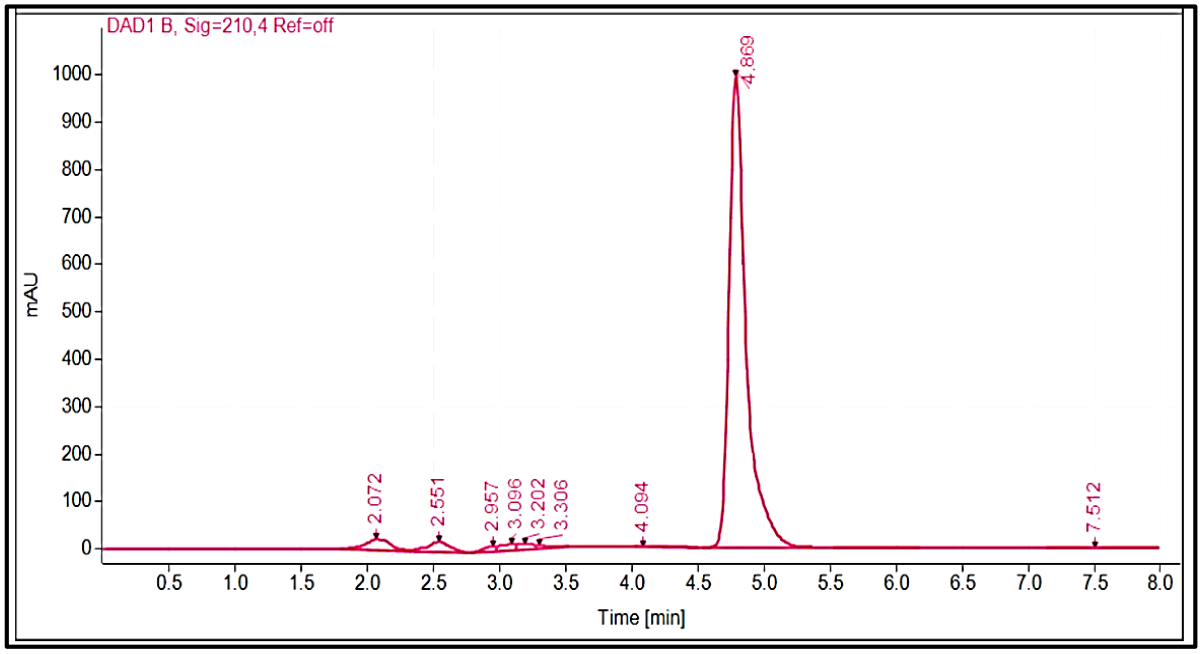


FIGURE 3.9 Chromatogram of MTG with optimized chromatographic conditions

3.3.2.3.2 Analytical Method Validation

The developed method was validated as per ICH guidelines Q2R1 in terms of parameters like specificity, linearity and range, precision, accuracy, LOD and LOQ, robustness, system suitability.

h) Linearity and Range

For current study, the calibration curve of MTG was prepared in the range of 5-100 µg/ml. The good linear relationship in the range of the calibration curve constructed was showed by the linear regression data. The value of correlation coefficient (r^2) was found to be 0.999 indicating that method is linear. A straight line equation of y= 90.168x+172.95 was obtained and used for the determination of MTG in dissolution samples. The range in which method is linear was found to be 5-10 µg/ml, suggesting that the analysis in this range can be done with accuracy.

i) Precision

Repeatability of developed method was found to be 0.76% RSD indicating that method gives reproducible results. Intermediate precision for the developed method was found to be 0.67 % RSD. Values of precision lies within the acceptance limit concluding that method is precise.

j) Accuracy

Accuracy is the degree of agreement between the observed value and true value. The accuracy of developed method was assessed by performing recovery study. The mean % recovery at three different concentration was found to be in range of 99.25 ± 0.68 and 101.45 ± 0.99. The results of recovery study fall within the acceptance limit indicating that developed method is accurate for estimation of MTG in dissolution samples.

k) Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is the minimum concentration of an analyte that can be detected by method and LOQ is the lowest concentration of an analyte that can be quantify by method with accuracy and precision. The LOD for the developed method was found to be 0.30µg/ml and LOQ was found to be 0.91µg/ml. The value of LOD and LOQ suggests that the method is sensitive to quantify lower concentration of MTG.

l) Specificity

No interference was observed at retention time of MTG from the placebo formulation using developed chromatographic method. The absence of any interference concludes that method is specific for estimation of MTG.

m) Robustness of method

Robustness of the developed method was determined to measure the capacity of method to remain unaffected by small, but deliberate changes brought about in method parameters. Robustness is an indication of the reliability of method during routine use.

Robustness of the method was studies by changing amount of water mobile phase (± 2ml), flow rate (±0.2 ml) and pH (±0.02) of mobile phase. % RSD was calculated for every change in the method and it was found to be less than 0.5 % indicating that developed method is robust and can be used for the routine analysis of MTG in dissolution media.

n) System suitability parameters

TABLE 3.8 Summary of analytical method validation of MTG by RP-HPLC method

Validation parameter	**Results obtained**			**Acceptance Criteria**
Accuracy (% Mean ± SD)	98.42 ±0.68 to 100.53 ±0.99			98 - 102
Repeatability precision (% RSD)	0.76 %			< 1
Intermediate precision (% RSD)	0.84 %			< 2
Linearity (r^2)	≥ 0.999 (Mean = 0.999 ± 0.0001166) (Intercept = 172.95 ±7.54) (Slope= 90.16±0.60)			> 0.999
LOD (µg/mL)	0.30			S/N ratio should be 3:1
LOQ (µg/mL)	0.91			S/N ratio should be 10:1
Robustness (% RSD)	Amount of water in mobile phase (mL)	47	0.117	< 2
		45	0.204	
		43	0.119	
	Flow rate (mL/min)	0.8	0.117	
		1	0.118	
		1.2	0.119	
	pH of mobile phase	2.13	0.119	
		2.15	0.205	
		2.17	0.119	
System suitability	Peak asymmetry (10 %)	1.367		T < 2
	Theoretical plates/meter	48790.6		N > 2000
	RSD for 6 injections Rt	4.88 ± 0.118		RSD < 1%

Tailing factor for MTG was found to be 1.367 which depicts that shape of MTG peak was symmetrical. Theoretical plates were found to be 48790.6 theoretical plates/meter, which show the column efficiency. The results for validation of analytical method are represented in Table 3.8 along with their acceptance criteria.

3.3.2.4 Bioanalytical Method of MTG in Rat Plasma

In the present study, Glipizide was used as an IS as it was well resolved from Mitiglinide with sharp symmetrical peak shape. For extraction of MTG from rat plasma, acetonitrile was used to precipitate protein. The sample preparation method gave the chromatogram free from endogenous interference at the retention time of MTG (5.69 min) and GLP (3.70 min).

3.3.2.4.1 Optimization of chromatographic conditions

Following chromatographic conditions were selected for the determination of Mitiglinide Calcium Hydrate in rat plasma.

TABLE 3.9 Optimized chromatographic conditions for estimation of MTG for bioanalytical method

Equipment	Hitachi L-2400 equipped with pump L-2130 with guard column
Mobile phase	Acetonitrile: water(60:40) (pH adjusted to 3.5 with o-phosphoric acid)
Column	C_{18} (150X4.6mm,3.5 µ)
Column temperature	Ambient
Injection volume	20 µl
Flow rate	1.2ml/min
Wavelength	210 nm
Diluent	Acetonitrile: water(60:40)

3.3.2.4.2 Validation of Bioanalytical Method for Estimation of MTG in Rat Plasma

Developed bioanalytical method for estimation of MTG in rat plasma was validated as per USFDA guidelines.

a) Linearity

The calibration curve for plasma was constructed using seven point calibration standards within the concentration range of 200-20000 ng/mL. The calibration curve was obtained by plotting the best fit of peak area ratios (MTG to IS) vs. concentration and fitted to y = mx+c. The calibration curve showing linearity is depicted in Fig.3.10. The slope for the calibration plot was found to be 0.00004 and intercept was 0.0916 ± 0.001. Correlation coefficient was found to be 0.9809 which is above 0.98 limit for bioanalytical method.

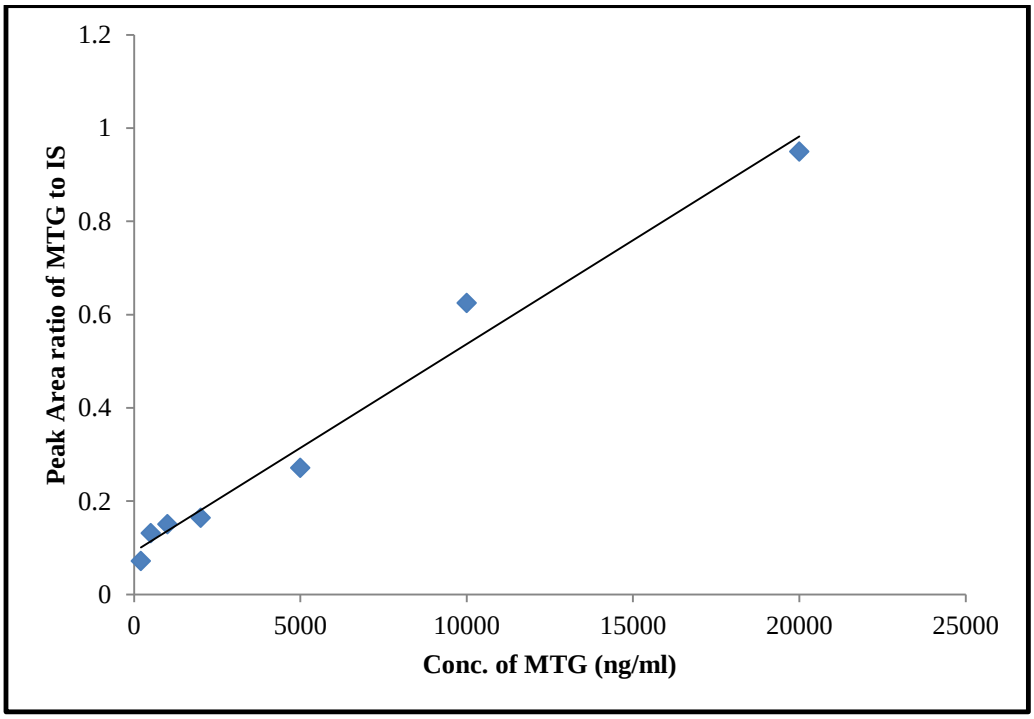


FIGURE 3.10 Calibration curve of MTG concentration vs. peak area ratio of MTG to IS

b) Precision and accuracy

The precision and accuracy of all QC samples, LQC, MQC and HQC samples were measured (n=6) for inter-day (2.31 to 3.86 %RSD) and intraday (2.05 to 3.95) studies. The results obtained were found to be in the acceptable limit. The data thus obtained demonstrates that method is accurate and precise for the quantification of MTG from rat plasma.

c) Extraction Recovery

The extraction recovery was performed to evaluate extraction procedure used to extract MTG from rat plasma in the developed bioanalytical method. The overall mean recovery of MTG was found to be 99.12± 1.98%. Extraction recovery of IS was found to be 86.56± 1.23%, indicating that the extraction procedure employed is suitable for measurement of MTG from blank plasma.

d) Selectivity

To assess selectivity of the method, six blank rat plasmas were injected and chromatograms were obtained. From the chromatogram, it was evident that at the retention time of MTG and GLP no interference from the endogenous substance was seen. The MTG and GLP were well separated from the plasma proteins using the developed chromatographic conditions with retention time of 5.67 and 3.70min. Representative chromatogram of plasma and that of MTG is depicted in fig 3.11 and 3.12 respectively.

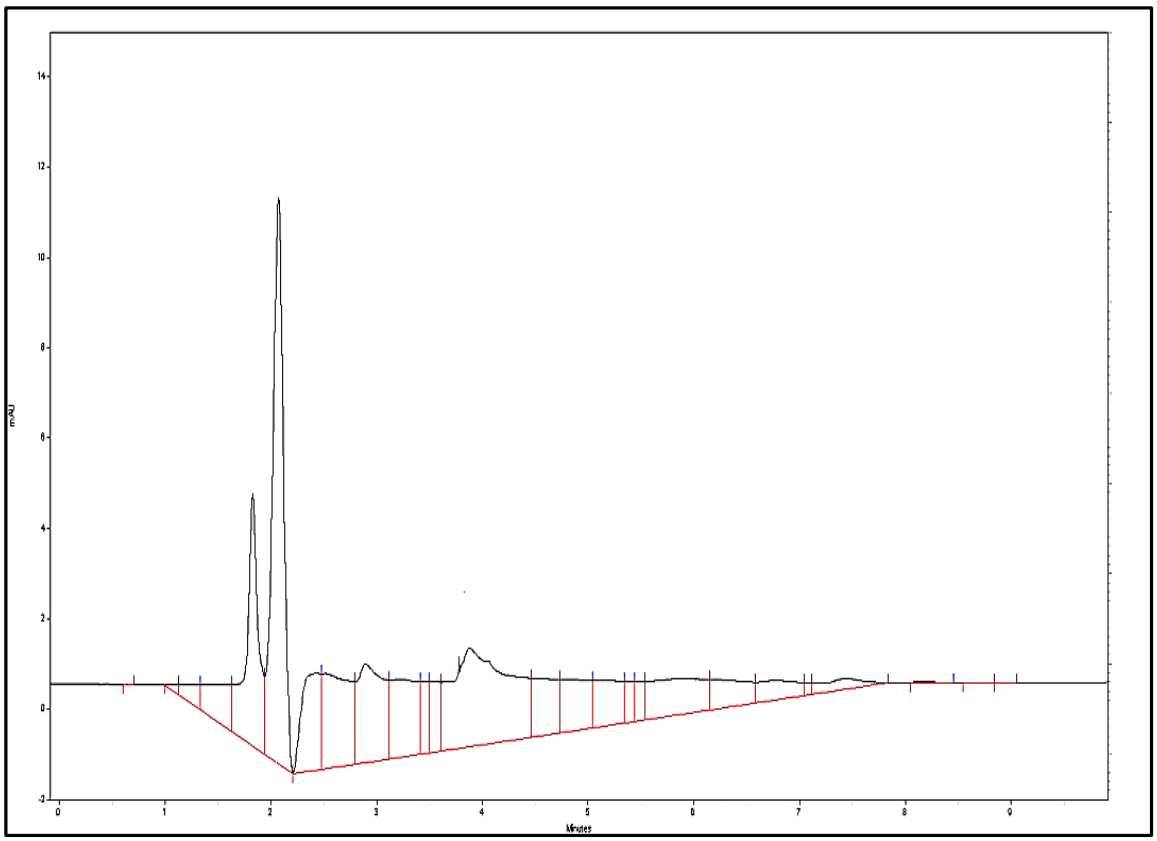


FIGURE 3.11 Representative chromatogram of blank plasma

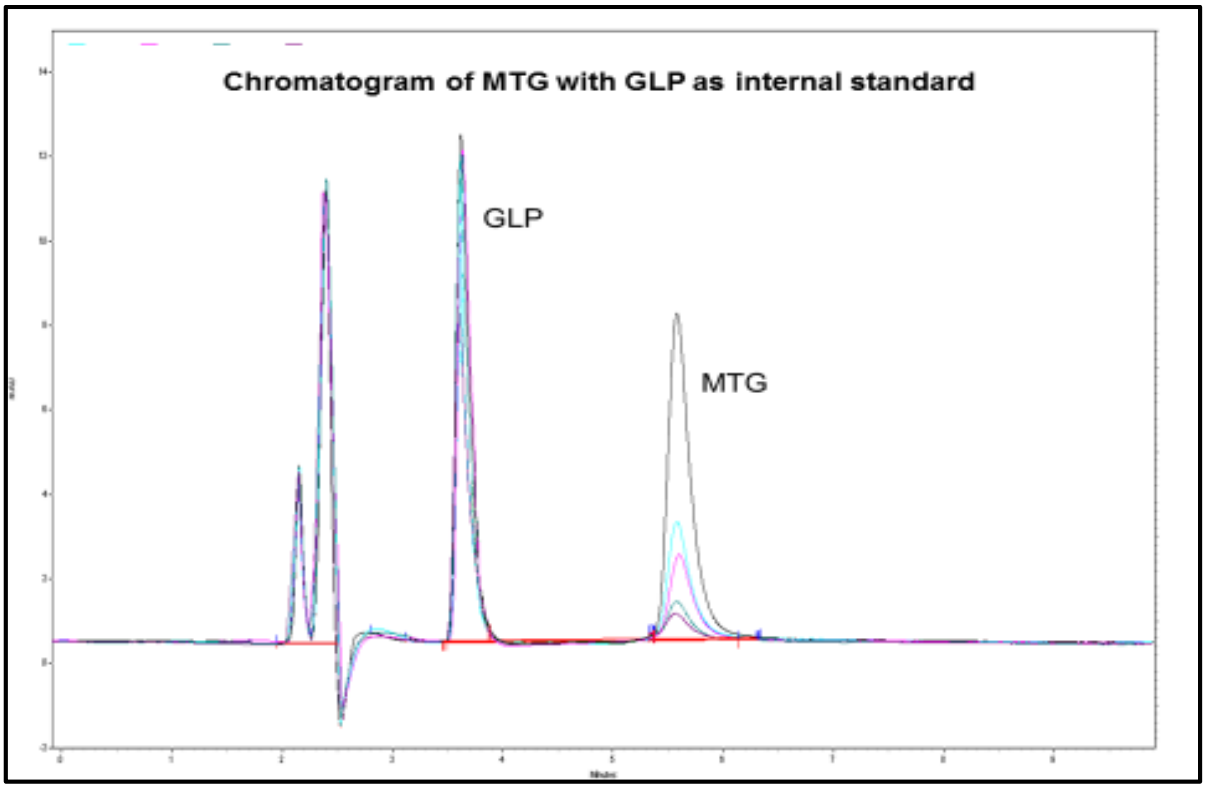


FIGURE 3.12 Overlay of calibration chromatograms of MTG and IS

The developed method could analyse 94.56ng/mL so it was selected as LLOQ. The results of selectivity conclude that the developed method is accurate and precise.

e) Stability

Freeze thaw stability and Bench top stability was performed for MTG at two levels of QC of LQC and HQC. MTG was found to be stable in rat plasma when stored in frozen condition for one month. Also it was found stable with bench top stability studies. The results of bioanalytical method validation along with acceptance criteria is summarized in Table 3.10

TABLE 3.10 Summary of results for Bioanalytical method validation for MTG in rat plasma

11	Observed Results	Acceptance Criteria
Calibration range (Coefficient determination)	200 - 20000 ng/mL $r^2 \geq 0.9809$ Mean (0.9809 ±0.0023) Slope (0.00004) Intercept (0.0916±0.0002)	> 0.98 with consistency

<table>
<tr><td>System suitability</td><td colspan="5">%CV (Area ratio) ≤ 1.13
%CV (RT-analyte) ≤ 0.561
%CV (RT-IS) ≤ 0.791</td><td>%CV ≤ 2.0 for area ratio and Rt of analyte</td></tr>
<tr><td>Sensitivity</td><td colspan="5">%CV = 6.23
Mean % nominal Conc= 94.56</td><td>%CV≤ 20.0 Mean % nominal conc should be 80-120%</td></tr>
<tr><td rowspan="4">Within day Precision and accuracy (N=6)</td><td colspan="2">Levels</td><td colspan="2">%RSD</td><td>%Recovery</td><td rowspan="4">± 15 % deviation in RSD</td></tr>
<tr><td colspan="2">LQC</td><td colspan="2">3.12</td><td>95.86-99.86</td></tr>
<tr><td colspan="2">HQC</td><td colspan="2">3.86</td><td>96.05-101.25</td></tr>
<tr><td colspan="2">MQC</td><td colspan="2">2.31</td><td>97.75- 100.25</td></tr>
<tr><td rowspan="7">Between the batches Precision and accuracy (N=6)</td><td>Batch</td><td>Levels</td><td colspan="2">%RSD</td><td>%Recovery</td><td rowspan="7">± 15 % deviation in RSD</td></tr>
<tr><td rowspan="3">Batch A</td><td>LQC</td><td colspan="2">3.54</td><td>94.91-99.91</td></tr>
<tr><td>HQC</td><td colspan="2">3.95</td><td>98.93-100.90</td></tr>
<tr><td>MQC</td><td colspan="2">2.05</td><td>94.87-101.81</td></tr>
<tr><td rowspan="3">Batch B</td><td>LQC</td><td colspan="2">3.54</td><td>98.39-100.69</td></tr>
<tr><td>HQC</td><td colspan="2">3.95</td><td>99.16-102.57</td></tr>
<tr><td>MQC</td><td colspan="2">2.05</td><td>99.13-102.64</td></tr>
<tr><td>Recovery analyte Recovery IS</td><td colspan="5">MTG: 99.12± 1.98%
IS : 86.56± 1.23%</td><td>Consistent recovery</td></tr>
<tr><td rowspan="3">Stability (N=6)</td><td rowspan="2">Types</td><td rowspan="2">Level</td><td colspan="2">% Normalization</td><td colspan="2">% Change</td><td rowspan="3">85 to 115 %.</td></tr>
<tr><td>%RSD</td><td>%Accuracy</td><td>%RSD</td><td>%Accuracy</td></tr>
<tr><td>Freeze Thaw Stability</td><td>HQC
LQC</td><td>1.11
0.98</td><td>98.51
99.01</td><td>0.624
1.26</td><td>97.52
96.99</td></tr>
<tr><td>Bench top Stability</td><td>HQC
LQC</td><td>0.87
0.65</td><td>99.35
99.27</td><td>0.51
1.67</td><td>98.62
97.99</td></tr>
</table>

3.3.2.5 Development of UV Spectrophotometric Method for Estimation of Glipizide

3.3.2.5.1 Standard Calibration Curve of GLP in Methanol as a Solvent

Calibration curve of GLP was prepared in methanol at 274.4 nm in the concentration range of 5-25 µg/ml. The overlay spectra and calibration curve are depicted in Fig. 3.13 (a) and (b). Data obtained for the calibration is shown in Table 3.11. The regression analysis for line y=0.0358x-0.015 was performed and correlation coefficient of 0.9993 was obtained for the calibration curve.

TABLE 3.11 Calibration Data for GLP in Methanol

S.N.	Conc. (µg/ml)	Abs at 274.4 nm * (n=3)
1.	5	0.173 ± 0.0014
2.	10	0.338 ± 0.0056
3.	15	0.513 ± 0.0071
4.	20	0.702 ± 0.0098
5.	25	0.887 ± 0.0087

* **Results are expressed as Mean ± SD**

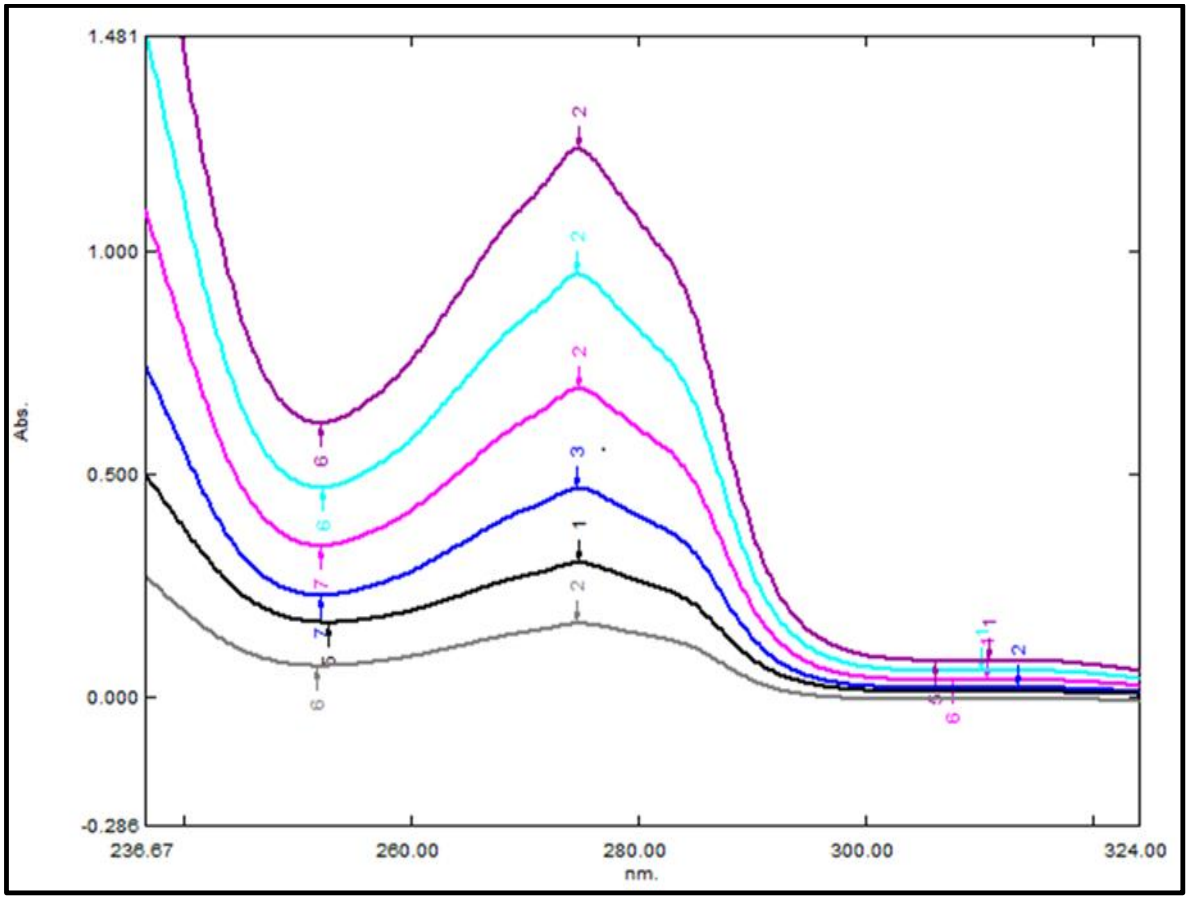


FIGURE 3.13(a) Overlay UV spectra of GLP in Methanol

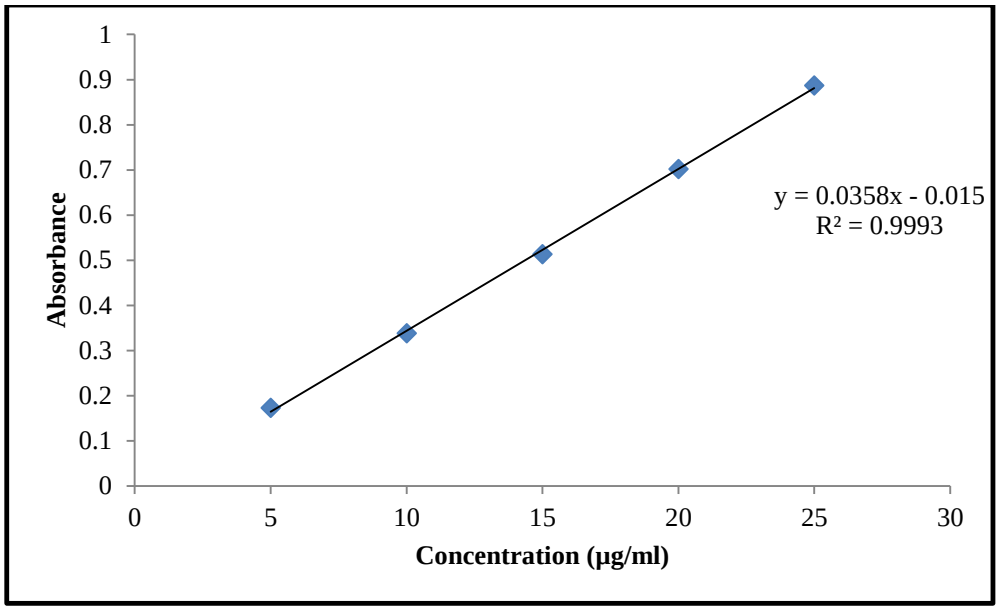


FIGURE 3.13 (b) Calibration curve of GLP in Methanol

3.3.2.5.2 Standard Calibration Curve of GLP in 0.1 N HCl as a Solvent

Calibration curve of GLP was prepared in 0.1 N HCl at 275nm in the concentration range of 5-40 µg/ml. The calibration curve is depicted in Fig. 3.14. Data obtained for the calibration is shown in Table 3.12. The regression analysis for line y=0.0238x-0.013 was performed and correlation coefficient of 0.999 was obtained for the calibration curve.

TABLE 3.12 Calibration Data for GLP in 0.1 N HCl

S.N.	Conc. (µg/ml)	Abs at 275nm * (n=6)
1.	5	0.121±0.0014
2.	10	0.211±0.0032
3.	15	0.342±0.0048
4.	20	0.468±0.0068
5.	25	0.581±0.0087
6.	30	0.692±0.0086
7.	35	0.824±0.0121
8.	40	0.947±0.0111

*** Results are expressed as Mean ± SD**

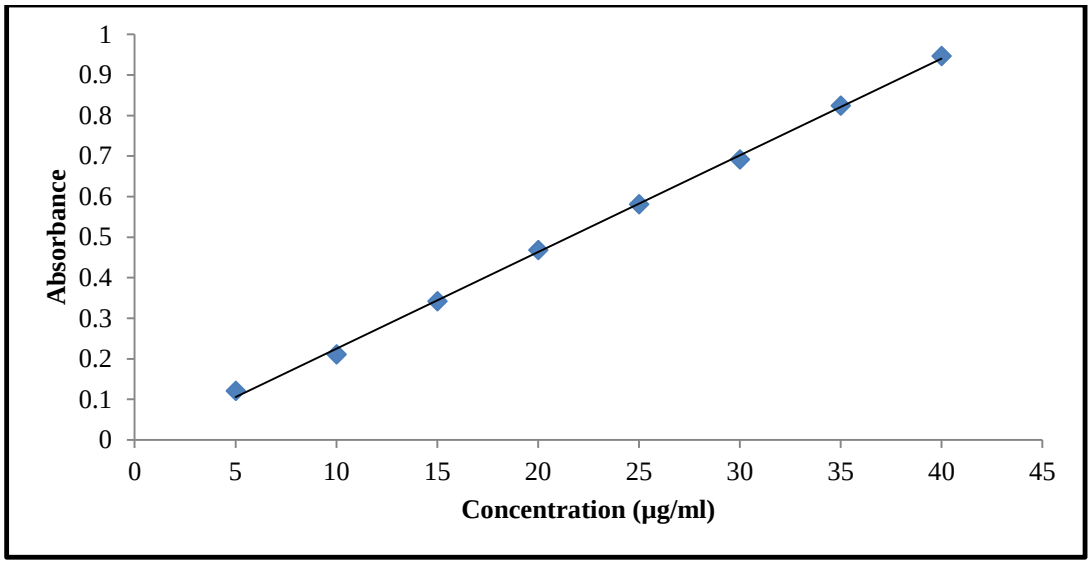


FIGURE 3.14 Calibration curve of GLP in 0.1 N HCl

3.3.2.6 Bioanalytical Method of Glipizide in Rat Plasma

In the present study, Glibenclamide was used as an IS as it was well resolved from Glipizide with sharp symmetrical peak shape. For extraction of GLP from rat plasma, acetonitrile was used to precipitate protein. The sample preparation method gave the chromatogram free from endogenous interference at the retention time of GLP (3.64 min) and GLB (7.18 min).

3.3.2.6.1 Optimization of Chromatographic Conditions

Following chromatographic conditions were selected for the determination of Glipizide in rat plasma.

TABLE 3.13 Optimized chromatographic conditions for estimation of GLP

Equipment	Hitachi L-2400 equipped with pump L-2130
Mobile phase	Acetonitrile: water(55:45) (pH adjusted to 3.5 with o- phosphoric acid)
Column	C_{18} (150X4.6mm,3.5 µ)
Column temperature	Ambient
Injection volume	20 µl
Flow rate	1ml/min
Wavelength	240 nm
Diluent	Acetonitrile: water(55:45)

3.3.2.6.2 Validation of Bioanalytical Method for Estimation of GLP in Rat Plasma

Developed bioanalytical method for estimation of GLP in rat plasma was validated as per USFDA guidelines.

f) Linearity

The calibration curve for plasma was constructed using six point calibration standards within the concentration range of 100-3200 ng/mL. The calibration curve was obtained by plotting the best fit of peak area ratios (GLP to IS) vs. concentration and fitted to y=mx+c. The slope for the linear fitted graph was found to be 0.0106 and intercept was 2.87 with correlation coefficient of 0.991.

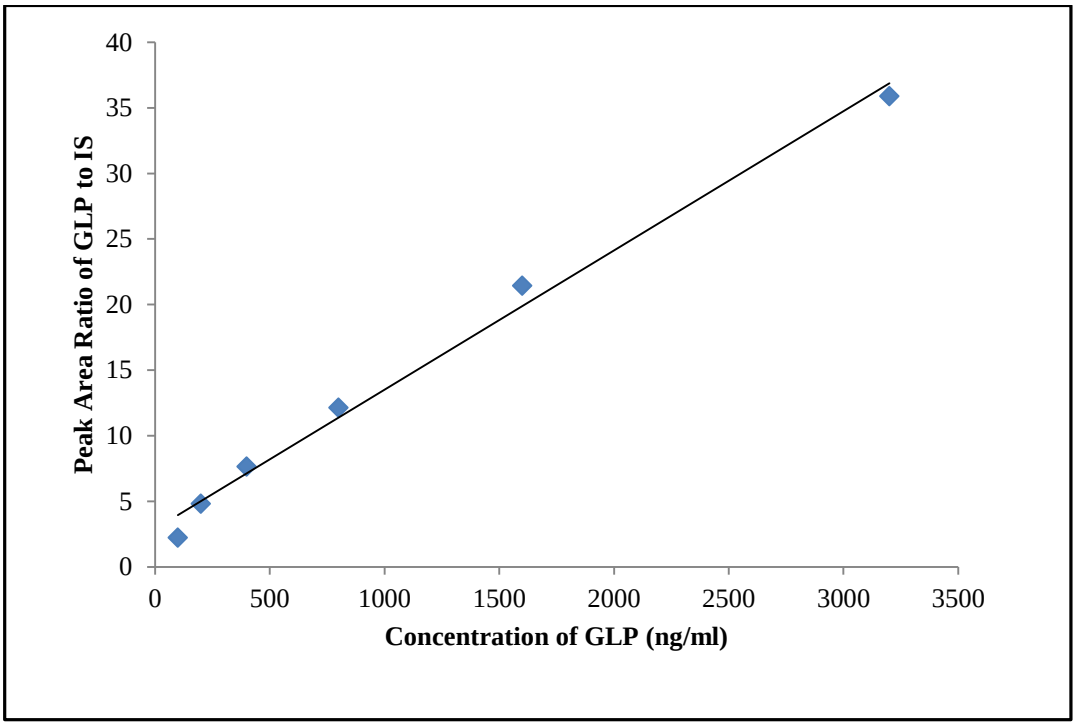


FIGURE 3.15 Calibration plot of Concentration of GLP to Peak area ratio of GLP to IS

g) Precision and accuracy

The precision and accuracy of all QC samples, LQC, MQC and HQC samples were measured (n=6) for inter-day and intraday studies. The results obtained were found to be in the acceptable limit. The data thus obtained demonstrates that method is accurate and precise for the quantification of GLP from rat plasma.

h) Extraction Recovery

The extraction recovery was performed to evaluate extraction procedure used to extract GLP from rat plasma in the developed bioanalytical method. The overall mean recovery of GLP was found to be 89.62 ± 2.13 %. Extraction recovery of IS was found to be 68.56± 1.70 %, indicating that the extraction procedure employed is suitable for measurement of GLP from blank plasma.

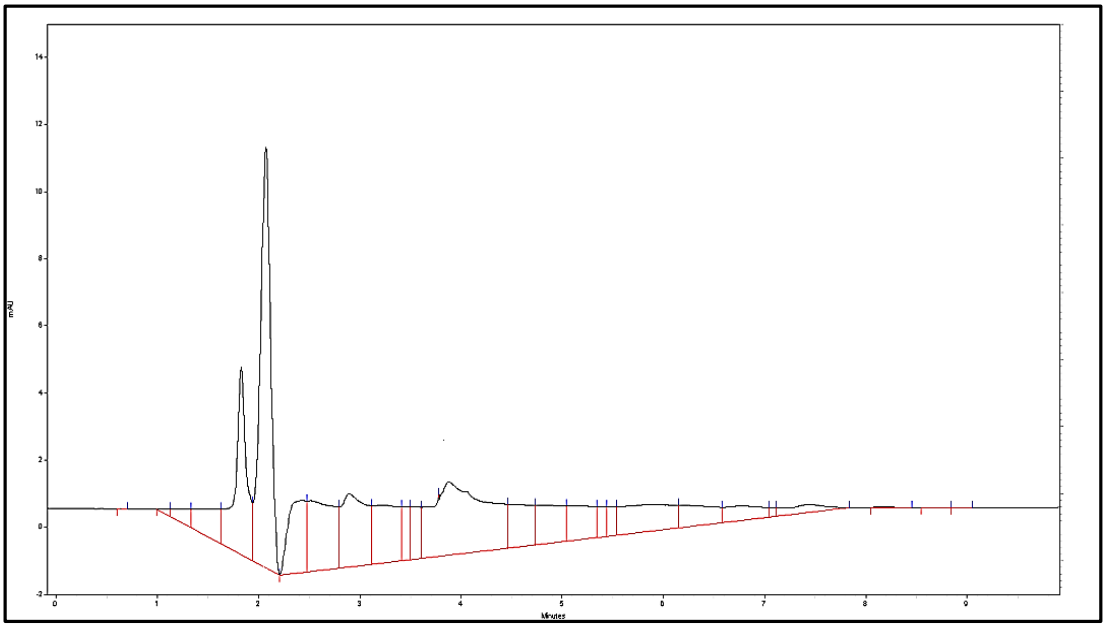


FIGURE 3.16 Representative chromatogram of blank plasma

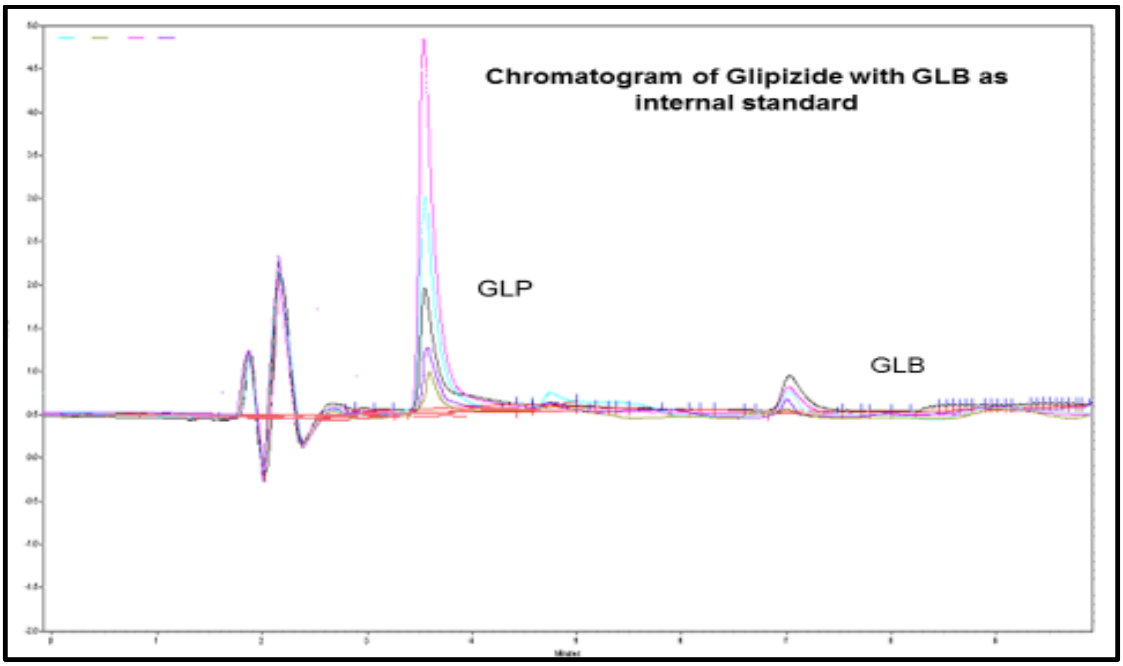


FIGURE 3.17 Overlay of calibration chromatograms of GLP and IS

i) Selectivity

To assess selectivity of the method, six blank rat plasmas were injected and chromatograms were obtained. From the chromatogram, it was evident that at the retention time of GLP and GLB no interference from the endogenous substance was seen. The GLP and GLB were well separated from the plasma proteins using the developed chromoatographic conditions with retention time of 3.64 and 7.18 min. The developed method could analyse 91.21ng/mL so it was selected as LLOQ. The results of selectivity conclude that the developed method is accurate and precise.

j) Stability

Freeze thaw stability and Bench top stability was performed for GLP at two levels of QC of LQC and HQC. GLP was found to be stable in rat plasma when stored in frozen condition for one month. Also it was found stable with bench top stability studies.

The results of bioanalytical method validation along with acceptance criteria is summarized in Table 3.14

TABLE 3.14 Summary of results for Bioanalytical method validation for GLP in rat plasma

Validation Parameters	**Observed Results**			**Acceptance Criteria**
Calibration range (Coefficient determination)	100 - 3200 ng/mL $r^2 \geq 0.985$ Mean (0.987 +- 0.0019) Slope (0.0105) Intercept (3.17+- 0.1634)			> 0.98 with consistency
System suitability	%CV (Area ratio) ≤ 0.713 %CV (RT-analyte) ≤ 0.215 %CV (RT-IS) ≤ 0.351			%CV ≤ 2.0 for area ratio and Rt of analyte
Sensitivity	%CV = 5.23 Mean % nominal Conc= 91.21			%CV≤ 20.0 Mean % nominal conc should be 80-120%
	Levels	**%RSD**	**%Recovery**	
	LQC	2.80	97.86-102.86	

<table>
<tr><td rowspan="2">Within day Precision and accuracy (N=6)</td><td>HQC</td><td colspan="2">3.86</td><td colspan="2">94.05-101.95</td><td rowspan="2">± 15 % deviation in RSD</td></tr>
<tr><td>MQC</td><td colspan="2">2.39</td><td colspan="2">95.75- 98.84</td></tr>
<tr><td rowspan="8">Between the batches Precision and accuracy</td><td>Batch</td><td>Levels</td><td colspan="2">%RSD</td><td>%Recovery</td><td rowspan="8">± 15 % deviation in RSD</td></tr>
<tr><td rowspan="3">Batch A (N=6)</td><td>LQC</td><td colspan="2">2.68</td><td>92.91-98.61</td></tr>
<tr><td>HQC</td><td colspan="2">3.16</td><td>91.93-97.98</td></tr>
<tr><td>MQC</td><td colspan="2">2.69</td><td>96.53-99.81</td></tr>
<tr><td rowspan="3">Batch B (N=6)</td><td>LQC</td><td colspan="2">2.68</td><td>94.39-99.77</td></tr>
<tr><td>HQC</td><td colspan="2">3.16</td><td>97.16-100.21</td></tr>
<tr><td>MQC</td><td colspan="2">2.69</td><td>98.13-99.56</td></tr>
<tr><td>Recovery analyte Recovery IS</td><td colspan="5">GLP : 89.62+- 2.13%
IS : 68.56 +- 1.70%</td><td>Consistent recovery</td></tr>
<tr><td rowspan="5">Stability (N=6)</td><td rowspan="2">Types</td><td rowspan="2">Levels</td><td colspan="2">% Normalization</td><td colspan="2">% Change</td><td rowspan="5">± 15 % deviation in RSD</td></tr>
<tr><td>%RSD</td><td>%Accuracy</td><td>%RSD</td><td>%Accuracy</td></tr>
<tr><td>Freeze Thaw Stability</td><td>HQC</td><td>0.435</td><td>99.054</td><td>0.458</td><td>98.61</td></tr>
<tr><td></td><td>LQC</td><td>0.426</td><td>99.47</td><td>0.722</td><td>98.64</td></tr>
<tr><td>Bench top Stability</td><td>HQC</td><td>0.56</td><td>99.56</td><td>0.46</td><td>98.79</td></tr>
<tr><td></td><td>LQC</td><td>1.02</td><td>99.89</td><td>1.76</td><td>96.79</td></tr>
</table>

3.4 Conclusion

The preformulation studies of MH, MTG and GLP was performed, before developing their gastroretentive formulations. Identification of the drugs was done by performing their physical evaluation and also by conducting the Fourier transform infrared spectroscopy (FTIR) study. The results indicated that the procured drugs were pure. 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 λmax and was further used for the preparation of calibration curve of drug in 0.1N HCl and methanol. The λmax value of MH and GLP, in 0.1N was found to be 230nm and 275nm respectively. The λmax value of MTG and GLP in methanol was found as 259nm and 274.4nm. The 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 during dissolution studies. 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. The method was used for analysis of drug after dissolution study. The equation, y = 90.168x + 172.95 was used to calculate the concentration of the drug in dissolution fluid.

The bioanalytical method for MTG and GLP was developed for pharmacokinetic estimation of drug from rat plasma. The mobile phase used for MTG was Acetonitrile:HPLC water (60:40), pH adjusted to 3.5 with o-phosphoric acid. The GLP was used as internal standard for the bioanalytical method development for MTG with detected wavelength 210 nm. The retention time of MTG and GLP was found as 5.67min and 3.70min, respectively. The concentration range in calibration curve was 200-20000 ng/mL and the slope was found to be 0.00004 with intercept as 0.0916 ± 0.001. Correlation coefficient was found to be 0.9809 which is above 0.98 limit for bioanalytical method.

The bioanalytical method for glipizide was done by using mobile phase, Acetonitrile:HPLC water (55:45), pH adjusted to 3.5 with o-phosphoric acid. Glibenclamide (GLB) was used as internal standard with detected wavelength was 240 nm. The retention time GLP and GLB was found to be 3.64 min and 7.18 min, respectively. The calibration curve for plasma was constructed using six point calibration standards within the concentration range of 100-3200 ng/mL. The slope for the linear fitted graph was found to be 0.0106 and intercept was 2.87 with correlation coefficient of 0.991. These bioanalytical methods developed for MTG and GLP, were used for the estimation of respective drugs in the rat plasma and the data was used for generating pharmacokinetic data of the drugs.

3.5 References

1. The United States Pharamcopoeial convention, (2011), Standards A. General Notices and Requirements. USP 34; 6.

2. Martindale. The complete drug reference, 36th edition, 2009, RPS Publication, pp 1324

3. Knox, C., Law, V., Jewison, T., Liu, P., Ly, S., Frolkis, A., Pon, A., Banco, K., Mak, C., Neveu, V. and Djoumbou, Y., 2010. DrugBank 3.0: a comprehensive resource for 'omics' research on drugs. *Nucleic Acids Research*, *39*(1), pp.D1035-D1041.

4. The National Toxicology Program (NTP) Interagency Center for the Evaluation of, Alternative Toxicological Methods (NICEATM). Test Method Protocol for Solubility Determination Phase III. 2003:1-10.

5. Dole, M.N., Patel, P.A., Sawant, S.D. and Shedpure, P.S., 2011. Advance applications of Fourier transform infrared spectroscopy. *International Journal of Pharmaceutical Science. Reviews and. Research*, *7*(2), pp.159-166.

6. Mishra, K., Soni, H., Nayak, G., Patel, S.S. and Singhai, A.K., 2011. Method development and validation of metformin hydrochloride in tablet dosage form. *Journal of Chemistry*, *8*(3), pp.1309-1313.

7. Kancharla, S., Perumal, R.V. and Kumar, P., 2012. Development and Validation of UV Spectrophotometric Method for Quantitative Estimation of Mitiglinide Calcium Hydrate in Bulk and Pharmaceutical Dosage form. *Inventi Rapid: Pharm Analysis & Quality Assurance*, 3, pp.1-4.

8. Sheth, B.K., Sagar, G.V., 2012. HPLC Development and Validation of RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride And Mitiglinide Calcium Dihydrate In Combined Dosage Form. *Pharma Science Monitor*, pp.2681-2693.

9. Srinivas, N.R., 2006. Applicability of bioanalysis of multiple analytes in drug discovery and development: review of select case studies including assay development considerations. *Biomedical Chromatography*, *20*(5), pp.383-414.

10. Shah, V.P., 2007. The history of bioanalytical method validation and regulation: evolution of a guidance document on bioanalytical methods validation. *The AAPS Journal*, *9*(1), pp.E43-E47.

11. Srinivas, N.R., 2008. Changing need for bioanalysis during drug development. *Biomedical Chromatography*, *22*(3), pp.235-243.

12. Pragst, F., Herzler, M. and Erxleben, B.T., 2004. Systematic toxicological analysis by high-performance liquid chromatography with diode array detection (HPLC-DAD). *Clinical Chemistry and Laboratory Medicine*, *42*(11), pp.1325-1340.

13. Ashri, N.Y. and Abdel-Rehim, M., 2011. Sample treatment based on extraction techniques in biological matrices. *Bioanalysis*, *3*(17), pp.2003-2018.

14. Żwir-Ferenc, A. and Biziuk, M., 2006. Solid Phase Extraction Technique--Trends, Opportunities and Applications. *Polish Journal of Environmental Studies*, *15*(5).

15. Xiao, S.H., Wei, G.L. and Liu, C.X., 2007. Determination of mitiglinide in human plasma by liquid chromatography with electrospray ionization tandem mass spectrometry. *Asian Journal of Pharmacodynamics and Pharmacokinetics*, 7(2), pp.145-152.

16. Lushan, Y. and Su, Z., 2006. Determination of mitiglinide in rat plasma by high-performance liquid chromatography with UV detection. *Journal of Chromatography B*, *834*(1), pp.204-207.

17. Choi, Y.H., Kim, S.G. and Lee, M.G., 2006. Dose-independent pharmacokinetics of metformin in rats: Hepatic and gastrointestinal first-pass effects. *Journal of pharmaceutical sciences*, *95*(11), pp.2543-2552.

18. Wanjari, M.M., There, A.W., Tajne, M.R., Chopde, C.T. and Umathe, S.N., 2008. Rapid and simple RPHPLC method for the estimation of metformin in rat plasma. *Indian Journal of Pharmaceutical Sciences*, *70*(2), p.198.

19. Karkhanis, V.V., Captain, A.D. and Patel, P.H., 2013. Development and validation of UV spectrophotometric method for estimation of glipizide in bulk and pharmaceutical dosage forms. *International Journal of Pharmaceutical Sciences and Research*, *4*(5), p.1865.

20. Yin, F., Zhang, C.X., Hu, J., Zhang, Q., Su, X.J. and Tang, C.R., 2014. A rapid and simple UPLC-MS/MS method for determination of Levofloxacin in human plasma and its application to bioequivalence study. *Latin American Journal of Pharmacy*, *33*(2), pp.197-203

21. Atif, M., Khalid, S.H., Kit, G.O., Sulaiman, S.A.S., Asif, M. and Chandersekaran, A., 2013. Development and validation of RP-HPLC-UV method for the determination of Glipizide in human plasma. *Journal of Young Pharmacists*, *5*(1), pp.26-29.

CHAPTER 4

Gastroretentive Floating Matrix Tablet of Metformin Hydrochloride (MH)

4.1 Introduction

Metformin hydrochloride (MH) is an antihyperglycemic agent, belonging to biguanide class, and used for the management of non-insulin dependent diabetes mellitus. Stepensky et al., established PK-PD rationale for the development of metformin CR formulations and it was concluded that GRDDS of this drug can be clinically advantageous[1]. The exhaustive literature research elucidates that several approaches have been tried for the preparation of gastro retentive metformin formulations.

An important aspect 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. Drug dissolution from hydrophilic matrix systems is related to the entry of water into the matrices. Li et al., suggested that many physicochemical phenomena occur simultaneously during dissolution[2]. The release mechanism of MH 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 system[3-5]. Gastroretentive drug delivery system of MH can be developed successfully by using the combination of various release retarding polymers. 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. Dorozynski et al., used carrageenans and their mixtures with HPMC for preparing gastro retentive drug delivery systems of l-dopa. The formulations showed linear increase in the releasing rate constantly. In such formulations, carrageenans can modify the properties of polymeric matrices, to obtain tailormade materials for drug delivery systems[6]. In another attempt, the effect of different viscosity grade HPMC polymers was checked on the Gastro retentive dosage form of metformin HCl[7].

Present research work has been done to check the combined effect of HPMC and other polymers on the release and gastro retentive properties of the metformin formulation. The final optimization of the gastroretentive floating matrix tablet of MH was done by simplex

centroid design. The dose of metformin for a gastro retentive SR formulation was taken as 500 mg. Dose calculation was done by using the equation given by Rawlins with the available pharmacokinetic data given by Defang et al.[8-10]

4.2 Experimental Studies

4.2.1 Method of Preparation of MH Floating Matrix Tablets

Tablets containing 500mg of MH were made by direct compression technique[11]. The active ingredient, Metformin, release-retarding polymer(s) (HPMC K15M and sodium alginate/kappa carrageenan/ pullulan/ xanthan gum/ poloxamer 188/ carbopol 934 P), a gas-forming agent, $NaHCO_3$, were passed through sieve no. 20, individually. Different powder blends were prepared and mixed in a mortar and pestle for 10 minutes (Table 4.1). Microcrystalline cellulose and magnesium stearate were then added to the mixed powders. Mixing was continued for another minute and the mixed blend was studied for pre-compression parameters. Finally, required quantity of mixture was weighed and fed into the die of Rotary tablet compression machine manually, with capsule shaped punch die set to produce caplet tablets (Dimensions - 8mm x 17mm with breakline).

4.2.2 Preliminary Studies

The gastroretentive floating matrix tablet of MH were prepared using the polymer hydroxyl propylmethyl cellulose (HPMC K15M) because the literature proved HPMC to be a good release retarding polymer. For optimizing the quantity of HPMC K15M various batches of floating matrix tablet of metformin were prepared. The amount of HPMC K15M was varied from 10% to 30% and the quantities of other additives were fixed (metformin 50%, sodium bicarbonate ($NaHCO_3$) 18%, Magnesium stearate 1%). The weight of Microcrystalline cellulose was adjusted to keep the total weight of the tablet as 1000mg. Prepared formulations were evaluated for the parameters of gastroretentive floating matrix tablet.

The results obtained for the batches prepared for the screening of HPMC K15M helped in fixing the concentration of release retarding polymer in the formulation. Further studies were conducted by preparing the formulation with the combination of HPMC K15 M with other ionic and non-ionic polymers. The amount of HPMC K15M was fixed and the quantities of other polymers were varied. The pre-compression studies like bulk density, tapped density,

carr's index, hausner's ratio and angle of repose, of powder blends were performed. Tablets containing 500 mg of metformin were prepared, according to the design depicted in table 4.1, by direct compression technique. The tablets were prepared using different release-retarding polymer(s) (HPMC K15M and sodium alginate/kappa-carrageenan/pullulan/xanthan gum/poloxamer 188).

TABLE 4.1 Composition (in percentage) of metformin HCl Floating Matrix Tablets*

Sr No	Ingredients	F1	F2	F3	F4	F5	F6
1	Metformin	50	50	50	50	50	50
2	HPMC K15M	17	17	17	17	17	17
3	Sodium bicarbonate	18	18	18	18	18	18
4	Sodium Alginate	8	-	-	-	-	-
5	κ-Carrageenan	-	8	-	-	-	-
6	Pullulan	-	-	8	-	-	-
7	Xanthan gum	-	-	-	8	-	-
8	Poloxamer 188	-	-	-	-	8	-
9	MCC	6	6	6	6	6	14
10	Magnesium stearate	1	1	1	1	1	1

***Total weight of the tablet was 1000mg**

The **prepared** formulations were evaluated for finding the best polymer combination to get the desired flotation and release pattern of MH from the gastroretentive tablet.

4.2.3 Evaluation of Gastroretentive Floating Matrix Tablet

4.2.3.1 Weight Variation

Twenty tablets were randomly selected and accurately weighed. The results were expressed as mean values ± SD[12].

4.2.3.2 Drug Content

Ten tablets were individually weighed and crushed. A quantity of powder equivalent to the mass of one tablet 1000 mg was extracted in 100 ml of 0.1N HCl. The solution was filtered through a cellulose acetate membrane (0.45 µm). The drug content was determined by UV spectroscopy (Shimadzu UV 1800 Double beam spectrometer, Shimadzu Corporation, Japan) at a wavelength of 230 nm after a suitable dilution with 0.1 N HCl[12].

4.2.3.3 Friability Studies

According to the IP specifications, 10 tablets were randomly selected from each batch and placed in the drum of a tablet friability test apparatus (DBK instruments, Electro quip Inst., Ahmedabad). The drum was adjusted to rotate 100 times in 4 min[12]. The percentage friability of the tablets was calculated by measuring the weight loss by the tablets during the rotations, using following equation:

$$\% \, friability = \frac{W_1 - W_2}{W_1} \; X \; 100$$

Where, W_1 is intital weight of 10 tablets and W_2 is weight of dusted tablets after 100 rotations.

4.2.3.4 Swelling Ability

The swelling behaviour of the tablets was determined, in triplicate, according to the method described by Dorozynski et al.[13]. Briefly, a tablet was weighed (W1) and placed in the petridish with 20 ml of HCl (0.1 N), maintained at 37 ± 0.5 ºC. After 8 hours the tablets were removed from the petridish and the swollen tablet was then reweighed (W2)[14]. The swelling index (SI) was calculated using following formula.

$$Swelling\ Index = \frac{(W2 - W1)}{W1}$$

Where, W2 is the weight of the swollen tablets, and W1 is the initial weight of the tablets. Size of tablets, before and after swelling, was also measured.

4.2.3.5 *In vitro* Buoyancy Studies

The floating behavior of the tablets was visually determined, in triplicate, according to the floating lag time method described by Rosa et al.[15]. Briefly, a tablet was placed in a glass

beaker, containing 200 ml of 0.1 N HCl, maintained in a water bath at 37 ± 0.5 ºC. The floating lag time, ‘‘the time between tablet was placed in a glass beaker with HCl and its buoyancy” and total floating duration, ‘‘the time during which tablet remains buoyant”, were recorded.

4.2.3.6 Adhesion Retention Period

The adhesion retention period of the tablets was evaluated, in triplicate, by an *in vitro* method reported by Nakamura et al. Briefly, an agar plate (2%, w/w) was prepared in 0.1 N HCl (pH 1.2). A side of the tablet was wetted with 0.1 N HCl and attached to the centre of agar plate by applying a light force with a fingertip[17].

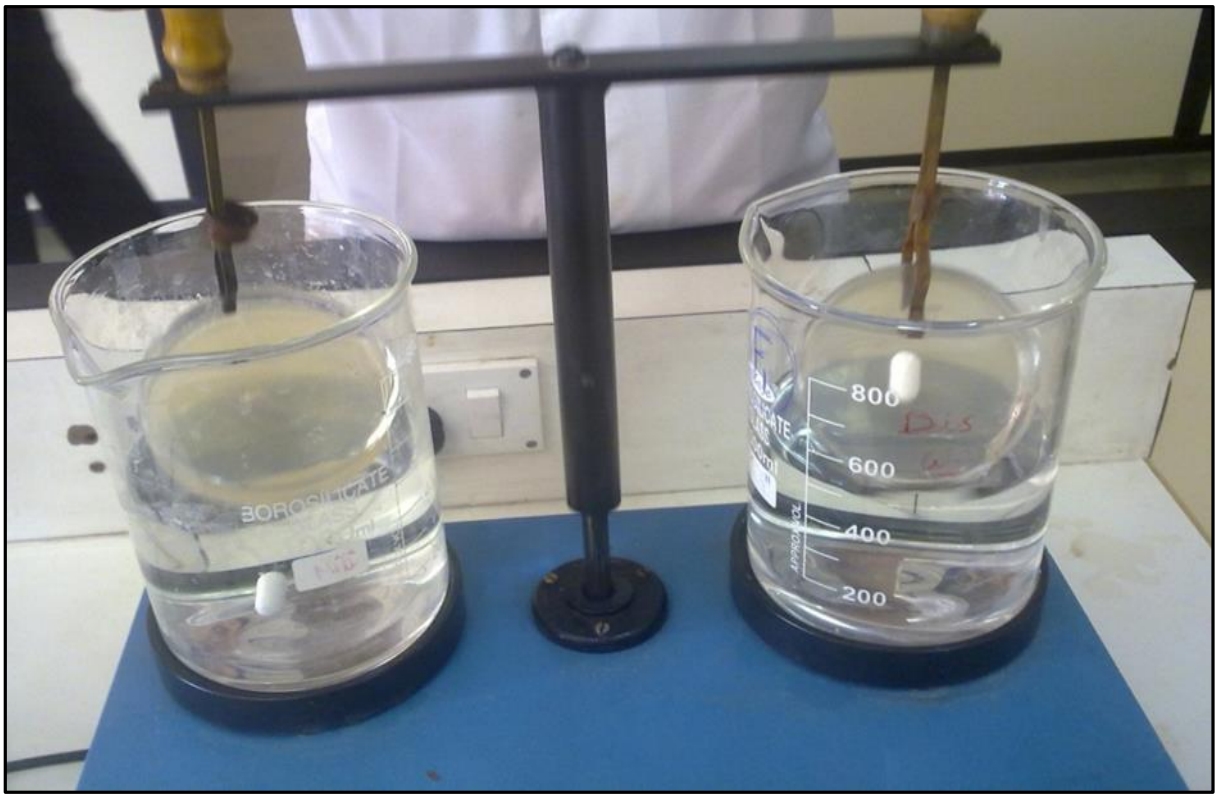


FIGURE 4.1 *In vitro* adhesion study of MH floating matrix tablets, in USP disintegration test apparatus

Five minutes later, the agar plate was attached to a USP disintegration test apparatus and moved up and down in 0.1 N HCl (pH 1.2) at 37 ± 0.5 ºC (Fig. 4.1). The tablet adhered on the plate was immersed into the solution at the lowest point and got out of the solution at the highest point. The retention period of the tablet on the plate was noted visually.

4.2.3.7 Drug Release Studies

Drug release studies of the prepared floating tablets were performed, in triplicate, in a USP Dissolution Tester Apparatus, type- II (Paddle method) at 37 ± 0.5ºC. The paddles were rotated at a speed of 100 rpm, as given in USP (Figure 4.2). The tablets were placed into 900 ml of 0.1N HCl solution (pH 1.2). Aliquots of 5 ml were withdrawn from the dissolution apparatus at different time intervals and filtered through a cellulose acetate membrane (0.45 µm). The drug content was determined spectrophotometrically at a wavelength of 230 nm, as mentioned before. At each time of withdrawal, 5 ml of fresh medium was replaced into the dissolution flask, to maintain the sink condition. The release of the prepared gastroretentive formulations was compared with the theoretical release of the drug using model independent method by calculating similarity and dissimilarity factor[18,19].

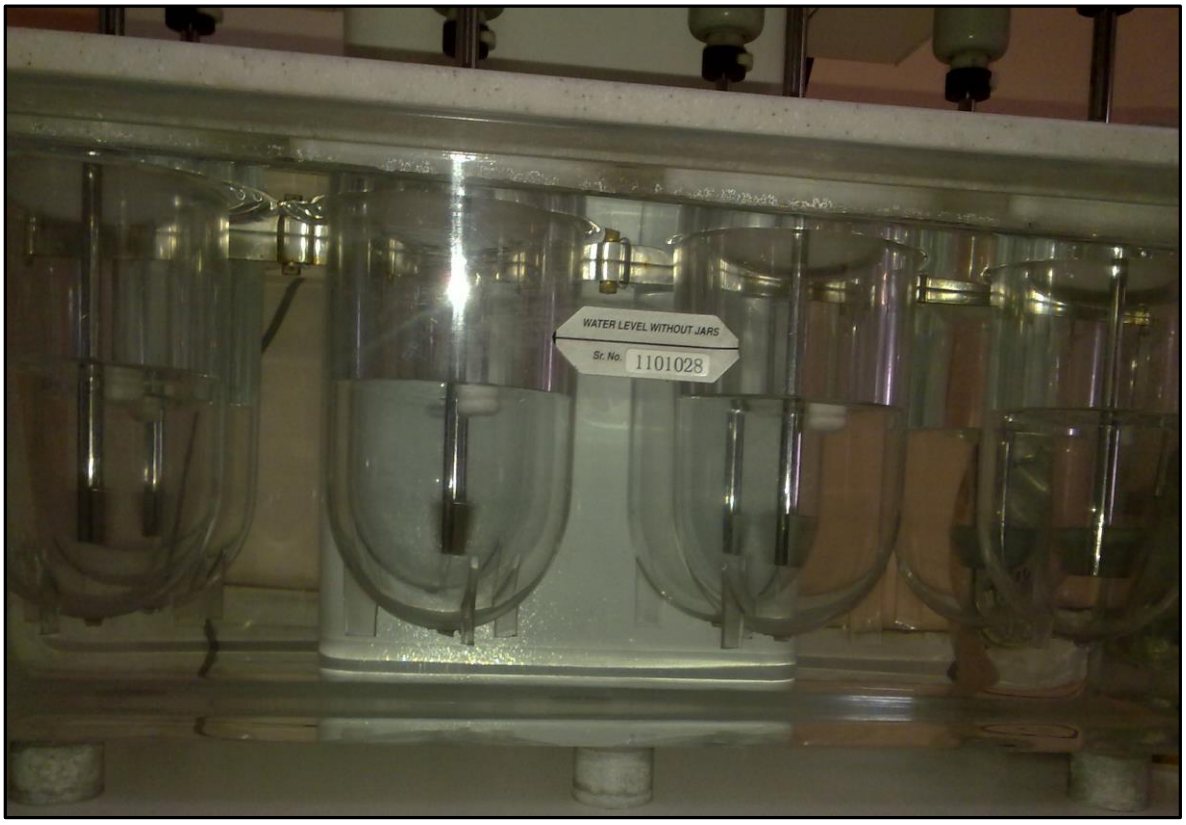


FIGURE 4.2 *In vitro* dissolution of MH floating matrix tablets, in USP dissolution apparatus

Similarity factor means the comparison of resemblance in the release pattern of two comparative formulations. Generally a similarity factor in the range of 50-100 is acceptable according to the US FDA. It can be calculated using the following equation:

$$f2 = 50.\log\{[1 + \frac{1}{n}\sum_{t=1}^{n} Rt - Tt^2]^{-0.5}\, x\, 100\}$$

where, n is the number of dissolution sample times, Rt and Tt are the individual or the mean percent dissolved at each time point, t, for the reference and test dissolution profiles, respectively. It is 100 when two comparative groups of reference and test are identical and approaches 0 as the dissimilarity increases. The dissimilarity factor is calculated by following equation:

$$f1 = \{[S_{t=1}^{n} |R_t - T_t|] / [S_{t=1}^{n} R_t]\} \times 100$$

Dissimilarity factor calculates the difference in percent dissolved between reference and test at various time intervals.

4.2.3.8 Drug Release Kinetics[20]

There are various type of kinetic models, which can be used to analyze the release pattern of the drug from the prepared formulations. The *in vitro* drug release data, for all the formulations prepared by applying SCD design, were graphed for finding release mechanism by zero-order, first-order, Higuchi and Korsmeyer–Peppas kinetic models. The model with the highest correlation coefficient was considered to be the best fitting one.

4.2.4 Drug Excipient Compatibility Study[21]

There is always the possibility of drug polymer interaction in any formulation. To check any such kind of interaction, Fourier-transform infrared spectroscopy (FTIR) study was conducted. The FTIR scan of pure drug (metformin Hydrochloride), polymers (HPMC K15M and kappa carrageenan) and physical mixture of drug-polymer were taken. The pure drug, polymer and physical mixture were separately mixed with IR grade KBr. This mixture was then scanned over a wave number range of 4000 to 400 cm^{-1}.

4.2.5 Experimental Design-Mixture Design

The evolution of a new pharmaceutical formulation by trial and error technique is very much time consuming and also calls for high cost. Due to these causes, the maturation of a novel drug molecule has diverted the pharmaceutical industry to investigate various strategies in the evolution of novel drug delivery systems[22]. The optimization techniques, on the foundation of a few experiments and statistical analysis of the resolutions can provide an effective and economical method for the prognostication of the optimal composition.

Recently, the use of Design of experiment (DoE) has increased immensely in R&D of drug due to its practical applicability in the industry. Even for the development of gastroretentive formulations, statistical designs are applied for the optimization of dosage form. Recent example is the application of DoE for the optimization of floating drug delivery prepared by hot melt extrusion[23]. In the present research, the optimization of metformin gastroretentive matrix tablet was done by applying mixture design. Mixture design has been already explored for the optimization of various type of pharmaceutical preparations[24-28].

4.2.5.1 Simplex Centroid Design

The preliminary studies suggested that floating matrix tablets prepared with the combination of HPMC K15 M and κ-Carrageenan, as release retarding polymers, were releasing the drug for 8hrs and had desired floating characteristics. Hence, these polymers were considered for the formulation of floating matrix tablet of MH. The levels of the independent variable was decided based on the literature survey and by the experimentation done during the preliminary studies.

TABLE 4.2 Factors and their examined levels in Simplex Centroid Design for MH

Independent Variables /Levels	**Amount of HPMC K15M**	**Amount of k-Carrageenan**	**Amount of sodium bicarbonate**
	X_1 (mg)	**X_2 (mg)**	**X_3 (mg)**
Low	150	50	150
High	200	100	200
Dependent Variables	**Y_1** - Floating lag time (sec)(F_{lag}) **Y_2** - Drug released after 1 hour (%) **Y_3** - Time required for 90% (t_{90})		
No. of replicates 4			

Mixture design was applied to optimize the formulations with HPMC K15 M, κ-Carrageenan and sodium bicarbonate as independent elements. Simplex centroid design was applied as the technique for optimization by changing the amount of three factors concurrently and keeping their total concentration constant.

The Simplex Centroid design (SCD) for three-component system is presented by an equilateral triangle in two-dimensional space. In this study, the amounts of matrixing agent,

HPMC K4 M (X_1), release retarding polymer, kappa-Carrageenan (X_2), gas-generating agent, sodium bicarbonate (X_3), were chosen as independent variable. The floating lag time (F_{lag}), drug released after 1 hour and time required for 90% drug release, were claimed as dependent variables (Table 4.2). The design was applied and evaluated using the Design-Expert® Software (version- 9.0.6, Stat-Ease) by running 14 experiments. The composition of the batches formulated by using this statistical design is given in table 4.3.

TABLE 4.3 Composition of floating matrix tablets of MH prepared by applying SCD

Runs	Batch code	Transformed Fractions of Variables*		
		X_1	X_2	X_3
1	M-SCD 1	175	50	175
2	M-SCD 2	158.33	58.33	183.33
3	M-SCD 3	150	100	150
4	M-SCD 4	150	50	200
5	M-SCD 5	183.33	58.33	158.33
6	M-SCD 6	166.67	66.67	166.67
7	M-SCD 7	175	75	150
8	M-SCD 8	158.33	83.33	158.33
9	M-SCD 9	175	75	150
10	M-SCD 10	150	75	175
11	M-SCD 11	150	100	150
12	M-SCD 12	200	50	150
13	M-SCD 13	150	50	200
14	M-SCD 14	200	50	150

***In all the batches, each tablet contained 500mg Metformin, 90mg microcrystalline cellulose and 10mg magnesium stearate. X_1 represents the amount of HPMC K15M (mg); X_2 represents the amount of kappa-carrageenan (mg); X_3 represents the amount of sodium bicarbonate (mg)**

4.2.5.2 Validation of Model

Additional three formulations, suggested by the design expert, were formulated to check and validate the reliability of the mathematical models built here with Simple centroid design.

TABLE 4.4 Formula for validation runs (MH-SCD)

Factors	Composition		
	F 1	F 2	F 3
X_1 : Amount of HPMC K15M (mg)	**191.82**	**185.14**	**183.33**
X_2 : Amount of k-Carrageenan (mg)	**56.73**	**64.62**	**66.66**
X_3 : Amount of sodium bicarbonate (mg)	**151.45**	**150.24**	**150.01**

The prepared formulation was evaluated and the experimentally obtained results were compared to those predicted values obtained by the mathematical models. Table no. 4.4 shows the values of the selected factors used for development of the validation batch, taken from the software, keeping the amount of all other ingredients constant.

4.2.6 Stability Studies

Physical stability study of optimized formulation M-SCD 7 was conducted according to International Conference on Harmonization (ICH) guidelines[29]. Accelerated stability studies were performed at 40ºC ±2ºC and 75 ± 5% relative humidity (RH), for six months (sampling interval 0, 3 & 6 months). After specified time, the tablets were examined for any statistical difference in their physical characteristics, floating characteristics and release pattern (comparison of drug release was done by calculating similarity factor *f*2).

4.3 Result and Discussion

4.3.1 Preliminary Studies

For optimizing the quantity of release retarding polymer, various batches of floating matrix tablet of metformin were prepared, varying amount of HPMC K15M from 10% to 30%, keeping the quantities of drug, microcrystalline cellulose, sodium bicarbonate constant. Results indicated that batches formulated with low concentration of HPMC K15M (10%), got disintegrated in 0.1N HCl and the tablets prepared with highest polymer concentration (30), could not float. It was found that HPMC K15M was giving satisfactory results from the concentration ranging 15-25%. The tablets prepared with 15% of HPMC could float for 3hrs with 10sec lag time. It was concluded that as the concentration of HPMC was increased,

the floating lag time as well as floating time was also increasing. Hence, it was decided to take 17% of HPMC K15M, for further studies to determine the effect of the combination of other polymers with HPMC on gastroretention and drug release of the tablets. The floating matrix tablets of MH were prepared with the composition, as per the table number 4.1. These preliminary batches were evaluated and the results are discussed below.

4.3.2 Evaluation of Preliminary batches

4.3.2.1 Physical Properties of Floating Tablet

The batches prepared as per the table no. 4.1, were evaluated and the results indicated that all the formulations had acceptable physical characteristics. Hardness of all the batches was found to be in the range of 4-5.7 kg/cm^2 (Table 4.5).

TABLE 4.5 Results of the physical evaluation of preliminary batches of MH floating matrix tablets

Batch code	Weight variation	Hardness* (kg/cm^2)	Drug content* (%)	Friability* (%)
F1	Complies	5.7±0.95	98.56±1.25	0.25±0.09
F2	Complies	4.2±0.62	100.94±0.94	0.23±0.12
F3	Complies	4.0±0.28	98.73±1.37	0.13±0.10
F4	Complies	4.7±0.54	102.46±0.59	0.29±0.20
F5	Complies	4.6±0.65	100.98±0.94	0.44±0.16
F6	Complies	5.1±0.98	99.57±0.99	0.13±0.11

***n=3, average of three determinations±SD**

The assay for drug content indicated acceptable content uniformity in the prepared tablets. Drug content were found to be within the limits given in Indian pharmacopoeia (IP). The percentage friability for all batches was less than 1%, indicating good mechanical resistance.

4.3.2.2 *In vitro* Buoyancy Studies

All the formulations had floating lag time less than 31 seconds and floating time, more than 8hrs, as shown in Table 4.6. Previous literature has reported that viscosity of the gel-forming polymer influences the *in vitro* buoyancy[30]. Also, the earlier studies suggests that strength of gel layer changes with the increase in polymer proportion, which in turn will affect

flotation of the tablet[31]. In the present study, variation of the polymers used along with HPMC had no effect on the floating properties of the tablets. This indicates the flotation property of tablets is dependent on the amount HPMC K 15M and sodium bicarbonate. As the amount of these two was same in all the formulations, the floating properties were also similar.

4.3.2.3 Swelling Ability

The swelling indices of all the preliminary batches were found to be in range of 1.734 to 3.864, as shown in table 4.6. Formulation 2, with kappa carrageenan exhibited highest swelling index, which was in agreement to the findings of Dorozynski et al., who revealed that application of mixtures of carrageenan and HPMC, increase the swelling capacity of HBS formulations and suggested that the combination can be directly utilized as a starting point in the development of various controlled release formulations[6].

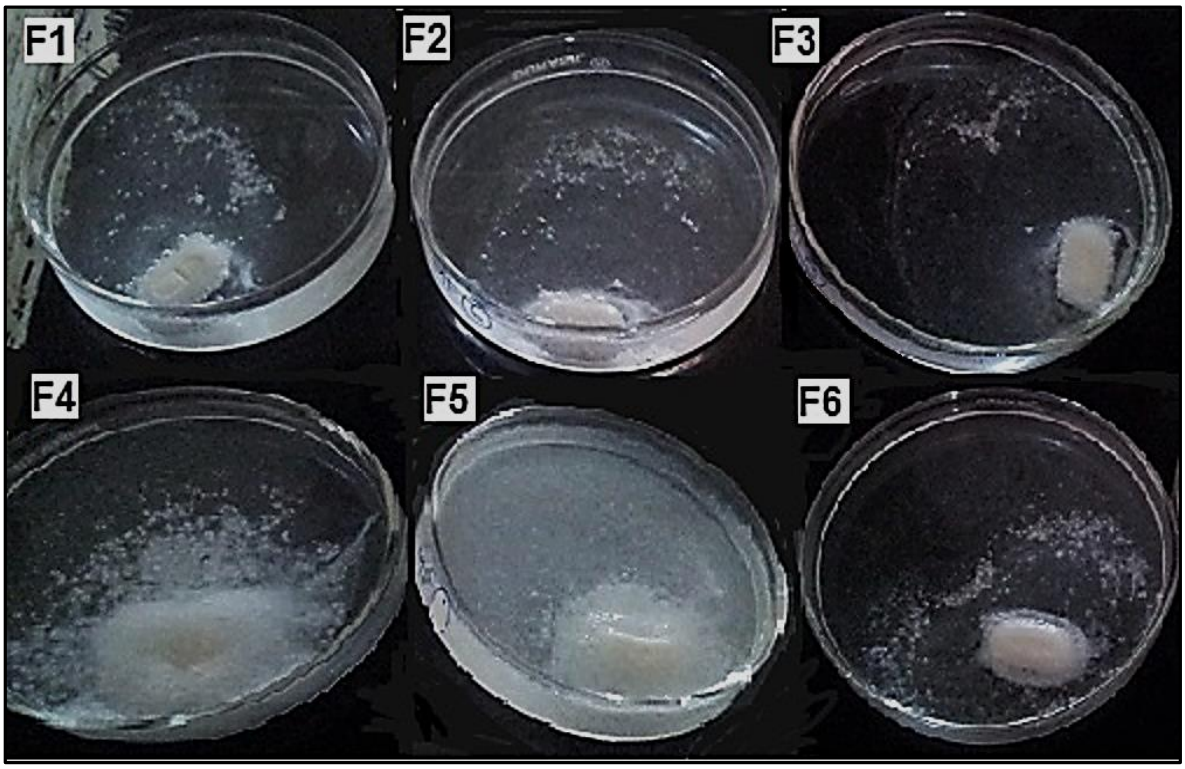


FIGURE 4.3 Swelling index study of preliminary batches of MH floating matrix tablets

This formulation was found to be intact for 8hrs and deformed after 24hrs, showing that the formulation would eventually go out of the stomach after the release of the drug, which is desirable. Minimum swelling index was found for the formulation with sodium alginate which means that the polymer doesn't promote water uptake by polymeric matrices

containing HPMC. This can be attributed to the pH dependent solubility of sodium alginate as suggested by Timmins et.al, where it was proved that sodium alginate hydrates and swells in alkaline pH and doesn't form the gel layer in the stomach[32]. This formulation was intact even after 24 hours, which suggests that formulation will not get eroded eventually (Figure 4.3).

4.3.2.4 Tablet Adhesion Retention Period

Agar plates were used to check the comparative adhesion retention of the prepared formulations as it has negatively charged ions, alike mucin covering the mucous membrane[16]. Agar gel contains large numbers of negative charges because of the presence of carboxyl and sulfate groups present in it.

TABLE 4.6 Results table for buoyancy, swelling ability and tablet retention period for preliminary floating matrix tablets of MH

Formulation	Lag Time*(s)	Floating Time*(h)	Tablet adhesion retention period* (min.)	Swelling index (ratio)	Physical appearance of the tablet after swelling	
					8 h (width)	24 h
F1	15.25 ± 1.20	> 8	18.25 ± 2.41	1.734	Intact 2cm	intact
F2	**10.71 ± 2.36**	**> 8**	**93.50** ± 3.36	**3.864**	**2.2cm**	**deformed**
F3	30.50 ± 3.17	> 8	66.41 ± 3.42	2.755	1.8cm	deformed
F4	12.07 ± 1.70	> 8	42.12 ± 4.25	2.851	deformed	deformed
F5	15.65 ± 2.20	> 8	17.10 ± 2.45	2.501	deformed	deformed
F6	30.75 ± 4.96	> 8	21.41 ± 2.15	2.827	1.7cm	deformed

***n=3, average of three determinations±SD**

The formulations gave the tablet retention between the range of 18 to 94 minutes, as shown in table no 4.6. On performing the comparative adhesion retention period study for prepared tablets, it was found that tablets prepared with HPMC K15M and kappa-carrageenan gave maximum adhesion retention of 93.50 minutes and formulation prepared using sodium alginate and HPMC K15M exhibited minimum adhesion retention period. Formulation with

kappa-carrageenan was retained on the agar plate for a longer period of time as compared to other formulations. The reason is that carrageenans are a high molecular weight sulfated polysaccharides and due to hydrogen bonding or ionic interaction with agar it gives high adhesion period as compared to other polymers[33]. Formulations prepared with poloxamer 188 and sodium alginate showed a minimum retention period as they have lesser ability to interact with agar.

4.3.2.5 Drug Release Studies

The drug release data of the prepared floating matrix tablet is given in table no. 4.7 and the graphical representation of the same is shown in figure no. 4.4. The similarity and dissimilarity factor of the release data was also calculated. The general finding were that, formulations F1, F3 and F6, prepared with HPMC K15M & sodium alginate, HPMC K15M & pullulan and only HPMC K15M, respectively, showed the delayed release of the drug from the matrix. Only 85% of the drug was released from these formulations in 8hrs. Formulation F4 (with xanthan gum) and F5 (with polaxamer 188), released 50 % drug within two hours. Among all the formulations, F2 (with kappa-carrageenan) could sustain the release of the drug for 8hrs.

The results indicated that formulation F2 (formulation with HPMC K15M and kappa-carrageenan), had same release pattern as that of a theoretical release patern of the drug. The f_2 and f_1 value for the formulation F2 was found to be 92% and 1%, respectively. These finding are credited towards the properties of kappa-carrageenan because literature suggests that incorporation of anionic polymers, in HPMC matrices is useful for developing a pH-independent release profile for weakly basic drugs[34]. The present study also revealed that incorporation of kappa-Carrageenan, a poly anionic polymer, in a HPMC matrix of metformin could achieve sustained release of the drug with desired buoyancy.

Another formulation prepared with xanthan gum (F4), could not sustain the release of the drug for more than 6 hours and also had out of range values of f_2 and f_1. These findings were not suppored by the literature. Singh et. al., presented the release behavior of drugs from different natural polymers and gums[35]. The authors reported that the presence of xanthan gum in the formulation can retard the release of the drug. Whereas, during the present study, xanthan gum could not sustain the release of the drug at the concentrations studied.

TABLE 4.7 Results table for *in vitro* drug release and f_2 & f_1 values for preliminary batches of MH matrix tablet*

Time (hrs)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	Theoretical release (%)
0	0	0	0	0	0	0	0
1	33.23±1.23	32.65±0.93	29.16±1.14	33.31±0.76	40.44±0.39	26.75±1.54	35.12±0.52
2	48.49±1.54	44.41±2.01	35.84±0.89	51.23±2.11	52.30±1.67	39.71±2.43	44.38±1.31
3	55.28±1.11	53.70±1.54	45.28±0.87	60.29±0.59	55.90±0.98	54.03±2.44	53.64±0.94
4	63.98±2.18	62.37±2.9	55.34±1.44	71.83±0.92	61.83±0.75	62.72±1.26	62.9±0.91
5	73.19±1.04	71.18±1.1	58.83±2.16	101.98±1.73	69.19±1.11	65.38±1.04	72.16±0.84
6	75.86±2.64	80.94±3.21	68.58±0.78	83.79±0.83	99.15±0.78	70.45±1.52	81.42±2.03
7	80.12±1.54	91.70±0.95	77.32±0.83	79.78±1.23	99.65±1.43	78.97±1.18	90.68±1.54
8	85.56±1.13	100.04±0.82	89.32±0.18	75.9±1.13	90.67±1.32	85.76±1.14	99.94±1.14
Similarity factor (f_2) (%)	58	92	49	41	53	53	-
Dissimilarity factor (f_1) (%)	7	1	15	17	10	11	-

***n=3, average of three determinations±SD**

Further, formulation F5 prepared with poloxamer 188, showed the same release pattern as F4, but the similarity factor was found to be 53%. The inability of poloxamer 188, to sustain the release of the drug, may be because it lacks the ability to form cohesive gel barrier for drug release[36]. Both F5 and F4 released 50% of the drug after 2 hours. Hence, both these polymers doesn't have a good release retarding properties for preparing matrix formulations of hydrophilic drug, at the concentrations studied. This may be because both the polymers and the drug used in the formulation are hydrophilic, hence couldn't withstand the dissolution conditions.

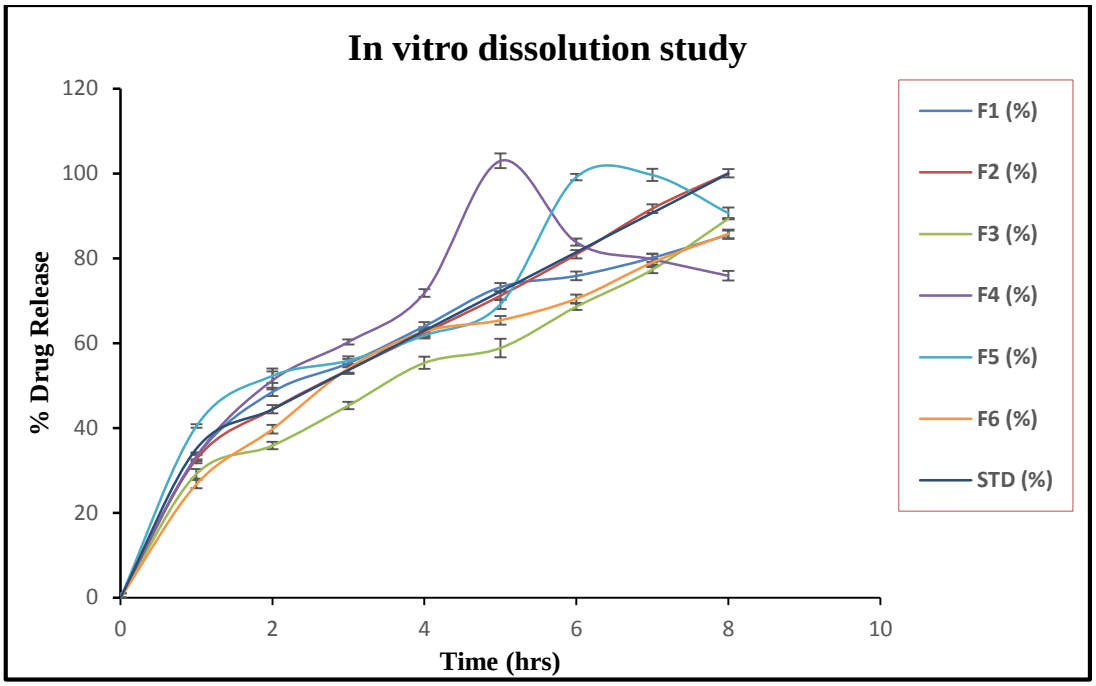


Figure 4.4 *In vitro* dissolution profiles of preliminary batches of floating matrix tablets of MH in 0.1 N HCl*

Next formulation, F6 prepared with only HPMC K15M, showed delayed release of the drug with f_2 as 53%. The findings were contradictory to the earlier outcomes, where it was reported that weakly basic drugs gives high release at lower pH when prepared with HPMC matrix alone[37].

Formulation prepared with HPMC and sodium alginate showed delayed release with $f2$ value as 58%. This may be because of less hydration of sodium alginate and also because in acidic pH it doesn't contribute to the matrix erosion and hence release of the drug[32]. Even though kappa carrageenan and sodium alginate, both are anionic polymers, the release pattern of drug was found to be different in these polymers, which could be due to the change in polymer properties at acidic pH.

Formulation F3 prepared with pullulan did not pass the similarity ratio limit. Literature suggested that Pullulan can be used for various coatings of the formulation[38]. In present research an attempt was made to check the ability of pullulan as release regarding polymer for floating formulation, but Formulations F3, with pullulan also showed delayed release of the drug which was almost same as that of formulation F6, this means that the presence of pullulan doesn't have much effect on the release pattern of metformin from the polymeric matrix system.

Although, all the combinations tried can be optimized by changing the amount of significant variables in the formulations, the batches containing kappa-carrageenan polymer showed

better release as compared to all other formulations. Moreover, the swelling index and adhesion retention of this formulation was better than all other formulations, which ensures the possibility of retention of the formulation in the stomach. Hence, it was decided to optimize the gastroretentive floating matrix tablet of MH using HPMC K 15 M and k-carrageenan as release retarding polymers, by simplex centeroid design.

4.3.3 Drug Excipient Compatibility Study

The FTIR scan of drug, polymers and physical mixture of drug and polymer is recorded in figure 4.5.

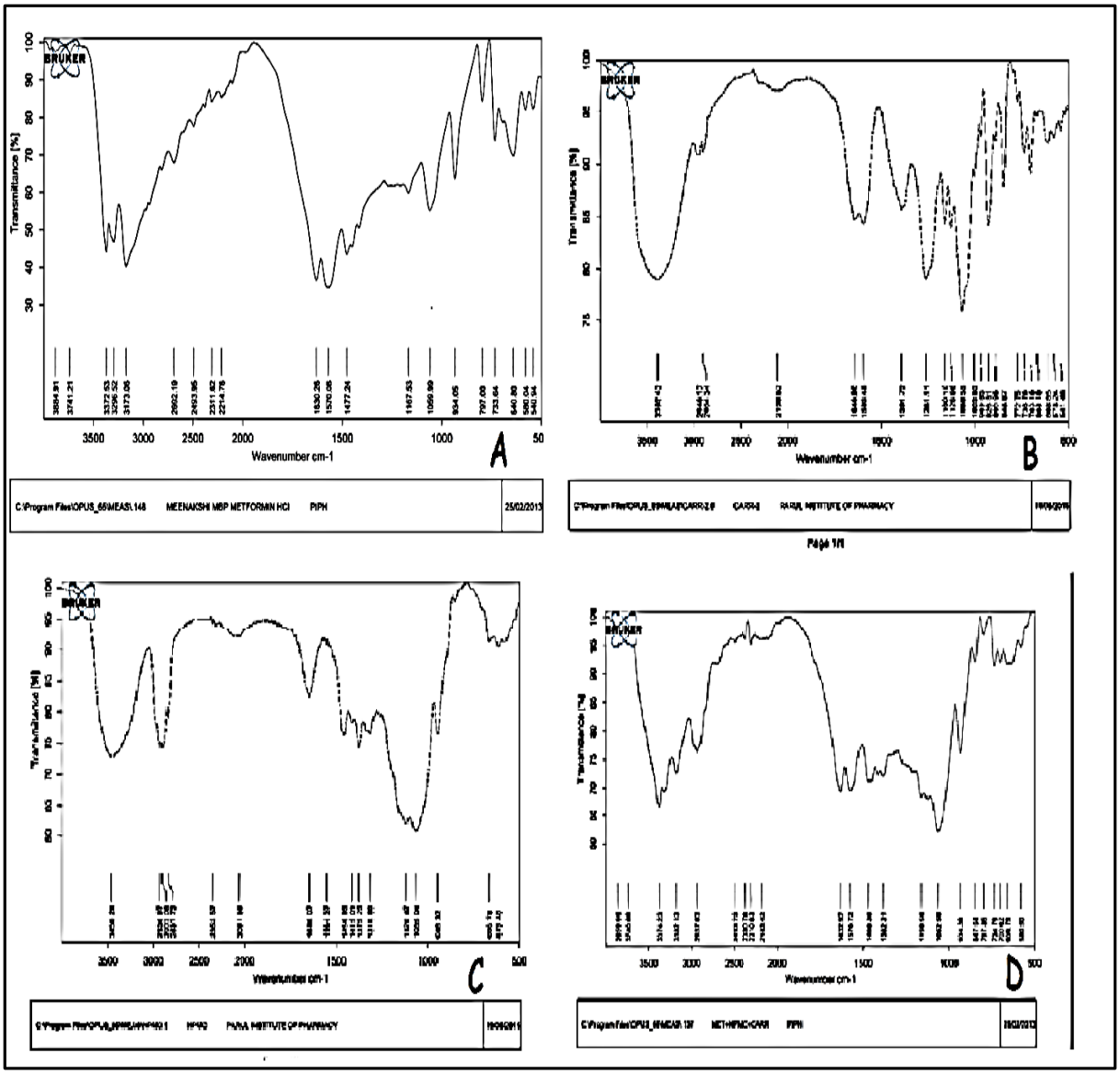


FIGURE 4.5 FTIR scan obtained for metformin hydrochloride (A), kappa carrageenan (B), HPMC K15M (C) and optimized formulation M-SCD 7 (D)

FTIR scan of metformin showed characteristic peaks at 3372.53 cm^{-1}, 3296.52 cm^{-1} and 3173.06 cm^{-1} for N-H asymmetric stretching, N-H symmetric stretching and symmetric N-H stretching, respectively. There was insignificant shift in the peaks observed in the FTIR scan of drug and polymer blend. It showed characteristic peaks at 3374.23 cm^{-1} and peaks at 3182.13 cm^{-1} for N-H asymmetric stretching and symmetric N-H stretching, respectively. Peak at 2692.19 cm^{-1} indicates CH_3 symmetric stretching. Peaks at 1630.26 cm^{-1} and 1477.24 cm^{-1} correspond to CH_3 asymmetric deformation and C = N stretching, respectively. All these peaks were observed in the infrared spectra obtained from drug-polymer blend, which demonstrates that there is no incompatibility observed between the drug and the other polymers.

4.3.4 Mixture Design - Simplex Centroid Design

Based on the results obtained after preliminary studies, it was decided to apply mixture design, for the optimization of gastroretentive floating matrix tablet of metformin. A simplex lattice is an arrangement of equally spaced points on a simplex (Lachman et al., 1970). When described by a polynomial equation the lattice can be referred to as {q, m}, where, q = Number of components, m = Degree of the polynomial, or in other words, the number of proportions assumed by each part. The simplex centroid design (SCD) is based on the same rules as the simplex lattice design, with the exception that the design points are not only equally spaced but now also appear either in equal proportions or zero (on the boundaries). The number of design points is determined by 2q – 1. The design also has an overall centroid containing equal proportions, equally spaced. The results obtained after the evaluation of the batches prepared by applying SCD is discussed further and the statistical analysis of the achieved results was also done. The results of pre-compression studies of all the formulations showed that the powder blends had good flow properties.

4.3.5 Physical Properties of Floating Tablets of MH

The results of the physical evaluation of MH floating tablets, prepared by applying SCD, are recorded in table 4.8. All the prepared formulations complied the weight uniformity study. The hardness of all the batches was found to be in the range of 4.1 to 5.7. Drug content of all the batches was within the limits prescribed by IP. The percentage friability for all formulae was less than 1%, indicating good mechanical resistance. Tablets from all the

prepared batches floated for more than 8 hours, but formulations M-SCD 12 and M-SCD 14 were found to sink in between the flotation time study, this may be due to the high concentration of HPMC K15M and minimum level of the gas generating agent.

The tablet adhesion retention time was in the range of 64.22 to 120.30 minutes. It was found that as the amount of kappa carrageenan increased in the formulations, the tablet retention also increased, which was expected because Carrageenan is high molecular weight sulfated polysaccharides and its high adhesion period may be due to hydrogen bonding or ionic interaction with agar. However, increased levels of sodium bicarbonate decreased the tablet adhesion retention period.

TABLE 4.8 Results of the physical properties of the MH floating matrix tablets prepared by applying SCD*

Batch code	Weight variation	Hardness (kg/cm^2)	Drug content (%)	Friability (%)	Floating Time (hrs.)	Tablet adhesion retention period (min.)	Swelling index (ratio)
M-SCD 1	Conforms	4.1±0.28	98.92±0.94	0.23±0.16	> **8**	74.25±2.54	2.36
M-SCD 2	Conforms	5.7±0.95	100.91±0.43	0.22±0.17	> **8**	73.37±4.43	2.32
M-SCD 3	Conforms	5.7±0.43	99.04±0.74	0.29±0.08	> **8**	120.30±3.67	3.41
M-SCD 4	Conforms	5.2±0.55	99.62±0.31	0.29±0.21	> **8**	69.52±2.44	2.23
M-SCD 5	Conforms	5.2±0.95	99.43±0.65	0.13±0.14	> **8**	81.41±2.31	2.56
M-SCD 6	Conforms	4.7±0.54	99.56±0.42	0.19±0.11	> **8**	88.43±1.53	2.82
M-SCD 7	Conforms	4.7±0.38	100.83±0.27	0.23±0.10	> **8**	97.52± 5.42	3.16
M-SCD 8	Conforms	4.8±0.75	99.56±0.29	0.24±0.08	> **8**	102.34± 2.42	2.91
M-SCD 9	Conforms	4.6±0.65	100.18±0.54	0.31±0.28	> **8**	96.55±1.96	3.10
M-SCD 10	Conforms	4.2±0.62	99.16±0.63	0.23±0.12	> **8**	85.27±1.42	2.98
M-SCD 11	Conforms	5.2±0.67	100.54±0.54	0.32±0.09	> **8**	118.24± 2.09	3.51
M-SCD 12	Conforms	4.8±0.34	100.3±0.51	0.32±0.18	> **8**	85.20± 2.43	3.01
M-SCD 13	Conforms	5.6±0.95	101.17±0.41	0.14±0.10	> **8**	64.22±3.72	2.18
M-SCD 14	Conforms	4.6±0.62	100.46±0.95	0.32±0.13	> **8**	80.21±1.87	2.94

***n=3, average of three determinations±SD**

Swelling index was found to be in the range of 2.18 to 3.51. In this case, it was also observed that the increase in amount of kappa carrageenan increased the swelling index. This is

credited to the capacity of the polymer to get hydrated quickly and high water uptake, which eventually cause the swelling of the polymeric matrix.

4.3.6 *In vitro* Drug Release Study

The *in vitro* drug release study was performed for all the matrix floating tablets prepared by applying simplex centroid design. The drug release data is given in table no. 4.9 and the graphical representation of the same is shown in figure no. 4.6. The similarity and dissimilarity factor for all the formulations was also calculated. It was observed that formulation M-SCD 3, 8 and 10 could sustain the release of the drug for 8 hours but the release pattern was different than that of the theoretical release pattern of the drug. The similarity factor was below 50 %. All these formulations had lower amount of HPMC K15M and higher amount of k-carrageenan, which indicates that the amount of HPMC K15M should be more (+1 coded value) to get the desired release pattern of drug from the formulation.

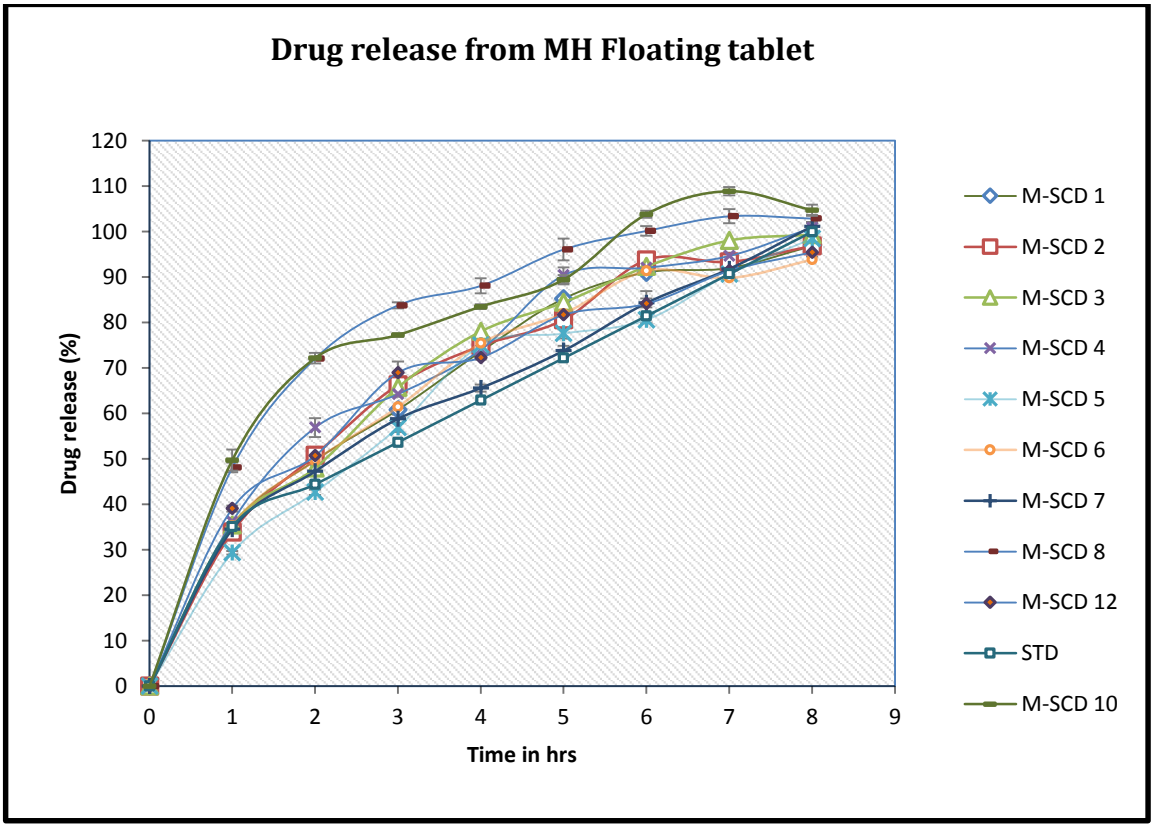


FIGURE 4.6 *In vitro* dissolution profiles of SCD batches of MH, in 0.1 N HCl

TABLE 4.9 Results Table for *in vitro* Drug Release and f_2 & f_1 Values for SCD Batches of MH Matrix Tablet*

Time	M-SCD 1 (%)	M-SCD 2 (%)	M-SCD 3 (%)	M-SCD 4 (%)	M-SCD 5 (%)	M-SCD 6 (%)	M-SCD 7 (%)	M-SCD 8 (%)	M-SCD 10 (%)	M-SCD 12 (%)	Theoretical release (%)
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1	35.06±1.23	33.92±0.93	35.47±1.14	36.20±0.76	29.37±0.39	35.27±0.43	34.54±1.05	48.09±1.06	49.75±2.31	39.09±0.66	35.12±0.52
2	49.63±0.72	50.76±2.01	47.98±0.89	56.88±2.11	42.67±0.58	49.63±0.72	47.25±2.43	72.05±0.29	72.16±1.18	50.69±0.72	44.38±1.31
3	60.78±0.94	66.27±0.79	65.74±0.87	64.24±0.59	56.92±0.98	61.40±1.73	58.80±0.94	83.77±0.69	77.25±0.39	68.96±2.43	53.64±0.94
4	73.57±0.52	74.84±0.83	78.04±1.44	74.00±0.92	74.99±0.75	75.43±0.52	65.59±0.77	88.07±1.65	83.49±0.52	72.27±0.44	62.9±0.91
5	85.22±1.04	80.58±0.92	84.42±2.16	90.40±1.73	77.58±1.11	81.92±1.04	73.80±1.04	96.06±2.36	89.42±1.04	81.70±0.93	72.16±0.84
6	90.98±0.78	93.75±0.42	92.32±0.78	92.04±0.83	80.59±0.78	91.37±1.52	84.29±0.99	100.14±1.03	103.83±0.78	84.13±2.81	81.42±2.03
7	91.94±1.54	93.30±0.95	98.05±0.83	94.64±0.71	90.64±0.79	89.77±1.32	91.80±1.29	103.37±1.54	108.87±0.93	91.39±1.54	90.68±1.54
8	96.81±1.13	96.82±0.82	99.32±0.18	100.86±1.13	98.46±1.18	93.80±1.14	101.15±0.99	102.87±0.82	104.69±1.26	95.46±0.92	99.94±1.14
(f_2) (%)	58	56	33	52	66	58	79	36	37	57	-
(f_1) (%)	9	11	23	13	6	10	3	29	29	10	-

Formulations M-SCD 11, 13, 9 and 14 were duplicate batches of M-SCD 3, 4, 7, 12, respectively. Hence, their *in vitro* drug release data is not presented in the table.
***n=3, average of three determinations±SD**

All other formulations had the similarity and dissimilarity factor in the standard range. Among all the formulations M-SCD 7 had maximum similarity 79% and minimum dissimilarity factor 3%. The formulation had medium (0 coded value) amount of X_1 (HPMC K15M) and X_2 (kappa-carrageenan) and minimum (-1 coded value) amount of X_3 (sodium bicarbonate) factor. The results are further illustrated in statistical analysis. Batches M-SCD 9, 11, 13 and 14 were duplication batches, hence the release profile of those batches is not given in the table 4.9 and the graphical representation of the same is given in the figure 4.6.

4.3.7 *In vitro* Drug Release Kinetics

Model dependent release kinetics describes the mechanisms of overall release of drug from the dosage forms. The results for the analysis of model-dependent drug release kinetics is given in table 4.10.

TABLE 4.10 Results table for *in vitro* drug model-dependent kinetics of MH tablets prepared by applying SCD

Batch code	Higuchi model (R_H)	Korsmeyer Peppas model (R_P)	Hixson Crowell model (R_{HC})	First order (R_1)	Zero order (R_0)
M 1	0.9913	0.9965	0.9923	0.9765	0.9365
M 2	0.9879	0.9938	0.9772	0.9663	0.9257
M 3	0.9917	0.9898	0.9939	0.9201	0.9282
M 4	0.9883	0.9838	0.9747	0.9284	0.9094
M 5	0.9873	0.9788	0.9651	0.8529	0.9203
M 6	0.9869	0.997	0.9904	0.9774	0.9305
M 7	0.9956	0.9962	0.9769	0.8147	0.9138
M 8	0.9483	0.9636	0.9188	0.9584	0.8112
M 9	0.9646	0.9618	0.7576	0.9144	0.8202
M 10	0.9876	0.9782	0.9541	0.9733	0.8643

The model dependent approaches evaluated for the drug release kinetics were zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas[39-42]. The release from these floating matrix tablets of MH was found to follow Higuchi diffusion model with R^2 value

close to 1, for the period of 8hours. This model is generated by plotting cumulative percentage drug release versus square root of time and is applicable for modified dosage forms especially to matrix drug delivery systems.

4.3.8 Statistical Analysis

The result of all the dependent variables is given in table 4.11. A statistical model incorporating 14 interactive terms was used to assess the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3$$

Where, Y is the dependent variable, b_0 is the arithmetic mean response of the 14 runs, and bi is the estimated coefficient for the factor Xi. The main effects (X_1, X_2, and X_3) represent the average result of changing one element at a time from its low to high value. The interaction terms (X_1X_2, X_2X_3, X_1X_3, and $X_1X_2X_3$) give the information about how the response changes when two or more factors are simultaneously modified.

TABLE 4.11 Results of dependent factors chosen for SCD design for MH floating matrix tablets*

Runs	Batch code	Floating lag time (sec)	Drug released after 1 hr (%)	Time required for 90% (hrs)
1	M-SCD 1	10.31±1.43	35.05±1.23	5.2±0.72
2	M-SCD 2	5.88±1.19	33.92±0.93	5.76±0.22
3	M-SCD 3	5.32±1.73	35.47±1.14	5.84±0.31
4	M-SCD 4	8.54±1.32	36.20±0.76	4.99±0.42
5	M-SCD 5	15.17±1.65	29.37±0.39	6.95±0.58
6	M-SCD 6	12.43±2.11	35.27±0.43	5.91±0.27
7	M-SCD 7	15.77±1.98	34.54±1.05	6.86±0.32
8	M-SCD 8	10.43±1.92	48.09±1.06	4.68±0.53
9	M-SCD 9	12.71±1.58	34.32±1.05	6.82±0.35
10	M-SCD 10	42.49±3.17	49.75±2.31	5.03±0.46
11	M-SCD 11	10.53±1.49	34.77±1.14	5.92±0.30
12	M-SCD 12	45.66±2.43	39.09±0.66	6.89±0.63
13	M-SCD 13	5.76±1.08	36.84±0.76	5.03±0.41
14	M-SCD 14	45.91±3.12	38.79±0.66	6.76±0.63

***n=3, average of three determinations±SD**

The values for F_{lag}, a drug released in 1 hour, and t_{90} for all 14 batches (M-SCD1- M-SCD14) is presented in table 4.11.

The outcomes indicated that the values of subject variables are dependent on the chosen independent variables. All the formulations gave satisfactory floating lag time in the range of 5 to 46 seconds, which means that the chosen independent variables had no significant effect on the floating lag time. The formulations gave percentage drug release in 1 hour in the range of 29.37 to 49.75%. The formulations released 90% of the drug in the time range of 4.68 to 6.95hrs.

TABLE 4.12 Analysis of Variance table for dependent variables with Simple centroid design model for MH floating matrix tablets

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	P-value
Floating lag time (sec) (F_{lag})					
Model	2801.58	6	466.93	29.89	0.0001
Residual	109.34	7	15.62		
Corrected Total	2910.93	13			
Drug released after 1 hour (%)					
Model	384.88	8	48.11	1932.94	< 0.0001
Residual	0.12	5	0.025		
Corrected Total	385.01	13			
Time to release 90% of drug (t_{90})					
Model	8.92	8	1.11	354.38	< 0.0001
Residual	0.016	5	3.146E-003		
Corrected Total	8.93	13			

Using analysis of variance (ANOVA), the significance ($p < 0.05$) of the ratio of mean square variation due to the regression coefficient, and the residual error were tested (Table 4.12). The special Cubic Mixture model was found to be significant for floating lag time, whereas Special Quartic Mixture model was followed by other two responses. The high values of correlation coefficients for F_{lag} ($R^2 = 0.9624$), drug release at 1hr ($R^2 = 0.9997$), and t_{90} ($R^2 = 0.9982$) indicated a good fit (ie, good agreement between the dependent and independent variables). Lack of Fit F-value for Y_1, Y_2 and Y_3 was found to be about 0.0675, 0.4934 and

0.4358 respectively, which suggests the desirable, insignificance of Lack of Fit. The detailed statistical analysis of all the responses was done individually, as discussed further.

4.3.8.1 Floating Lag Time

The result can be expressed for model analysis by Special Cubic Mixture model. The fitted equation for the responses are given as follows:

$$F_{lag} = +45.17X_1 + 7.22X_2 + 5.73X_3 - 51.21X_1X_2 - 66.59X_1X_3 + 133.70X_2X_3 - 388.68X_1X_2X_3$$

The polynomial equations can be applied to find the conclusions after looking at the magnitude of coefficient and the mathematical sign it carries (i.e. positive or minus). By looking into the above equation, it is apparent that all the three factors, Amount of HPMC K15M (X_1), kappa-carrageenan (X_2) and sodium bicarbonate (X_3) show positive effects on floating lag time of the formulated floating tablets of metformin. But it was observed that X_1 had significant effect on the lag floating time. This means, more the concentration of HPMC K15M, more time floating time is experienced by the formulation. The three way interaction was found to be more significant and the proper combination of the three variables is required to get the desired minimum floating lag time. Observed and predicted values of the floating lag time were found to be comparable, which additional validates the suitability of the model. The three dimensional response surface graphs for a floating lag time are given in Figure 4.7 shows the obtained contour plot (1a) and response surface plots (1b). This gives the information about the main and interaction effects of the independent components.

Looking into the results of F statistics, it was observed that model probability was greater than F value i.e. 29.89, which confirms the significance of the model. There is only a 0.12% chance that an F-value this large could occur due to noise. Significance of the model was also proved by the p-value less than 0.0500. In this case X_1, X_1X_2, X_2X_3, $X_1X_2X_3$ are significant model terms.

4.3.8.2 Drug released After 1 hour

The results of ANOVA for the applied model on percentage drug released after 1 hour are shown in Table 4.12. Looking into the results of F statistics, it was observed that model probability was greater than F value i.e. 1932.94, which confirms the significance of the

model. There is only a 0.01% chance that an F-value this large could occur due to noise. Significance of the model was also proved by the p-value less than 0.0500.

In this case X_1, X_2, X_3, X_1X_2, X_2X_3, $X_1^2X_2X_3$, $X_1X_2^2X_3$, $X_1X_2X_3^2$ are significant model terms. The predicted and adjusted R^2 values were found to be 0.9941 and 0.9992 respectively, that means the difference was less than 0.2, which shows the good agreement between dependent and independent variables. The result can be expressed for model analysis by Special Quartic Mixture model using following equation:

$$Drug\ released\ after\ 1hr\ = +38.88X_1 + 35.47X_2 + 36.10X_3 - 10.60X_1X_2 - 9.71X_1X_3 + 55.91X_2X_3 - 699.29X_1^2X_2X_3 + 916.03X_1X_2^2X_3 - 656.40X_1X_2X_3^2$$

There is strongest synergistic effect shown by a ternary interaction of $X_1X_2X_3$ at higher level of kappa carrageenan (X_2). This means that as the concentration of X_2 is more in the three dimensional plane, the percentage of drug released after 1hr will increase, which can be attributed to the rapid hydration and erosion of kappa carrageenan. The regression coefficient obtained for Y_2 was 0.9997, which shows that the model is best fitted. Here also, observed and predicted values for percentage drug released after 1 hour were found to be comparable, which additional validates the suitability of the model. The three dimensional response surface graphs for percentage drug released after 1 hour are given in Figure 4.7. It shows the obtained contour plot (2a) and response surface plots (2b). This give the information about the main and interaction effects of the independent components.

4.3.8.3 Time to Release 90% of Drug

The results of ANOVA for the applied model on time to release 90% of drug are shown in Table 4.11. Looking into the results of F statistics, it was observed that model probability was greater than F value i.e. 354.38, which confirms the significance of the model. There is only a 0.01% chance that an F-value this large could occur due to noise. Significance of the model was also proved by the p-value less than 0.0500. In this case X_1, X_2, X_3, X_1X_2, X_2X_3, $X_1^2X_2X_3$, $X_1X_2^2X_3$, $X_1X_2X_3^2$ are significant model terms.

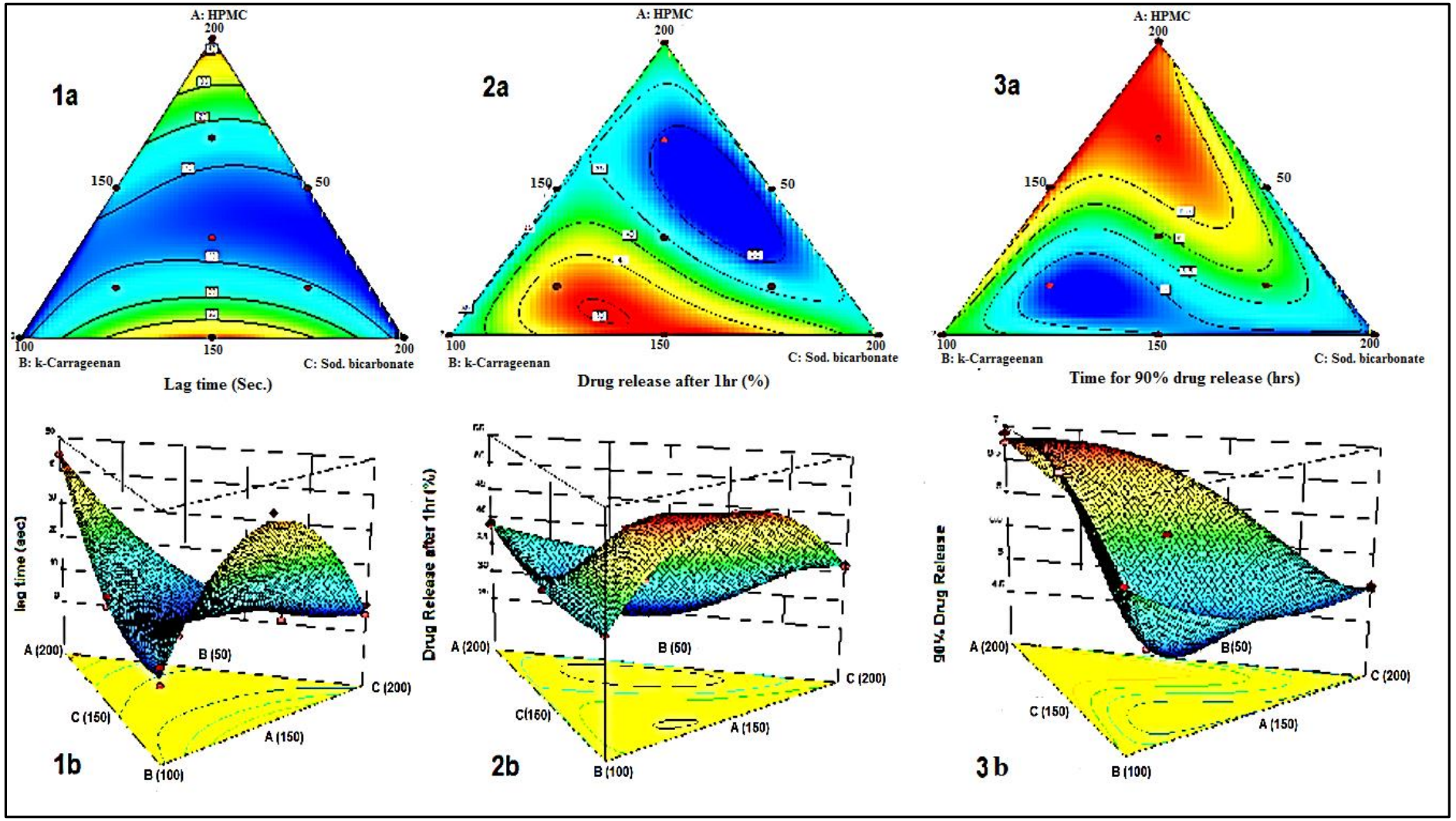


FIGURE 4.7 Contour plot and Response surface plot for MH-SCD batches: (1) lag time (2) Drug released after 1hr (3) Time to release 90% of drug; a-contour plot; b-surface response plot.

The predicted and adjusted R^2 values were found to be 0.9609 and 0.9954 respectively, that means the difference was less than 0.2, which shows the good agreement between dependent and independent variables. The result can be expressed for model analysis by Special Quartic Mixture model using following equation:

$$t_{90} = +6.82X_1 + 5.88X_2 + 5.01X_3 + 1.94X_1X_2 - 2.89X_1X_3 - 1.68X_2X_3 + 74.85X_1^2X_2X_3 - 130.17X_1X_2^2X_3 - 76.96X_1X_2X_3^2$$

By looking into the above equation, it is evident that all the three factors, Amount of HPMC K15M (X_1), kappa-carrageenan (X_2) and sodium bicarbonate (X_3) show positive effects on time to release 90% of drug of the prepared floating tablets of metformin. There is strongest antagonistic effect shown by a ternary interaction of $X_1X_2X_3$ at higher level of kappa carrageenan (X_2). This means that as the concentration of X_2 is more in the three dimensional plane, the time required for the release of 90% drug will decrease, which means early release of the drug from the formulation due to rapid hydration and erosion property of kappa carrageenan. The regression coefficient obtained for Y_3 was 0.9997, which shows that the model is best fitted. The three dimensional response surface graphs for time to release 90% of drug are given in Figure 4.7, shows the obtained contour plot (3a) and response surface plots (3b). This give the information about the main and interaction effects of the independent components.

4.3.9 Validation of Model

Additional three formulations, suggested by the design expert, were formulated to check and validate the reliability of the mathematical models built here with Simple centroid design. These check point batches were prepared according to the formula given by design expert and then evaluated for getting the experimental values of responses. The comparison between the experimental and predicted values was done. The actual and predicted values of the responses is shown in Table no. 4.13 and it is evident that the predicted values were close to the actual values which validates the model successfully.

TABLE 4.13 Predicted and actual values of the responses for validation run (Check point batches) in SCD design for MH

Responses	F1		F2		F3	
	Predicted values	**Actual values**	**Predicted values**	**Actual values**	**Predicted values**	**Actual values**
Floating lag time (sec)	30.786	33.74	22.946	24.33	21.145	22.74
Drug released after 1 hr (%)	35.560	35.31	35.516	35.19	35.392	34.97
Time required for 90% (hrs)	6.940	6.89	6.940	6.79	6.940	6.84

To optimize all the above responses with different targets, a numerical optimization technique by the desirability function and a graphical optimization technique by the overlay plot was used. The overlay plot gives the regions not meeting the specifications as greyed out, leaving an operating window or sweet spot in yellow colour (Figure 4.8). This means that within the yellow region the formulation prepared will give desired lag time and release profile. The optimized formulation was obtained by applying constraints on dependent variable responses and independent variables. The constraints for the responses, floating lag time, drug released after 1hr and t_{90} were set as minimum, 34% to 36% and 6-7 hrs, respectively. The recommended concentrations of the independent variables were calculated by the Design Expert software from the plots with highest desirability near to 1.0. It is evident from the overlay plot that the (-1) coded value of gas generating agent, coded value of 0 to 1, of HPMC K15 M and 0 (coded value) of kappa carrageenan is fulfilling the desirability criteria and the formulations prepared in this region would give the desired gastroretention and sustained release of MH. However, other studies showed that the presence of high amount kappa carrageenan increases the adhesion retention period and swelling index of the formulation, which insures the presence of the formulation in stomach even with low level of fluid by enabling swelling and mucoadhesion technique.

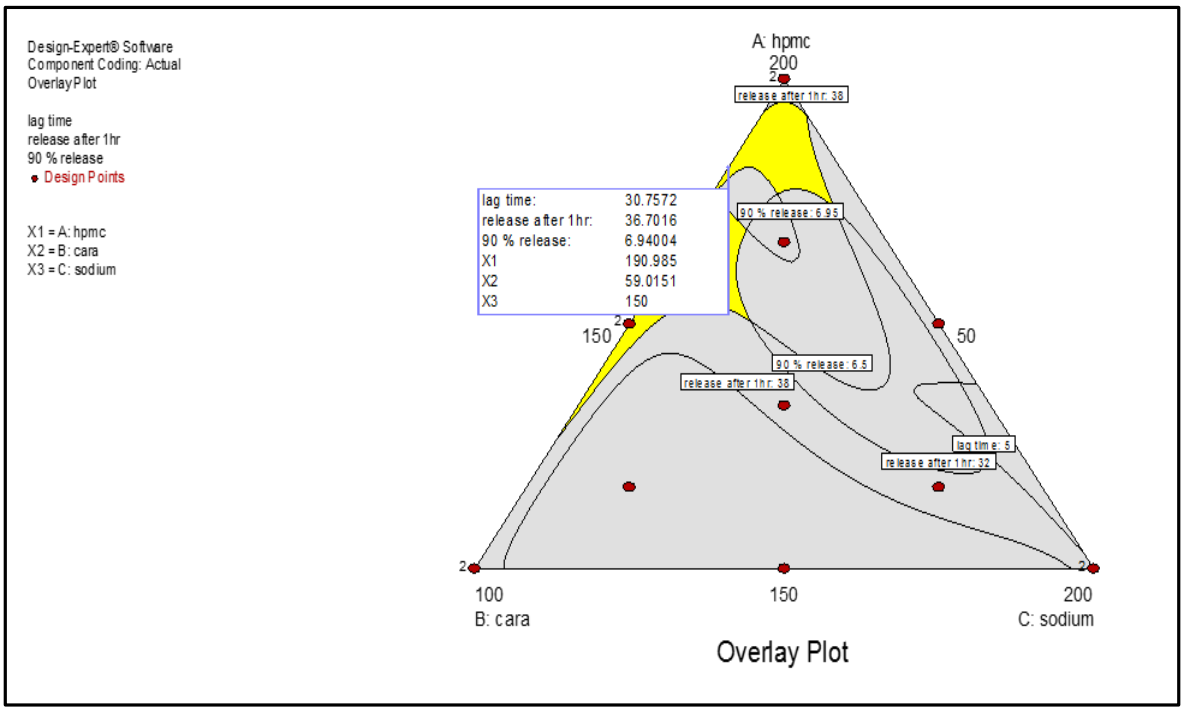


FIGURE 4.8 Overlay plot of MH floating tablets from SCD design

It was found that the fomulation M-SCD 7 and M-SCD 9 (with same composition) fullfilled the desiarablity criteria and hence can be considered as optimized formulation. Moreover, the formulation showed resonably high adhesion retention period and swelling index desirable for ensuring the retetion of formulation in stomach.

4.3.10 Stability Studies

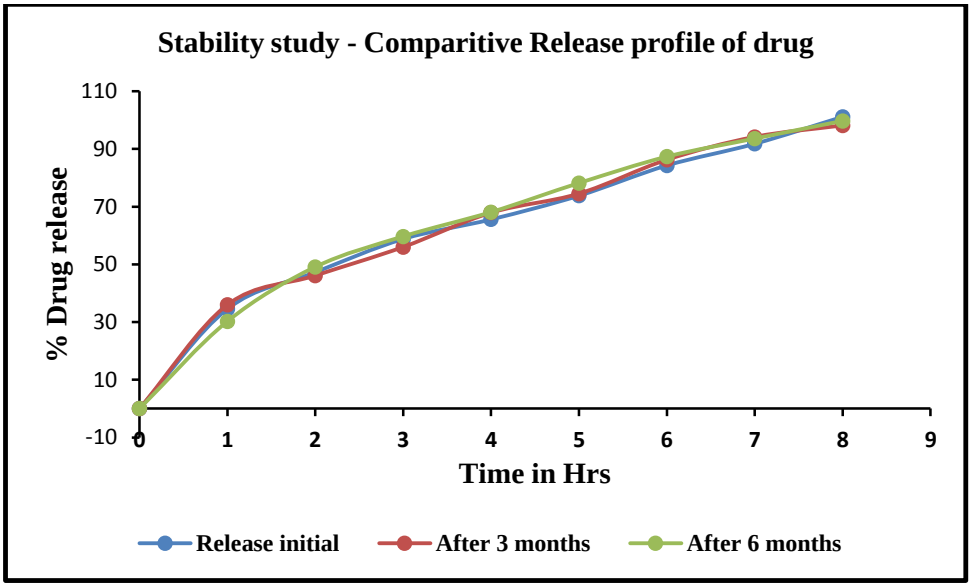


FIGURE 4.9 Comparison of drug release from MH optimized formulation M-SCD7 Initially, after 3 and 6 months of accelerated stability study.

The results obtained after three and six months of accelerated stability study of optimized MH gastroretentive floating matrix tablet showed no significant change in the physical properties. The release pattern of the formulation, before and after the stability study is given in figure 4.9. The release showed that the release pattern of the optimized formulation had 90% similarity ($f2$) with the formulation after three and six months of stability study (Acceptable range of $f2$ is 50-100%). The variation in the release pattern was insignificant. Hence, it can be concluded that formulation M-SCD7 has good stability when stored at 40ºC under 75% RH for 6 months.

4.4 Conclusion

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 like, sodium alginate, pullulan, kappa carrageenan, xanthan gum, poloxamer 188. Prepared formulations were evaluated for swelling, floating adhesive period and drug release. All the tablets showed acceptable physicochemical properties but, the formulation F_2 (prepared with HPMC K15M and kappa carrageenan) showed 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.

The basic mechanism of the gastroretention for the formulation is floatation but in case of low level of fluid in the stomach, the mechanisms like mucoadhesion and swelling can retain the formulation at the required site, which can be better achieved by formulation with kappa-carrageenan. It has been already reported that the gastrorentive formulations prepared by using the carrageenans can modify the properties of polymeric matrices, to obtain tailor-made materials for drug delivery systems. Hence, this combination of polymers was further evaluated by applying a statistical mixture design, using HPMC K15M, kappa carrageenan and sodium bicarbonate as independent variables.

A simplex centeroid design was applied to inspect the combined effect of the three variables in the formulations.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. Results revealed that there was strongest synergistic and antagonistic effect shown by a ternary interaction of $X_1X_2X_3$ at higher level of kappa carrageenan (X_2) on amount of drug released in 1hr and t_{90}, respectively. The overlay plot displayed that (-1) coded value of gas generating agent, coded value of 0 to 1, of HPMC K15 M and 0 (coded value) of kappa carrageenan is fulfilling the desirability criteria and the formulations prepared in this region would give the desired gastroretention and sustained release of MH.

Formulation M-SCD 7 with the quantities as X_1 175mg, X_2 75mg, X_3 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 of 97.52 ± 5.42 minutes and good swelling index as 3.16, which ensures the retention of formulation in the stomach. Hence, it was concluded that the mixture of kappa carrageenan and HPMC K 15M could give the desired release pattern of the drug by changing the polymer concentration. 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.

4.5 References

1. Stepensky, D., Friedman, M., Srour, W., Raz, I. and Hoffman, A., 2001. Preclinical evaluation of pharmacokinetic–pharmacodynamic rationale for oral CR metformin formulation. *Journal of Controlled Release*, 71(1), pp.107-115.

2. Li, H., Hardy, R.J. and Gu, X., 2008. Effect of drug solubility on polymer hydration and drug dissolution from polyethylene oxide (PEO) matrix tablets. *AAPS PharmSciTech*, *9*(2), pp.437-443.

3. Colombo, P., Bettini, R., Massimo, G., Catellani, P.L., Santi, P. and Peppas, N.A., 1995. Drug diffusion front movement is important in drug release control from swellable matrix tablets. *Journal of Pharmaceutical Sciences*, *84*(8), pp.991-997.

4. Pillay, V. and Fassihi, R., 2000. A novel approach for constant rate delivery of highly soluble bioactives from a simple monolithic system. *Journal of Controlled Release*, *67*(1), pp.67-78.

5. Bettini, R., Catellani, P.L., Santi, P., Massimo, G., Peppas, N.A. and Colombo, P., 2001. Translocation of drug particles in HPMC matrix gel layer: effect of drug solubility and influence on release rate. *Journal of Controlled Release*, *70*(3), pp.383-391.

6. Dorożyński, P., Kulinowski, P., Mendyk, A. and Jachowicz, R., 2011. Gastroretentive drug delivery systems with l-dopa based on carrageenans and hydroxypropylmethylcellulose. *International Journal of Pharmaceutics*, *404*(1), pp.169-175.

7. Kshirsagar, R.V., Jain, V. and Wattamwar, S., 2009. Effect of different viscocity grade HPMC polymers on gastroretantive drug delivery of Metformin. *International Journal of Applied Pharmaceutics*, 1(1), pp. 44-50.

8. Mandal, U. and Pal, T.K., 2008. Formulation and *in vitro* studies of a fixed-dose combination of a bilayer matrix tablet containing metformin HCl as sustained release and glipizide as immediate release. *Drug Development and Industrial Pharmacy*, *34*(3), pp.305-313.

9. Rawlins EA. Bentley's text book of pharmaceutics. London, England: Cassell and Collier Macmillan. 1977.

10. Defang, O., Shufang, N., Wei, L., Hong, G., Hui, L. and Weisan, P., 2005. *In vitro* and *in vivo* evaluation of two extended release preparations of combination metformin and glipizide. *Drug Development and Industrial Pharmacy*, *31*(7), pp.677-685.

11. Tadros, M.I., 2010. Controlled-release effervescent floating matrix tablets of ciprofloxacin hydrochloride: Development, optimization and *in vitro–in vivo* evaluation in healthy human volunteers. *European Journal of Pharmaceutics and Biopharmaceutics*, *74*(2), pp.332-339.

12. The Indian Pharmacopoeia, Indian Pharmacopoeia Commission, Ministry of Health & family welfare, Government of India, 2007, pp.193.

13. Dorożyński, P., Jachowicz, R., Kulinowski, P., Kwieciński, S., Szybiński, K., Skorka, T. and Jasiński, A., 2004. The macromolecular polymers for the preparation of hydrodynamically balanced systems—methods of evaluation. *Drug Development and Industrial Pharmacy*, *30*(9), pp.947-957.

14. Patel, A., Modasiya, M., Shah, D. and Patel, V., 2009. Development and *in vivo* floating behavior of verapamil HCl intragastric floating tablets. *AAPS PharmSciTech*, *10*(1), pp.310-315.

15. Jiménez-Castellanos, M.R., Zia, H. and Rhodes, C.T., 1994. Design and testing *in vitro* of a bioadhesive and floating drug delivery system for oral application. *International Journal of Pharmaceutics*, *105*(1), pp.65-70.

16. Nakamura, F., Ohta, R., Machida, Y. and Nagai, T., 1996. *In vitro* and *in vivo* nasal mucoadhesion of some water-soluble polymers. *International Journal of Pharmaceutics*, *134*(1-2), pp.173-181.

17. Perioli, L., Ambrogi, V., Pagano, C., Scuota, S. and Rossi, C., 2009. FG90 chitosan as a new polymer for metronidazole mucoadhesive tablets for vaginal administration. *International Journal of Pharmaceutics*, *377*(1), pp.120-127.

18. Yuksel, N., Kanık, A.E. and Baykara, T., 2000. Comparison of *in vitro* dissolution profiles by ANOVA-based, model-dependent and-independent methods. *International Journal of Pharmaceutics*, *209*(1), pp.57-67.

19. Costa, P. and Lobo, J.M.S., 2001. Modeling and comparison of dissolution profiles. *European Journal of Pharmaceutical Sciences*, *13*(2), pp.123-133.

20. Dash, S., Murthy, P.N., Nath, L. and Chowdhury, P., 2010. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica*, 67(3), pp.217-23.

21. Pani, N.R., Nath, L.K., Acharya, S. and Bhuniya, B., 2011. Application of DSC, IST, and FTIR study in the compatibility testing of nateglinide with different pharmaceutical excipients. *Journal of Thermal Analysis and Calorimetry*, *108*(1), pp.219-226.

22. Colombo, P., Bettini, R., Massimo, G., Catellani, P.L., Santi, P. and Peppas, N.A., 1995. Drug diffusion front movement is important in drug release control from swellable matrix tablets. *Journal of Pharmaceutical Sciences*, *84*(8), pp.991-997.

23. Vo, A.Q., Feng, X., Morott, J.T., Pimparade, M.B., Tiwari, R.V., Zhang, F. and Repka, M.A., 2016. A novel floating controlled release drug delivery system prepared by hot-melt extrusion. *European Journal of Pharmaceutics and Biopharmaceutics, 98*, pp.108-121.

24. Duangjit, S., Mehr, L.M., Kumpugdee-Vollrath, M. and Ngawhirunpat, T., 2014. Role of simplex lattice statistical design in the formulation and optimization of microemulsions for transdermal delivery. *Biological and Pharmaceutical Bulletin*, *37*(12), pp.1948-1957.

25. Gohel, M.C., Parikh, R.K., Aghara, P.Y., Nagori, S.A., Delvadia, R.R. and Dabhi, M.R., 2009. Application of simplex lattice design and desirability function for the formulation development of mouth dissolving film of salbutamol sulphate. *Current Drug Delivery*, *6*(5), pp.486-494.

26. Suhesti, T.S., Fudholi, A. and Martien, R., 2016. Application of Simplex Lattice Design for the Optimization of the Piroxicam Nanosupensions Formulation using Evaporative Antisolvent Technique. *International Journal of Pharmaceutical and Clinical Research*, *8*(5), pp.433-439.

27. Mandlik, S.K., Adhikari, S. and Deshpande, A.A., 2012. Application of Simplex Lattice Design in Formulation and Development of Buoyant Matrices of Dipyridamole. *Journal of Applied Pharmaceutical Science*. 2(12): pp.107-111.

28. Thakkar, A., Patel, N., and Patel, M.B., 2014. Formulation and optimization of acyclovir floating tablet. *Journal of Pharmaceutical Science and Bio-scientific Research, 4*, pp.216-221.

29. Matthews, B.R., 1999. Regulatory aspects of stability testing in Europe. *Drug Development and Industrial Pharmacy*, *25*(7), pp.831-856.

30. Rao, M.R., Sonar, G.S., Mandsaurwale, R.R. and Vanshiv, S.D., 2014. Evaluation of effervescent floating matrix tablet formulations of salbutamol sulfate using full factorial design. *Asian Journal of Pharmaceutics,* 3(1), 43-49.

31. Puthoori, H., Murthy, T.E.G.K. and Kaushik, A., 2012. Formulation and evaluation of floating tablets of niacin for sustained release. *Asian Journal of Pharmaceutics*, *6*(1), pp.31-37.

32. Timmins, P., Delargy, A.M. and Howard, J.R., 1997. Optimization and characterization of a pH-independent extended-release hydrophuic matrix tablet. *Pharmaceutical Development and Technology*, *2*(1), pp.25-31.

33. Campo, V.L., Kawano, D.F., da Silva, D.B. and Carvalho, I., 2009. Carrageenans: Biological properties, chemical modifications and structural analysis–A review. *Carbohydrate Polymers*, *77*(2), pp.167-180.

34. Tatavarti, A.S., Mehta, K.A., Augsburger, L.L. and Hoag, S.W., 2004. Influence of methacrylic and acrylic acid polymers on the release performance of weakly basic drugs from sustained release hydrophilic matrices. *Journal of Pharmaceutical Sciences*, *93*(9), pp.2319-2331.

35. Singh, A., Sharma, P.K. and Malviya, R., 2011. Release behavior of drugs from various natural gums and polymers. *Polimery w Medycynie*, *41*(4), pp.73-80.

36. Nayak, A.K., Malakar, J. and Sen, K.K., 2010. Gastroretentive drug delivery technologies: Current approaches and future potential. *Journal of Pharmaceutical Education and Research*, *1*(2), pp.1-10.

37. Tatavarti, A.S. and Hoag, S.W., 2006. Microenvironmental pH modulation based release enhancement of a weakly basic drug from hydrophilic matrices. *Journal of Pharmaceutical Sciences*, *95*(7), pp.1459-1468.

38. Izutsu, Y., Sogo, K., Okamoto, S. and Tanaka, T., Dainippon Pharmaceutical Co., Ltd., 1987. *Pullulan and sugar coated pharmaceutical composition*. U.S. Patent 4,650,666.

39. Franz, R.M., Browne, J.E. and Lewis, A.R., 1988. Experiment design, modeling and optimization strategies for product and process development. *Pharmaceutical Dosage Forms: Disperse Systems*, 1, pp.427-519.

40. Higuchi, T., 1963. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *Journal of Pharmaceutical Sciences*, 52(12), pp.1145-1149.

41. Hixson, A.W. and Crowell, J.H., 1931. Dependence of reaction velocity upon surface and agitation. *Industrial & Engineering Chemistry*, 23(10), pp.1160-1168.

42. Korsmeyer, R.W., Gurny, R., Doelker, E., Buri, P. and Peppas, N.A., 1983. Mechanisms of solute release from porous hydrophilic polymers. *International Journal of Pharmaceutics*, 15(1), pp.25-35.

CHAPTER 5A

Gastroretentive Floating Matrix Tablet of Mitiglinide (MTG)

5A.1 Introduction

Meglitinide analog, Mitiglinide is a mildly acidic drug with the pKa 4.45. It remains unionized in acidic environment, hence gets absorbed from the stomach. Recently, it was found that mitiglinide is better absorbed via the stomach and the gastric absorption was delayed when the abdominal pH was higher than 5 pH[1,2]. Hence, it has a strong rationale for preparing the gastroretentive formulation to maintain the level of drug in the blood for improved treatment of type II diabetes mellitus.

In the present study, an attempt was made to prepare the floating matrix tablets of MTG by effervescence mechanism. The tablets were prepared using the combination of HPMC K15M with other ionic and anionic polymeric substances. The approach applied, was same as used for the formulation and optimization of floating matrix tablet of metformin. Various anionic and non-ionic polymers used in the present work are sodium alginate, kappa carrageenan, pullulan, xanthan gum and poloxamer 188.

The final optimization of floating MTG tablet was done by applying 3^2 full factorial design using sodium alginate and HPMC K15M as independent variable. Full factorial design has been explored by many researchers for the optimization of floating gastroretentive tablets[3,4]. This statistical design allows the investigator to study the effect of individual factor on the response variable, as well as the effects of interactions between factors on the response variable. With the help of factorial designs, the time required to develop a pharmaceutical dosage form is effectively decreased by limiting the number of experimental trials. It encompasses study of all factors in maximum possible combinations and thereby the most effective method to measure influence of individual and combination of variables in minimum number of experiments[5]. With the factorial designs one can control the

independent variables while the responses obtained are dependent. The design was employed and evaluated using the Design-Expert® Software (version- 9.0.6, Stat-Ease) by running 10 experiments.

5A.2 Experimental Studies

5A.2.1 Method of Preparation of MTG Floating Matrix Tablets

Tablets containing 10mg of mitiglinide calcium dihydrate (MTG) were made by direct compression technique[6-8]. Using the ingredients and their quantities mentioned in table 5A.1. The required quantity of drug, cross linking polymers (HPMC K15M and sodium alginate/kappa carrageenan/ pullulan/ xanthan gum/ poloxamer 188) and PVP K30 (dry binder) and gas generating agent (sodium bicarbonate), were sieved through sieve number #80 to break the lumps and also for proper blending of powder. The powder blends were thoroughly mixed in a mortar by following geometric order. Then, the required quantities of microcrystalline cellulose and magnesium stearate were added and the mixture was filled in plastic bottle. These bottles were placed in double cone blender and the equipment was run for 1 minute. The powder blends were evaluated for pre-compression characteristics and finally the powder was compressed to prepare tablets, on rotary tablet compression machine using 7 mm round and flat punches with the hardness of 5 kg/sq.cm.

5A.2.2 Preliminary Studies

Selection of a suitable polymer for the development of floating matrix drug delivery system is challenging. The polymer should have the capacity to make the tablet float over the gastric fluid. Moreover, it should be able to make a cohesive gel barrier and also possess the power to provide the sustained release of drugs[9]. The effervescent floating system is widely being used for developing floating matrix drug delivery system[10, 11]. The matrices are made with gas generating agent, which in the presence of acidic environment, liberates carbon dioxide. Carbon dioxide gets entrapped in the gel forming hydrocolloid polymers, present in the formulation. This produces an upward motion of the dosage form and polymeric matrix maintains its buoyancy[12].

The release of the drug, from floating matrix tablet, is controlled by the entry of water into the matrices and ability of the polymer to form cohesive gel barrier. Hydroxy propyl methyl

cellulose (HPMC) has been widely used to prepare the polymeric floating matrix systems. In present research work, formulations were prepared using HPMC K15M as release retarding and gel forming polymer. The quantity of the polymer was taken based on the previous studies carried out by the researcher and the available literature review[13, 14]. The combination of HPMC K15M with other release retarding polymers (sodium alginate, kappa carrageenan, pullulan, xanthan gum, poloxamer 188), was tried for preparing floating matrix tablet of MTG. The composition of preliminary batches is given in table 6A.1. The prepared formulations were evaluated for post compression characteristics of floating gastroretentive matrix tablet.

TABLE 5A.1 Composition (in mg) of preliminary batches of MTG Floating Matrix Tablets.

Sr No	Ingredients	MT1	MT2	MT3	MT4	MT5	MT6
1	MTG	10	10	10	10	10	10
2	PVP K30	10	10	10	10	10	10
3	HPMC K15M	60	60	60	60	60	80
4	Sodium bicarbonate	15	15	15	15	15	15
5	Sodium Alginate	20	-	-	-	-	-
6	κ-Carrageenan	-	20	-	-	-	-
7	Pullulan	-	-	20	-	-	-
8	Xanthan gum	-	-	-	20	-	-
9	Poloxamer 188	-	-	-	-	20	-
10	MCC	33.5	33.5	33.5	33.5	33.5	33.5
11	Mg stearate	1.5	1.5	1.5	1.5	1.5	1.5

5A.2.3 Evaluation of Gastroretentive floating matrix tablet

Same as that of chapter 4

5A.2.4 Drug Excipient Compatibility Study[15]

There is always the possibility of drug polymer interaction in any formulation. To check any such kind of interaction, Fourier-transform infrared spectroscopy (FTIR) study was conducted. The FTIR of pure drug (MTG), polymers (HPMC K15M and sodium alginate) and optimized formulation of MTG floating matrix tablet were taken. The pure drug, polymer and physical mixture were separately mixed with IR grade KBr. This mixture was then scanned over a wave number range of 4000 to 400 cm^{-1}.

5A.2.5 Experimental Design – Two Factor Three Level Full Factorial Design

An important issue in the development of gastroretentive floating matrix tablet, with sustained release of drug, is to design an optimized pharmaceutical formulation within a short time period and with minimum trials. For this purpose, a computer optimization technique, based on a response surface methodology (RSM) utilizing a polynomial equation can be helpful[16,17]. RSM was developed by Box and Wilson to access the significance of important process parameter on the responses[18]. Response surface methodology uses contour plot (2-D visual) and responses surfaces (3-D visual) representation facilitate optimization with the help of empirical model equation relating the independent variables with dependent variables[19-21]. It was decided to apply two factor and three level full factorial design of experiment to optimize the gastroretentive floating matrix tablets of MTG. Statistically, a full factorial design consists of the experimental design of two or more factors, each with discrete possible values or "levels", and whose experimental units take on all possible combinations of these levels across all such factors. This design helps the researcher to study the effect of each factor on the response variable, as well as the effects of interactions between factors on the response variable. In the present research, the Design expert software was used to run the experiment and to obtain the optimized formulation.

5A.2.6 Optimization of Floating Matrix Tablet of MTG by 3^2 Full Factorial Design

The preliminary studies suggested that controlled release formulations prepared with the combination of HPMC K15 M and sodium alginate, as release retarding polymers were giving satisfactory release, hence these polymers were considered for the formulation of buoyant matrix tablet of MTG. The levels of the independent variable was decided based on the literature survey and by the experimentation done during the preliminary studies. For the

optimization of floating matrix tablet of MTG, a full factorial design was applied to study the effect of independent variables on dependent variables[22].

TABLE 5A.2 Levels of process parameters used in 3^2 full factorial design for MTG

Independent Variable	Level		
	Upper level (1)	Medium level (0)	Lower level (-1)
Concentration of HPMC K 15 M (X_1)	60 mg	55 mg	50 mg
Concentration of Sodium alginate (X_2)	30 mg	25 mg	20 mg
Dependent Variables: **Y_1 – Floating Lag time** **Y_2 - time required for 50% drug release (t_{50})** **Y_3 - time required for 90% drug release (t_{90})**			

TABLE 5A.3 Coded and actual values of the MTG floating tablets prepared by applying 3^2 full factorial design

Formulation	Coded value		Actual value	
	X_1	X_2	HPMC K15M X_1 (mg)	Sodium alginate X_2 (mg)
M 1	1	0	60	25
M 2	-1	0	50	25
M 3	1	1	60	30
M 4	-1	-1	50	20
M 5	-1	1	50	30
M 6	0	0	55	25
M 7	1	-1	60	20
M 8	0	1	55	30
M 9	0	0	55	25
M 10	0	-1	55	20

All the batches contained 10 mg MTG, 10 mg PVP K 30, 15mg sodium bicarbonate, 1.5 mg magnesium stearate and microcrystalline cellulose q.s. to make total weight of 150mg per tablet

A 3^2 factorial design with three level and two factor was applied for the optimization of gastroretentive floating microsponges of MTG. 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 10 experiments (5A.2).

The levels of factors were decided based on the preliminary studies and the literature available. The levels of the process parameters are given in Table 5B.2. The coded and actual values of the batches prepared by applying 3^2 full factorial design is given in table 5A.3.

5A.2.7 Validation of Model – 3^2 Factorial Design for MTG Floating Matrix Tablets

An additional formulation, suggested by the design expert, was formulated to check and validate the reliability of the mathematical models built here with full factorial design. The check point batch was prepared with the level of X_1 (HPMC K15M) and X_2 (Sodium alginate) as -0.60 and 1, respectively. The quantities of other ingredients were kept same as that of the batches prepared in factorial design. The check point batch was evaluated and the results obtained experimentally were compared to those predicted by the mathematical models. To validate the chosen experimental design, the experimental values of the responses were quantitatively compared with predicted values and, the relative error (%) was calculated using the following equation.

$$Relative\ error\ (\%) = \frac{Predicted\ value - Experimental\ value}{Predictive\ value}\ X\ 100$$

5A.2.8 Stability Studies

Physical stability study of optimized formulation M-3 was conducted according to International Conference on Harmonization (ICH) guidelines[23]. Accelerated stability studies were performed at 40ºC ±2ºC and 75 ± 5% relative humidity (RH), according to the current International Conference on Harmonization (ICH) guidelines for six months. After specified time, the tablets were examined for any statistical difference in their physical characteristics, floating characteristics and release pattern.

5A.2.9 *In vivo* Radiographic Studies

The gastroretentive formulation has to be evaluated for its gastroretentive property *in vivo*. There are various techniques like, radiographic study, gastroscopy, gamma scintillography, magnetic marker monitoring, etc. available to confirm the gastroretention of the formulation[24]. The *in vivo* radiographic studies were conducted on healthy albino rabbits (n=3) weighing 2.0 kg to 2.2 kg. The protocol (BIP/IAEC/2015/05) for this 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). Gastroretentive floating matrix tablet was prepared by incorporating the X-ray opaque material in the optimized formula by replacing MTG with barium sulphate and keeping all other ingredients constant[25]. The amount of the X-ray opaque material in the optimized formula was kept sufficient to ensure visibility by X-ray, but at the same time the amount of barium sulphate was low enough to enable the formulation to float. After overnight fasting, the formulation was given to albino rabbit for *in vivo* X-ray imaging study. A radiograph was taken just before the administration of the tablet, at zero hour, to ensure the absence of radio-opaque material in the stomach. During the study the rabbit was not allowed to eat, but water was available freely and the X-ray images were snapped after 4hrs and 12hrs to monitor the gastroretention of optimized floating matrix tablets[26, 27].

5A.3 Results and Discussion

5A.3.1 Preliminary Studies

The gastroretentive floating matrix tablet of MTG was prepared with HPMC K15M as release retarding polymer in combination with other polymers. The effect of the polymer blend was checked on the floatation characteristics of the tablets and also on the release pattern of the drug from the matrix.

The effect of the physical evaluation of the prepared dosage forms is presented in table 5A.4. All the formulations, except MT5 complied with the weight variation study. Formulation MT5, prepared with HPMC K15M and Poloxamer 188 was forming flakes during the punching, hence did not pass the weight variation study. Hardness of all the batches was found to be in the range of 4.8 - 5.4 kg/cm^2 except for MT5, which was bending during the hardness testing. The drug content in all the batches was found to be in the range of 89.43%

to 100.86%. The minimum drug content was observed in the tablet containing poloxamer 188. The friability was found to be less than 0.5% that complies the limits given in IP (Table 5A.4).

TABLE 5A.4 Results of preliminary batches of MTG gastroretentive floating matrix tablets

Batch code	Weight variation	Hardness* (kg/cm^2)	Drug content* (%)	Friability* (%)	Lag Time*(s)	Floating Time*(h)
MT1	Complies	4.9±0.35	99.32±1.74	0.28±0.17	30.43 ± 2.87	> 12
MT2	Complies	5.2±0.73	100.86±1.65	0.27±0.39	15.76 ± 1.72	3
MT3	Complies	5.4±0.23	100.73±1.43	0.13±0.10	15.34 ± 3.43	8†
MT4	Complies	4.8±0.29	100.31±0.98	0.39±0.07	20.76 ± 3.87	10
MT5	Doesn't Comply	bending not breaking	89.43±2.87	0.15±0.03	22.64 ± 2.97	> 12
MT6	Complies	5.3±0.36	99.53±1.78	0.24±0.06	120.4 ± 4.71	> 12

***n=3, average of three determinations±SD, † Tablets were sinking in between the study**

5A.3.1.1 *In vitro* Floatation Studies

The *in vitro* floating lag time of all the formulations was found to be in the range of 15.34±3.43 to 120.4±4.71 seconds. The formulation MT6, prepared with only HPMC K15 M, had the maximum floating lag time, probably because of delayed gel layer formation due to slow hydration of the polymer[28,29]. All other formulations had reasonably same floating lag time.

The flotation time of all the formulations was found to be ranging from 3 hours to 12 hrs, which means that the polymers had significant effect on the floating time of tablets. The unanticipated results obtained was in the formulation MT2, prepared with HPMC K15M and kappa carrageenan. The formulation disintegrated within 3 hours, hence could not float

beyond that time, the same is visible in figure 5A.1 - D. The same combination was used for preparing the optimized floating gastroretentive matrix tablet of MH and with MTG the properties changed completely. The reason for such finding was supported by the literature, which suggests that kappa carrageenan forms brittle and stiff gel in the presence of calcium ions. Hermansson et.al., checked the effect of potassium, sodium and calcium on the microstructure and rheological behavior of kappa-carrageenan gels and found that calcium-kappa-carrageenan formed relatively weak gels in a limited calcium concentration range, whereafter salting-out effects were observed[30]. As the antidiabetic drug used in the present work, is in calcium salt form, the calcium ions react with kappa carrageenan and hence disrupts its gelling property.

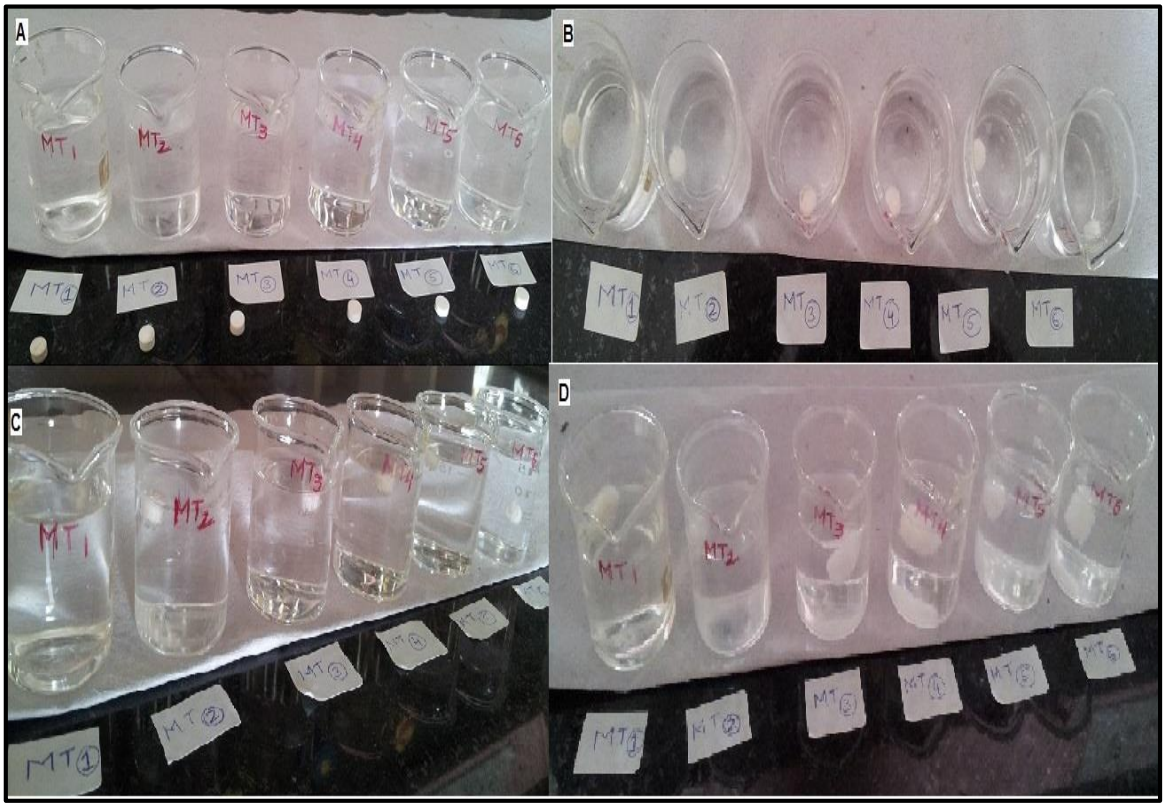


FIGURE 5A.1 *In vitro* floatation study of preliminary batches of MTG floating matrix tablets

Formulation MT3, prepared with HPMC K15M and pullulan could float for only 8 hours. Moreover, the tablets were sinking on and off during the study duration, which may be because pullulan was not able to get hydrated and swell properly. Formulations, MT1 (prepared with HPMC K15M and sodium alginate), MT5 (prepared with HPMC K15M and xanthan gum) and MT6 (prepared with only HPMC K15M), gave maximum floating time of 12hrs. Overall, it was apparent from the buoyancy studies that the presence of other

release retarding polymer in combination with HPMC K15M had a drastic effect on the flotation behavior of formulations, as indicated in Table 5A.4. The pictorial representation of *in vitro* floatation behavior of preliminary batches of MTG floating matrix tablets is shown in figure 5A.1.

5A.3.1.2 Drug Release Studies of Preliminary Batches

The release study of the preliminary batches of MTG floating matrix tablet, prepared with combination of polymers was performed. The release study was conducted in 500ml of 0.1N HCl under all the standard conditions prescribed by Indian Pharmacopoeia (IP).
At regular intervals the samples of the dissolution fluid were withdrawn and analyzed by high performance liquid chromatography (HPLC). The details of the method is given in chapter 3 of preformulation studies. The sink condition was maintained in the dissolution apparatus by replacing the withdrawn dissolution fluid with fresh 0.1N HCl. The tabulated release from the preliminary batches is given in table 5A.5 and the graphical representation of the same is given in Figure 5A.2.

The results of *in vitro* drug release studies of MTG matrix tablet were different than the findings of floating matrix tablet of metformin, prepared using same polymer combination. This change can be attributed to the properties of drug, for which the formulation is developed. Results of formulation MT2 (formulation with HPMC K15M and kappa-carrageenan) were totally opposite to that of findings of chapter 4, where this combination of polymers showed the best floating and sustained release characters. Antagonistically, in present study, this polymer blend could not withstand the conditions of dissolution medium and got dissolved completely after first hour of drug release study. After, rigorous literature search, the reason for such findings was understood. Such outcome was probably because of the salt form of the mitiglinide, the presence of calcium ions was responsible for this result. The literature supporting the finding has been given during the discussion of floatation studies of the same batches.

Formulation MT3 was prepared with HPMC K15M and pullulan as literature supported the release retarding property of pullulan[31]. During the present study the formulation could sustain the release of the drug till 7 hours. This outcome may be attributed to the gelling capacity of release retarding polymer, as the literature suggests that difference in the release

pattern of the drug is because of the type and amount of the adjuvants[32]. At our experimental concentration of polymers, the MT3 could not sustain the release beyond the said duration.

TABLE 5A.5 *In vitro* drug release data of preliminary batches of floating matrix tablets of MTG*

Time (hrs)	MT 1 (%)	MT 2 (%)	MT 3 (%)	MT 4 (%)	MT 5 (%)	MT 6 (%)
0	0	0	0	0	0	0
1	12.23 ± 1.53	43.47 ± 3.54	11.32 ± 2.89	17.43 ± 1.04	10.03 ± 1.93	11.43 ± 1.54
2	16.78 ± 2.92	98.32 ± 2.71	25.34 ± 1.59	26.98 ± 1.11	13.44 ± 1.99	16.43 ± 1.03
3	32.1 ± 2.79	100.43 ± 3.04	30.99 ± 2.91	40.49 ± 2.43	32.43 ± 2.73	27.43 ± 1.19
4	39.37 ± 1.49	-	64.97 ± 3.17	52.36 ± 2.71	30.54 ± 1.08	33.54 ± 0.73
5	44.54 ±1.32	-	83.24 ± 1.06	60.29 ± 1.04	33.75 ± 2.45	38.57 ± 0.57
6	57.2 ± 1.6	-	95.43 ± 3.79	83.55 ± 3.77	42.64 ± 0.88	43.91 ± 3.09
7	63.41 ± 1.18	-	100.43±0.75	92.43 ± 2.46	45.34 ± 1.53	53.54 ± 2.67
8	74.32 ± 2.17	-	-	100.54 ± 1.91	54.65 ± 0.92	66.34 ± 1.12
10	84.32 ± 0.92	-	-	-	60.62 ± 2.21	71.32 ± 1.18
12	92.32 ± 0.89	-	-	-	65.32 ± 0.79	80.43 ± 1.68

*** n=3, average of three determinations±SD**

Formulation, MT4, prepared with HPMC K15M and xanthan gum gave the sustained release of the drug for 8hours. The sustained release effect of the combination was better than observed for floating matrix tablets MH in chapter 4. The findings might be due to the interaction of xanthan gum with calcium ions present in the drug. The literature gave the mixed review, some studies ascertained that calcium ions increase the release of the drug from the xanthan gum matrix and some researchers suggests that the release of the drug from the matrix, made by xanthan gum, is delayed in presence of calcium ions[33].

MT5, formualtion prepared with HPMC K15M and poloxamer could sustain the release of the drug till 12 hours. However, only 65.32% of the drug was released in the said duration, as the drug content was 89.43%. Formulation MT6, prepared with only HPMC K15 M as release retarding polymer, could sustain the release of the drug for 12 hours. However, only 80% of drug was released in that duration. The result is supported by the liturature, which proved that higher concentration of HPMC reduces the release rate of drug[34]. Also, the literatures specified that the drug release from the tablet containing a high amount of HPMC alone was incomplete[35].

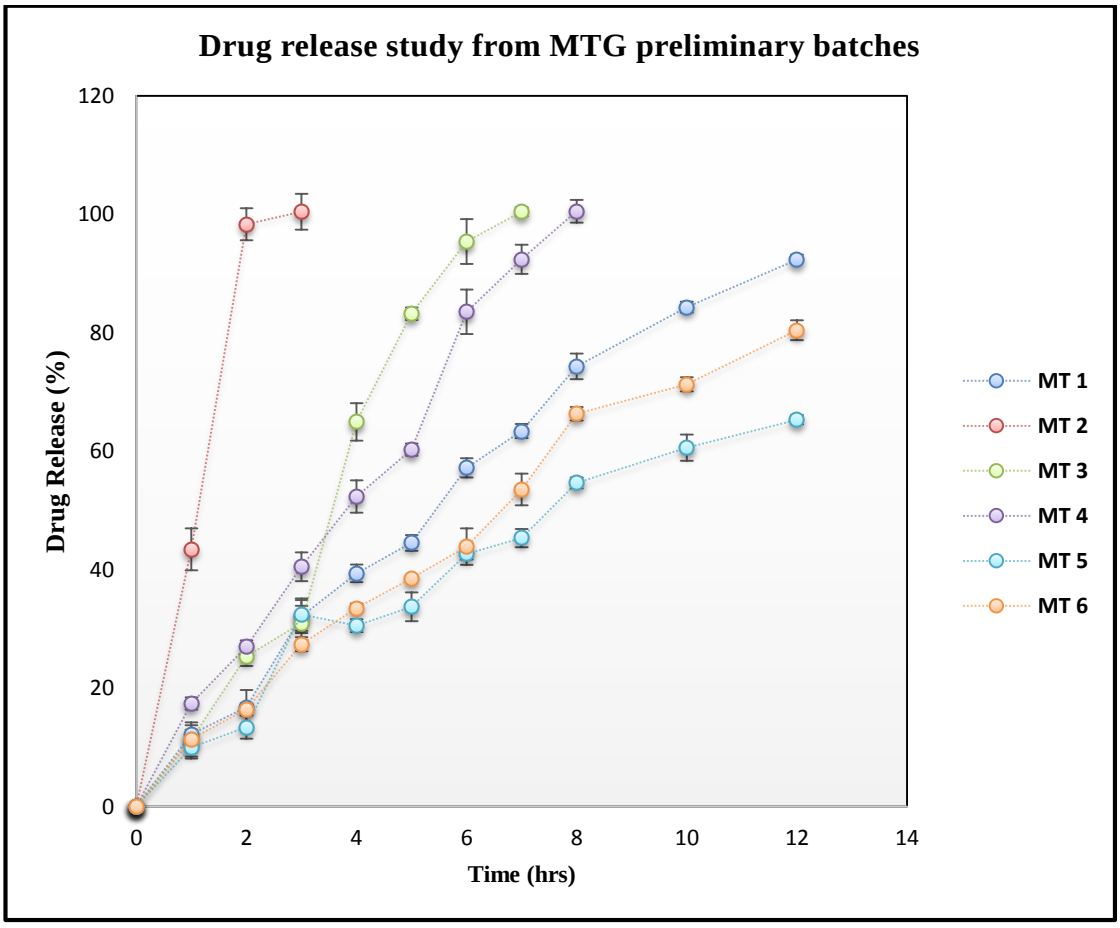


FIGURE 5A.2 *In vitro* dissolution profiles of different floating tablet of MTG in 0.1 N HCl
***n=3, average of three determinations±SD**

The *in vitro* dissolution results, obtained for batch MT1 prepared with HPMC K15M and sodium alginate showed sustained release of the drug from the polymeric matrix for 12hrs. Sodium alginate has been explored for achieving sustained release of many drugs[36,37]. In present study, cumulative drug released at the end of 12 hours was 92.32%, which were

different than the results obtained for the floating matrix tablet of MH (chapter 4). The same polymer combination used in chapter 4, released upto 90% of the drug in 6hours only, with poor swelling property and the findings were supported by the literature[38].

The justification for such finding in the batch MT1 is due to the presence of calcium ions in the drug. Sodium Alginate is a natural polysaccharide which forms gel in the presence of calcium. As soon as sodium alginate comes in the contact with calcium ions, a gel forms as the sodium ions (Na+) are exchanged with calcium ions (Ca2+) and the polymers become crosslinked[34,39]. The same mechanism is used in the preparation of sodium alginate beads[40]. Hence, this is the reason delayed release rate of the drug from the polymeric matrix of formulation MT1.

5B.3.2 Drug Excipient Compatibility Study by FTIR

Fourier transform infrared spectroscopy **(**FTIR) scan of MTG, HPMC K15M, sodium alginate and optimized formulation of MTG (M-1) was taken (Figure 5A.3).

The peaks corresponding to the characteristics bands of the MTG were found to be preserved in the spectra of the optimized MTG matrix tablet (M 3), there were no such peaks present in the scan of polymers, HPMC K15M and sodium alginate. This indicates that no chemical interaction between drug and polymer has been taken place during the preparation of the formulations. The IR peaks observed in the scan of pure MTG were: 3537.48 (O-H stretch); 3416.55 (N-H stretch, amide); 3082.21, 3060.87, 3026.62 (C-H stretch); 2924.24, 2869.21, 2850.93 (C-H stretch); 1649.83 (C=O stretch, amide merged); 1622.60 (N-H bend); 1544.75 (C=C stretch, aromatic). Same peaks, with slight change in intensity, were found to be present in M 3. As there was no change and shifting of characteristic peaks of MTG in M 3, it indicated no significant drug-polymer interaction. Hence, MTG is compatible with the polymer used in the formulation of its floating matrix tablet.

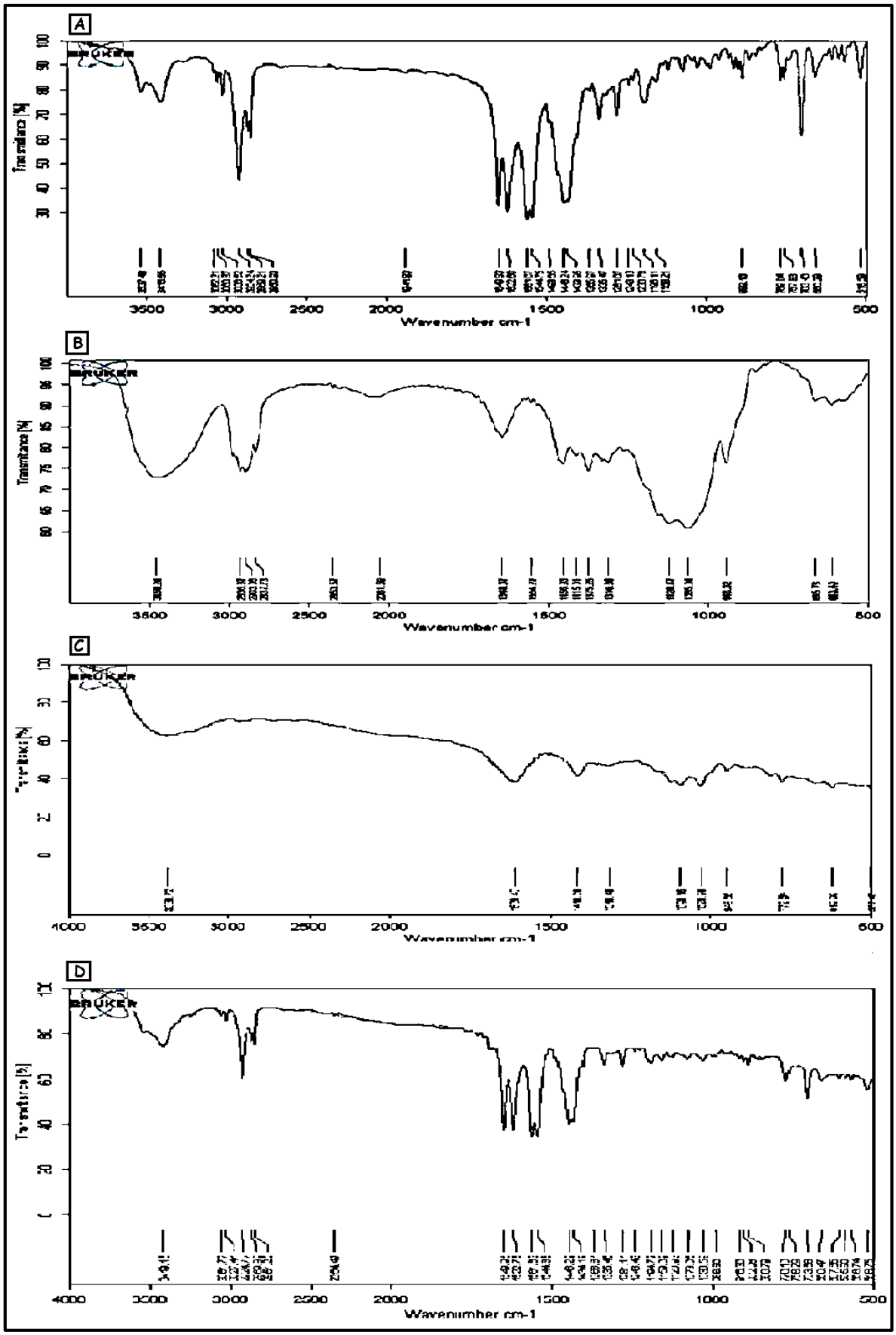


Figure 5A.3 FTIR scan obtained for mitiglinide calcium dihydrate (A) HPMC K15M, (B) Sodium alginate (C) and optimized formulation, M 3 (D)

5A.3.3 Experimental Design – Two Factors and Three Level Full Factorial Design

The results of preliminary batches of MTG floating matrix tablets, prepared with HPMC K15M and other ionic and anionic polymers, revealed that formulations prepared with the combination of HPMC K15 M and sodium alginate, as release retarding polymers were giving satisfactory release, hence these polymers were considered for the formulation of floating matrix tablet of MTG. The optimization of the formulation was done by applying a two factor, three level full factorial design. The levels of the factors were decided based on the preliminary studies and the literature review. 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 10 experiments (5A.2). The prepared formulations were evaluated to study the effect of independent variables on dependent variables. The results of the evaluation studies are discussed below.

5A.3.4 Physical Properties of Floating Tablet of Factorial Batches

All the prepared formulations complied the weight uniformity study. The hardness of all the batches was found to be in the range of 4.2 to 5.7 kg/cm^2. Drug content of all the 10 batches was found between the ranges of 99.44 to 101.97%, which is in the limits specified in Indian Pharmacopeia. Friability of all the batches was found to be less than 0.5%, which indicates the good mechanical strength of the formulations. All the prepared batches could float for more than 12 hours.

The tablet adhesion retention time was in the range of 24.32 to 115.22 minutes (Table 5A.6). It was found that as the amount of both the independent variables increased (1 coded value) in the formulation, tablet adhesion retention time increased. The tablet adhesion of the tablets prepared with the minimum amount (-1 coded value) of X_1 and X_2 had the direct effect on the tablet adhesion of tablets. As the polymer concentration decreased the tablet retention period also decreased. Our findings were in accordance with the finding of Yong et. al. and Derle et.al., who reported that as the concentration of sodium alginate and HPMC increases in the polymeric matrix, the adhesive properties of the tablet increases[41,42].

TABLE 5A.6 Results of the physical properties of the MTG floating matrix tablets prepared by applying full factorial design*

Batch code	Weight uniformity	Hardness (kg/cm^2)	Drug content (%)	Friability (%)	Floating Time (hrs.)	Tablet adhesion retention period (min.)
M 1	Complies	5.1±0.28	101.92±0.98	0.13±0.16	> 12	98.27 ± 2.43
M 2	Complies	4.7±0.95	100.67±0.44	0.22±0.17	> 12	45.23 ± 3.98
M 3	Complies	4.7±0.03	101.32±0.53	0.21±0.08	> 12	115.22 ± 2.54
M 4	Complies	5.2±0.55	100.75±0.31	0.21±0.54	> 12	24.32 ± 3.34
M 5	Complies	5.2±0.15	101.97±0.65	0.13±0.14	> 12	53.41 ± 4.92
M 6	Complies	5.7±0.54	100.47±1.01	0.19±0.11	> 12	85.38 ± 4.75
M 7	Complies	4.7±0.38	99.83±0.27	0.17±0.10	> 12	60.43 ± 6.21
M 8	Complies	4.8±0.75	99.43±1.16	0.22±0.08	> 12	104.63 ± 4.76
M 9	Complies	5.6±0.85	100.18±1.04	0.21±0.07	> 12	85.38 ± 4.75
M 10	Complies	4.2±0.62	100.21 ±1.14	0.23±0.12	> 12	48.53 ± 5.71

***n=3, average of three determinations±SD**

5A.3.5 In vitro Drug Release Study of Factorial Batches

The release study of all the batches of MTG floating matrix tablet, prepared by applying two factor three level factorial design. The release study was conducted in 500ml of 0.1N HCl under all the standard conditions prescribed by Indian Pharmacopoeia (IP). At regular intervals of time the samples of the dissolution fluid were withdrawn and analyzed by high performance liquid chromatography (HPLC).

TABLE 5A.7 *In vitro* drug release profile and model dependent kinetics of MTG floating matrix tablets prepared by 3^2 full factorial design†

Time (hrs)	M1 (%)	M2 (%)	M3 (%)	M4 (%)	M5 (%)	M6 (%)	M7 (%)	M8 (%)	M9 (%)	M10 (%)
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1	13.91±1.54	21.73±0.94	15.91±1.54	20.54±1.12	12.68±1.03	19.64±1.43	22.53±1.25	14.61±3.02	18.64±1.43	25.73±1.43
2	19.43±0.97	30.54±1.54	25.13±3.11	35.42±1.54	27.59±0.89	30.53±0.97	34.82±1.18	23.76±2.89	31.43±0.97	33.64±0.79
3	30.24±2.02	39.64±1.11	28.22±2.71	50.43±3.03	38.62±1.43	46.73±1.04	40.32±0.79	37.72±1.13	47.77±1.04	49.82±1.56
4	48.34±1.43	47.32±1.78	43.21±1.54	58.39±2.43	47.09±1.67	50.64±1.73	55.43±2.08	44.72±0.93	52.37±1.73	54.72±1.04
5	58.51±0.67	59.32±2.87	54.21±1.53	67.92±1.16	59.41±0.83	62.02±2.01	69.53±3.21	57.93±0.94	64.03±2.01	68.95±2.87
6	62.54±2.43	75.32±0.79	65.32±0.84	74.19±0.92	72.67±1.43	68.52±0.82	77.93±2.7	71.53±1.03	69.34±0.82	77.92±3.1
7	69.92±3.54	83.29±3.14	75.32±0.91	88.38±1.02	87.54±0.89	73.38±1.82	86.32±1.32	79.09±1.43	71.36±1.82	81.52±0.89
8	82.54±1.76	90.09±1.15	84.27±0.79	91.41±1.89	90.43±1.11	88.41±1.21	99.43±0.79	87.06±1.5	87.2±1.21	92.63±1.02
10	95.53±0.92	98.43±0.79	92.84±1.03	100.42±0.68	98.32±1.23	98.31±1.11	98.99±1.43	92.72±0.58	97.2±1.11	98.02±1.89
12	101.19±1.09	100.65±0.71	100.43±1.78	99.77±0.59	101.53±0.86	100.53±0.86	98.10±2.6	94.32±0.33	99.67±0.86	100.79±0.79

†**Determination of mean with ±SD**

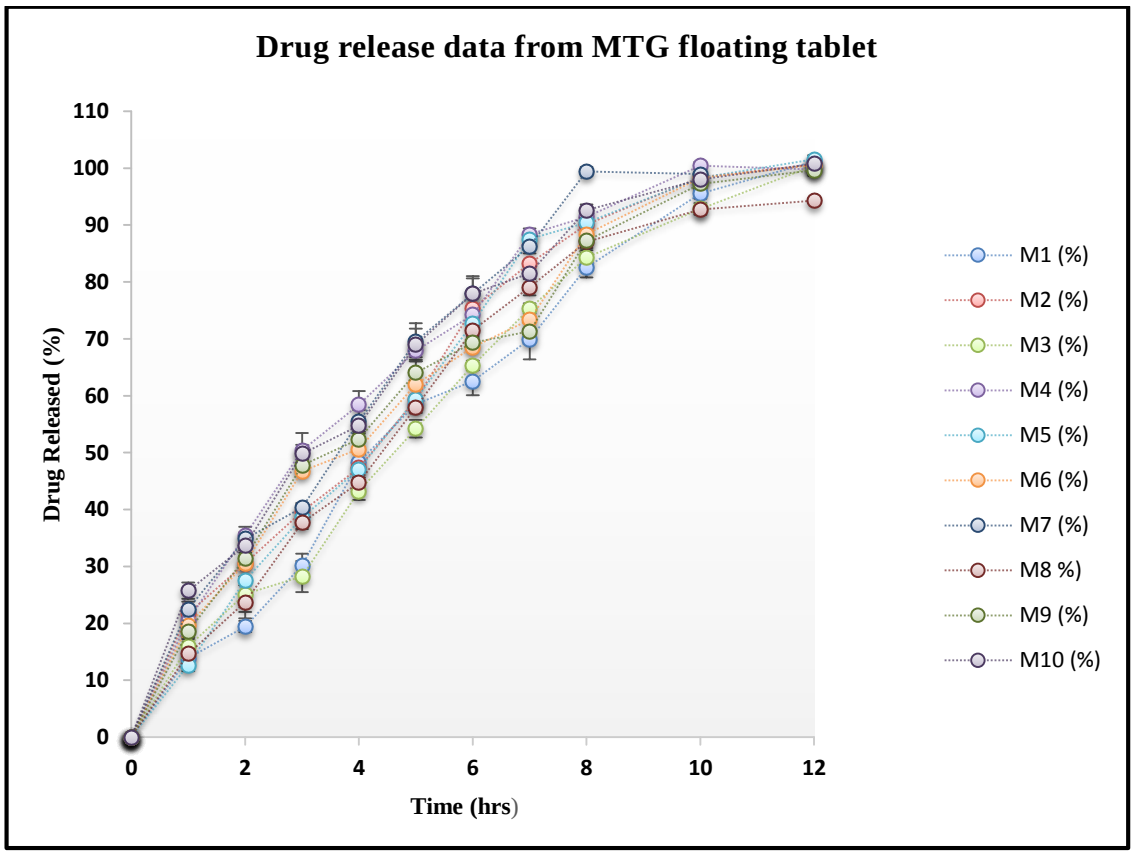


FIGURE 5A.4 Graphical representation of *in vitro* release profile of MTG floating matrix tablets prepared by 3^2 full factorial design

The details of the HPLC method for *in vitro* study are given in chapter 3 (Preformulation studies). The sink conditions were maintained in the dissolution apparatus by replacing the withdrawn dissolution fluid with fresh 0.1N HCl. The tabulated release from the gastroretentive floating matrix tablets of MTG batches is given in table 5A.7 and the graphical representation of the same is given in Figure 5A.4.

5A.3.6 *In vitro* Drug Release Kinetics of Factorial Batches

Model dependent release kinetics describes the mechanisms of overall release of drug from the dosage forms. The model dependent approaches evaluated for the drug release kinetics were zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas. The release from batches M4, M6, M7, M9, M10 of MTG floating matrix tablets was found to follow R_{HC} model with R^2 value close to 1, for the period of 12 hours. R_{HC} model data is obtained from *in vitro* drug release studies plotted as cube root of drug percentage remaining in matrix versus time. This model applies to tablets where dissolution occurs in all the planes equally and the initial geometrical of the tablet remains constant[43]. The release from rest of the

batches of floating matrix tablets was found to follow R_0 model with R^2 value close to 1, for the period of 12 hours. The data is obtained from *in vitro* drug release studies, plotted as cumulative amount of drug released versus time. This relationship is used to describe the drug dissolution of matrix tablets with low soluble drugs[44]. The results for the analysis of model-dependent drug release kinetics are given in table 5A.8.

TABLE 5A.8 Results table for *in vitro* drug release analysis, by model-dependent kinetics for MTG Floating matrix tablets

Batch code	Higuchi model (R_H)	Korsmeyer Peppas model (R_P)	Hixson Crowell model (R_{HC})	First order (R_1)	Zero order (R_0)
M 1	0.9469	0.9606	0.9766	0.7634	0.9809
M 2	0.9627	0.9693	0.9621	0.8491	0.9776
M 3	0.9466	0.9576	0.9726	0.7765	0.9845
M 4	0.9774	0.9929	0.9951	0.8618	0.9637
M 5	0.9505	0.9943	0.9851	0.8619	0.996
M 6	0.9776	0.9892	0.9899	0.8034	0.9666
M 7	0.9593	0.9766	0.9823	0.8224	0.9807
M 8	0.9518	0.9912	0.9729	0.871	0.9952
M 9	0.9796	0.9892	0.9905	0.8051	0.966
M 10	0.9815	0.9753	0.9859	0.8736	0.9679

5A.3.7 Statistical Analysis

The full factorial analyses, describes the quadratic or linear effects of the variables on the responses. A statistical model incorporating interactive and polynomial terms were utilized to evaluate responses. The polynomial equation generated under 3^2 full factorial design using Design expert software is as follows:

$$Y = b0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

Where, Y is the dependent variable, b_0 is the intercept, and b_1 to b_{22} are regression coefficient. The master effects (X_1 and X_2,) represent the average result of changing one

element at a time from its low to high value. X_1X_2, represents the interaction terms and X_1^2 and X_2^2 represents quadratic effect.

The results of all the dependent variables are shown in table 5A.9. All the formulations gave satisfactory floating lag time in the range of 6.83 to 45.41 seconds, which means that the chosen independent variables had no significant effect on the dependent variables. The formulations released 50% of the drug in the time range of 3.01 to 4.47hrs and 90% of the drug was released in the time range of 7.24 to 9.71hrs. The statistical analysis of the obtained results was done by Design Expert, which is further explained in the chapter.

TABLE 5A.9 Results of dependent variables of MTG floating matrix tablets prepared by applying 3^2 full factorial design*

Runs	Batch code	Floating lag time (sec)	Time required for 50% (t_{50}) (hrs)	Time required for 90% (t_{90}) (hrs)
1	M 1	35.46 ± 2.43	4.14 ± 0.26	9.42 ± 1.01
2	M 2	45.41 ± 3.21	4.23 ± 0.31	7.99 ± 0.81
3	M 3	10.33 ±1.91	4.63 ± 0.42	9.69 ± 0.91
4	M 4	35.12 ± 2.19	3.01 ± 0.18	7.87 ± 0.43
5	M 5	40.18 ± 3.28	4.25 ± 0.29	7.96 ± 0.66
6	M 6	10.23 ± 0.93	3.95 ± 0.37	9.1 ± 0.37
7	M 7	33.54 ± 2.43	3.6 ± 0.15	7.24 ± 0.52
8	M 8	6.83 ± 0.79	4.47 ± 0.25	9.71 ± 1.01
9	M 9	11.28 ± 1.02	3.82 ± 0.27	9.2 ± 0.45
10	M 10	15.41 ± 1.73	3.65 ± 0.19	7.77 ± 0.42

***n=3, average of three determinations±SD**

The summary of Analysis of Variance table for response parameters for 3^2 full factorial design for MTG gastroretentive floating microsponges, is given in table 5A.10. Using analysis of variance (ANOVA), the significance ($p < 0.05$) of the ratio of mean square variation due to the regression coefficient, and the residual error were tested. The model was

proved to be significant after observing the P value for the response parameters[45]. The P value for F_{lag}, t_{50} and t_{90} were found to be 0.0230, 0.0039 and 0.0430, respectively, which is less than 0.0500 indicating the significance of model terms. The Quadratic model was found to be significant for floating lag time (F_{lag}) and time to release 90% of the drug (t_{90}), whereas the response, time to release 50% of the drug (t_{50}) followed linear model. The high values of correlation coefficients for F_{lag} (R^2 = 0.9634), t_{50} (R^2 = 0.8420), and t_{90} (R^2 = 0.9437) indicated a good fit (ie, good agreement between the dependent and independent variables). Figure 5A.6 represents the contour plots and surface response curves for each response.

TABLE 5A.10 ANOVA table of response parameters for 3^2 full factorial design for Gastroretentive floating matrix tablets MTG

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	P-value
Floating lag time (sec) (F_{lag})					
Model	1732.44	5	346.49	15.80	0.0230
Residual	65.78	3	21.93		
Corrected Total	1798.22	8			
Time to release 50% of drug (t_{50})					
Model	1.72	2	0.86	15.98	0.0039
Residual	0.32	6	0.054		
Corrected Total	2.04	8			
Time to release 90% of drug (t_{90})					
Model	6.86	5	1.37	10.07	0.0430
Residual	0.41	3	0.14		
Corrected Total	7.27	8			

5A.3.7.1 Floating Lag Time

The floating lag time was found to be in the range of 6.83 to 45.42 seconds. R^2 was found to be equal to 0.9634. The F-value was found to be 15.80, which implies the model is significant. There is only a 2.30% chance that an F-value this large could occur due to noise.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable and the ratio found for the present model was 10.724, which indicates an adequate signal. This model can be used to navigate the design space. The other information obtained from ANOVA table was about the variables. The P value for both factors, X_1 and X_2 was found to be 0.0352 and 0.0999, respectively, indicating that only X_1 has significance in the model. Interaction effect and quadratic effect of X_2 were found to be insignificant, whereas quadratic effect of X_1 was significant. The result can be expressed for model analysis by Quadratic model. The fitted equation for the responses are given as follows:

$$F_{lag} = +14.89 - 7.00X_1 - 4.50X_2 - 7.00X_1X_2 + 22.67X_1^2 - 6.83X_2^2$$

The polynomial equations can be applied to find the conclusions after looking at the magnitude of coefficient and the mathematical sign it carries (i.e. positive or minus). By looking into the above equation, it is evident that both the factors, Amount of HPMC K15M (X_1) and sodium alginate (X_2) have negative effects on floating lag time of the formulated floating matrix tablets of MTG, which means that, as the concentration of both the variable increase, the floating lag time decreases, which is desirable. This may be attributed to the gelling capability of both the polymers, which makes the matrix formulation less dense[46-48]. Additionally, the observation was that the antagonistic effect of X_1 was more significant than X_2. Above all, the quadratic effect of X_1 was highly significant with agonistic effect on the response. A quadratic effect is an interaction term where a factor interacts with itself and its significance indicates that the optimal levels of X are not in the extremes of the experimental region but inside it[49]. These effects were further illustrated in contour and surface response plots (Figure 5A.5-a). The relationship between the dependent and independent variables was elucidated by contour plots. The plots were found to be curvy indicating a nonlinear relationship between X_1 and X_2 and quadratic effect of HPMC K15M was also spotted.

5A.3.7.2 Time to Release 50% of Drug (t_{50})

The t_{50} was found to be in the range of 3.01 to 4.63 hrs. R^2 was found to be equal to 0.8420. The F-value was found to be 15.98, which implies the model is significant. There is only a 0.39% chance that an F-value this large could occur due to noise.

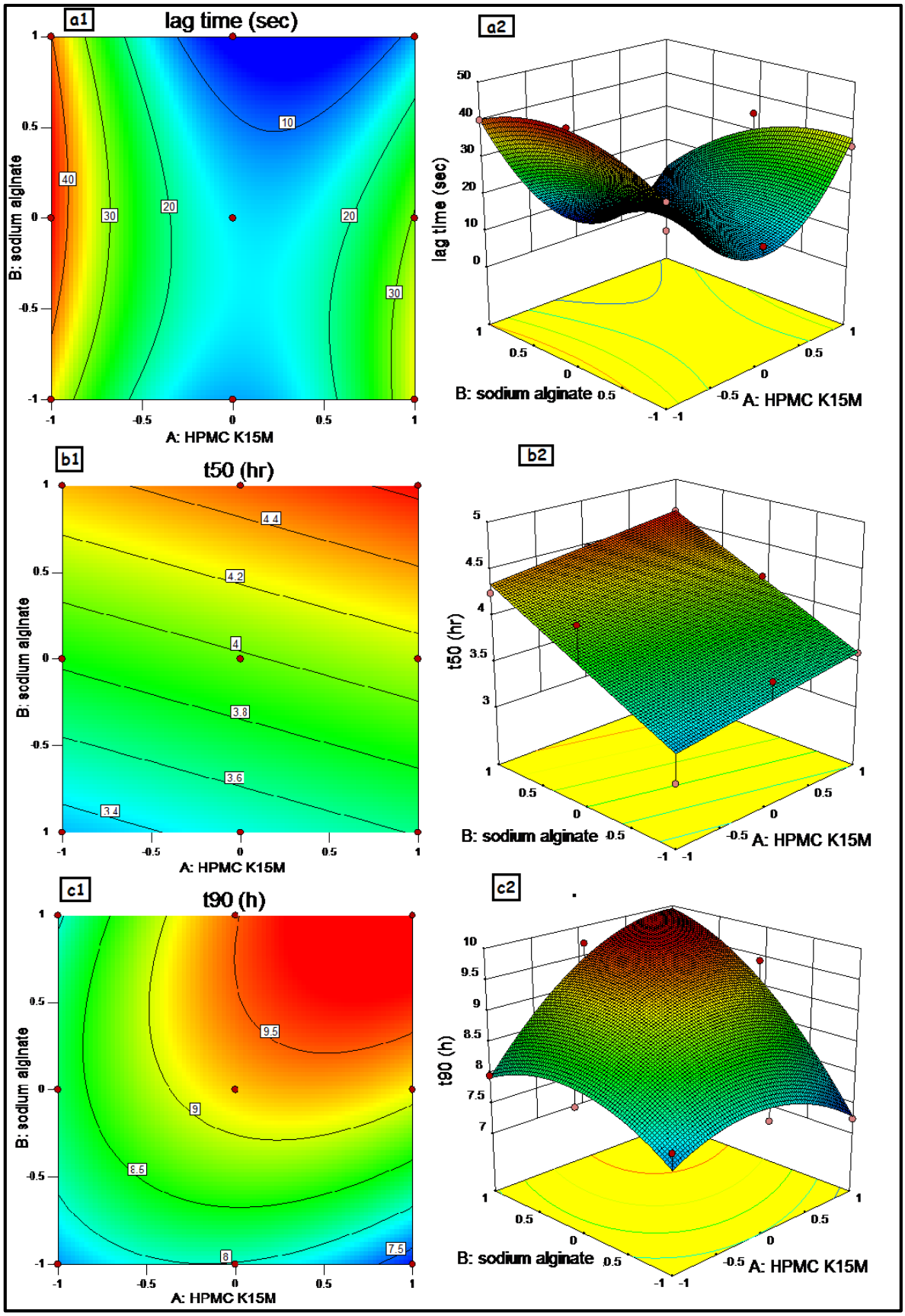


FIGURE 5A.5 Contour plot and Response surface plot for MTG tablet: (a) lag time (2) Time to release 50% of drug (3) Time to release 90% of drug; 1-contour plot; 2-surface response plot.

The difference between predicted and adjusted R^2 values was less than 0.2, which shows the good agreement between dependent and independent variables. "Adeq Precision" measures the signal to noise ratio.

A ratio greater than 4 is desirable and the ratio found was for present model was 9.880, which indicates an adequate signal. This model can be used to navigate the design space. The other information obtained from ANOVA table was about the variables. The P value for both factors, X_1 and X_2 was found to be 0.1725 and 0.0016, respectively, indicating that only X_2 has significance effect on time required to release 50% of drug from the formulation. As the response, t_{50} followed linear model, there were no interaction and quadratic effect observed between the independent factors on the response. The result can be expressed for model analysis as linear model. The fitted equation for the responses are given as follows:

$$t_{50} = +3.98 + 0.15X_1 + 0.52X_2$$

By looking into the above equation, it is apparent that both the factors, Amount of HPMC K15M (X_1) and sodium alginate (X_2) have a positive effect on t_{50} of the formulated floating matrix tablets of MTG, which signifies that, as the absorption of both the variable increase, the t_{50} increases. This may be attributed to the release retarding property of both the polymers, which can hold up the release of the drug from the matrix system. This effect obtained by sodium alginate, on $t_{50,}$ were contradictory to the findings of Prajapati et al[37]. The authors found that the increased amount of sodium alginate decreased the t_{50} of domperidone. The solution was freed based on the poor water affinity of sodium alginate. In the present work the result was contradictory to the previous finding, probably because of the fact that the drug is in its salt form. The sodium alginate interacts with the calcium ions of the salt form of the drug, thus enhances the gelling property of sodium alginate. The effect obtained by HPMC K15M was in accordance to the earlier findings, where the increased amount of HPMC increased the time to release the 50% of the drug from the formulation[50]. These effects were further illustrated in contour and surface response plots (Figure 5A.5-b). The plots were found to be flat indicating a linear relationship between X_1 and X_2 with no interactions between the variables.

5A.3.7.3 Time to Release 90% of Drug (t_{90})

The t_{90} was found to be in the range of 7.24 to 9.71hrs. R^2 was found to be equal to 0.9437. The F-value was found to be 10.07, which implies the model is significant. There is only a 4.30% chance that an F-value this large could occur due to noise. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable and the ratio found was 8.866, which indicates an adequate signal. This model can be used to navigate the design space. The other information obtained from ANOVA table was about the variables. The P value for both factors, X_1 and X_2 was found to be 0.0680 and 0.0158, respectively, indicating that only X_2 has significance in the model. Interaction effect was found to be significant and quadratic effect of X_1 and X_2 were found to be insignificant. The result can be expressed for model analysis by Quadratic model. The fitted equation for the responses are given as follows:

$$t_{90} = +9.22 + 0.42X_1 + 0.75X_2 + 0.59X_1X_2 - 0.53X_1^2 - 0.50X_2^2$$

By looking into the above equation, it is evident that both the factors, Amount of HPMC K15M (X_1) and sodium alginate (X_2) have agonistic effects on t_{90} of the formulated floating matrix tablets of MTG, which means that, as the concentration of both the variable increase, the t_{90} also increases. The justification for obtaining such results is same as that of the results obtained for t_{90}. Additionally, the observation was that the positive effect of X_2 was more significant than X_1. The interaction effect between X_1 and X_2 was highly significant with agonistic effect on the response. A quadratic effect of both the independent variables had antagonistic effect on time to release 90% of the drug. This directs that the optimal levels of X are not in the extremes of the experimental region but inside it. These effects were further illustrated in contour and surface response plots (Figure 5A.5-c). The relationship between the dependent and independent variables was elucidated by contour plots. The plots were found to be curvy indicating a nonlinear relationship between X_1 and X_2. It was evident that t_{90} was near 9.5hrs with maximum amount of both the variables.

5A.3.8 Validation of 3^2 – Full Factorial Experimental Design

An additional formulation, suggested by the design expert, was formulated to check and validate the reliability of the mathematical models built here with full factorial design. The check point batch was prepared with the level of X_1 (HPMC K15M) and X_2 (Sodium alginate) as -0.60 and 1, respectively. The predicted values of responses for the check point

batch, given by the design expert software, were found to be 12.9207sec for floating lag time, 4.40547hrs for t_{50} and 8.68384hrs for t_{90}. The batch was formulated and evaluated to get the actual values of the responses. The experimental response values for the formulated batch were found to be 13.21 sec for floating lag time, 4.21hrs for t_{50} and 8.38hrs for t_{90}. The relative errors (%) between the predicted and experimental values for each response were found to be 2.23% (F_{lag}), 4.436% (t_{50}) and 3.498% (t_{90}), which was within 5%. This indicates that the experimental values were in agreement with the predicted values which confirm the predictability and validity of the model.

5A.3.9 Selection of Optimized Formulation

The multivariate combination and interaction of independent variables and process parameters that have been demonstrated to provide assurance of quality is termed as design space. So, for creating design space and to optimize all the responses, a numerical optimization technique by the desirability function and a graphical optimization technique by the overlay plot was used (Figure 5A.6-1 and 2). The overlay plot gives the regions not meeting the specifications as greyed out, leaving an operating window or sweet spot in yellow colour.

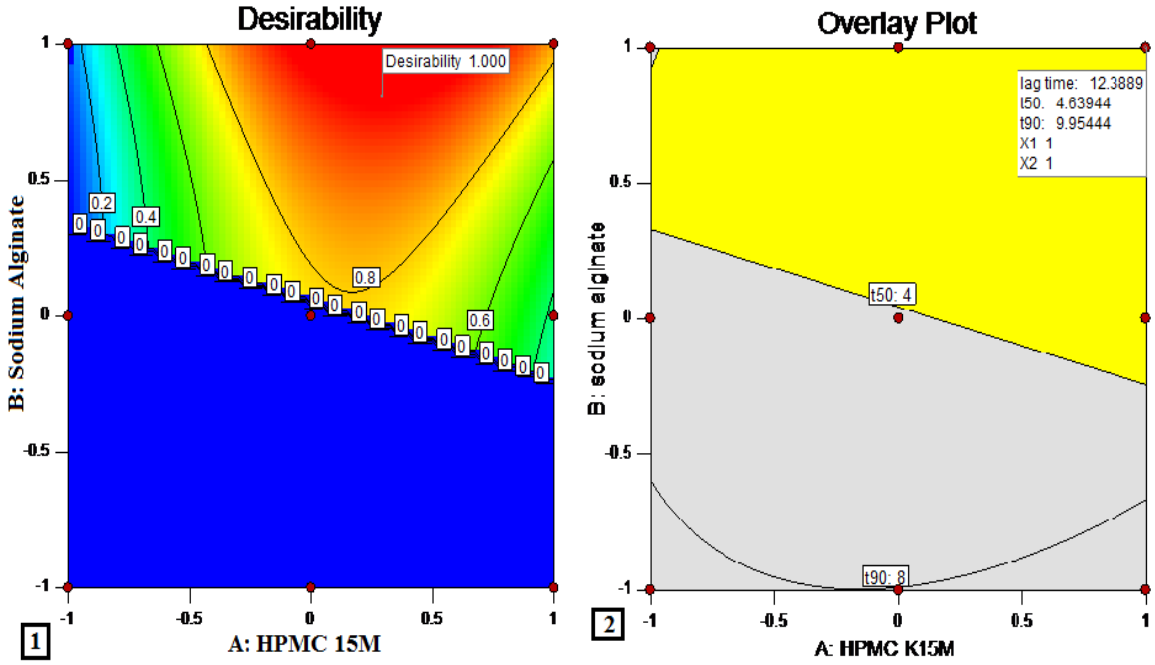


FIGURE 5A.6 Optimization of MTG floating matrix tablet 1: Desirability function, 2: Overlay Plot

The optimized formulation was obtained by applying constraints on dependent variable responses and independent variables. The constraints for the responses, floating lag time, t_{50} and t_{90} were set as minimum, between 4-5hrs and 8-10hrs respectively. The recommended concentrations of the independent variables were calculated by the Design Expert software from the plots with highest desirability near to 1.0. The optimized region for getting the desired values of responses was obtained in the entire range of X_1 and between the range of 0.3 to 1 of X_2. Hence, formulation M 3 was considered to be the optimized formulation as it falls in the yellow portion of overlay plot, with the desirability equal to 1.

As shown in the figure no 5A.6, optimized formulation had the level of X_1 and X_2 equal to 1 with the predicted responses as 12.38 sec, 4.63hrs and 9.954hrs for floating lag time, t_{50} and t_{90} respectively. The observed values of floating lag time (10.33 ± 0.91), t_{50} (4.63 ± 0.42) and t_{90} (9.69 ± 1.91) were in close agreement to the model predictions values.

5A.3.10 Stability Studies of Formulation M3

The results obtained after three and six months of accelerated stability study of optimized MTG gastroretentive floating matrix tablet (M3), showed no significant change in the physical properties and buoyancy parameters.

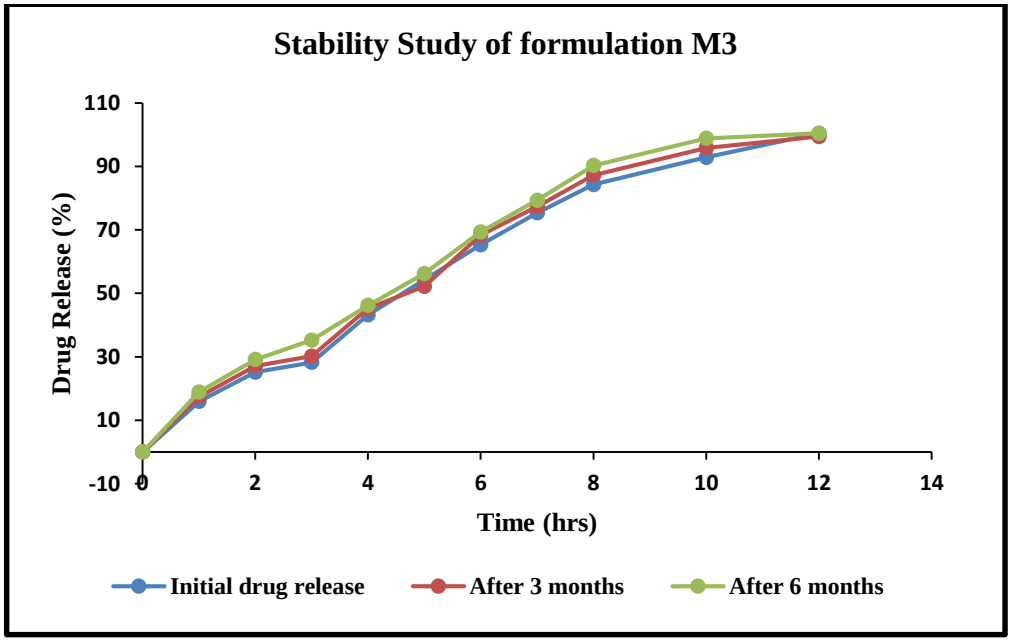


FIGURE 5A.7 Comparison of drug release from MTG optimized formulation M3 Initially, after 3 and 6months of accelerated stability study.

The release pattern of the formulation, before and after the stability study is given in figure 5A.7. The release showed that the release pattern of the optimized formulation had 90% similarity with the formulation after three and six months of stability study. The variation in the release pattern was insignificant. Hence, it can be concluded that formulation M3 has good stability when stored at 40 ºC under 75% RH for 6 months.

5A.3.11 Radiographic Study

To determine the retention time of the optimized floating matrix tablets of MTG inside the body, radiographic studies were conducted. The barium Sulfate loaded tablets, prepared with optimized formula of matrix tablet, were given to rabbits. Barium sulphate is a radio opaque material, which obstructs the passage of radiant energy, such as x-rays, the representative areas appearing light or white on the exposed film. This visibility provides the contrast needed to accurately locate or position the device inside the body during critical procedure.

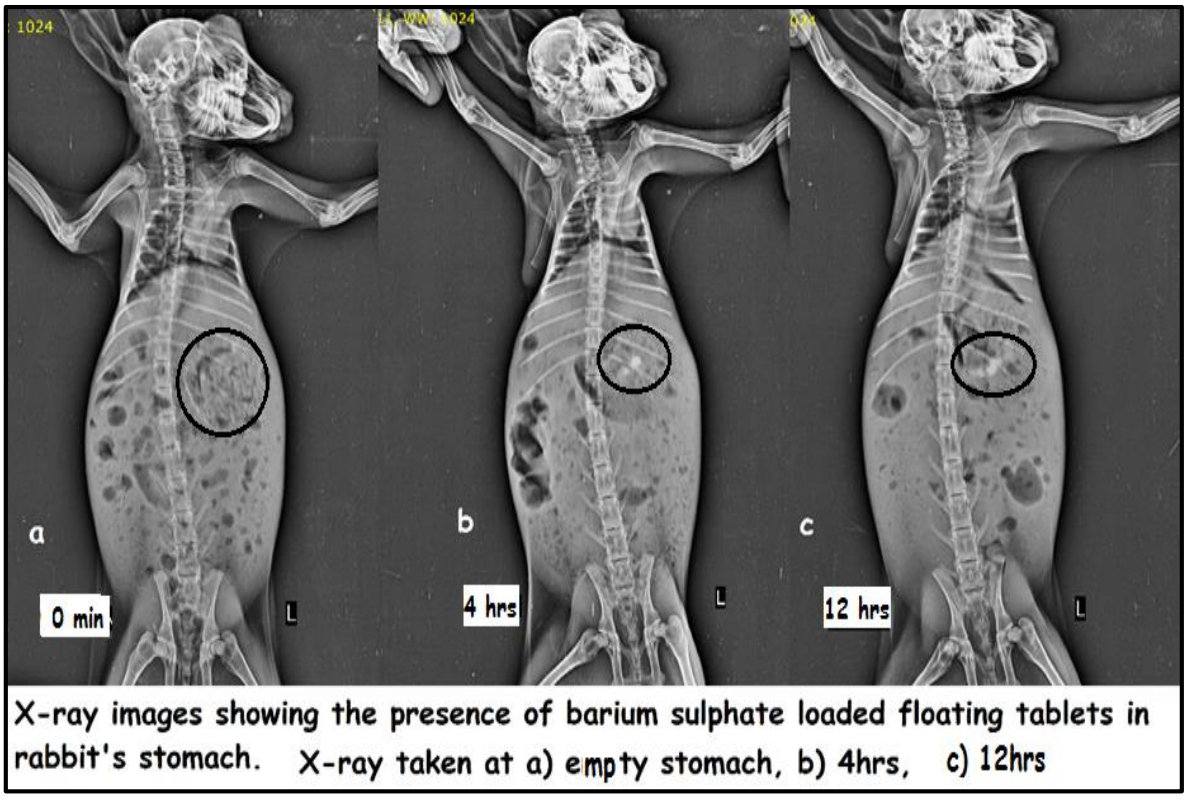


FIGURE 5A.8 X-ray images showing the presence of barium sulfate-loaded floating matrix tablet in the rabbit's stomach. a) 0 min b) 4hrs c) 12hrs

The X-ray photomicrographs were taken before and after administering the barium sulphate tablet to rabbits. Figure 5A.8 shows the X-ray images taken at 0, 4 and 12 hrs, time period. The images clearly showed white spots which indicated that the tablets remained afloat in gastric fluid for up to 12 h in the stomach of rabbit. Hence, the study confirms the gastroretentive behavior of the developed floating matrix tablet of MTG.

5A.4 Conclusion

The floating matrix tablets of MTG were prepared by direct compression technique. The preliminary batches were prepared using HPMC K15M, as release retarding polymer along with other ionic and anionic polymeric substances. The prepared formulations were evaluated for floatation behavior and drug release. The results revealed that tablets prepared using HPMC K15M and sodium alginate, as release retarding polymers were giving satisfactory sustained release property and acceptable floatation behavior. Hence, 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. 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 and drug release study. Results showed that M-3 formulation containing maximum amount of both variables released the MTG for the period of 12hrs and was falling in the yellow region of overlay plot. Hence, it was considered as optimized gastroretentive floating matrix tablet of MTG. The radiographic study of the barium sulphate loaded tablets of this optimized formula, confirmed the gastroretention of the developed formulation

5A.5 References

1. Urashima, Y., Kayano, E., Kato, R., 2012. Effect of gastric pH on Mitiglinide absorption. *Clinical Pharmacy and Clinical Pharmacokinetics*, 29(2), pp.41-46.

2. Prop, I.N.N.M., 2000. Mitiglinide Calcium Hydrate. *Drugs of the Future*, *25*(10), pp.1034-1042.

3. Menon, A., Ritschel, W.A. and Sakr, A., 1994. Development and evaluation of a monolithic floating dosage form for furosemide. *Journal of Pharmaceutical Sciences*, *83*(2), pp.239-245.

4. Acharya, S., Patra, S. and Pani, N.R., 2014. Optimization of HPMC and carbopol concentrations in non-effervescent floating tablet through factorial design. *Carbohydrate Polymers*, *102*, pp.360-368.

5. Singh, B. and Ahuja, N., 2002. Development of controlled-release buccoadhesive hydrophilic matrices of diltiazem hydrochloride: optimization of bioadhesion, dissolution, and diffusion parameters. *Drug Development and Industrial Pharmacy*, *28*(4), pp.431-442.

6. Reddy, G.S., Nayak, U.Y., Deshpande, P.B. and Mutalik, S., 2014. Gastroretentive Pulsatile Release Tablets of Lercanidipine HCl: Development, Statistical Optimization, and *In vitro* and *in vivo* Evaluation. *The Scientific World Journal*, 2014, pp 1-13.

7. Raval, J.A. and Patel, M.M., 2011. Formulation and characterization of gastroretentive discs containing famotidine. *Brazilian Archives of Biology and Technology*, *54*(2), pp.293-300.

8. Gaikwad, V.D., Yadav, V.D. and Jadhav, P.D., 2013. Formulation and evaluation of bilayer floating matrix tablets for captopril. *International Journal of Current Pharmaceutical Review and Research*, *4*(2), pp.27-35.

9. Strübing, S., Metz, H. and Mäder, K., 2008. Characterization of poly (vinyl acetate) based floating matrix tablets. *Journal of Controlled Release*, *126*(2), pp.149-155.

10. Pare, A., Yadav, S.K. and Patil, U.K., 2008. Formulation and evaluation of effervescent floating tablet of amlodipine besylate. *Research Journal of Pharmacy and Technology*, 1(4), pp.526-530.

11. Srivastava, A.K., Wadhwa, S., Ridhurkar, D. and Mishra, B., 2005. Oral sustained delivery of atenolol from floating matrix tablets—formulation and *in vitro* evaluation. *Drug Development and Industrial Pharmacy*, 31(4-5), pp.367-374.

12. El Gamal, S.S., Naggar, V.F. and Allam, A.N., 2011. Optimization of acyclovir oral tablets based on gastroretention technology: factorial design analysis and physicochemical characterization studies. *Drug Development and Industrial Pharmacy*, *37*(7), pp.855-867.

13. Ford, J.L., Rubinstein, M.H. and Hogan, J.E., 1985. Formulation of sustained release promethazine hydrochloride tablets using hydroxypropyl-methylcellulose matrices. *International Journal of Pharmaceutics*, 24(2-3), pp.327-338.

14. Sung, K.C., Nixon, P.R., Skoug, J.W., Ju, T.R., Gao, P., Topp, E.M. and Patel, M.V., 1996. Effect of formulation variables on drug and polymer release from HPMC-based matrix tablets. *International Journal of Pharmaceutics*, 142(1), pp.53-60.

15. Pani, N.R., Nath, L.K., Acharya, S. and Bhuniya, B., 2011. Application of DSC, IST, and FTIR study in the compatibility testing of nateglinide with different pharmaceutical excipients. *Journal of Thermal Analysis and Calorimetry*, *108*(1), pp.219-226.

16. Sonaglio, D., Bataille, B., Ortigosa, C. and Jacob, M., 1995. Factorial design in the feasibility of producing Microcel MC 101 pellets by extrusion/spheronization. *International Journal of Pharmaceutics*, *115*(1), pp.53-60.

17. Bouckaert, S., Massart, D.L., Massart, B. and Remon, J.P., 1996. Optimization of a granulation procedure for a hydrophilic matrix tablet using experimental design. *Drug Development and Industrial Pharmacy*, *22*(4), pp.321-327.

18. Box, G.E.P. and Wilson, K.B., 2012. On the Experimental Attainment of Optimum Conditions. *Breakthroughs in Statistics: Methodology and Distribution*, p.270.

19. Kincl, M., Turk, S. and Vrečer, F., 2005. Application of experimental design methodology in development and optimization of drug release method. *International Journal of Pharmaceutics*, 291(1), pp.39-49.

20. Mehta, A.K., Yadav, K.S. and Sawant, K.K., 2007. Nimodipine loaded PLGA nanoparticles: formulation optimization using factorial design, characterization and *in vitro* evaluation. *Current Drug Delivery*, 4(3), pp.185-193.

21. Patil, S.B. and Sawant, K.K., 2009. Development, optimization and *in vitro* evaluation of alginate mucoadhesive microspheres of carvedilol for nasal delivery. *Journal of Microencapsulation*, 26(5), pp.432-443.

22. Patel, D.M., Patel, N.M., Patel, V.F. and Bhatt, D.A., 2007. Floating granules of ranitidine hydrochloride-gelucire 43/01: formulation optimization using factorial design. *AAPS PharmSciTech*, *8*(2), pp.E25-E31.

23. Matthews, B.R., 1999. Regulatory aspects of stability testing in Europe. *Drug Development and Industrial Pharmacy*, 25(7), pp.831-856.

24. Parikh, D.C. and Amin, A.F., 2008. *In vitro* and *in vivo* techniques to assess the performance of gastro-retentive drug delivery systems: a review. *Expert Opinion on Drug Delivery*, *5*(9), pp.951-965.

25. Tadros, M.I., 2010. Controlled-release effervescent floating matrix tablets of ciprofloxacin hydrochloride: Development, optimization and *in vitro–in vivo* evaluation in healthy human volunteers. *European Journal of Pharmaceutics and Biopharmaceutics*, 74(2), pp.332-339.

26. Patel, A., Modasiya, M., Shah, D. and Patel, V., 2009. Development and *in vivo* floating behavior of verapamil HCl intragastric floating tablets. *AAPS PharmSciTech*, 10(1), pp.310-315.

27. Sharma, O.P., Shah, M.V., Parikh, D.C. and Mehta, T.A., 2015. Formulation optimization of gastroretentive drug delivery system for allopurinol using experimental design. *Expert Opinion on Drug Delivery*, 12(4), pp.513-524.

28. Shakya, R., Thapa, P. and Saha, R.N., 2013. *In vitro* and *in vivo* evaluation of gastroretentive floating drug delivery system of ofloxacin. *Asian Journal of Pharmaceutical Sciences*, *8*(3), pp.191-198.

29. Baumgartner, S., Kristl, J., Vrečer, F., Vodopivec, P. and Zorko, B., 2000. Optimisation of floating matrix tablets and evaluation of their gastric residence time. *International Journal of Pharmaceutics*, *195*(1), pp.125-135.

30. Hermansson, A.M., Eriksson, E. and Jordansson, E., 1991. Effects of potassium, sodium and calcium on the microstructure and rheological behaviour of kappa-carrageenan gels. *Carbohydrate Polymers*, *16*(3), pp.297-320.

31. Kulkarni, A., Mandhare, T., Aloorkar, N., 2017. Application of novel natural polymer for controlling the release of fenoverine from controlled release matrix tablets. *International Journal of Applied Pharmaceutics,* 9(2), pp.1-9.

32. Gohel, M.C. and Panchal, M.K., 2002. Novel use of similarity factors f 2 and S d for the development of diltiazem HCl modified-release tablets using a 3^2 factorial design. *Drug Development and Industrial Pharmacy*, *28*(1), pp.77-87.

33. Baumgartner, S., Pavli, M. and Kristl, J., 2008. Effect of calcium ions on the gelling and drug release characteristics of xanthan matrix tablets. *European Journal of Pharmaceutics and Biopharmaceutics*, *69*(2), pp.698-707.

34. Nokhodchi, A. and Tailor, A., 2004. In situ cross-linking of sodium alginate with calcium and aluminum ions to sustain the release of theophylline from polymeric matrices. *Il Farmaco*, *59*(12), pp.999-1004.

35. Huang, Y.B., Tsai, Y.H., Yang, W.C., Chang, J.S. and Wu, P.C., 2004. Optimization of sustained-release propranolol dosage form using factorial design and response surface methodology. *Biological and Pharmaceutical Bulletin*, *27*(10), pp.1626-1629.

36. Prajapati, S.T., Patel, L.D. and Patel, D.M., 2009. Studies on formulation and *in vitro* evaluation of floating matrix tablets of domperidone. *Indian Journal of Pharmaceutical Sciences*, 71(1), pp.19-23.

37. Prajapati, S., Patel, L. and Patel, D., 2008. Gastric floating matrix tablets: design and optimization using combination of polymers. *Acta Pharmaceutica,* 58(2), pp.221-229.

38. Timmins, P., Delargy, A.M. and Howard, J.R., 1997. Optimization and characterization of a pH-independent extended-release hydrophuic matrix tablet. *Pharmaceutical Development and Technology*, 2(1), pp.25-31.

39. Potter, K., Balcom, B.J., Carpenter, T.A. and Hall, L.D., 1994. The gelation of sodium alginate with calcium ions studied by magnetic resonance imaging (MRI). *Carbohydrate Research*, 257(1), pp.117-126.

40. Badwan, A.A., Abumalooh, A., Sallam, E., Abukalaf, A. and Jawan, O., 1985. A sustained release drug delivery system using calcium alginate beads. *Drug Development and Industrial Pharmacy*, *11*(2-3), pp.239-256.

41. Yong, C.S., Jung, J.H., Rhee, J.D., Kim, C.K. and Choi, H.G., 2001. Physicochemical characterization and evaluation of buccal adhesive tablets containing omeprazole. *Drug Development and Industrial Pharmacy*, 27(5), pp.447-455.

42. Derle, D., Joshi, O., Pawar, A., Patel, J. and Jagadale, A., 2009. Formulation and evaluation of buccoadhesive bi-layer tablet of propranolol hydrochloride. *International Journal of Pharmacy and Pharmaceutical Sciences*, 1(1), pp.206-212.

43. Chen, S., Zhu, J. and Cheng, J., 2007. Preparation and *in vitro* evaluation of a novel combined multiparticulate delayed-onset sustained-release formulation of diltiazem hydrochloride. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, *62*(12), pp.907-913.

44. Freitas, M.N. and Marchetti, J.M., 2005. Nimesulide PLA microspheres as a potential sustained release system for the treatment of inflammatory diseases. *International Journal of Pharmaceutics*, *295*(1), pp.201-211.

45. Nayak, A. K., & Pal, D., 2011. Development of pH-sensitive tamarind seedpolysaccharide–alginate composite beads for controlled diclofenac sodiumdelivery using response surface methodology. *International Journal of Biological Macromolecules*, 49, pp.784–793.

46. Ukawala, R., Singhvi, G., Jain, S., Shukla, V., Yadav, N. and Sharma, S., 2012. Design and characterization of controlled release gastro-retentive floating tablet of an atypical psychotropic agent. *Journal of Pharmacy & Bioallied Sciences*, 4(1), pp.S88-S89.

47. Mohapatra, S., KumarKar, R., Mohapatra, D.K., Sahoo, S.K. and Barik, B.B., 2012. Cefuroxime axetil loaded gastroretentive floating tabletsbased on hydrophilic polymers:

preparation and *in vitro* evaluation. *Brazilian Archives of Biology and Technology*, 55(2), pp.269-275.

48. Mandal, S., Basu, S.K. and Sa, B., 2009. Sustained release of a water-soluble drug from alginate matrix tablets prepared by wet granulation method. *AAPS PharmSciTech*, 10(4), pp.1348-1356.

49. Gharti, K.P., Budhathoki, U., Thapa, P. and Bhargava, A., 2012. Formulation *in vitro* evaluation of floating tablets of hydroxypropyl methylcellulose and polyethylene oxide using ranitidine hydrochloride as a model drug. *Journal of Young Pharmacists*, 4(4), pp.201-208.

50. Singh, I. and Saini, V., 2016. Formulation and optimization of floating matrix tablets of clarithromycin using simplex lattice design. *Pakistan Journal of Pharmaceutical Sciences*, 29(2), pp.511-519.

CHAPTER 5B

Gastroretentive Floating Microsponges of MTG

5B.1 Introduction

The Microsponge Delivery System (MDS) is a patented polymeric system consisting of porous microspheres[1]. They are tiny sponge like spherical particles that consist of a numerous interlocking cavities within a non-collapsible structure with a large porous surface through which active ingredient are released in a controlled manner. Recently their use has also being investigated for oral drug delivery. The size of the microsponge's ranges from 5-300μm in diameter with upto 250000 pores. This results in a large reservoir within each microsponge, which can be loaded with up to its own weight of active agent[2-3].

Microsponges were not explored for low density gastro retentive system until Arya et. al., developed targeted floating curcumin microsponges for improved site specific absorption for gastric cancer[4]. This study proved that microsponges have floating ability and can be used for the gastroretention of the drugs. Hence, floating microsponge is a novel approach of preparing the gastroretentive formulations of antidiabetic drugs, which needs to be present in the upper division of GIT for giving better therapeutic action.

Mitiglinide calcium dihydrate (MTG) has a good rationale for preparing gastroretentive formulation, as explained in previous chapters. After preparing the floating matrix tablet of MTG, it was decided to prepare its floating multiparticulate system as microsponges, because of all the advantages offered by this delivery system[6].

Microsponges were prepared by quasi-emulsion solvent diffusion method[7]. Preliminary batches were prepared for the screening of levels of excipient and process variables for the formulation of MTG microsponges. Simple screening process investigates each factor separately by keeping all the remaining factors constant and it cannot uncover the interaction effects between the elements. Hence, the final optimization of dosage form was done by applying 3^2 full factorial design[8]. In full factorial design, an experimental run is performed at every combination of the factor levels. The sample size is the product of the numbers of levels of the factors. In this experiment all combinations of elements are investigated in each

replicate of the experimentation. Full factorial experiments are the only means to completely and systematically study interactions between factors in addition to identifying significant factors.

5B.2 Experimental Studies

5B.2.1 Method of Preparation of MTG Microsponges

Gastroretentive floating Microsponges were prepared by quasi-emulsion solvent diffusion method using an external phase containing distilled water and polyvinyl alcohol (PVA)[8,9.] The internal phase was prepared by adding ethyl cellulose in the organic solvent system consisting of ethanol as good solvent and Dichloromethane (DCM) as a bridging liquid[10]. To this organic phase, MTG was added and dissolved completely. At last triethylcitrate (TEC) which was added in organic phase to facilitate the plasticity. Then, the inner phase was poured into outer phase with stirring. After emulsification, the mixture was continuously stirred on mechanical stirrer, for a specified time and at a specific temperature. Once the process was finished, the product was immediately filtered to separate the microsponges. The product was washed and dried at room temperature for 24 h.

5B.2.2 Preliminary Studies

For the preparation of microsponges, it was necessary to determine the effect of different process and formulation variables on the development of formulation. Hence, the screening of the variables was done by trial and error technique. Here, the variables were screened, one by one, by keeping the other variables constant and the prepared formulations were evaluated for the physical appearance, using trinocular microscope.

5B.2.2.1 Determination of Effect of Drug Polymer Ratio

Literature revealed that concentration of polymer plays an important role in the formulation of microsponges. Hence, trail batches of MTG microsponges were formulated using ethyl cellulose in various ratios. Formulations with five different ratios of drug-polymer (MTG:Ethyl cellulose) were prepared (1:1, 1:2, 1:3, 1:4, 1:5) to determine the effects of

drug-polymer ratio on morphology of microsponges (Table 5B.1). For the screening of other variable, the drug-polymer ratio, 1:3 was fixed.

TABLE 5B.1 Composition of preliminary batches for determination of effect of drug-polymer ratio on floating microsponges of MTG

Ingredients	MTG 1	MTG 2	MTG 3	MTG 4	MTG 5
MTG: Ethyl cellulose *	1:1	1:2	1:3	1:4	1:5
Polyvinyl alcohol (%)	1	1	1	1	1
Triethyl citrate (%)†	20	20	20	20	20
Ethanol (ml)	20	20	20	20	20
Dichloro methane (ml)	5	5	5	5	5
Distilled water (ml)	300	300	300	300	300
Stirring speed (rpm)	700	700	700	700	700
Temperature (ºC)	25	25	25	25	25
Stirring time (hrs)	4	4	4	4	4

***Amount of MTG was 500mg; † TEC: % of polymer concentration**

5B.2.2.2 Determination of Effect of Temperature

With the purpose of determining the effect of temperature on the floating microsponges of MTG, different batches of microsponges was prepared by varying the temperature during the development of formulation. The batches were prepared at three temperatures, i.e. 30ºC, 40ºC, 50 ºC with fixed drug polymer ratio as 1:3, remaining composition was same as given in table 5B.1. The effect of temperature on the morphology of microsponges was checked.

5B.2.2.3. Determination of the Effect Speed of Agitation

During the preparation of microsponges, the organic phase containing drug is dispersed in an aqueous phase, which needs vigorous stirring using mechanical stirrer. The effects of different stirring speeds on the physical features of the microsponges were investigated. Different agitation speeds (300, 700, 1000 and 1500 rpm) were employed to a chosen ratio of drug to polymer (1:3) with remaining composition similar to that of table 5B.1.

5B.2.2.4. Determination of the Effect of Plasticizer

Triethyl citrate (TEC) is added in the formulation as plasticizer. To check its effect on the formation of microponges, different batches containing the varying amount of TEC were prepared. The formulations were prepared with amount of TEC as 10, 20 and 30 % of polymer concentration, with remaining composition similar to that of table 5B.1.

5B.2.2.5 Determination of the Effect of Polyvinyl Alcohol (PVA)

The effect of the concentration of PVA was checked on the formulation, by preparing the microsponges with varying its concentration as 1%, 2% and 3%. The prepared formulations were evaluated for their physical characteristics.

5B.2.2.6 Equilibrium Solubility Study of Drug

The equilibrium solubility study of MTG in the presence of ethyl cellulose and PVA was performed by shake flask method. Weighed amount of excipients were added separately in three different conical flasks containing 10 ml of double distilled water followed by excess amount of drug. The flasks were allowed to shake for 72 h at 25 ºC in a mechanical shaker. At the end of 72 h the samples were filtered through whattman filter paper, diluted with methanol and assayed spectrophotometrically at 259 nm. This study was conducted to check the effect of various excipients on the solubility of MTG, as the solubility is very important criteria in the formulation of microsponges[4].

5B.2.3 Optimization by 3^2 Full Factorial Design

From the preliminary studies, it was found that there are two very important factors i.e concentration of ethyl cellulose and polyvinyl alcohol (PVA), which effect the microsponges. Hence, it was decided to apply full factorial design to study the effect of independent variables on dependent variables[11]. A two factor with three levels, full factorial design was applied for the optimization of gastroretentive floating microsponges of MTG.

TABLE 5B.2 Levels of process parameters in 3^2 full factorial design for MTG floating microsponges

Independent Variable	Level		
	Upper level (1)	Medium level (0)	Lower level (-1)
Concentration of Ethyl cellulose (X_1)	2 gm	1.5 gm	1 gm
Concentration of PVA (X_2) (%w/v)	1.5	1	0.5
Dependent Variables - Y_1 - % Yield Y_2 - % Buoyancy Y_3 - % Entrapment efficiency Y_4 - % CDR_{12} (cumulative drug release after 12hrs)			

The low and high levels of factors were adopted from the preliminary studies and the medium levels were set as the midpoint of low and high levels (Table 5B.2).

TABLE 5B.3 Coded and actual values of the batches prepared by applying 3^2 full factorial design for floating microsponges of MTG

Formulation	Coded value		Actual value	
	X_1	X_2	X_1(gm)	X_2 (%)
F-1	1	1	2	1.5
F-2	1	0	2	1
F-3	1	-1	2	0.5
F-4	0	1	1.5	1.5
F-5	0	0	1.5	1
F-6	0	-1	1.5	0.5
F-7	-1	1	1	1.5
F-8	-1	0	1	1
F-9	-1	-1	1	0.5

Design-Expert® software (trial version 9.0.6, Stat-Ease) was used to apply the design and total 9 runs were formulated. Coded and actual values of batches of gastroretentive floating

microsponges of MTG, prepared by applying 3^2 full factorial design is given in Table 5B.3. The actual composition of the prepared batches is given in table 5B.4.

TABLE 5B.4 Composition of MTG loaded microsponges prepared by applying 3^2 factorial design

Ingredients	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9
MTG (mg)	500	500	500	500	500	500	500	500	500
Ethyl cellulose (mg)	2000	2000	2000	1500	1500	1500	1000	1000	1000
Polyvinyl alcohol (%)	1.5	1	0.5	1.5	1	0.5	1.5	1	0.5
Triethyl citrate (%)*	20	20	20	20	20	20	20	20	20
Ethanol (ml)	20	20	20	20	20	20	20	20	20
Dichloro methane (ml)	5	5	5	5	5	5	5	5	5
Distilled water (ml)	300	300	300	300	300	300	300	300	300
Stirring speed (rpm)	1000	1000	1000	1000	1000	1000	1000	1000	1000
Stirring time (hrs)	3	3	3	3	3	3	3	3	3

*** TEC: % of polymer concentration**

5B.2.4 Evaluation of MTG Floating Microsponges

5B.2.4.1 Microscopic Study and Particle Size

The morphology and the particle size of microsponges were determined by optical microscopy. The sample was mounted on a slide and placed on the mechanical stage. The shapes of the microsponges were observed visually and the size of the microsponges was automatically calculated and displayed on the computer attached with trinocular microscope (Primostar, Carl Zeiss). The mean particle size was calculated by measuring more than 100 particles of microsponges.

5B.2.4.2 Product Yield and Measurement of Bulk Density

Product yield of microsponges was calculated by dividing the weight of microsponges to total amount of drug and excipients taken for the preparation of microsponges[12]. All the batches of prepared microsponges were placed in graduated measuring cylinder to find its bulk density using following formula:

$$\text{Bulk density} = \frac{\text{Weight of microsponges}}{\text{Initial volume}}$$

5B.2.4.3 Entrapment Efficiency

MTG loaded microsponges theoretically equivalent to 10 mg of MTG were weighed, crushed and extracted with 10 ml of methanol. The sample was centrifuged at 2000 rpm for 10 min, filtered and after appropriate dilution, assayed spectrophotometrically at 259 nm. Entrapment efficiency was determined by dividing the practically entrapped amount of drug to the total amount of drug[13].

5B.2.4.4 *In vitro* Buoyancy

In vitro buoyancy of microsponges was performed by spreading 100mg microsponges over the surface of 200 ml of 0.1N HCl. The medium was stirred magnetically at a speed of 100 rpm at 37 ± 0.5°C for 12 h. After 12 hrs, the floating and non-floating microsponges were filtered separately and dried. Both the portions of dried microsponges were weighed. The percent buoyancy was calculated by ratio of the floating microsponges to the total number of microsponges (floating and sinking)[14].

$$Buoyancy\ (\%) = \frac{W_f}{(W_f + W_{nf})}\ X\ 100$$

Where W_f are floating microsponges and W_{nf} is non floating microsponges. All the determinations were made in triplicates.

5B.2.4.5 *In vitro* Drug Release[15]

The release of MTG from the microsponges was determined by filling microsponges weight equivalent to 10mg in an empty capsule shell and then the dissolution was carried out in USP

dissolution apparatus II. The dissolution medium used was 500 ml of 0.1N HCl, which was maintained at 37 ± 0.5ºC at 75rpm. Aliquot samples of 5 ml were withdrawn at every hour till 12 hrs. The withdrawn samples were replaced with an equal volume of fresh medium to maintain a constant volume and a perfect sink condition. The samples were filtered through membrane filter (0.45 µm) and assayed by HPLC. (Method given in chapter 3)

FIGURE 5B.1 *In vitro* drug release study of MTG floating microsponges

5B.2.4.6 Drug Release Kinetics[16]

There are various type of kinetic models, which can be used to analyze the release pattern of the drug from the prepared formulations. The *in vitro* drug release data, for all the floating microsponges of MTG formulations, prepared by applying 3^2 full factorial design, were graphed for finding release mechanism by zero-order, first-order, Higuchi and Korsmeyer–Peppas kinetic models. The model with the highest correlation coefficient was considered to be the best fitting one.

5B.2.5 Validation of Experimental Design

To validate the chosen experimental design, an extra batch of floating gastroretentive microsponges of MTG was prepared with highest desirability value, as suggested by the Design Expert software. This batch was termed as F-0 with the composition as X_1: 0.47362 and X_2: - 0.151682. The prepared formulation was evaluated for finding the experimental values of all dependent responses. The relative errors (%) between the predicted and experimental values for each response were calculated using the following equation:

$$Relative\ error\ (\%) = \frac{Predicted\ value - Experimental\ value}{Predictive\ value}\ X\ 100$$

5B.2.6 Physicochemical Characterization of Optimized Formulation

5B.2.6.1 Scanning Electron Microscopy (SEM)

Surface morphology of optimized formulation was visualized by scanning electron microscope from Sophisticated Instrumentation Centre for Applied Research & Testing (SICART), Anand, Gujarat, India. The optimized batch of MTG floating microsponges F-0, was coated with gold under argon atmosphere using gold sputter module in a high vacuum evaporator and observed under various magnifications (100–1000X) with direct data capture of the image[17,18].

5B.2.6.2 Differential Scanning Calorimetry (DSC)

The Differential scanning calorimetry (DSC) thermograms are taken to characterize the physical state of drug in the polymer matrix and to assess incompatibility, if any. In the present work, thermal behavior of MTG, ethyl cellulose, physical mixture of drug and polymer and optimized formulation, F-0 was estimated by DSC (Schimadzu DSC 60). The samples was sealed in aluminum pans heated in an atmosphere of nitrogen and thermograms was obtained by heating at a constant heating rate of 10ºC/min in the range of 0–300ºC. A nitrogen purge (40 ml/min) was maintained during the run[19,20].

5B.2.6.3 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopic analysis (FTIR) was performed for the pure drug, the polymer and the drug-polymer physical mixture, placebo formulation and optimized floating microsponge of MTG. FTIR study gives the information about the interaction between the drug and polymer[21]. The samples and potassium bromide (IR grade) were mixed in a glass pestle mortar and discs were prepared by punching the powder at 20 psi for 10 min using potassium bromide press. The prepared disc was placed in the sample compartment and scanned at transmission mode in the region of 4000-400 cm^{-1}. The peaks corresponding to the characteristics bands of the drug should be conserved in the spectra of the microsponges to ensure that no chemical interaction or changes took place during the preparation of the formulations.

5B.2.6.4 Powder X-ray Diffraction (XRD)

Powder X-ray diffraction (XRD) for pure drug and optimized MTG microsponges, F-0 was performed, to investigate the effect of polymerization on crystallinity of the drug as the peaks present in the XRD scan of crystalline drug vanishes if the drug is completely transformed to amorphous form[22]. The samples were analyzed over the angle range (2θ) 0º - 60º.

5B.2.6.5 Residual Solvent Analysis

Residual solvents are the left over organic solvents in the final finished pharmaceutical product, when such solvents are used for the preparation of the formulation. The ICH guidelines "Q3C" for the residual solvents, has given the permitted daily exposure (PDE) and concentration limit in ppm for these solvents[23]. In the present work, dichloromethane (DCM) was used in the preparation of MTG microsponges. Hence, the Gas Chromatographic technique was applied to determine the amount of DCM (limit is upto 600ppm) in the optimized MTG microsponges (F-0). Formulation F-0 was tested by a 7697A Headspace (Agilent; Santa Clara, CA, USA) gas chromatograph (GC) with a DB 624 column (30 m × 450 µm × 2.55 µm) and flame ionization detector. For this study, 10 mg of optimized microsponges MTG, F-0, were dissolved in 5 mL of Dimethyl sulfoxide (DMSO) and transferred to the GC system. For calculations, a standard solution of dichloromethane in DMSO (20 ppm) was also analyzed[24].

5B.2.7 Stability Study of Optimized Formulation as per ICH Guidelines

Optimized floating microsponges of MTG (F-0) equivalent to 10 mg of MTG were filled in hard gelatin capsules. The filled capsules were manually packed in blister and the samples were maintained in a stability chamber under accelerated storage conditions, 40 ± 2°C and 75 ± 5% relative humidity for six months with humidity and temperature control. The samples were analyzed for physical changes, buoyancy, % drug content and % CDR_{12} at three and six months of stability study[4].

5B.2.8 *In vivo* Studies

To investigate the actual effect of the optimized MTG gastroretentive floating microsponges (F-0) inside the body, various *in vivo* studies were conducted which are explained below.

5B.2.8.1 Radiographic Study

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). Floating microsponges was prepared by incorporating the X-ray opaque material in the optimized formulation by replacing MTG with barium sulphate. The amount of the X-ray opaque material in the optimized formula of microsponge was kept sufficient to ensure visibility by X-ray, but at the same time the amount of barium sulphate was low enough to enable the formulation to float. This formulation was given to albino rabbit for *in vivo* X-ray imaging study. During the study the rabbit was not allowed to eat, but water was available freely[25].

5B.2.8.2 Pharmacodynamic Study on Diabetic Rats

For conducting the *in vivo* studies of the optimized floating microsponges of MTG, a protocol was approved by the Institutional Animal Ethical Committee (IAEC). Pharmacodynamic effect of optimized microsponges of MTG (F-0) 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. For inducing type 2 diabetes, rats were feeding with High Fat Diet (58%

fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) for the initial period of 2 weeks. Then the rats were injected intraperitoneally (i.p.) with low dose of streptozocin, STZ (35 mg kg^{-1}) kg^{-1})[26]. The rats with the non-fasting PGL of ≥130 mg dl−1 were considered diabetic and were selected for further pharmacological studies.

For conducting a pharmacodynamic study of the floating optimized microsponges of MTG (F-0), the diabetic rats were separated into two groups (n=3 in each group). The dose calculation was done based on the bases of weight of rats in accordance to surface area, as given by Dr. M.N. Ghosh in 1984, the human adult dose multiplied by 0.018 gave the dose for rat weighing 200g[27]. Considering maximum daily dose of MTG as 20 mg, the calculated dose for rat was found to be 1.8 mg/kg.

FIGURE 5B.2 Image of Pharmacodynamic study of MTG microsponges performed on diabetic rat

One group of rats was given pure MTG drug and other group was given the optimized MTG microsponges. Then, after regular interval of time till 24 hrs, the blood sugar level of the rats was recorded using the Glucose Kit (Accu-Chek* Active Blood Glucose Monitor System)

by puncturing tail vein. The percent reduction in blood glucose level, recorded as mean ± SEM was plotted against the time.

5B.2.8.3 Pharmacokinetic Study of MTG[28]

Pharmacokinetic study was conducted on twelve Albino Wistar rats, either sex, of average weight of 200-250g. These rats were fasted for 24 h (but with free access to water) before being randomly assigned into two groups with six rats in each group. Before administering the drug the blood was collected for blank calculation. One set of group was administered pure MTG and other group was given Optimized gastroretentive microsponges of MTG (F-0) orally. Blood samples, (0.5 ml) were collected at regular intervals of time from the retro-orbital puncture. The blood samples were introduced into heparinized micro centrifuge tubes, and then plasma was separated by centrifugation. The samples were stored at -20ºC till further analysis. Plasma samples obtained from the rats were analysed by developed RP-HPLC method described in Chapter 3 using Kinetica 5.0 software.

Following pharmacokinetic parameters were calculated.

Maximum Plasma Concentration (C_{max})

It is defined as observed maximum plasma or serum concentration after administration
It can be determined from the plasma concentration profile.

Time for maximum plasma concentration (t_{max})

It is defined as the time after administration of a drug when the maximum plasma concentration is reached.

It can be determined from the plasma concentration profile.

Area under plasma concentration curve (AUC)

The area under the plasma drug concentration-time curve (AUC) reflects the actual body exposure to drug after administration of a dose of the drug and is expressed in mg*h/L.

AUC $_{(0-t)}$: It is the area under plasma concentration curve for time zero to t. It can be calculated using trapezoidal rule in which area under curve from t_2 to t_1 is calculated by following equation.

$$AUC_{t_1}^{t_2} = \frac{C_1+C_2}{2} X(t_2 - t_1)$$

AUC $_{(0-\infty)}$: It is Area under the concentration-time curve from zero up to ∞ with extrapolation of the terminal phase and calculated by following formula.

$$AUC_{t_0}^{t_\infty} = \Sigma AUC_{t_{n-1}}^{t_n} + \frac{C_{last}}{k}$$

Where,

C_{last} = last observed plasma concentration at t_n
k= slope obtained from the terminal portion of the graph

AUMC: It is the Area under the first moment of the concentration-time curve from zero up to ∞ with extrapolation of the terminal phase. It can be calculated as the area under curve for graph plotted of C_{last}*t versus t.

K_{el}: The elimination rate constant is defined as the fraction of drug in an animal that is eliminated per unit of time, e.g., fraction/h. It can be calculated from the slop of terminal linear portion obtained with log plasma concentration versus time.

***$t_{1/2}$*:** It is the elimination half-life and defined as the time required for the amount of drug (or concentration) in the body to decrease by half. It is calculated from following formula

$$t_{\frac{1}{2}} = \frac{0.693}{K_{el}}$$

MRT: It is Mean residence time of the unchanged drug in the systemic circulation. It can be calculated by following equation.

$$MRT = \frac{AUMC}{AUC}$$

Kinetica 5.0 software was used to perform the non-compartmental pharmacokinetic analysis of the obtained results[29,30]. With the trapezoidal rule C_{max}, T_{max}, AUC $_{(0-t)}$, AUC $_{(0-\infty)}$, K_{el}, $t_{1/2}$, V_d and MRT. All data for pharmacokinetic analysis are reported as mean ± SD.

5B.3 Results and Discussion

5B.3.1 Preliminary Studies

Preliminary studies included the screening of the process variable and other parameters required for the formulation of floating microsponges of MTG. To find the effect of drug-polymer ratio on the physical properties of microsponges, trial batches of microsponges were prepared with varying drug:polymer ratios (1:1,1:2,1:3, 1:4, 1:5). The results revealed that at 1:1 drug-polymer ratio, with minimum amount of polymer, the microsponges were not formed. At 1:2 ratio, the microsponges were formed but were of irregular shape. As the polymer concentration increased the physical properties of the formulation improved. At the ratio 1:3 and higher, the spherical spongy microspheres were prepared, as observed by trinocular microscope. Hence, it was considered to be the critical factor in the development of the formulation and for further screening of other variables 1:3 ratio was fixed.

To explore the effect of temperature on the formulation of microsponges the formulations were prepared at different temperature conditions. At 50°C, the microsponges formed aggregates and at 30°C, the formulated microsponges were formed but the shape was not perfectly spherical. The best microsponges with uniform spherical shape and porous nature were formed at 40ºC.

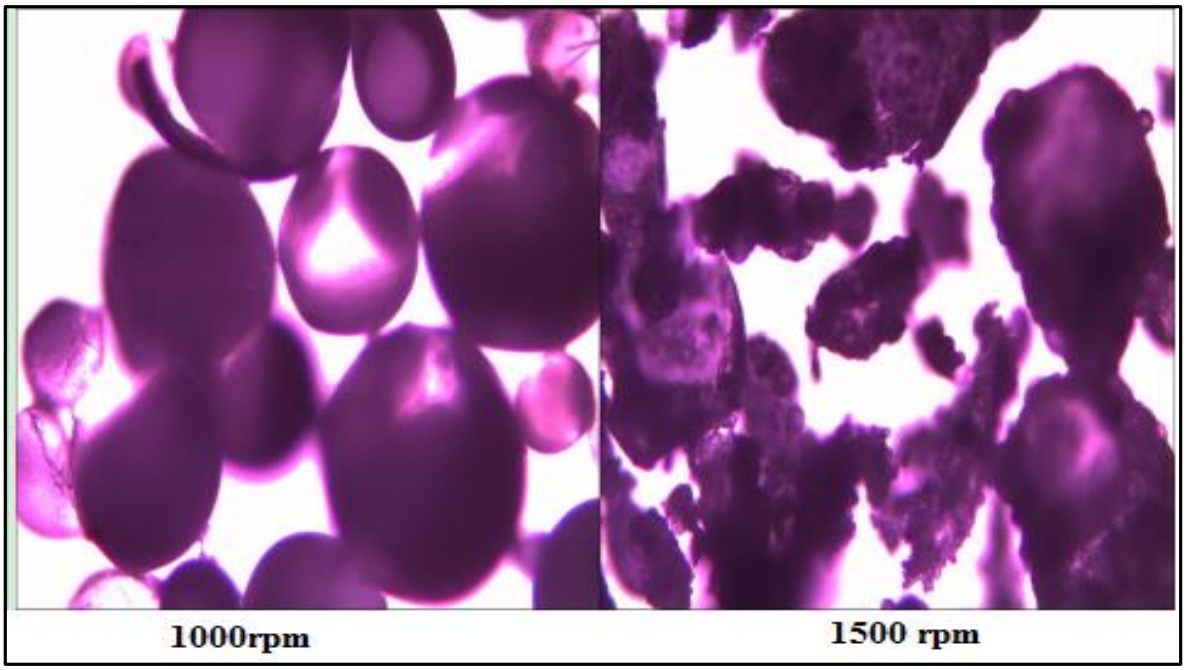


FIGURE 5B.3 Trinocular microscope images of the microsponges prepared at different stirring speed

This reason behind such observation at different temperatures is that temperature affects the rate of solvent removal which influences the solidification rate of the dispersed phase as well as morphology of the resulting microspheres. From present study, 40°C was considered as optimum temperature for obtaining spherical and uniform microsponges.

Another factor evaluated was the concentration of TEC and the observation was that microsponges could be prepared successfully, only with TEC as 20% of polymer concentration. At low concentration of TEC, microsponges were not formed and at high concentration, product formed was sticky.

The effect of PVA on the formulation of microsponges was also checked. The results showed that the amount of PVA should be kept below 2% beyond this concentration the froth formation increases and it becomes impossible to formulate microsponges.

The effects of different stirring speeds, on the physical characteristics of the microsponges were investigated. The optimum speed for getting spherical and uniform microsponge was found to be 1000rpm. As the literature survey suggests, the stirring speed has inverse effect on the yield of the drug[31]. Hence, the speed of agitation can't be increased beyond the limit.

5B.3.1.1 Equilibrium Solubility Study

The literature survey recommended that solubility of drug is a very important factor to be considered during the formulation of microsponges[4]. The drug with low solubility has to be dissolved in organic phase and then that phase is added to aqueous phase, in which the drug is not soluble. For the drug, to get entrapped in the polymeric matrix, the fulfilment of this criterion is essential. If the excipients have the capability to change the solubility pattern of the drug in both the phases, it can affect the yield, entrapment efficiency and drug release from the dosage form. Hence, equilibrium solubility of MTG was determined in presence of the excipients used in the formulation of floating microsponges.

MTG has a limited aqueous solubility of 70.08 µg/ml, hence, the effect of ethyl cellulose and PVA, was checked on solubility of drug. The solubility of MTG was decreased to 50.72 µg/ml, in presence of 1% w/v concentration of ethyl cellulose. The solubility reduced to 21.12 µg/ml, in presence of 1% w/v concentration of PVA. The solubility study data showed that PVA and ethyl cellulose had noticeable effect on solubility of MTG, hence they were selected as independent variables in the optimization of the MTG floating microsponges.

The preliminary screening of the formulation and process variables of microsponges and the solubility studied of the drug suggested that concentration of ethyl cellulose and polyvinyl alcohol are important factors in the formulation of floating microsponge of MTG. Hence, these factors were considered as key variables. With these independent factors, a 3^2 full factorial design was applied to check their effect on the dependent variables like yield (%), entrapment efficiency (%), buoyancy (%) and CDR_{12} (%) of floating microsponges of MTG.

5B.3.2 Optimization of MTG Microsponges by 3^2 Full Factorial Design

The results presented in table 5B.5 shows that the percent yield for all the batches was in the range of 66.3% to 87.3%. The particle size was found to be in the orbit of 250.42 to 361.53 µm, which comes in the standard range (5-300µm) of microsponges as mentioned by Nacht and Katz[32]. All the formulations had almost similar size, which shows that the chosen independent variable had no significant effect on the particle size of prepared MTG microsponges.

TABLE 5B.5 Result table of MTG microsponges prepared by applying 3^2 full factorial design*

Formulation	% Yield	Particle size (µm)	Bulk density (g/cc)	% Buoyancy	% Entrapment Efficiency	% CDR_{12}
F-1	78.8	257.29±13.6	0.294	98.3 ± 2.6	81.3 ± 2.6	72.3 ± 1.2
F-2	87.3	316.83±15.9	0.123	95.6 ± 1.8	85.5 ± 3.5	83.7 ± 1.6
F-3	85.2	294.43±11.6	0.134	90.3 ± 0.8	89.5 ± 1.9	53.2 ± 2.1
F-4	71.5	354.21±17.3	0.092	91.9 ± 1.6	87.6 ± 4.7	84.2 ± 1.7
F-5	79.4	250.42±10.1	0.283	93.8 ± 2.4	94.7 ± 2.8	93.4 ± 0.9
F-6	83.7	335.27±10.3	0.104	88.3 ± 2.9	92.4 ± 2.6	83.2 ± 0.6
F-7	66.3	235.73±13.8	0.213	80.3 ± 2.4	65.9 ± 3.1	45.3 ± 1.4
F-8	76.2	361.53±15.7	0.088	79.3 ± 1.5	70.7 ± 1.8	63.2 ± 0.5
F-9	78.5	330.42±12.5	0.110	73.6 ± 2.8	80.6 ± 1.7	56.2 ± 1.8

***Determination of mean with ±SD**

5B.3.2.1 Micromeritic Properties of MTG Microsponges

The Bulk density was found to be in the range of 0.088 to 0.294 g/cc. There was somewhat direct relation observed between bulk density and particle size, as the particle size increased the bulk density decreased. The outcomes were in accordance with the results obtained by Arya et.al. which says that density of microsponges decreased with increase in particle size[4]. The result of the morphological characteristics of the drug is given in the figure 5B.4. This figure shows the spherical nature and spongy behavior of the prepared microsponges. The formulations prepared with minimum concentration of ethyl cellulose lacked the spherical symmetry, as they were not orbicular in shape.

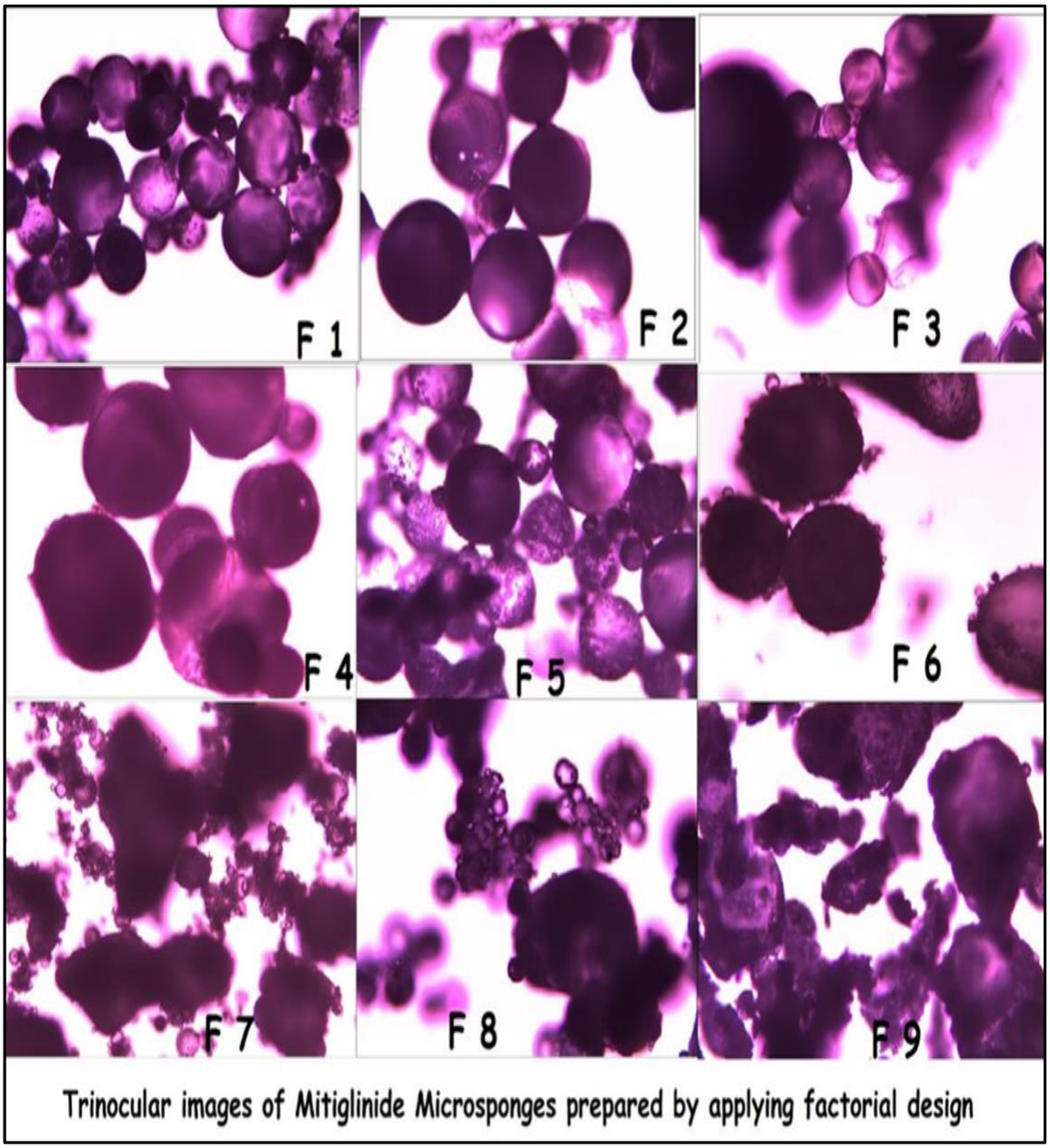


FIGURE 5B.4 Trinocular microscopic images of MTG microsponges

5B.3.2.2 *In vitro* Drug Release

The drug release data shows the release pattern of all the formulations prepared after applying 3^2 full factorial design (F-1 to F-9), for the optimization of MTG floating gastroretentive microsponges. It also displays the release pattern of the optimized formulation (F-0) of MTG microsponges, as obtained by the design expert software. The *in vitro* data is given in table no 5B.6 and the graphical representation of the same is given in figure 5B.5. The release study of all the batches showed the wide variation in the pattern of the drug release. This shows the significant outcome of the chosen independent variables along the drug release from the polymeric matrix.

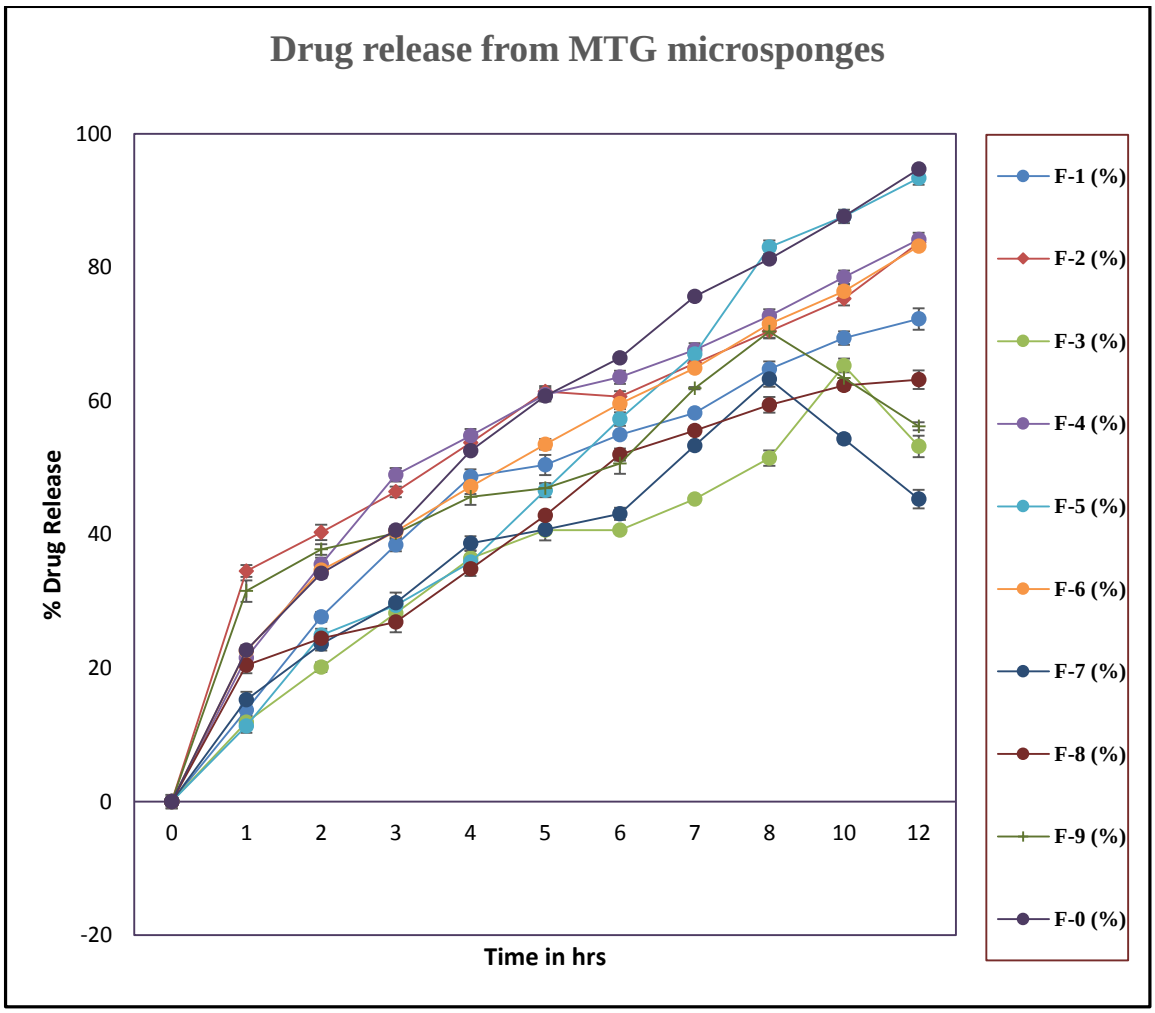


FIGURE 5B.5 Graphical representation of *in vitro* release profile of MTG microsponges

TABLE 5B.6 *In vitro* drug release profile of MTG microsponges prepared by 3^2 full factorial design†

	F-1 (%)	F-2 (%)	F-3 (%)	F-4 (%)	F-5 (%)	F-6 (%)	F-7 (%)	F-8 (%)	F-9 (%)	F-0 (%)*
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1	13.7±1.53	34.54±0.92	11.91±0.79	21.54±0.49	11.32±1.32	22.56±1.6	15.25±1.18	20.43±2.17	31.53±0.92	22.68±0.89
2	27.68±0.54	40.33±0.71	20.13±1.04	35.53±1.39	24.91±1.16	34.65±0.79	23.58±0.94	24.43±0.75	37.77±1.07	34.19±1.16
3	38.47±0.93	46.41±0.99	28.22±0.73	48.95±1.08	29.37±0.45	40.47±0.88	29.79±1.53	26.91±0.92	40.18±0.21	40.65±0.79
4	48.68±1.6	53.76±1.1	36.45±0.89	54.81±0.59	35.87±0.91	47.19±1.17	38.68±1.06	34.87±0.79	45.63±0.61	52.55±1.09
5	50.42±0.88	61.43±1.53	40.64±0.92	60.93±0.21	46.61±0.79	53.52±0.77	40.75±0.46	42.87±0.91	46.95±1.16	60.73±0.79
6	54.97±0.79	60.67±0.49	40.66±1.32	63.56±1.6	57.31±1.18	59.62±1.53	43.12±0.92	51.98±0.57	50.61±0.79	66.45±0.82
7	58.21±0.16	65.66±0.31	45.32±0.22	67.65±0.26	67.04±0.13	64.93±0.17	53.32±0.17	55.55±0.16	61.92±0.31	75.68±0.22
8	64.77±1.39	70.45±1.16	51.44±0.53	72.75±0.69	83.05±0.78	71.56±1.03	63.31±1.19	59.43±0.73	70.41±0.57	81.26±0.99
10	69.42±1.02	75.34±1.02	65.32±0.52	78.54±0.91	87.65±0.42	76.43±0.89	54.32±0.67	62.32±0.88	63.45±0.45	87.65±1.02
12	72.3±1.2	83.7±1.6	53.2±2.1	84.2±1.7	93.4±0.9	83.2±0.6	45.3±1.4	63.2±0.5	56.2±1.8	94.75±0.09

***F-0 is the release pattern of the optimized gastroretentive floating microsponges of MTG as given by design expert software**
†Determination of mean with ±SD

5B.3.2.3 Drug Release Kinetics

The results for the analysis of model-dependent drug release kinetics, for the *in vitro* release of MTG from floating microsponges, is given in table 5B.7. The model dependent approaches evaluated for the drug release kinetics were zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas. The drug release from the floating microsponges of MTG, batches F-1, F-2, F-4, F-6 and F-8 followed Higuchi diffusion model with R^2 value close to 1. This model is generated by plotting cumulative percentage drug release versus square root of time and is applicable for modified dosage forms especially to matrix drug delivery systems. Other batches, F-3, F-7, F-9 and F-0 followed Korsmeyer Peppas model (R_P). This data is obtained from *in vitro* drug release studies by plotting log cumulative percentage drug release versus log time. Only one batch, F-5 followed Zero-order drug release kinetics.

TABLE 5B.7 Results table for *in vitro* drug release model-dependent kinetics of MTG microsponges

Batch code	Higuchi model (R_H)	Korsmeyer Peppas model (R_P)	Hixson Crowell model (R_{HC})	First order (R_1)	Zero order (R_0)
F-1	0.9781	0.9702	0.9778	0.8905	0.9419
F-2	0.9798	0.9651	0.9041	0.7751	0.8201
F-3	0.9653	0.9828	0.9727	0.9174	0.9438
F-4	0.9863	0.9791	0.9697	0.8435	0.9164
F-5	0.9337	0.9794	0.9805	0.7916	0.9855
F-6	0.9974	0.9955	0.9743	0.8117	0.9229
F-7	0.9494	0.9878	0.9716	0.9452	0.9274
F-8	0.9666	0.8919	0.9438	0.9024	0.9408
F-9	0.867	0.9862	0.8951	0.9243	0.7355
F-0	0.9904	0.9931	0.9888	0.8118	0.9575

5B.3.3 Statistical Analysis

The result of all the dependent variables is given in table 5B.5. The full factorial analyses, describes the quadratic effects of the variables on the responses. A statistical model incorporating interactive and polynomial terms were utilized to evaluate responses. The polynomial equation generated under 3^2 full factorial design using Design expert software is as follows:

$$Y = b0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

Where, Y is the dependent variable, b_0 is the intercept, and b_1 to b_{22} are regression coefficient. The master effects (X_1 and X_2,) represent the average result of changing one element at a time from its low to high value. X_1X_2, represents the interaction terms and X_1^2 and X_2^2 represents quadratic effect.

The results of statistical analysis of experimental design batches obtained by Design Expert software are shown in table 5B.5. Figure 5B.6 represents the contour plots and surface response curves for each response.

TABLE 5B.8 ANOVA table for response parameters for 3^2 full factorial design for MTG floating gastroretentive microsponges

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	P-value
Yield (%)					
Model	346.47	5	69.29	30.06	0.0091
Residual	6.91	3	2.30		
Corrected Total	353.38	8			
Buoyancy (%)					
Model	553.76	5	110.75	50.21	0.0043
Residual	6.62	3	2.21		
Corrected Total	560.38	8			
Entrapment Efficiency (%)					
Model	714.42	5	142.88	13.11	0.0299
Residual	32.68	3	10.89		
Corrected Total	747.10	8			
CDR_{12} (%)					
Model	2193.91	5	438.78	26.83	0.0108
Residual	49.06	3	16.35		
Corrected Total	2242.98	8			

The summary of Analysis of Variance table for response parameters is given in table 5B.8. The model was proved to be significant after observing the P value for the response parameters. Model simplification was carried out by eliminating non-significant terms ($p > 0.05$) in polynomial equations[33]. The P value for yield, entrapment efficiency, buoyancy and cumulative drug release in 12 hrs (CDR_{12}) were found to be 0.0091, 0.0299, 0.0043 and 0.0108, respectively, which is less than 0.0500 indicating the significance of model terms.

5B.3.3.1 Effect of Independent Variables on Percentage Yield of Microsponges

The percent yield was found to be in the range of 66.3 to 87.3%. R^2 was found to be equal to 0.9804. The Model F-value of 30.06 implies the model is significant. The difference between "Pred R-Squared" and "Adj R Squared" value was found to be less than 0.2, which is desirable. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here, the ratio of 16.430 indicates an adequate signal. This model can be used to navigate the design space. The other information obtained from ANOVA table was about the variables. The P value for both factors, X_1 and X_2 was found to be 0.0039 and 0.0037, respectively, indicating their significance in the model. Interaction effect and quadratic effect of X_1 were found to be insignificant, whereas quadratic effect of X_2 was significant. The fitted final equation can be used to make predictions about the response for given levels of each factor. The equation for the response percentage yield was as follow:

$$\% \text{ Yield} = + 80.62 + 5.05X_1 - 5.13X_2 + 1.45X_1X_2 + 0.52X_1^2 - 3.63X_2^2$$

The equation indicates that X_1 i.e polymer concentration had positive effect on yield of microsponges which indicates that the higher amount of ethyl cellulose contributes to increase in %yield. Factor X_2 i.e concentration of PVA had negative effect on the yield of microsponges, which was contradictory to the finding of Moin et al.[34]. But this result was in agreement with the observation of Singh et al., the reason of may be that high concentration of PVA creates hydrophobic region in association with polymer which consecutively can dissolve some portions of drug resulting in a reduction in production yield within microsponge formulation[35]. These effects were further illustrated in contour and surface response plots Figure 5B.6-A. The relationship between the dependent and independent variables was elucidated by contour plots. The plots were found to be linear up to 75% yield, but above this value, the plots were found to be nonlinear indicating a nonlinear relationship between X_1 and X_2. It was determined from the contour plot that a higher value of Yield

(≥85%) could be obtained with an X_1 level range from 0.6 to 1 and an X_2 level range from –0.1 to 0.12. It is evident from the contour that the high level of X_1 and low level of X_2 favors percentage yield of microsponges of MTG.

5B.3.3.2 Effect of Independent Variables on Buoyancy

The percent Buoyancy was found to be in the range of 73.6 to 98.3%. R^2 was found to be equal to 0.9882. The Model F-value of 50.21 implies the model is significant. The difference between "Pred R-Squared" and "Adj R Squared" value was found to be less than 0.2, which is desirable. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here, the ratio of 19.049 indicates an adequate signal. This model can be used to navigate the design space. The other information obtained from ANOVA table was about the variables. The P value for both factors, X_1 and X_2 was found to be 0.0008 and 0.0151, respectively, indicating their significance in the model. Interaction effect and quadratic effect of X_2 were found to be insignificant, whereas quadratic effect of X_1 was significant. The fitted final equation can be used to make predictions about the response for given levels of each factor. The equation for the response percentage buoyancy was as follow:

$$\% \text{ Buoyancy} = + 92.97 + 8.50X_1 + 3.05X_2 + 0.32X_1X_2 - 5.10X_1^2 - 2.45X_2^2$$

The equation indicates that the magnitude of coefficient of both X_1 and X_2 shows positive and significant effect on buoyancy of the floating microsponges. As the concentration of ethyl cellulose and PVA increased, the buoyancy of the microsponges increased. The reason for the more buoyancy due to high concentration of ethyl cellulose may be attributed to the low density of polymer, as reported in the literature[36]. However, an antagonistic quadratic effect of concentration of ethyl cellulose was observed, which means that optimal levels of X are not in the extremes of the experimental region but inside it. These effects were further illustrated in contour and surface response plots (Figure 5B.6 -B). The plots were found to be curvilinear up to 90% buoyancy, but above this value, the plots were found to be nonlinear indicating a nonlinear relationship between X_1 and X_2. It was determined from the contour plot that a higher value of Buoyancy (≥95%) could be obtained with an X_1 level range from 0.25 to 1 and an X_2 level range from –0.3 to 1.

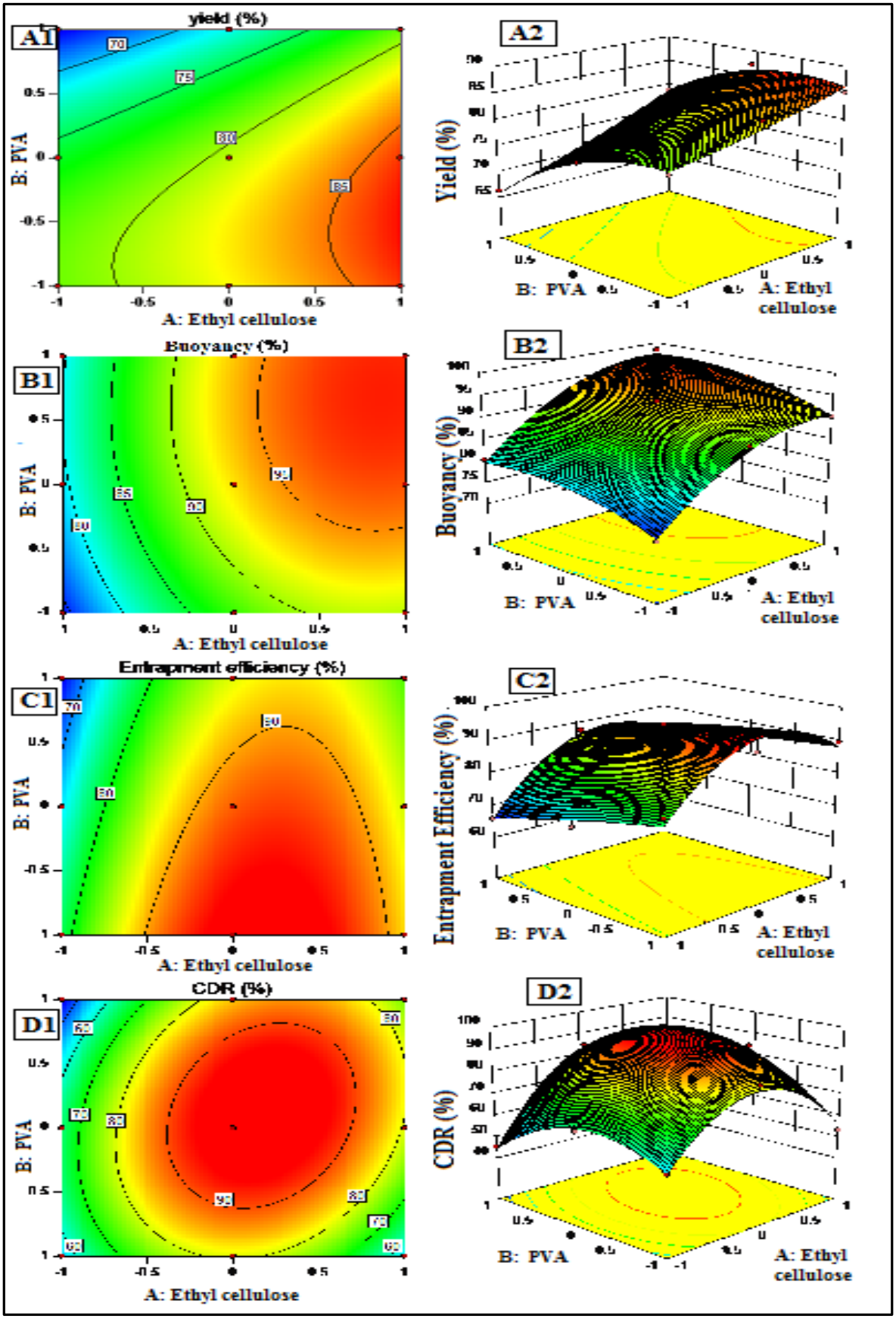


FIGURE 5B.6 Contour plot and Response surface plot for MTG floating microsponges: (A): Yield (B): Buoyancy (C): entrapment efficiency (D): CDR_{12}; 1-Contour plot; 2-Surface response plot.

It is evident from the contour that the high level of X_1 and X_2 favors the buoyancy of microsponges of MTG, due to making the formulation less dense.

5B.3.3.3 Effect of Independent Variables on Entrapment Efficiency

The percent entrapment efficiency was found to be in the range of 65.9 to 94.7%. R^2 was found to be equal to 0.9563. The Model F-value of 13.11 implies the model is significant. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here, the ratio of 11.141 indicates an adequate signal. This model can be used to navigate the design space. The other information obtained from ANOVA table was about the variables. The P value for both factors, X_1 and X_2 was found to be 0.0169 and 0.0417, respectively, indicating their significance in the model. Interaction effect and quadratic effect of X_2 were found to be insignificant, whereas quadratic effect of X_1 was significant. The fitted final equation can be used to make predictions about the response for given levels of each factor. The equation for the response percentage entrapment efficiency was as follow:

$$\% EE = + 92.07 + 6.52X_1 - 4.62X_2 + 1.62X_1X_2 - 12.65X_1^2 - 0.75X_2^2$$

The equation indicates that X_1 i.e polymer concentration had positive effect on entrapment efficiency of microsponges which indicates that the higher amount of ethyl cellulose contributes to increase in entrapment of drug[37]. Factor X_2 i.e concentration of PVA had negative effect on the entrapment efficiency of microsponges[35]. The justification may be same as that for the low yield of microsponges. However, an antagonistic significant quadratic effect of concentration of ethyl cellulose was observed, which means that optimal levels of X are not in the extremes of the experimental region but inside it. These effects were further illustrated in contour and surface response plots (Figure 5B.6-C). The contour plots were found to be nonlinear. This signifies that there is no direct linear relationship among the selected independent variables. It was determined from the contour plot that a higher value of entrapment efficiency (≥90%) could be obtained with an X_1 level range from -0.5 to 0.9 and an X_2 level range from –0.1 to 0.6. It is evident from the contour that medium level of X_1 and minimum level of X_2 favors the entrapment efficiency of microsponges of MTG.

5B.3.3.4 Cumulative Drug Release (CDR 12 hrs)

Microsponges offer the controlled release of the drug as proven during earlier studies performed by Osmani, et al[2]. The percent CDR_{12} was found to be in the range of 45.3 to 93.4%. R^2 was found to be equal to 0.9781. The Model F-value of 26.83 implies the model is significant. The difference between "Pred R-Squared" and "Adj R Squared" value was found to be less than 0.2, which is desirable. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here, the ratio of 15.859 indicates an adequate signal. This model can be used to navigate the design space. The other information obtained from ANOVA table was about the variables. The P value for factors, X_1 was found to be 0.0206, indicating its significance in the model, whereas the P value for X_2 was found to be insignificant. Interaction effect and quadratic effect of X_1 and X_2 were found to be significant. The fitted final equation can be used to make predictions about the response for given levels of each factor. The equation for the response % CDR_{12} was as follow:

$$\% CDR_{12} = + 96.51 + 7.42X_1 + 1.53X_2 + 7.50X_1X_2 - 24.62X_1^2 - 14.37X_2^2$$

The equation indicates that X_1 i.e. polymer concentration had positive effect on % CDR_{12} of microsponges which indicates that the higher amount of ethyl cellulose contributes to increase in % CDR_{12}. The highest magnitude of significant antagonistic quadratic effect was observed for the X_1 factor, which means that optimal levels of X are not in the extremes of the experimental region but inside it. These effects were further illustrated in contour and surface response plots (Figure 5B.6-D). The contour plots were found to be nonlinear. This signifies that there is no direct linear relationship among the selected independent variables. It was determined from the contour plot that a higher value of percent CDR_{12} ($\geq$90%) could be obtained with medium levels of both X_1 and X_2.

5B.3.4 Evaluation and Validation of the Optimized Formulation

To optimize all the above responses with different targets, a numerical optimization technique by the desirability function and a graphical optimization technique by the overlay plot was used (Figure 5B.7-1 and 2). The optimized formulation was obtained by applying constraints on dependent variable responses and independent variables. The constraints for all the dependent variables were set as 80% to 100%. The recommended concentrations of

the independent variables were calculated by the Design Expert software from the above plots which has the highest desirability near to 1.0. 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 (Formulation F-0). The theoretical values of Y1, Y2, Y3 and Y4 were found to be 83.72%, 95.31%, 92.88%, 93.40%, respectively were found to be in close agreement with the practical values.

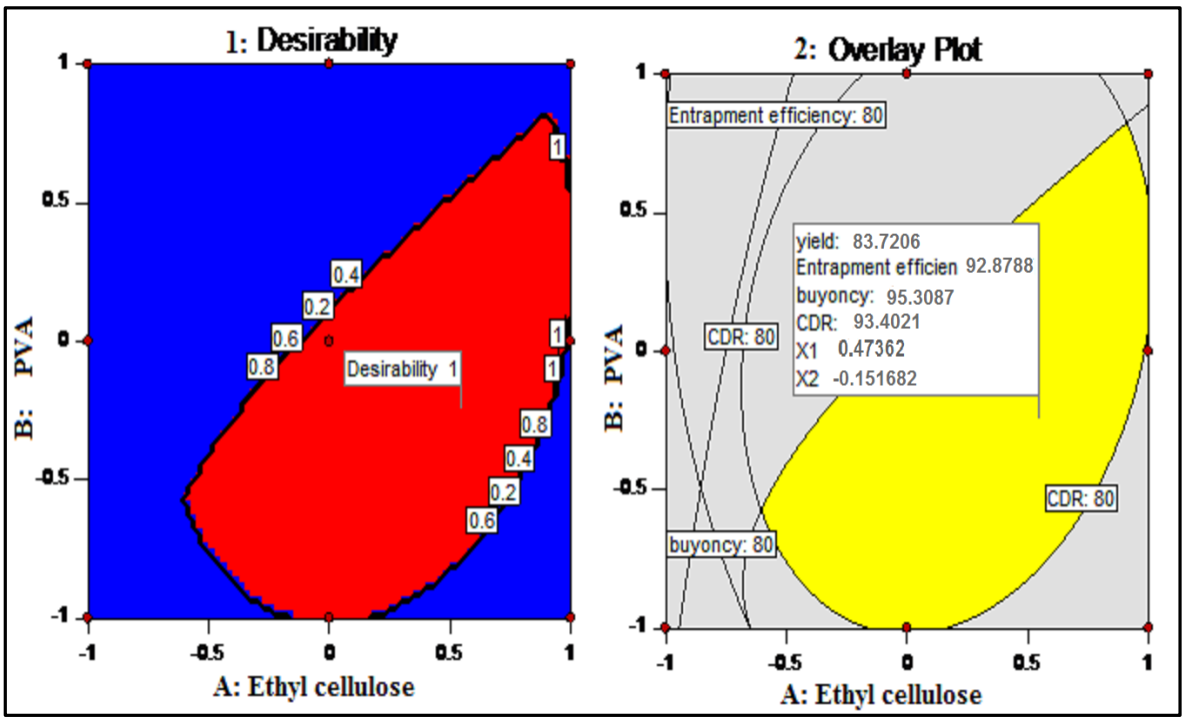


FIGURE 5B.7 Optimization of MTG floating microsponges 1: Desirability function, 2: Overlay Plot

The statistically optimized formulation of MTG floating microsponges (F-0) fulfilled all the physicochemical criteria. The formulation was evaluated for finding the experimental values of all the dependent variables to confirm the theoretical estimate. The observed value of yield (82.95 %), buoyancy (94.62 %), entrapment efficiency (92.29%) and CDR_{12} (94.75%) were in close agreement with the model predictions of yield (83.7206 %), buoyancy (95.3087%), entrapment efficiency (92.8788%) and CDR_{12} (93.40%). The relative errors (%) between the predicted and experimental values for each response were calculated and the values found to be within 5%. The experimental values were in agreement with the predicted values confirming the predictability and validity of the model. This formulation was considered to be the optimized formulation of MTG floating microsponges.

5B.3.5 Physicochemical Characterization of MTG Microsponges

5B.3.5.1 Scanning Electron Microscopy (SEM)

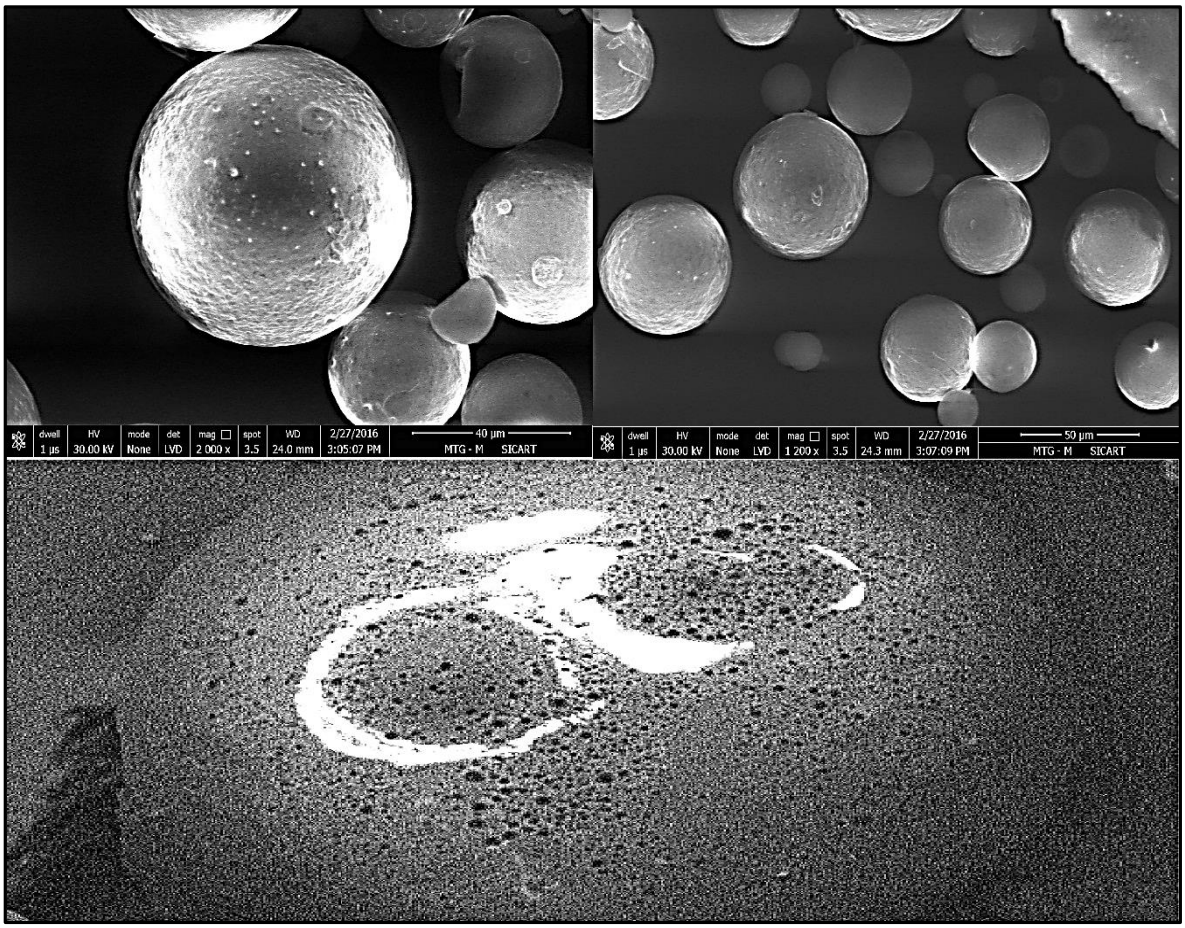


FIGURE 5B.8 SEM images of MTG floating microsponges (F-0)

SEM images of F-0, optimized batch of MTG microsponges were taken to get the topographical information about the formulation. The SEM images are shown in Figure 5B.8, which revealed that the optimized MTG microsponges are smooth surfaced spherical and highly porous in nature. Porosity was induced in the microsponges during the diffusion of solvent from surface of microsponges. Furthermore, no drug crystals were observed over the surface of formulation.

5B.3.5.2 Differential Scanning Calorimetry (DSC)

DSC provides the information about the crystalline and amorphous form of drug and possible interaction during the polymerization and formulation of microsponges[38]. The thermogram

of pure MTG exhibited a sharp endothermic peak at 172˚C equivalent to its melting point (179–185˚C), representing its crystalline nature (Figure 5B.9 - A).

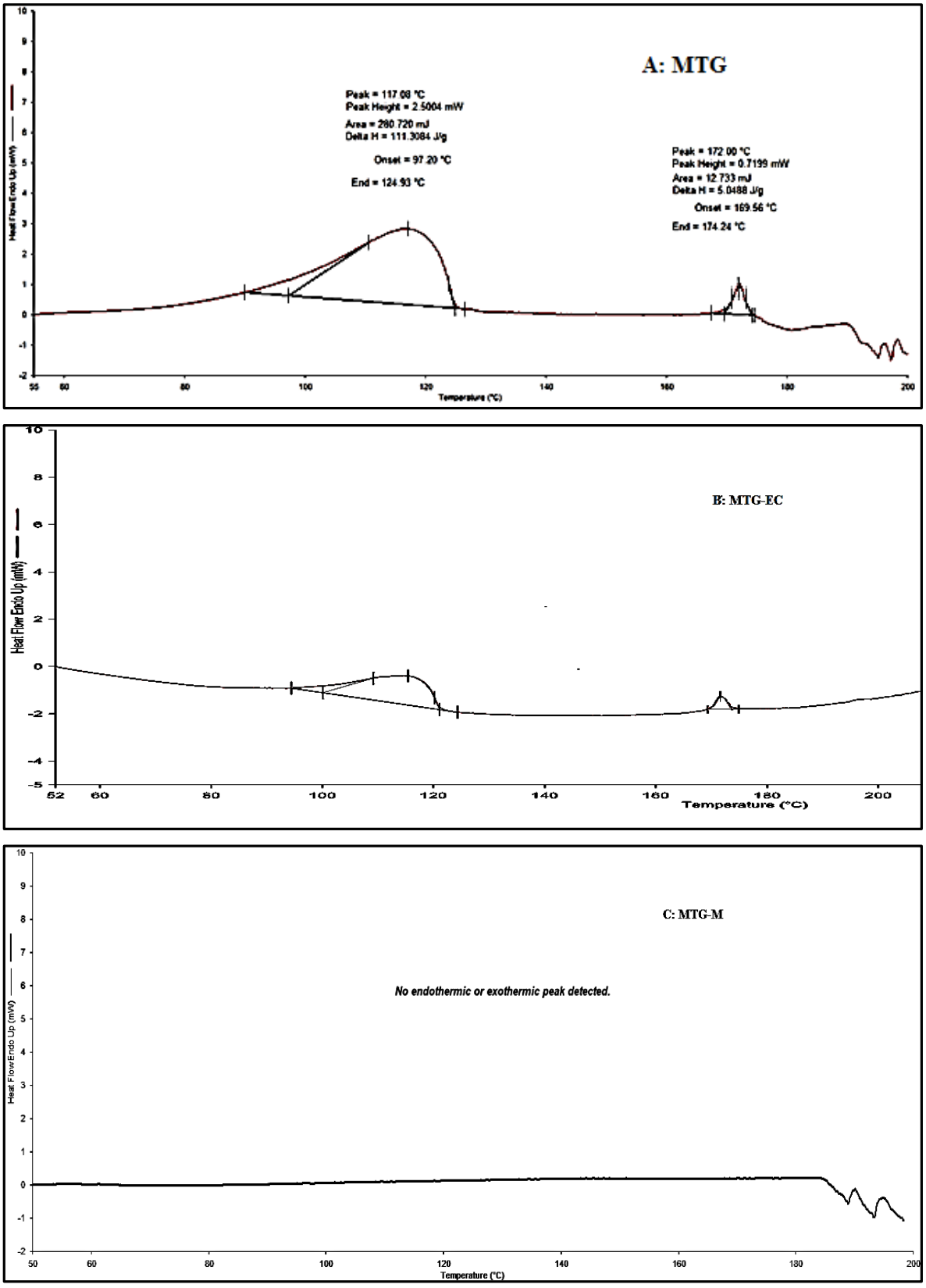


FIGURE 5B.9 DSC scan of A) MTG, b) Physical mixture of drug and polymer, c) Optimized formulation F-0

There was another peak observed at 117.08 ˚C, which was because of the presence of water molecules in the salt form of MTG. This is due of the fact that dihydrate shows two endothermic peaks, first peak corresponds to loss of water molecule and the second corresponds to melting of the crystalline drug[39-42]. The thermogram of physical mixture of polymer (EC) and drug (MTG) showed the peaks at 115.42 and 174.2˚C, corresponding to dihydrate and drug, respectively. There was decrease in the peak intensity and slight increase in the peak temperature at both peaks, which may be attributed to the presence of ethyl cellulose and the dispersion of drug in polymeric matrix. The thermogram of F-0 showed detectable loss of sharp peak for pure drug that signifies uniform molecular dispersion of MTG in polymer matrix of microsponges[43,44]. Appearance of no new peak and absence of any potential shift suggested compatibility of MTG with polymers and was confirmed by XRD and FTIR.

5B.3.5.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR scan of MTG, ethyl cellulose, physical mixture of drug and polymer and optimized formulation of MTG microsponges F-0 was taken (Figure 5B.10). The IR peaks observed in the IR scan of pure MTG were: 3537.48 (O-H stretch); 3416.55 (N-H stretch, amide); 3082.21, 3060.87, 3026.62 (C-H stretch); 2924.24, 2869.21, 2850.93 (C-H stretch); 1649.83 (C=O stretch, amide merged); 1622.60 (N-H bend); 1544.75 (C=C stretch, aromatic). Same peaks, with slight change in intensity, were found to be present in drug-polymer physical mixture and microsponge formulations. As there was no change and shifting of characteristic peaks of MTG in F-0, it indicated no significant drug-polymer interaction. Hence, MTG is compatible with the polymer in microsponge formulations.

The peaks corresponding to the characteristics bands of the drug were found to be preserved in the spectra of the microsponges which indicates that no chemical interaction between drug and polymer has been taken place during the preparation of the formulations.

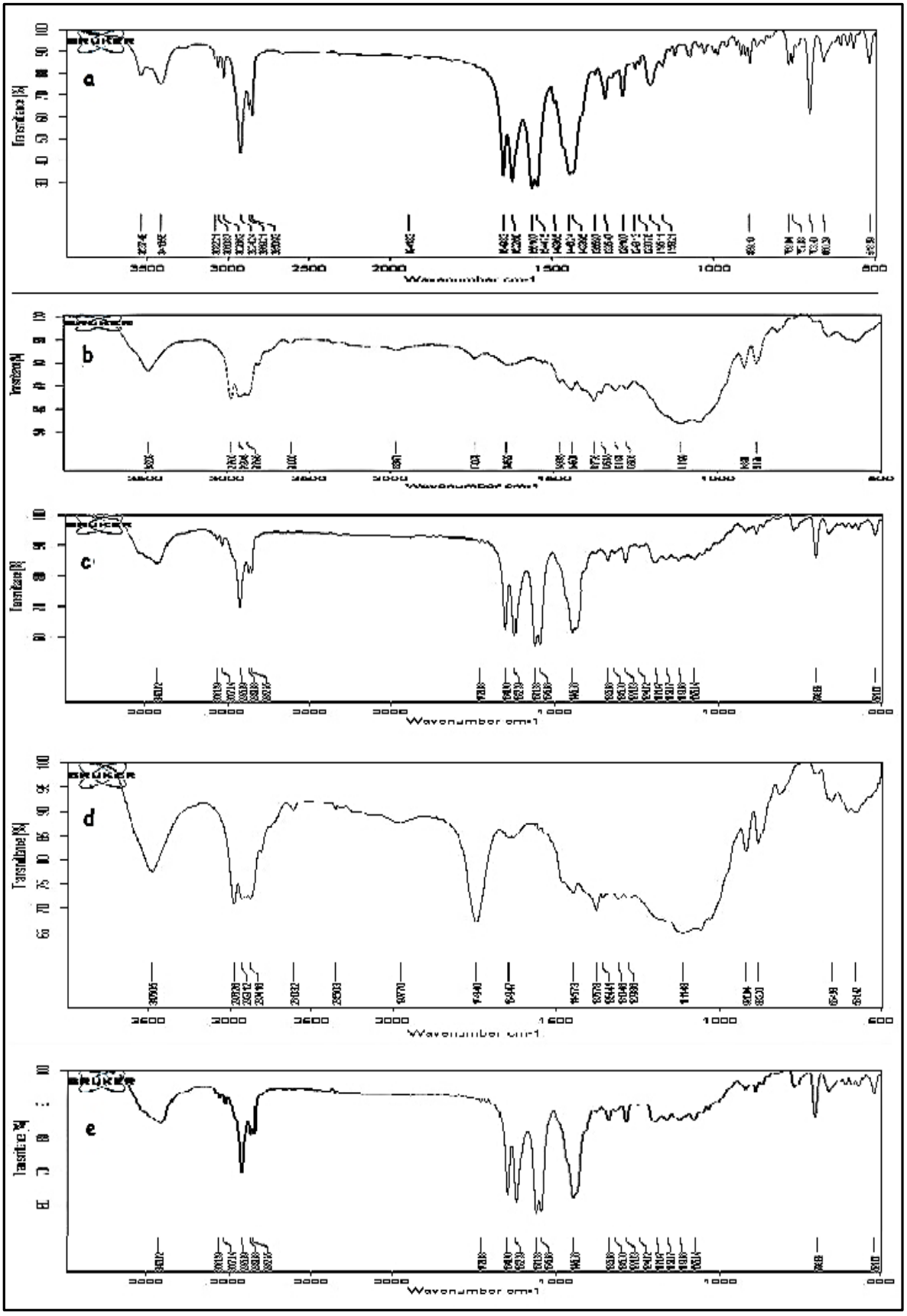


FIGURE 5B.10 FTIR scan of a) MTG b) Ethyl cellulose c) MTG-ethyl cellulose physical mixture d) Placebo microsponges e) MTG microsponges

5B.3.5.4 Powder X-ray Diffraction (XRD)

Powder X-ray diffraction (XRD) was performed for optimized microsponge formulation of MTG to investigate the effect of polymerization on crystallinity of the drug. The XRD scan of F-0, optimized floating microsponges of MTG was compared with the XRD scan of pure drug (Figure 5B.11).

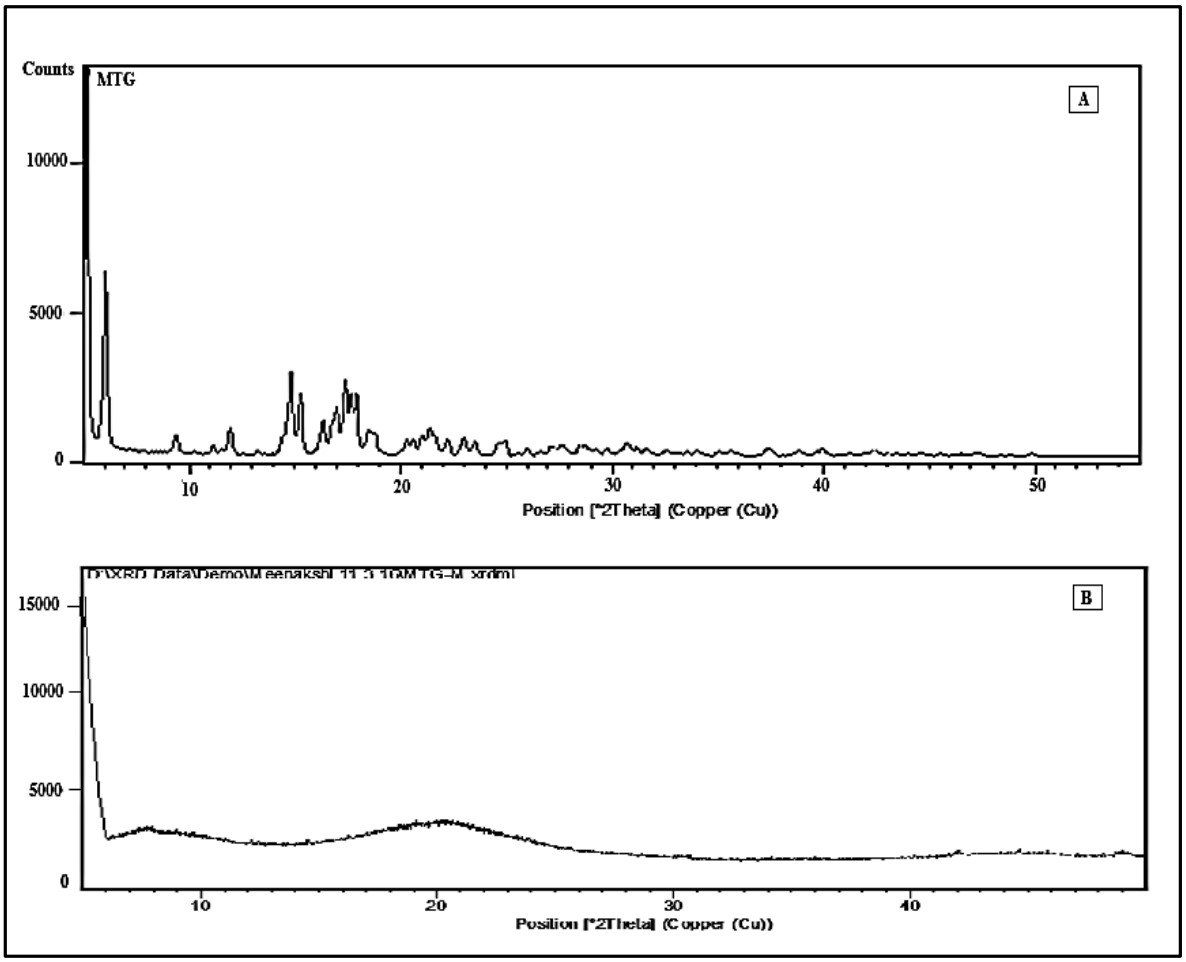


FIGURE 5B.11 XRD scan of A) MTG pure drug B) MTG Microsponges (F-0)

The distinct peaks can be observed in the XRD of pure MTG which shows the crystalline behavior of the drug. The disappearance of most of the characteristic peaks of the drug in the XRD scan of F-0 indicates that most of the drug has been converted to the amorphous form and drug is dispersed at a molecular level in the polymeric matrix. The disappearance of the characteristic peaks of the drug in the formulation indicate that the drug is dispersed at a molecular level in the polymer matrix[43,44]. The interpretation of the results achieved was matching with the XRD analysis obtained by Deshmukh, R[22].

5B.3.5.5 Residual Solvent Analysis

There are many volatile organic chemicals which are used in the preparation of pharmaceutical preparations. Some amount of those organic solvents might remain in the final formulation, which is called as residual solvents. Residual solvents cannot be entirely taken out by the standard manufacturing process and can remain in final finished product at low levels (ppm). These residual solvents are classified into three classes based on their potential risk to human health[14,45]. The ICH guidelines "Q3C" for the residual solvents, has given the permitted daily exposure (PDE) and concentration limit in ppm for such solvents[23]. The dichloromethane (DCM) was used in the preparation of microsponges and it belongs to class 1 residual solvents and its amount in the finished formulation should be within limit (up to 600 ppm).

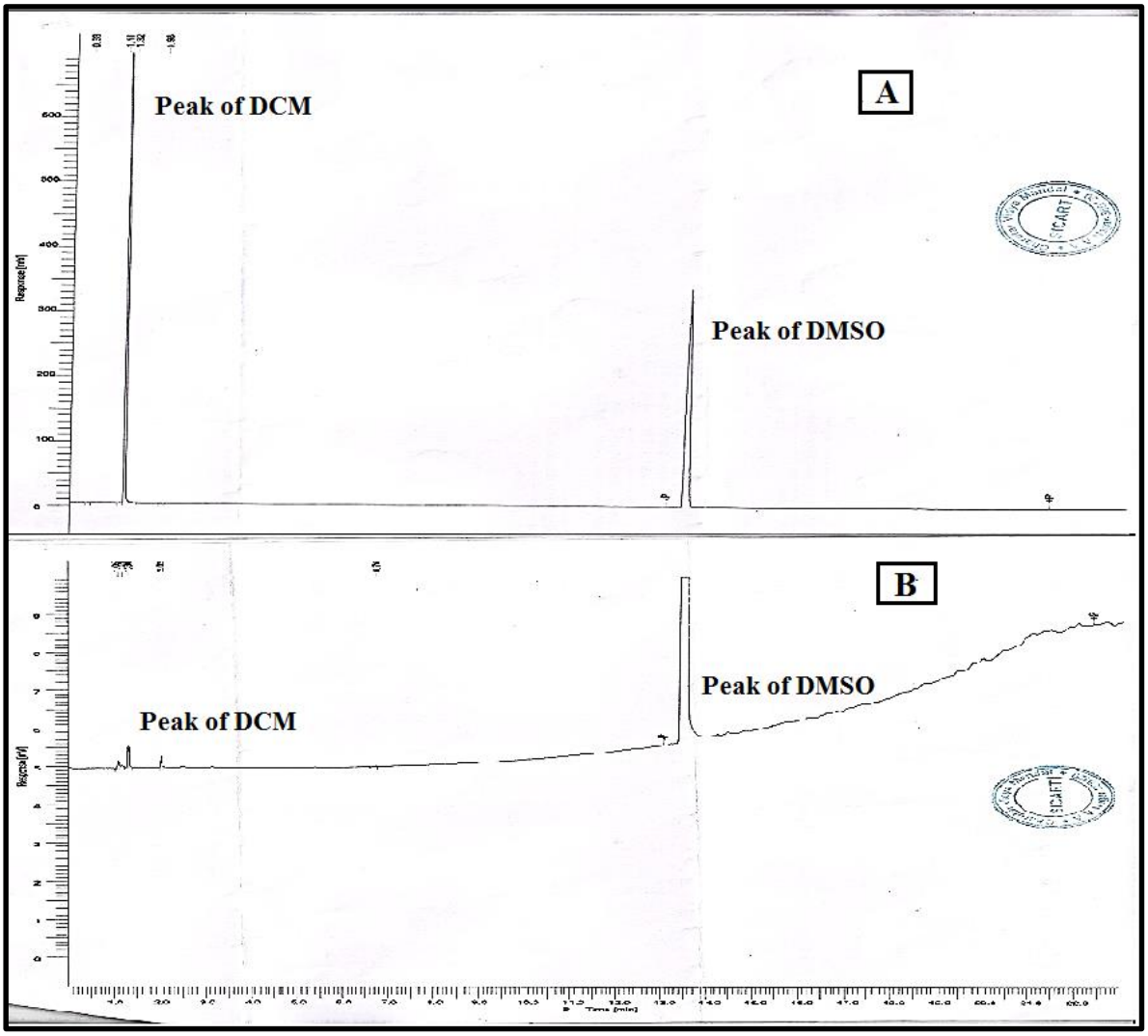


FIGURE 5B.12 GC scan for DCM residual solvent in MTG floating microsponges A) Scan of standard DCM solution; B) Scan of F-0 formulation, solution prepared in DMSO

The gas chromatogram of standard DCM solution in Dimethyl sulfoxide (DMSO) and F-0 dissolved in DMSO is shown in figure 5B.12. The retention time of dichloromethane was 1.181 min as observed in standard preparation of DCM. The peak of DMSO was observed at 13.4 minutes in both standard and sample preparation. The retention time of dichloromethane was 1.181 min and limit of quantitation for dichloromethane was found to be 15 ppm according to the signal-to-noise ratio (10:1) method. Dichloromethane residue was within the limits, in the MTG loaded microsponges. Hence, the prepared formulation F-0 is considered to be safe for human use.

5B.3.6 Stability Study of Optimized Formulation as per ICH Guidelines

Stability study conducted for the formulation F-0, optimized formulation of MTG floating microsponges, indicated the physical and chemical stability of the formulation for the period six months under test condition (5B.13). There was no change in the colour and shape of microsponges. There was no significant change observed in the drug content, drug content and drug release pattern of the drug on storage for six months.

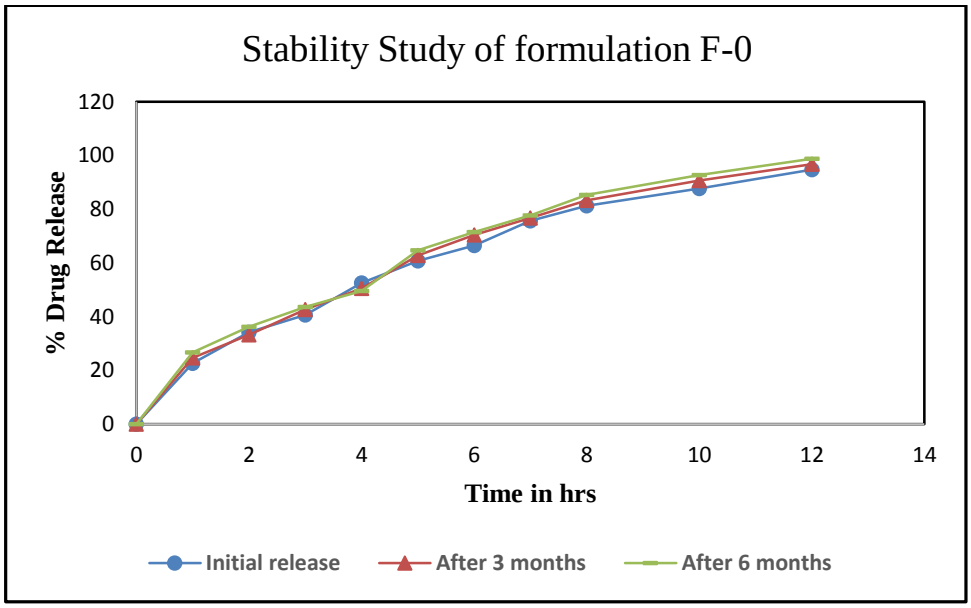


FIGURE 5B.13 Stability study of optimized floating microsponges of MTG (F-0)

5B.3.7 *In vivo* Studies

In vivo studies were executed to determine the residence of the F-0 in the stomach and also to go over its extended glucose lowering capacity as compared to pure MTG. The

pharmacokinetics studies of the optimized formulation of MTG floating microsponges and pure MTG were also done to determine the extent of the drug in the body which can achieve better treatment of the disease.

5B.3.7.1 Radiographic Study

To determine the retention time of the optimized floating microsponges of MTG, radiographic studies were conducted. The floating barium Sulfate loaded microsponges were given to rabbits. The X-ray photomicrographs were taken immediately at 0, 4 and 12 h and were recorded, as shown in Figure 5B.14. The *in vivo* X-ray imaging study clearly indicated that the optimized formulation of MTG microsponges, F-0, remained afloat in gastric fluid up to 12 h in the stomach of rabbit. Hence, it is believed that the developed gastroretentive microsponges of MTG will remain buoyant in the stomach of human being as well.

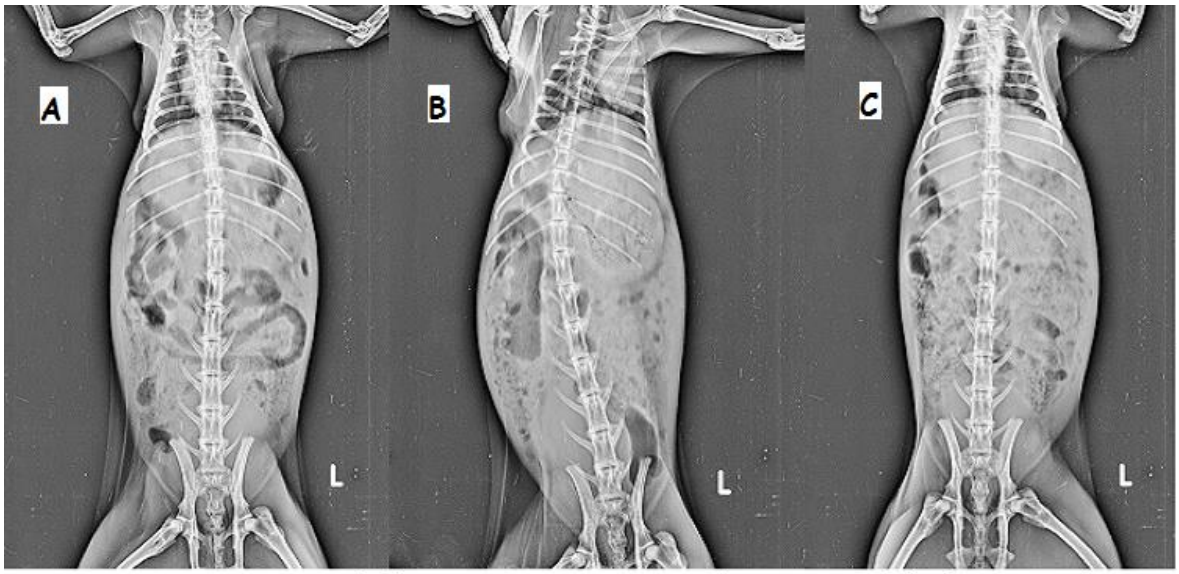


FIGURE 5B.14 X-ray images of floating MTG microsponges

5B.3.7.2 Pharmacodynamic Study on Diabetic Rats

The pharmacodynamics study of optimized MTG microsponge (F-0) and pure MTG was performed on diabetic wistar rats. The pharmacodynamics studies showed the reduction in the blood glucose level after the single dose administration of pure MTG drug, but it could

not maintain the blood glucose level for extended time. On the other hand, the gastroretentive floating microsponges of mitiglinide F-0 showed a significant decrease in blood glucose level over the period of 12 hrs, as shown as mean with ± S.E.M (Figure 5B.15). Hence, formulation F-0 is very efficient in controlling the body glucose level as compared to pure drug.

This indicates the capability of floating MTG microsponges to release the drug in the body over prolonged period of time. The single dose of pure drug could not control the blood sugar level for long period. Hence, the prepared MTG microsponges are efficiently controlling the body glucose level as compared to pure MTG drug, which can treat type II diabetes in superior manner.

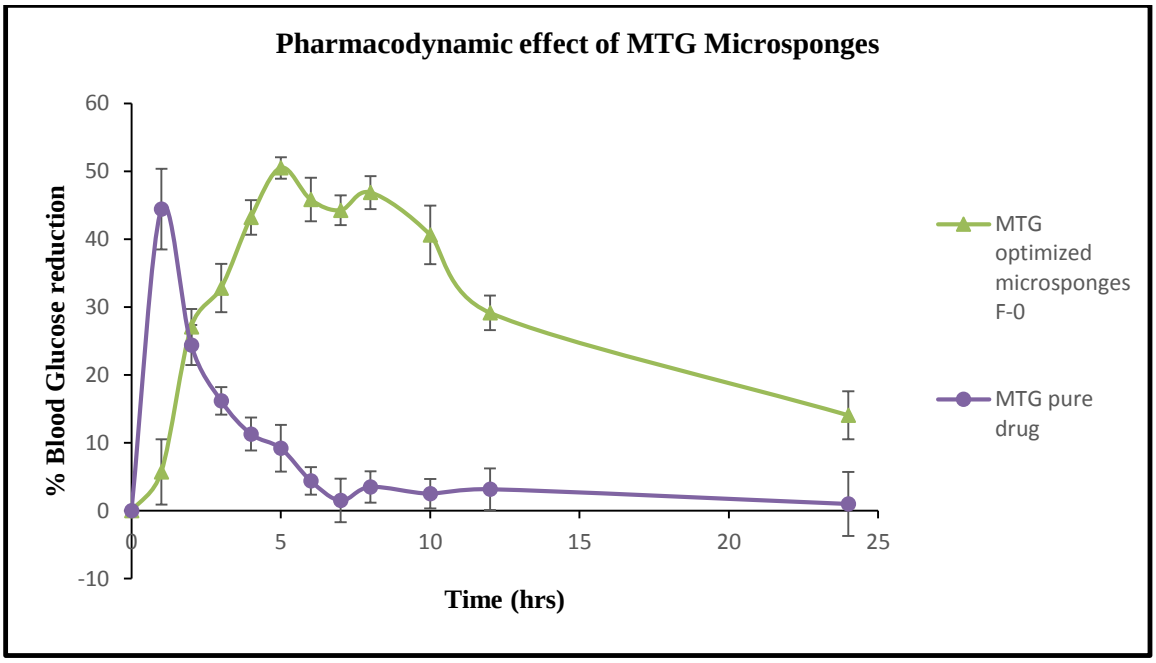


FIGURE 5B.15 Graphical representation of Pharmacodynamic study of MTG microsponges

5B.3.7.3 Pharmacokinetic Study

The *in vivo* study of the optimized batch of floating gastroretentive microsponges of MTG was conducted to obtain its pharmacokinetic data. The study was conducted on twelve healthy wistar rats by dividing them in two groups with equal number of rats (n=6) and the data was expressed in terms of ± S.E.M. The blood samples collected from the rats were extracted, following the method given in chapter 3 (Preformulation studies). The analysis of the plasma samples were performed on HPLC, according to the bioanalytical method of

MTG explained in chapter 3. The HPLC was carried out at room temperature and the stationary phase used was Agilent C 18 column (150 mm ×4.6 mm) with guard column. Acetonitrile:HPLC water (60:40) (pH adjusted to 3.5 with o-phosphoric acid) was used as mobile phase. Glipizide was used as internal standard and the flow rate was maintained as 1.2ml/min at detected wavelength 210 nm. The graphical representation of mean plasma concentration vs time profile of, single dose of, MTG pure drug and its gastroretentive floating microsponges F-0 are shown in Figure 5B.16.

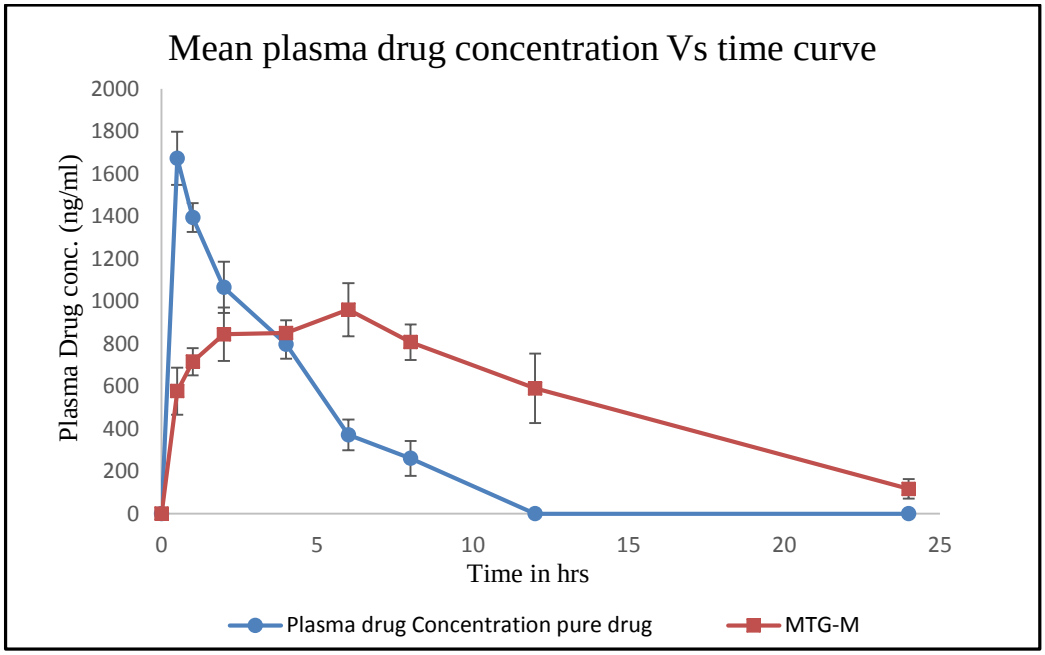


FIGURE 5B.16 Graphical representation of plasma time profile of single dose of mitiglinide pure drug and its gastroretentive floating microsponges F-0

The pharmacokinetic parameters such as C_{max}(µg/mL), t_{max} (hrs), $K_{el}(h^{-1})$, $t_{1/2}$(hrs), $AUC_{0-\infty}$ (µg*hr/mL) and MRT (hrs) of drug were determined from mean plasma concentration time profile, using Kinetica 5.0 software. The MRT of F-0 (8.847 ± 1.62hrs) was found to be increased significantly in comparison to pure MTG (4.29 ± 1.03hrs), which indicated the increased residence time of the optimized MTG microsponges at the site of absorption. The t_{max} value of F-0 was found to be 6hrs, which was increased in comparison to pure drug, where t_{max} was achieved in 0.5 hour. Considerable difference was found in the elimination half-life and elimination rate constant of MTG and F-0. Optimized microsponges (F-0) showed increased elimination half-life of the drug with diminished elimination rate, which indicates the prolonged residence of the drug in blood. A significant difference was observed

in the $AUC_{0-\infty}$ of MTG and F-0 as, 8.167 ± 1.73 µg/ml.h and 13.824 ± 1.48 µg/ml.h, respectively, which indicates the 1.7s fold increase in the relative bioavailability of the drug in formulation F-0.

TABLE 5B.9 Pharmacokinetic parameters of pure MTG and optimized floating microsponges of MTG (F-0)

Sr. No.	Pharmacokinetic parameters	Pure drug MTG	Optimized batch F-0
1	Peak plasma concentration C_{max} (µg/ml)	1.671 ± 0.25	0.960 ± 0.03
2	Time to reach peak plasma concentration T_{max} (h)	0.5	6
3	Elimination half (h) life $t_{1/2}$	2.79 ± 0.47	5.83 ± 0.93
4	Elimination rate constant K_e (h^{-1})	0.248 ± 0.061	0.119 ± 0.017
5	Area under the curve $AUC_{0-\infty}$ (µg/ml.h)	8.167 ± 1.73	13.824 ± 1.48
6	MRT (h)	4.29 ± 1.03	8.847 ± 1.62

5B.4 Conclusion

Gastroretentive floating microsponges of MTG were prepared successfully by quasi-emulsion solvent diffusion method. The results of preliminary studies 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. The solubility study conducted during the preliminary study, suggested that concentration of ethyl cellulose and PVA affects are important factors. 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. Gas chromatographic studies were performed to check the limit of residual solvent, dichloromethane (DCM) in F-0. The results indicated that DCM was within the limits (less than 600ppm), in the MTG loaded microsponges. Hence, the prepared formulation F-0 is considered to be safe for human use.

The optimized microsponges, incorporated with barium sulphate was put in capsule and 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 remained afloat in gastric fluid up to 12 h in the stomach of rabbit. Hence, it was concluded that the optimized MTG microsponges would probably show the similar gastroretention in the stomach of human beings.

The *in vivo* pharmacodynamic and pharmacokinetic studies of F-0 were conducted on diabetic rat and healthy wistar rat, respectively. This results indicated the capability of floating MTG microsponges to release the drug in the body over prolonged period of time and proves the formulation to be sustained release. The single dose of pure drug could not control the blood sugar level for long period of time as indicated by pharmacodynamics studies. Hence, the prepared mitiglinide microsponges are efficient in controlling the body glucose level as compared to pure MTG drug.

5B.5 References

1. Won, R., Advanced Polymer Systems, Inc., 1987. Method for delivering an active ingredient by controlled time release utilizing a novel delivery vehicle which can be prepared by a process utilizing the active ingredient as a porogen. U.S. Patent 4,690,825.

2. Osmani, A.M., H Aloorkar, N., S Kulkarni, A., K Kulkarni, P., Hani, U., Thirumaleshwar, S. and R Bhosale, R., 2015. Novel cream containing microsponges of anti-acne agent: Formulation development and evaluation. *Current Drug Delivery*, *12*(5), pp.504-516.

3. Embil, K. and Nacht, S., 1996. The microsponge® delivery system (MDS): a topical delivery system with reduced irritancy incorporating multiple triggering mechanisms for the release of actives. *Journal of Microencapsulation*, *13*(5), pp.575-588.

4. Arya, P. and Pathak, K., 2014. Assessing the viability of microsponges as gastro retentive drug delivery system of curcumin: Optimization and pharmacokinetics. *International Journal of Pharmaceutics*, *460*(1), pp.1-12.

5. Singh, B.N. and Kim, K.H., 2000. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. *Journal of Controlled Release*, *63*(3), pp.235-259.

6. Çomoğlu, T., Gönül, N. and Baykara, T., 2003. Preparation and *in vitro* evaluation of modified release ketoprofen microsponges. *Il Farmaco*, *58*(2), pp.101-106.

7. Gohel, M.C. and Amin, A.F., 1998. Formulation optimization of controlled release diclofenac sodium microspheres using factorial design. *Journal of Controlled Release*, *51*(2), pp.115-122.

8. Anderson, D.L., Chung-Heng, C. and Nacht, S., 1994. Flow characteristics of loosely compacted macroporous microsponge® polymeric systems. *Powder Technology*, *78*(1), pp.15-18.

9. Kawashima, Y., Niwa, T., Takeuchi, H., Hino, T. and Ito, Y., 1992. Control of prolonged drug release and compression properties of ibuprofen microsponges with

acrylic polymer, Eudragit RS, by changing their intraparticle porosity. *Chemical and Pharmaceutical bulletin*, *40*(1), pp.196-201.

10. Re, M.I. and Biscans, B., 1999. Preparation of microspheres of ketoprofen with acrylic polymers by a quasi-emulsion solvent diffusion method. *Powder Technology*, *101*(2), pp.120-133.

11. Patel, D.M., Patel, N.M., Patel, V.F. and Bhatt, D.A., 2007. Floating granules of ranitidine hydrochloride-gelucire 43/01: formulation optimization using factorial design. *AAPS PharmSciTech*, *8*(2), pp.E25-E31.

12. Kılıçarslan, M. and Baykara, T., 2003. The effect of the drug/polymer ratio on the properties of the verapamil HCl loaded microspheres. *International journal of pharmaceutics*, *252*(1), pp.99-109.

13. Orlu, M., Cevher, E. and Araman, A., 2006. Design and evaluation of colon specific drug delivery system containing flurbiprofen microsponges. *International Journal of Pharmaceutics*, *318*(1), pp.103-117.

14. Pandit, V., Pai, R.S., Yadav, V., Devi, K., Surekha, B.B., Inamdar, M.N. and Suresh, S., 2013. Pharmacokinetic and pharmacodynamic evaluation of floating microspheres of metformin hydrochloride. *Drug Development and Industrial Pharmacy*, *39*(1), pp.117-127.

15. Phutane, P., Lotlikar, V., Ghule, A., Sutar, S., Kadam, V. and Shidhaye, S., 2010. *In vitro* evaluation of novel sustained release microspheres of glipizide prepared by the emulsion solvent diffusion-evaporation method. *Journal of Young Pharmacists*, *2*(1), pp.35-41.

16. Dash, S., Murthy, P.N., Nath, L. and Chowdhury, P., 2010. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica*, *67*(3), pp.217-23.

17. Nokhodchi, A., Jelvehgari, M., Siahi, M.R. and Mozafari, M.R., 2007. Factors affecting the morphology of benzoyl peroxide microsponges. *Micron*, *38*(8), pp.834-840.

18. Solanki, A.B., Parikh, J.R. and Parikh, R.H., 2007. Formulation and optimization of piroxicam proniosomes by 3-factor, 3-level Box-Behnken design. *AAPS PharmSciTech*, *8*(4), pp.43-49.

19. Kawashima, Y., Niwa, T., Takeuchi, H., Hino, T., Itoh, Y. and Furuyama, S., 1991. Characterization of polymorphs of tranilast anhydrate and tranilast monohydrate when crystallized by two solvent change spherical crystallization techniques. *Journal of Pharmaceutical Sciences*, *80*(5), pp.472-478.

20. Jones, D.S. and Pearce, K.J., 1995. An investigation of the effects of some process variables on the microencapsulation of propranolol hydrochloride by the solvent evaporation method. *International Journal of Pharmaceutics*, *118*(2), pp.199-205.

21. Dole, M.N., Patel, P.A., Sawant, S.D. and Shedpure, P.S., 2011. Advance applications of Fourier transform infrared spectroscopy. *International Journal of Pharmaceutical Sciences Review and Research*, *7*(2), pp.159-166.

22. Deshmukh, R.K. and Naik, J.B., 2014. Aceclofenac microspheres: quality by design approach. *Materials Science and Engineering: C*, *36*, pp.320-328.

23. ICH Topic Q3C (R4) Impurities: Guideline for Residual Solvents, 2010. *European Medicines Agency*; pp.3–22.

24. Yerlikaya, F., Ozgen, A., Vural, I., Guven, O., Karaagaoglu, E., Khan, M.A. and Capan, Y., 2013. Development and Evaluation of Paclitaxel Nanoparticles Using a Quality-by-Design Approach. *Journal of Pharmaceutical Sciences*, *102*(10), pp.3748-3761.

25. Sharma, O.P., Shah, M.V., Parikh, D.C. and Mehta, T.A., 2015. Formulation optimization of gastroretentive drug delivery system for allopurinol using experimental design. *Expert Opinion on Drug Delivery*, *12*(4), pp.513-524.

26. Srinivasan, K., Viswanad, B., Asrat, L., Kaul, C.L. and Ramarao, P., 2005. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacological Research*, *52*(4), pp.313-320.

27. Pamidiboina, V., Razdan, R. and Hariprasad, M., 2010. Evaluation of the antihyperlipidemic, cardioprotective activity of a polyherbal formulation. *International Journal of Pharmacy and Pharmaceutical Sciences*, *10*, pp.86-91.

28. Lushan, Y. and Su, Z., 2006. Determination of mitiglinide in rat plasma by high-performance liquid chromatography with UV detection. *Journal of Chromatography B*, *834*(1), pp.204-207.

29. Wagner, J.G. and Nelson, E., 1964. Kinetic analysis of blood levels and urinary excretion in the absorptive phase after single doses of drug. *Journal of Pharmaceutical Sciences*, *53*(11), pp.1392-1403.

30. Wagner, J.G., 1970. "Absorption rate constants" calculated according to the one-compartment open model with first-order absorption: Implications in *in vivo-in vitro* correlations. *Journal of Pharmaceutical Sciences*, *59*(7), pp.1049-1050.

31. Jelvehgari, M., Siahi-Shadbad, M.R., Azarmi, S., Martin, G.P. and Nokhodchi, A., 2006. The microsponge delivery system of benzoyl peroxide: Preparation, characterization and release studies. *International Journal of Pharmaceutics*, *308*(1), pp.124-132.

32. Nacht, S. and Katz, M., 1990. The microsponge: a novel topical programmable delivery system. *Drugs and the Pharmaceutical Sciences*, *42*, pp.299-325.

33. Nayak, A.K. and Pal, D., 2011. Development of pH-sensitive tamarind seed polysaccharide–alginate composite beads for controlled diclofenac sodium delivery using response surface methodology. *International Journal of Biological Macromolecules*, *49*(4), pp.784-793.

34. Moin, A., Deb, T.K., Osmani, R.A.M., Bhosale, R.R. and Hani, U., 2016. Fabrication, characterization, and evaluation of microsponge delivery system for facilitated fungal therapy. *Journal of Basic and Clinical Pharmacy*, *7*(2),pp.39-48.

35. Singh, R.P. and Prajapati, S.K., 2014. Formulation and Evaluation of Prednisolone loaded Microsponges for Colon Drug Delivery: in vitro and Pharmacokinetic study. *International Journal of Pharmaceutical Sciences and Research*, *5*(5), pp.1994-2005.

36. Rowe, R.C., Sheskey, J.P., Owen, S.C., 2006. Handbook of Pharmaceutical Excipients, fifth ed. Pharmaceutical Press, London.

37. Pawar, A.P., Gholap, A.P., Kuchekar, A.B., Bothiraja, C. and Mali, A.J., 2015. Formulation and evaluation of optimized oxybenzone microsponge gel for topical delivery. *Journal of Drug Delivery*, 2015, pp.1-9.

38. Ford, J.L., Timmins, P., 1989. Pharmaceutical thermal analysis: techniques and applications. *Halsted Press*.

39. Ramana, G. and Chaitanya, A.K., 2011. Preparation and *In vitro* characterization of ethylcellulose coated pectin alginate microspheres of 5-fluorouracil for colon targeting. *Journal of Applied Pharmaceutical Science*, 1(8), pp.170-176.

40. Sawant K.D., Naik T.A., Naidu A.V., 2013. Preparation, Characterization of Hydrates and Anhydrous Forms of Anti migraine Drug - Frovatriptan Succinate. *Research Journal of Pharmaceutical Sciences.* 2(5), pp.1-10.

41. Cantor, S., 1979. DSC study of melting and solidification of salt hydrates. *Thermochimica Acta*, *33*, pp.69-86.

42. Zhang, Y., Debenedetti, P.G., Prud'homm, R.K. and Pethica, B.A., 2004. Differential scanning calorimetry studies of clathrate hydrate formation. *The Journal of Physical Chemistry B*, *108*(43), pp.16717-16722.

43. Bodmeier, R. and Chen, H., 1989. Preparation and characterization of microspheres containing the anti-inflammatory agents, indomethacin, ibuprofen, and ketoprofen. *Journal of Controlled Release*, *10*(2), pp.167-175.

44. Singla, A.K., Sharma, M.L. and Dhawan, S., 2001. Nifedipine loaded chitosan microspheres: characterization of internal structure. *Biotechnic & Histochemistry*, *76*(4), pp.165-171.

45. Witschi, C. and Doelker, E., 1997. Residual solvents in pharmaceutical products: acceptable limits, influences on physicochemical properties, analytical methods and documented values. *European Journal of Pharmaceutics and Biopharmaceutics*, *43*(3), pp.215-242.

CHAPTER 6A

Gastroretentive Floating Matrix Tablet of Glipizide (GLP)

6A.1 Introduction

The literature review suggested that Glipizide (GLP) is weakly acidic in nature with pKa value equal to 5.9, which means that the drugs remains unionized at acidic pH[1,2]. The unionization is the prerequisite for the drugs to get absorbed by passive diffusion mechanism. Hence, the gastroretentive dosage form of GLP is desired. The elimination half-life of GLP is 2–4 h, which demands frequent administration of drug, to maintain its level in the body for extended period of time. Gastroretentive dosage form overcomes that demerit by releasing the drug continuously in the upper part of gastrointestinal tract, thereby achieving the better control of plasma glucose level[3,4].

The exhaustive literature research elucidates that gastro retentive formulations of glipizide have been prepared using several approaches[5-8]. Present research involves the development of gastroretentive floating matrix tablet of GLP by effervescence mechanism. Floating matrix tablet of GLP was prepared using the combination of hydrophilic polymer HPMC K15M with anionic and non-ionic polymers. The approach used is same as that of used for the formulation and optimization of floating matrix tablet of metformin. Various anionic and non-ionic polymers used in the present work are sodium alginate, kappa carrageenan, pullulan, xanthan gum and poloxamer 188. The final optimization of floating GLP formulation was done by applying Simplex lattice design (SLD)[9] 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.

6A.2 Experimental Studies

6A.2.1 Method of Preparation of GLP Floating Matrix Tablets

Tablets containing 10mg of Glipizide were made by wet granulation technique[10,11]. The required quantity of drug, cross linking polymers and gas generating agent, were sieved through sieve number #80 and were thoroughly mixed in a mortar by following geometric order. Then, the required quantity of microcrystalline cellulose was added and the mixture was filled in plastic bottle. These bottles were placed in double cone blender and the equipment was run for 5minutes. After the set time, the powder blend was put in mortar and the granulation was performed using granulating fluid (polyvinyl pyrrolidone, PVP K30, dissolved in alcohol). The mixture was blended properly with granulating fluid to form a dough mass. The mass was passed through mesh No. 10 to obtain wet granules. The wet granules were dried by keeping in hot air oven at 60ºC for an hour. The dried granules were passed through mesh No. 16 to break aggregates and then sieved through sieve no. 40 to separate granules and fines. The magnesium stearate (1%) and (10%) fines were added to dry granules and blended in double cone blender after enclosing into a closed plastic bottle. The granules were then compressed into tablets on rotary tablet compression machine, using 7 mm round and flat punches with the hardness of 5 kg/sq.cm.

6A.2.2 Preliminary Studies

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. Drug dissolution from hydrophilic matrix systems is related to the entry of water into the matrices. The release mechanism of the drug from the polymeric matrix has been explained by many researchers, and in most of the studies, hydroxy propyl methyl cellulose (HPMC) is used as polymeric floating matrix system[12-14]. The combination of HPMC with other release retarding polymers can entrap the drug in a polymeric matrices, which can be helpful in obtaining the tailored drug release pattern from the formulation[15].
Initially five batches of gastroretentive floating matrix tablet of GLP were prepared by direct compression technique (as explained in chapter 4). The formulations were prepared with 50% HPMC K15M, 20% gas forming agent and 20 % of other release retarding polymers (sodium alginate, kappa carrageenan, pullulan, xanthan gum, poloxamer 188) in

combination with HPMC K15M. GLP 10mg (dose of GLP) and magnesium stearate 1% was also added. Finally, microcrystalline cellulose was added to make up the weight equal to 150mg. The prepared formulations were evaluated for lag time and release characteristics of drug. As the formulations were not giving the desired sustained release profile, hence it was decided to prepare the formulations by wet granulation technique using (PVP K30) as granulating agent.

The formulations were prepared by wet granulation technique using the composition as given in Table 6A.1. Prepared formulations were evaluated for checking the characteristics of floating matrix tablet.

TABLE 6A.1 Composition (in mg) of preliminary batches of Glipizide Floating Matrix Tablets

Sr No	Ingredients	G1	G2	G3	G4	G5
1	Glipizide	10	10	10	10	10
2	PVP K30	10	10	10	10	10
3	HPMC K15M	60	60	60	60	60
4	Sodium bicarbonate	15	15	15	15	15
5	Sodium Alginate	20	-	-	-	-
6	κ-Carrageenan	-	20	-	-	-
7	Pullulan	-	-	20	-	-
8	Xanthan gum	-	-	-	20	-
9	Poloxamer 188	-	-	-	-	20
10	MCC	33.5	33.5	33.5	33.5	33.5
11	Mg stearate	1.5	1.5	1.5	1.5	1.5

6A.2.3 Evaluation of Gastroretentive Floating Matrix tablet of GLP

Same as that of chapter 4

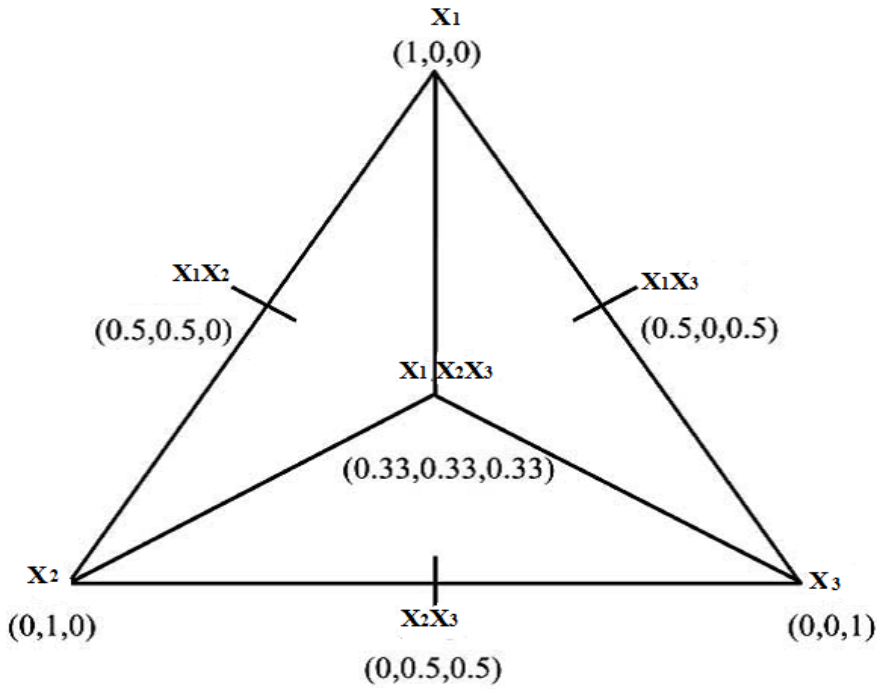
6A.2.4 Drug Excipient Compatibility Study[16]

There is always the possibility of drug polymer interaction in any formulation. To check any such kind of interaction, Fourier-transform infrared spectroscopy (FTIR) study was conducted. The FTIR scan of pure drug (Glipizide), polymers (HPMC K15M and kappa

carrageenan) and physical mixture of drug-polymer were taken. The pure drug, polymer and physical mixture were separately mixed with IR grade KBr. This mixture was punched to form a disc, which was scanned over a wave number range of 4000 to 400 cm^{-1}.

6A.2.5 Experimental Design-Mixture Design

The development of new pharmaceutical formulation by trial and error technique is very time consuming and also calls for high cost. Due to these reason, the pharmaceutical industry has turned to investigate various strategies in the advancement of novel drug delivery systems. The optimization techniques, by statistical analysis provides an effective and economical method for the prediction of the optimal composition of dosage form. That is the reason of tremendous increase in the use of Design of experiment (DoE), in research and development in pharmaceutical industries. Statistical designs, DoE, have been applied for the development and optimization of many pharmaceutical products[17].



6A.1 Pictorial Presentation of Simplex Lattice Design.

Literature revealed that DoE has also been applied for the development of various gastroretentive formulations[18-20]. In the present study, optimization of GLP gastroretentive matrix tablet has been done by mixture design. Mixture design have been already employed for the formulation and optimization of floating dosage form of Dipyridamole[21].

6A.2.6 Optimization of Floating Matrix Tablet of GLP by Simplex Lattice Design

The preliminary studies suggested that floating matrix tablets of GLP, prepared with the combination of HPMC K15 M and κ-Carrageenan, as release retarding polymers, were releasing the drug for 12hrs and had desired floating characteristics. Hence, these polymers were considered for the final optimization of floating matrix tablet of GLP. The levels of the independent variable was decided based on the literature survey and by the experimentation done during the preliminary studies.

Mixture design was used to optimize the formulations with HPMC K15 M, κ-Carrageenan and sodium bicarbonate as independent elements. **Simplex Lattice** design was applied as the technique for optimization by changing the amount of three factors concurrently and keeping their total concentration constant.

TABLE 6A.2 Factors and their examined levels in Simplex Lattice Design for GLP

Independent Variables /Levels	**Amount of HPMC K15M**	**Amount of k-Carrageenan**	**Amount of sodium bicarbonate**
	X_1 (mg)	**X_2 (mg)**	**X_3 (mg)**
Low	50	20	10
High	60	30	20
Dependent Variables	Y_1 – Similarity factor % Y_2 – Time required for 50% drug release (t_{50}) Y_3 - Time required for 90% drug release (t_{90})		
No. of replicates 4			

The Simplex Lattice design (SLD) for three-component system is presented by an equilateral triangle in two-dimensional space[22,23]. The general purpose of mixture experimentation is to describe the response as a function of the composition of the mixture, by means of a mathematical model from only a limited number of experiments[24]. The earlier studies have proved that SLD is a very efficient tool in optimization of gastroretentive matrix tablet[25]. In this study, the amounts of matrixing agent [HPMC K15 M (X_1)], release retarding polymer [kappa-Carrageenan (X_2)], gas-generating agent [sodium bicarbonate (X_3)], were chosen as independent variable with the total weight as 90mg. Similarity factor $f2$ (%), time required for 50% drug release (t_{50}) and time required for 90% drug release (t_{90}) were claimed as dependent variables (Table 6A.2). The design was applied and evaluated using the Design-

Expert® Software (version- 9.0.6, Stat-Ease) by running 14 experiments. The composition of the batches formulated by using this statistical design is given in table 6A.3.

TABLE 6A.3 Composition of GLP matrix tablets prepared by applying SLD

Runs	Batch code	Transformed Fractions of Variables*		
		X_1	X_2	X_3
1	G-SLD 1	50	20	20
2	G-SLD 2	56.6667	21.6667	11.666
3	G-SLD 3	55	20	15
4	G-SLD 4	55	25	10
5	G-SLD 5	60	20	10
6	G-SLD 6	60	20	10
7	G-SLD 7	50	20	20
8	G-SLD 8	50	30	10
9	G-SLD 9	51.66	21.66	16.666
10	G-SLD 10	50	25	15
11	G-SLD 11	51.66	26.66	11.666
12	G-SLD 12	55	25	10
13	G-SLD 13	50	30	10
14	G-SLD 14	53.33	23.333	13.333

***In all the batches, each tablet contained 10 mg Glipizide, 10 mg PVP K 30, 38.5 mg microcrystalline cellulose and 1.5 mg magnesium stearate. X_1 is amount of HPMC K15M (mg); X_2 is amount of kappa-carrageenan (mg); X_3 is amount of sodium bicarbonate (mg)**

6A.2.7 Validation of Model

Additional three formulations, suggested by the design expert, were formulated to check and validate the reliability of the mathematical models built here with Simple Lattice design. The check point batches were evaluated and the experimentally obtained results were compared to those predicted by the mathematical models. Table no. 6A.4 shows the values of the factors used for development of the validation batch, taken from the software, keeping the amount of all other ingredients constant

To validate the chosen experimental design, the experimental values of the responses were quantitatively compared with predicted values and, the relative error (%) was calculated using the following equation.

$$Relative\ error\ (\%) = \frac{Predicted\ value - Experimental\ value}{Predictive\ value}\ X\ 100$$

TABLE 6A.4 Formula for validation runs of SLD design for the optimization of GLP floating matrix tablets

Factors	Composition		
	F 1 (mg)	**F 2 (mg)**	**F 3 (mg)**
X_1 : Amount of HPMC K15M	52.03	55.81	58.51
X_2 : Amount of k-Carrageenan	23.33	23.33	21.49
X_3 : Amount of sodium bicarbonate	14.64	10.86	10.00

6A.2.8 Stability Studies

Physical stability study of optimized formulation G-SLD 8 was conducted according to International Conference on Harmonization (ICH) guidelines. Accelerated stability studies were performed at 40ºC ±2ºC and 75 ± 5% relative humidity (RH), for six months. After specified time (sampling interval was 0, 3, 6 months), the tablets were examined for any statistical difference in their physical characteristics, floating characteristics and drug release pattern.

6A.2.9 *In vivo* Radiographic Studies

Same as that of chapter 5A

6A.3 Result and Discussion

6A.3.1 Preliminary Studies

The gastroretentive floating matrix tablets of GLP were prepared with HPMC K15M as release retarding polymer in combination with other polymers to check the effect of the polymer blend on the release pattern of the drug from the matrix. The quantities of the ingredients were decided based on the literature survey and the previous work done on metformin, by the researcher. The initial batches were prepared by direct compression

technique, but they could not give the desired sustained release of the drug. The formulation prepared with Xanthan gum could remain intact in the dissolution fluid for an hour only. The formulation prepared with k-carrageenan had a lag time of 3 seconds, whereas the formulations prepared with pullulan and sodium alginate could not float for more than10 minutes. The grounds for this kind of result may be the less hydration of HPMC K15M and reduced swelling. The tablets prepared using poloxamer 188 was forming flakes during the punching, hence the tablet didn't pass the weight variation study. It was decided to not to try this polymer for further studies. The release from the GLP floating matrix tablets prepared by direct compression technique, could not sustain the release of the drug. Hence, it was decided to prepare the formulation with certain modifications, based on the results of preliminary studies.

The quantity of release retarding polymer HPMC K15M was increased so as to sustain the release of the drug from polymeric matrix. Literature suggests that the release of drug from the tablet can be significantly delayed with an increase in the concentration of HPMC in the tablet[26].

The low ability of sustaining the release of the drug is also attributed to the high quantity of the gas forming agent. Hence, in the new batches the quantity of sodium bicarbonate was decreased. Moreover, the technique of preparation was also changed to wet granulation using PVP K30 as granulating agent. Because, its proven that tablets prepared with wet granulation technique offers delayed release. Chowdary et al., did a comparative study of the effect of methods for the preparation of tablets on the dissolution rate of the drug[2]. They establish that the disintegration rate of the tablets prepared by direct compression technique was a lot more eminent than the tablets prepared by of wet granulation technique. This proves that wet granulation increases the cohesiveness between the particles and hence delays the release of the drug from the polymeric matrix. The granulation makes the polymeric matrix more compact and increases the adhesion between the particles. It also ensures the proper distribution of the contents in all the batches.

6A.3.2 Evaluation of Preliminary Batches of GLP Floating Matrix tablet

6A.3.2.1 Physical Properties of GLP Tablets Prepared by Wet Granulation Technique

The results of physical evaluation of the prepared dosage forms gave acceptable physical characteristics. Hardness of all the batches was found to be in the range of 4.7-5.3 kg/cm^2.

The assay for drug content indicated acceptable content uniformity in the prepared tablets. Drug content of the formulations were in the range of 98.82% to 101.56%, which is within the limits given by Indian Pharmacopoeia (Table 6A.5). The friability was found to be less than 0.25% for all the formulation, hence passes the test for friability.

TABLE 6A.5 Results of the physical evaluation of GLP tablet prepared by wet granulation technique

Batch code	Weight uniformity	Hardness* (kg/cm^2)	Drug content* (%)	Friability* (%)	Lag Time*(s)	Floating Time*(h)
G1	Complies	4.7±0.58	98.82±1.04	0.14±0.18	16.39 ± 3.53	8
G2	Complies	5.2±0.43	101.56±1.25	0.15±0.16	27.31 ± 3.41	12
G3	Complies	4.9±0.39	99.76±0.87	0.19±0.10	45.25 ± 2.20	5†
G4	Complies	4.8±0.71	100.06±0.79	0.20±0.18	120.74 ± 7.87	12

***n=3, average of three determinations±SD, †Tablet was going up and down during the study**

6A.3.2.2 *In vitro* Buoyancy Studies

The formulation G1, prepared using sodium alginate in combination with HPMC K15M, had the minimum floating lag time, but it could float for only 8hours. Formulations prepared with k-carrageenan, G2, had the floating lag time as 27.31 ± 3.41seconds and the formulation could float for 12hours, which was desired for present formulation. The formulations prepared with pullulan, G3, had acceptable floating lag time, but it had 5hrs of floating time. Moreover, during the flotation study, the tablet was sinking in between the said duration, which is non-satisfactory. The matrix tablets of GLP prepared with xanthan gum showed the flotation for 12 hours, but it took about 2 minutes to float. Overall, it was apparent from the buoyancy studies that the presence of other release retarding polymer in combination with HPMC K15M had a drastic effect on the flotation behavior of formulations, as indicated in Table 6A.5.

6A.3.2.3 Drug Release Studies

For checking the release pattern of the formulated gastroretentive matrix tablets of GLP, dissolution of marketed formulation of GLP, 10mg was also performed (GLYTOP-SR). The

aim was to get the release of the batches similar to that of marketed formulation. The graphical representation of drug release study for the preliminary batches of GLP floating tablets and marketed tablet is shown in Fig. 6A.2. The graph indicates that the formulation G1 (formulation with HPMC K15M and sodium alginate) could sustain the release of the drug till 8 hours only, whereas the reference sustained release tablet of GLP gave the sustained release of the drug till 12 hrs. This may be because of less hydration of sodium alginate and also because in acidic pH it doesn't contribute to the matrix erosion and hence release of the drug[27].

Formulations G3, prepared with pullulan could not delay the release of the drug, as the entire amount of drug was released within 4hrs. This means that pullulan doesn't have the ability to sustain the release of GLP from the polymeric matrix system. The formulation G2, (formulation with HPMC K15M and kappa-carrageenan) was giving almost same release pattern as that of a theoretical release patern of the drug with 62% similarity factor value. All other formulations couldn't have acceptable similarity factor value.

TABLE 6A.6 *In vitro* drug release data of preliminary batches of floating matrix tablets of GLP*

Time (hrs)	G1 (%)	G2 (%)	G3 (%)	G4 (%)	STD (%)
0	0	0	0	0	0
1	32.84±2.54	18.22±2.01	56.85±1.04	15.14±0.88	18.23±0.54
2	48.04±0.71	37.96±1.1	76.68±1.11	27.59±1.53	32.61±1.03
3	63.66±1.04	43.67±0.89	84.37±1.43	38.59±0.92	41.31±1.19
4	73.96±1.39	50.65±0.59	98.99±0.71	40.18±1.21	50.41±0.73
5	76.72±1.16	59.45±0.91	100.63±1.04	42.51±1.79	65.39±0.57
6	86.54±1.79	78.58±1.17	-	48.46±2.77	73.01±1.09
7	94.77±2.94	82.53±1.06	-	53.28±1.46	78.42±0.67
8	101.54±1.75	95.72±1.79	-	59.16±0.91	82.24±1.12
10	-	98.32±0.61	-	63.32±1.16	94.21±1.18
12	-	101.2±1.09	-	65.42±0.79	99.95±0.68

***n=3, average of three determinations±SD**

Tatavarti et. al., proved that incorporation of anionic polymers, in HPMC matrices is useful for developing a pH-independent release profile[28]. The present study also revealed that incorporation of kappa-Carrageenan, a poly anionic polymer, in a HPMC matrix of metformin showed best release pattern. This combination in G2 formulation showed an

almost similar release pattern as that of a theoretical release pattern of the drug with maximum $f2$ value.

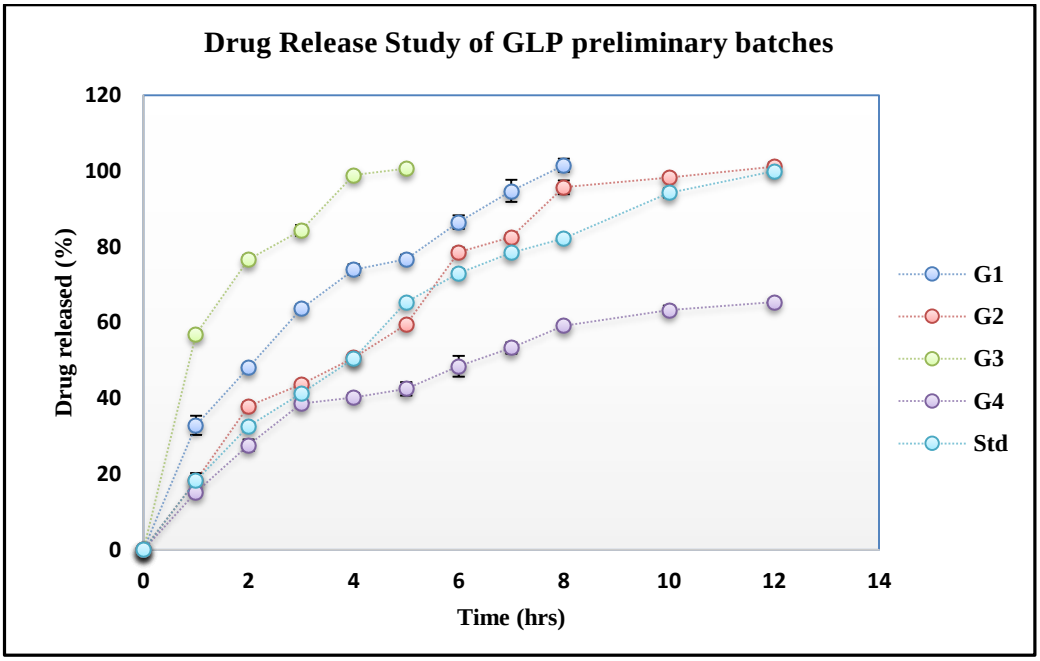


FIGURE 6A.2 Graphical representation of the drug release from preliminary floating tablets of GLP *n=3, average of three determinations±SD

The formulation G4, prepared with xanthan gum, could sustain the release of the drug for more than 12 hours, the rate of drug release was very slow. This result was similar to that of the study conducted by *Sankalia*, et al., which states that the higher xanthan gum content in the formulation, diminished the initial drug release and also the drug diffused slowly continuously for more than 12 h[29]. Singh *et. al.*, presented the release behavior of drugs from different natural polymers and gums[30]. They found that the presence of xanthan gum in the formulation can retard the release of the drug. In the present study also the researcher got the same result.

6A.3.3 Drug Excipient Compatibility Study

The FTIR scan of drug, polymers and physical mixture of drug and polymer was taken. FTIR scan of glipizide showed characteristic peaks at 1649, 2943.22, 3325.47, 1527.57, 1688.84 cm^{-1} corresponding to C ═ N aliphatic group, C ─ H_2 aliphatic, N ─ H stretching of NH_2, C ─ H aliphatic and C ═ O stretching respectively. No such peaks were observed in the FTIR scan of polymers. All these peaks were observed in the infrared spectra obtained from drug-

polymer blend, which demonstrates that there is no significant incompatibility between the drug and the other polymers (Fig. 6A.3).

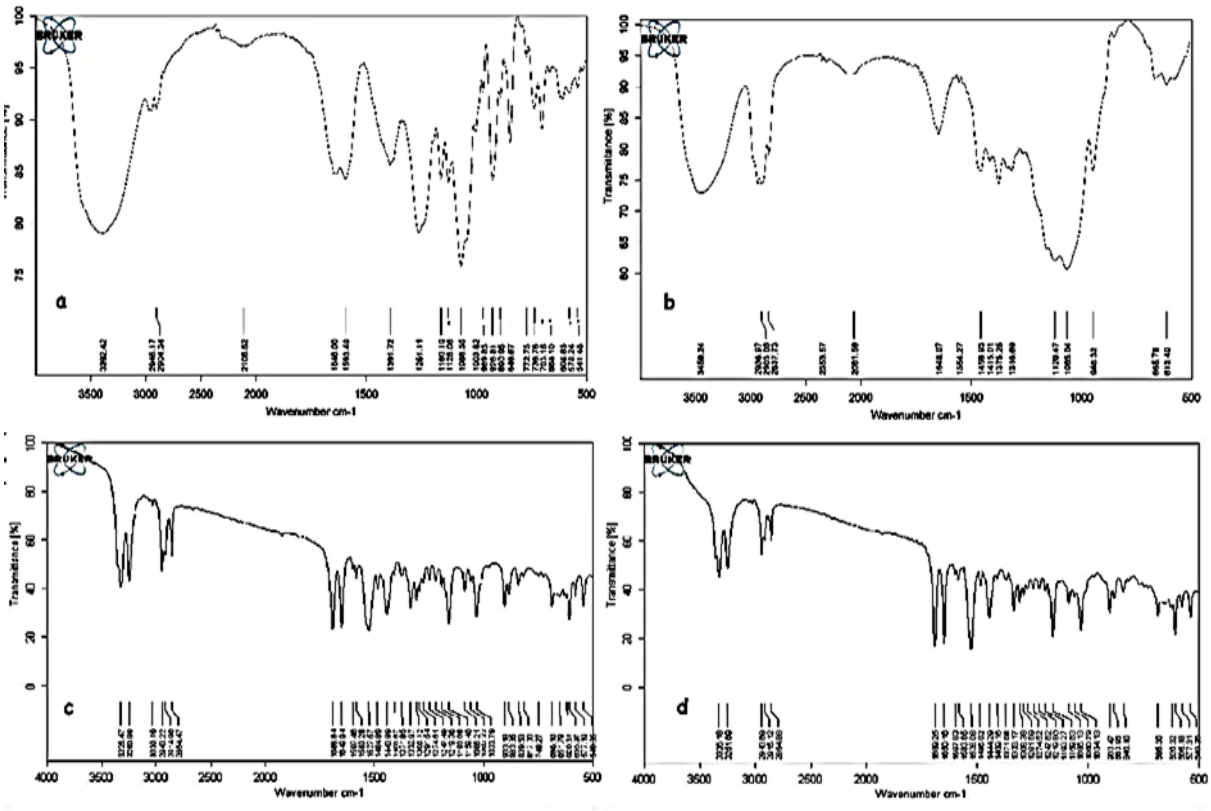


FIGURE 6A.3 FTIR scan obtained for kappa carrageenan (A), HPMC K15M (B), Glipizide (C) and optimized formulation, G-SLD 8 (D)

6A.3.4 Mixture Design - Simplex Lattice Design

Preliminary studies gave an idea about the polymers and their effect on the release pattern of the drug. The formulation prepared with the combination of HPMC K15M and k-carrageenan gave promising results, so it was decided to optimize the formulation of floating matrix tablet of GLP using these polymers. Mixture design was used to optimize the gastroretentive floating matrix tablet of Glipizide. A simplex lattice is an arrangement of equally spaced points on a simplex (Lachman et al., 1970). The experiments should be well distributed over the factor space because simplex designs provide an optimal distribution. The design indicates the experimenting points in the factor space that allows an easy estimation of the parameters. When described by a polynomial equation the lattice can be referred to as {q, m}, where, q = Number of components, m = Degree of the polynomial, or in other words, the number of proportions assumed by each part. In a {q,m} lattice, the

proportions used for each of the q components have (m + 1) equally spaced values from 0 to 1.

All possible mixtures with these proportions for each component are used[31, 32]. The number of points in a {q,m} lattice is equal to the number of parameters or terms in the model.

$$p = \frac{(m+q-1)!}{m!\,(q-1)!}$$

This equation can be used to calculate number of design points in the simplex lattice design.

6A.3.5 Physical Properties of Floating Tablet of GLP by applying SLD

The results of the physical properties of GLP floating matrix tablets prepared by applying SLD are shown in table 6A.6.

TABLE 6A.6 Results of the physical properties of GLP floating matrix tablet prepared by applying SLD*

Batch code	Weight uniformity	Hardness (kg/cm^2)	Drug content (%)	Friability (%)	Floating Time (hrs.)	Tablet adhesion retention period (min.)	Lag time (sec.)
G-SLD 1	Complies	5.6±0.25	99.35±0.83	0.25±0.07	> 12	46.34±4.19	12.35±3.21
G-SLD 2	Complies	4.8±0.46	100.91±0.73	0.31±0.10	> 12	84.37 ±3.76	39.16±2.54
G-SLD 3	Complies	4.9±0.17	98.87±0.82	0.22±0.09	> 12	53.32 ±3.43	8.63±2.31
G-SLD 4	Complies	5.2±0.49	100.94±0.93	0.31±0.11	> 12	120.52 ±4.54	90.43±4.52
G-SLD 5	Complies	5.1±0.32	99.43±0.77	0.32±0.07	> 12	63.51±3.56	83.53±5.12
G-SLD 6	Complies	4.9±0.62	100.43±0.54	0.29±0.06	> 12	62.48±4.32	85.53±4.21
G-SLD 7	Complies	5.5±0.53	100.23±0.65	0.19±0.04	> 12	47.52 ±5.26	13.87±1.63
G-SLD 8	Complies	5.1±0.56	99.46±0.43	0.28±0.07	> 12	139.21±5.43	20.42±1.12
G-SLD 9	Complies	4.6±0.85	98.96±0.74	0.38±0.12	> 12	74.55±3.65	9.77±1.43
G-SLD 10	Complies	4.2±0.62	99.38±0.78	0.35±0.08	> 12	104.43±3.95	22.40±2.19
G-SLD 11	Complies	5.2±0.67	99.64±0.79	0.27±0.09	> 12	118.54 ±3.67	40.22±3.55
G-SLD 12	Complies	5.2±0.53	101.27±0.93	0.25±0.08	>12	119.20 ±4.55	88.46±5.21
G-SLD 13	Complies	5.1±0.57	100.54±0.64	0.36±0.14	> 12	140.22±6.34	22.18±1.47
G-SLD 14	Complies	4.6±0.82	100.16±0.89	0.31±0.11	> 12	97.21±2.87	31.96±2.63

***n=3, average of three determinations±SD**

All the prepared formulations complied the weight uniformity study. The hardness of all the batches was found to be in the range of 4.2 to 5.6 kg/cm^2. Drug content of all the batches was within the limits prescribed by IP. The percentage friability for all formulae was less than 1%, indicating good mechanical resistance. All the prepared batches were floating for more than 12 hours.

The tablet adhesion retention time was in the range of 46.34 to 139.21 minutes. It was found that as the amount of kappa carrageenan increased in the formulations, the tablet retention also increased, which was expected because Carrageenan is high molecular weight sulfated polysaccharides and its high adhesion period may be due to hydrogen bonding or ionic interaction with agar. However, increased levels of sodium bicarbonate decreased the tablet adhesion retention period. The finding were same as that of the results found for the metformin floating matrix tablet, prepared with the combination of same release retarding polymers. The lag time for all the batches was found to be in the range of 8.63 to 90.43 seconds. General observation was that the batches with minimum amount of gas generating agents had maximum floating lag time.

6A.3.6 *In vitro* Drug Release Study

The *in vitro* dissolution study of all the batches of GLP floating matrix tablet, prepared by applying simplex lattice design was performed in 500ml 0.1N HCl. The drug release data is given in table 6A.7 and the graphical representation of the same is shown in Fig. 6A.4.

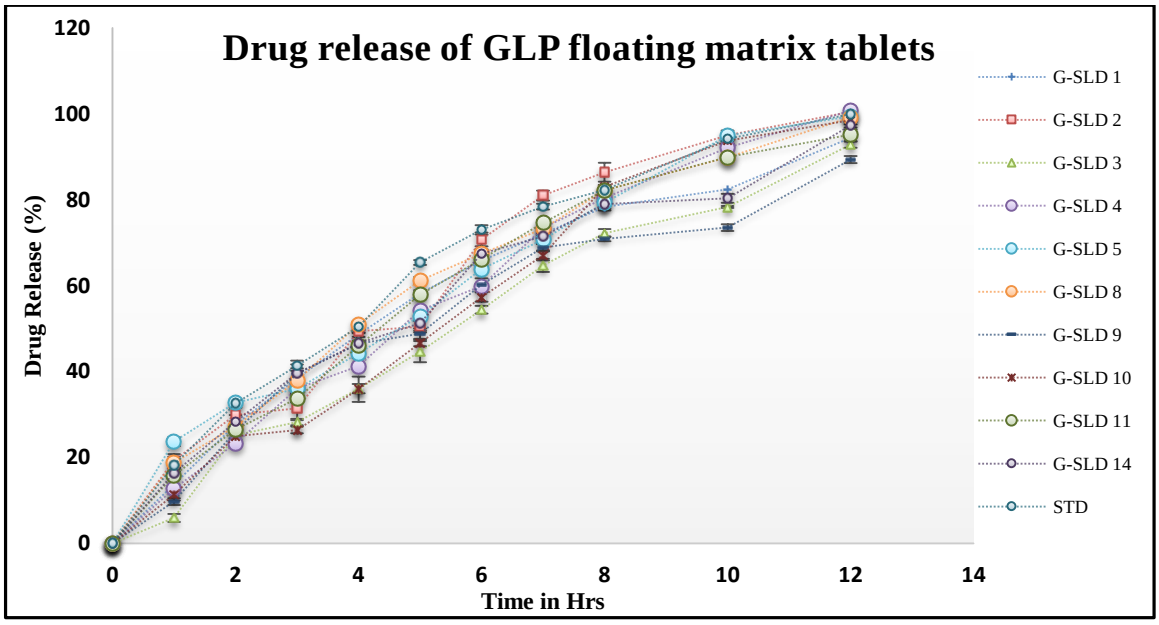


FIGURE 6A.4 Graphical representation of the drug release from floating tablets of GLP prepared by Simplex Lattice Design

TABLE 6A.7 Results of *in vitro* release of GLP floating matrix prepared by applying SLD*

Time (hrs)	G-SLD 1 (%)	G-SLD 2 (%)	G-SLD 3 (%)	G-SLD 4 (%)	G-SLD 5 (%)	G-SLD 8 (%)	G-SLD 9 (%)	G-SLD 10 (%)	G-SLD 11 (%)	G-SLD 14 (%)	STD (%)
0	0	0	0	0	0	0	0	0	0	0	0
1	13.7±1.09	19.27±1.53	5.91±0.93	12.68±1.02	23.68±1.03	18.75±1.39	9.71±0.79	11.32±1.39	15.85±0.89	16.37±1.18	18.23±0.54
2	27.68±2.67	29.96±2.92	25.13±0.99	23.34±1.02	32.79±0.34	27.23±1.16	26.14±0.49	24.91±1.16	26.54±0.59	28.33±1.53	32.61±1.03
3	38.47±1.12	31.43±2.79	28.22±0.73	36.02±0.52	35.94±1.04	37.95±0.53	39.46±1.32	26.37±0.79	33.71±0.91	39.64±2.02	41.31±1.19
4	48.68±1.18	49.41±0.49	36.01±1.08	41.24±0.91	44.26±1.11	51.02±0.69	46.47±1.6	35.87±2.94	46.03±1.17	46.54±2.54	50.41±0.73
5	58.21±0.92	50.45±1.32	44.64±2.45	54.21±2.42	52.93±0.43	61.18±0.78	48.81±1.18	46.61±0.75	57.9±1.06	51.23±1.79	65.39±0.57
6	65.82±0.82	70.75±1.6	54.37±0.88	59.89±0.89	63.85±0.71	67.44±1.03	60.19±1.53	57.31±1.07	66.17±0.79	67.43±0.92	73.01±1.09
7	72.05±1.53	80.97±1.18	64.71±1.53	73.38±0.67	70.68±1.04	73.26±1.19	68.86±0.92	67.04±1.16	74.61±0.75	71.44±0.88	78.42±0.67
8	78.33±1.29	86.4±2.17	72.24±0.92	80.4±0.88	79.26±1.39	82.2±0.73	70.9±0.57	83.05±0.39	82.16±1.07	78.99±1.49	82.24±1.12
10	82.41±1.42	95.03±0.92	78.33±0.21	92.12±0.45	94.94±1.16	89.91±0.57	73.52±0.79	93.77±0.75	89.98±1.16	80.35±1.03	94.21±1.18
12	94.43±1.11	100.47±0.89	92.87±0.79	100.75±1.02	99.4±0.79	99.16±0.99	89.36±0.82	98.5±0.91	95.19±1.63	97.33±0.98	99.95±0.68

Formulations G-SLD 6, 7, 12 and 13 were duplicate batches of G-SLD 5, 1, 4 and 8, respectively. Hence, their *in vitro* drug release data is not presented in the table.
***n=3, average of three determinations±SD**

6A.3.7 *In vitro* Drug Release Kinetics

Model dependent release kinetics describes the mechanisms of overall release of drug from the dosage forms. The model dependent approaches evaluated for the drug release kinetics were zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas. The release from batches G-SLD 1, G-SLD 3, G-SLD 4, G-SLD 8 and G-SLD 9 of GLP floating matrix tablets was found to follow R_{HC} model with R^2 value close to 1, for the period of 12 hours. R_{HC} model data is obtained from *in vitro* drug release studies plotted as cube root of drug percentage remaining in matrix versus time.

TABLE 6A.8 Results table for *in vitro* drug model-dependent kinetics for GLP Floating matrix tablets

Batch code	Higuchi model (R_H)	Korsmeyer Peppas model (R_P)	Hixson Crowell model (R_{HC})	First order (R_1)	Zero order (R_0)
G-SLD 1	0.9773	0.9956	0.9997	0.7602	0.9897
G-SLD 2	0.9494	0.9335	0.9380	0.8136	0.9563
G-SLD 3	0.9427	0.9232	0.9796	0.6888	0.9749
G-SLD 4	0.9551	0.9942	0.9946	0.7411	0.9892
G-SLD 5	0.9664	0.9563	0.9647	0.7484	0.9437
G-SLD 8	0.9785	0.9870	0.9947	0.7673	0.9858
G-SLD 9	0.9681	0.9542	0.9798	0.7117	0.9623
G-SLD 10	0.9220	0.9689	0.9749	0.7549	0.9823
G-SLD 11	0.9662	0.9915	0.9899	0.8245	0.9930
G-SLD 14	0.9727	0.9902	0.9753	0.7010	0.9756
STD	0.9807	0.9946	0.9908	0.8068	0.9858

This model applies to tablets where dissolution occurs in all the planes equally and the initial geometrical of the tablet remains constant. The release from batches G-SLD 2, G-SLD 10 and G-SLD 11, followed R_0 model. The data is obtained from *in vitro* drug release studies,

plotted as cumulative amount of drug released versus time. This relationship is used to describe the drug dissolution of matrix tablets with low soluble drugs. The drug release from G-SLD 2 followed R_H model and batch G-SLD 14 and STD formulation of GLP, followed R_P model The results for the analysis of model-dependent drug release kinetics is given in table 6A.8.

6A.3.8 Statistical Analysis

The result of all the dependent variables is given in table 6A.8. A statistical model incorporating 14 interactive terms was used to assess the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3$$

Where, Y is the dependent variable, b_0 is the arithmetic mean response of the 14 runs, and bi is the estimated coefficient for the factor Xi. The main effects (X_1, X_2, and X_3) represent the average result of changing one element at a time from its low to high value. The interaction terms (X_1X_2, X_2X_3, X_1X_3, and $X_1X_2X_3$) give the information about how the response changes when two or more factors are simultaneously modified. The values for Similarity factor $f2$ (Y_1), Time required for 50% drug release (t_{50}) (Y_2), Time required for 90% drug release (t_{90}) (Y_3) 14 batches (G-SLD1 - G-SLD14) is presented in table 6A.8. The outcomes indicated that the values of subject variables are dependent on independent variables.

All the formulations gave satisfactory floating lag time in the range of 8 to 90 seconds, which means that the chosen independent variables had no significant effect on the dependent variables. The formulations released 50% of the drug in the time range of 3.89 to 5.51 hours and released 90% of the drug in the time range of 9.48 to 12.24 hours.

Using analysis of variance (ANOVA), the significance ($p < 0.05$) of the ratio of mean square variation due to the regression coefficient, and the residual error were tested (Table 6A.9). The Special Cubic Mixture model was found to be significant for Y_1 and Y_2 responses, whereas Special Quartic Mixture model was followed by Y_3[24]. The high values of correlation coefficients for similarity factor $f2$ ($R^2 = 0.9443$), t_{50} ($R^2 = 0.9643$), and t_{90} ($R^2 = 0.9887$) indicated a good agreement between the dependent and independent variables. Lack of Fit F-value for Y_1, Y_2 and Y_3 was found to be about 0.5410, 0.1048 and 0.2216 respectively, which suggests the desirable, insignificance of Lack of Fit.

TABLE 6A.8 Results of dependent factors of GLP floating matrix tablets prepared by applying SLD*

Runs	Batch code	Similarity factor *f*2 (%)	Time required for 50 % (hrs)	Time required for 90% (hrs)
1	G-SLD 1	60	4.29±0.09	11.43±0.83
2	G-SLD 2	61	4.95±0.17	9.47±0.16
3	G-SLD 3	43	5.51±0.29	11.49±0.31
4	G-SLD 4	56	4.61±0.18	9.77±0.49
5	G-SLD 5	60	4.72±0.07	9.48±0.29
6	G-SLD 6	61	4.63±0.08	9.21±0.34
7	G-SLD 7	48	4.41±0.17	11.32±0.98
8	G-SLD 8	70	3.92±0.09	10.01±0.72
9	G-SLD 9	48	5.21±0.21	12.24±0.92
10	G-SLD 10	47	5.36±0.15	9.59±0.59
11	G-SLD 11	64	4.32±0.06	10.0±0.48
12	G-SLD 12	55	4.43±0.17	9.71±0.44
13	G-SLD 13	69	3.89±0.21	10.31±0.28
14	G-SLD 14	58	4.88±0.18	11.2±0.28

***n=3, average of three determinations±SD**

TABLE 6A.9 ANOVA table for response parameters for Simple Lattice design model for GLP gastroretentive floating matrix tablets

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	P-value
Similarity factor % (*f*2)					
Model	643.69	6	107.28	6.33	0.0042
Residual	118.67	7	16.95		
Corrected Total	762.36	13			
Time to release 50% of drug (t_{50})					
Model	3.05	6	0.51	31.54	0.0001
Residual	0.11	7	0.016		
Corrected Total	3.16	13			

Time to release 90% of drug (t_{90})					
Model	11.95	8	1.49	54.93	0.0002
Residual	0.14	5	0.027		
Corrected Total	12.09	13			

6A.3.8.1 Similarity Factor $f2$

The similarity factor, $f2$, given by Scale Up and Pose Approval Changes (SUPAC) guidelines for modified release dosage form was used to compare dissolution profiles of developed GLP floating matrix tablets with marketed sustained release formulation of GLP[33]. The dissolution profiles are considered to be similar when $f2$ is between 50 and 100. The method was first reported by Moore and Flanner[34].

The statistical analysis of the results obtained for the similarity factor, of all the prepared formulations, was done by Design Expert. The result can be expressed for model analysis by special cubic Mixture model, which is an extension of quadratic model. On looking into the results of F statistics, it was observed that model probability was greater than F value i.e. 6.33, which confirms the significance of the model. There is only 1.42% chance that an F-value this large could occur due to noise. Significance of the model was also proved by the p-value less than 0.0500. In this case A, B, C, AC, BC, ABC are significant model terms.

The fitted equation for the responses are given as follows:

$$f2 = +61.03X_1 + 67.46\,X_2 + 53.34\,X_3 - 31.01\,X_1\,X_2 - 57.75\,X_1\,X_3 - 55.18\,X_2\,X_3 + 415.85\,X_1\,X_2\,X_3$$

As the value of correlation coefficient was found to be high, the obtained polynomial equations can be used to get the inferences after considering the magnitude of coefficient and the mathematical sign that it carries. The polynomial equations can be applied to find the conclusions after looking at the magnitude of coefficient and the mathematical sign it carries (i.e. positive or minus). By looking into the above equation, it is apparent that all the three factors, Amount of HPMC K15M (X_1), kappa-carrageenan (X_2) and sodium bicarbonate (X_3) show positive effects on floating lag time of the formulated floating tablets of glipizide. It was observed that X_2 had more significant effect on the similarity factor. This means, more the concentration of k-Carrageenan, more the drug release pattern of prepared floating matrix tablets of glipizide was matching the release pattern of marketed formulation.

The interaction was found to be significant and the proper combination of the three variables is required to get the maximum $f2$ value.

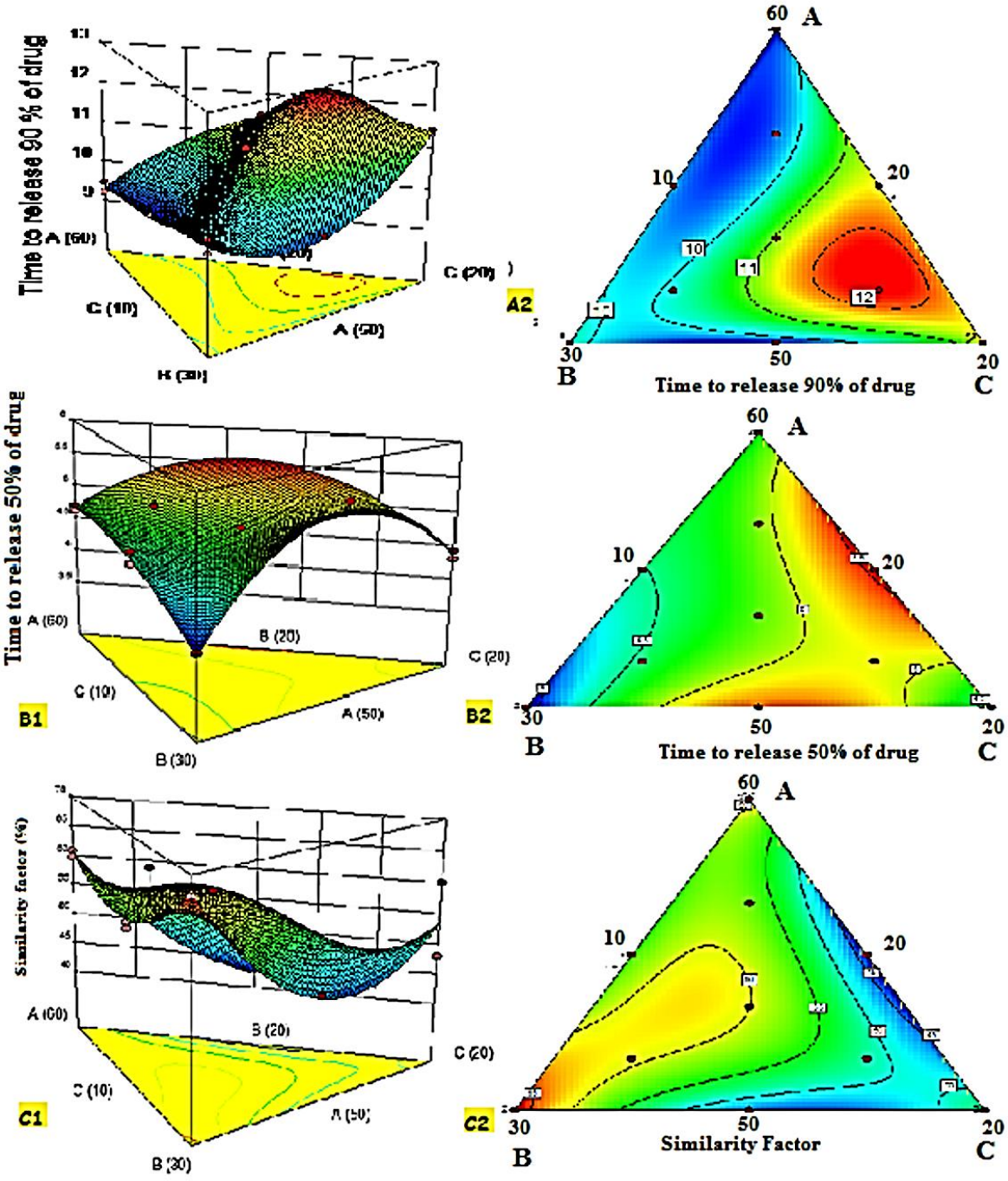


FIGURE 6A.5 Response surface plot and contour plot for GLP floating matrix tablet prepared by applying SLD
*** (In contour plot A, B, C stands for HPMC K15M, k-carrageenan and sodium bicarbonate respectively)**

There was antagonistic effect of variables in two dimensional plane indicating the significant interaction between the variables. This means that on changing the two variable simultaneously, the interaction was observed and that decreased the similarity factor value.

However, the most significant coefficient with highest magnitude was when all the three factors were modified simultaneously, it had agonistic effect on Y1. Observed and predicted values of the similarity factor were found to be comparable, which additional validates the suitability of the model. The three dimensional response surface graphs for similarity factor given in Fig. 6A.5, shows the obtained contour plot (C2) and response surface plots (C1). This gives the information about the main and interaction effects of the independent components. It can be clearly seen that maximum similarity value, above 65% is obtained in the portion with highest value of k-carrageenan. The results for Y_3 could have been better if the higher value of X_2 variable would have been increased beyond the existing level.

6A.3.8.2 Time to Release 50% of Drug

The results of ANOVA for the applied model, time to release 50% of drug, are shown in Table 6A.9. On looking into the results of F statistics, it was observed that model probability was greater than F value i.e. 31.54, which confirms the significance of the model. There is only a 0.01% chance that an F-value this large could occur due to noise. Significance of the model was also proved by the p-value less than 0.0500. In this case A, B, C, AC, BC, ABC are significant model terms. The result can be expressed for model analysis by special cubic model using following equation:

$$t_{50} = +4.68X_1 + 3.87\,X_2 + 4.87X_3 + 0.86\,X_1X_2 + 4.18\,X_1X_3 + 4.84\,X_2X_3 - 15.17\,X_1X_2X_3$$

As the value of correlation coefficient was found to be high, the obtained polynomial equations can be used to get the inferences after considering the magnitude of coefficient and the mathematical sign that it carries. By looking into the above equation, it is evident that all the three factors, Amount of HPMC K15M (X_1), kappa-carrageenan (X_2) and sodium bicarbonate (X_3) show positive effects on time to release 50% of drug of the prepared gastroretentive floating matrix tablets of glipizide. The agonistic effect of the factors on the time for 50% drug release was in the order of $X_3 > X_1 > X_2$. The most significant factor was the amount of gas forming agent. The positive interaction effect was observed between the independent variables. There is synergistic effect was shown by X_2 and X_3 and there was strongest antagonistic effect observed when all the three factors were modified simultaneously. The three dimensional response surface graphs for time to release 50% of

drug are given in Fig. 4.3. It shows the obtained response surface plots (B1) and contour plot (B2). This give the information about the main and interaction effects of the independent components. The contour plot indicated that maximum value for Y_2 is obtained with minimum quantity of k-carrageenan and HPMC K15M. However, the sweet spot will be obtained only after putting constrains for the responses.

6A.3.8.3 Time to Release 90% of Drug

The results of ANOVA for the applied model on time to release 90% of drug are shown in Table 4. On looking into the results of F statistics, it was observed that model probability was greater than F value i.e. 54.93, which confirms the significance of the model. There is only a 0.02% chance that an F-value this large could occur due to noise. Significance of the model was also proved by the p-value less than 0.0500. In this case A, B, C, AC, BC, A^2BC, ABC^2 are significant model terms. As the cubic model was aliased, the result can be expressed for model analysis by Special quartic model using following equation:

$$t_{90} = +\,9.34X_1 + 10.15\,X_2 + 11.37\,X_3 - \,0.081\,X_1X_2 + 4.43\,X_1X_3 - \,4.8\,X_2X_3 - \,87.10\,X_1^2X_2X_3 + 9.20X_1X_2^2X_3 + 144.92X_1X_2\,X_3^2$$

As the value of correlation coefficient was found to be high, the obtained polynomial equations can be used to get the inferences after considering the magnitude of coefficient and the mathematical sign that it carries. By looking into the above equation, it is evident that all the three factors, Amount of HPMC K15M (X_1), kappa-carrageenan (X_2) and sodium bicarbonate (X_3) show positive effects on time to release 90% of drug of the prepared floating matrix tablets of GLP. The agonistic effect of the factors on the time for 90% drug release was in the order of $X_3 > X_2 > X_1$. The interaction values in the equation indicated that there is non-significant effect when X_1 and X_2 are modified simultaneously. However, there was strong ternary antagonistic interactions observed at higher level of HPM K15M and strongest synergistic effect was shown by a ternary interaction of $X_1X_2X_3$ at higher level of sodium bicarbonate (X_3). The three dimensional response surface graphs for time to release 90% of drug are given in Fig. 6A.5, shows the obtained contour plot (3a) and response surface plots (3b). This give the information about the main and interaction effects of the independent components.

6A.3.9 Validation of Simplex Lattice Design

Additional three formulations, suggested by the design expert, were formulated to check and validate the reliability of the mathematical models built here with Simple Lattice design. These check point batches were prepared according to the formula given by design expert and then evaluated for getting the experimental values of responses. The comparison between the experimental and predicted values was done.

TABLE 6A.10 Predicted and actual values of the responses for validation run: SLD for GLP

Responses	F1		F2		F3	
	Predicted values	**Actual values**	**Predicted values**	**Actual values**	**Predicted values**	**Actual values**
Similarity factor % f_2 in %	56.5849	54.23	58.9587	61.54	58.0639	60.35
Time required for 50% drug release (t_{50}) in hrs	4.99175	4.84	4.64653	4.72	4.67158	4.61
Time required for 90% drug release (t_{90}) in hrs	11.8698	11.72	9.2678	9.41	9.44833	9.29

The actual and predicted values of the responses is shown in table no. 6A.10. The relative errors (%) between the predicted and experimental values for each response were calculated and the values were found to be within 5%, which confirms the validity of the model.

6A.3.10 Selection of Optimized Formulation

To optimize all the above responses with different targets, a numerical optimization technique by the desirability function and a graphical optimization technique by the overlay plot was used. The overlay plot gives the regions not meeting the specifications as greyed out, leaving an operating window or sweet spot in yellow colour (Fig. 6A.6). This means that within the yellow region the formulation prepared will give maximum similarity factor and better release profile. It is evident from the overlay plot that the minimum amount of HPMC K15 M and gas generating agent, sodium bicarbonate is sufficient to give the desired effect. Whereas, it is clear from the plot that high concentration of kappa carrageenan is required to get the maximum similarity with the release profile of marketed formulation. It

was found that the fomulation G-SLD 8 and G-SLD 13 (with same composition) fullfilled the desiarablity criteria and hence can be considered as optimized formulation.

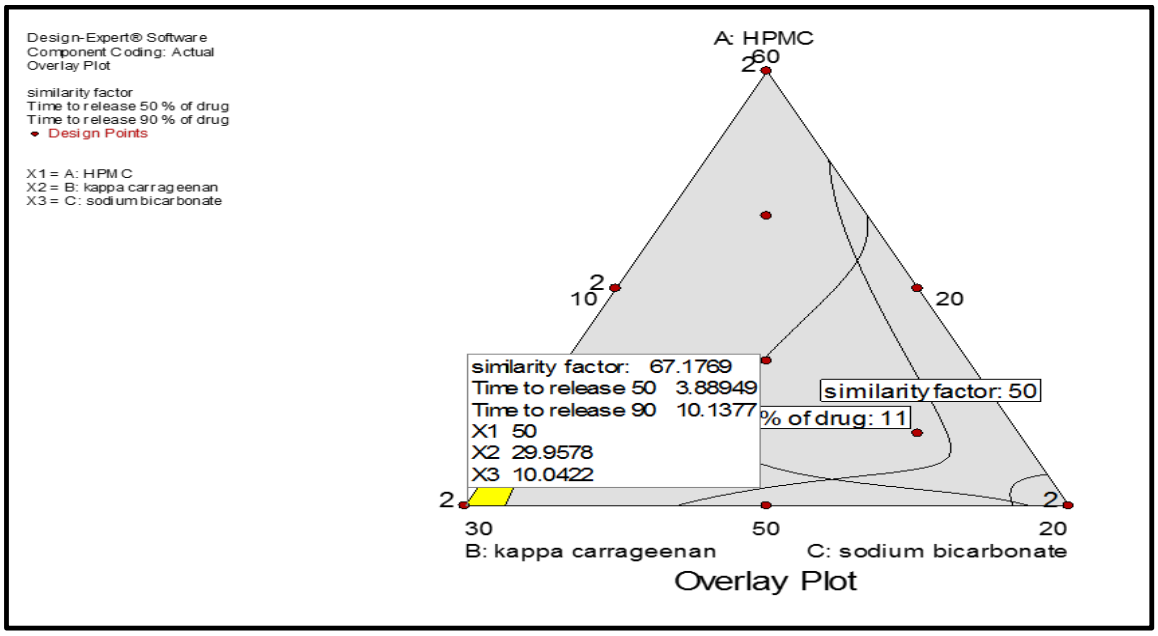


FIGURE 6A.6 Overlay plot of GLP formulations by SLD

6A.3.11 Stability Studies

The results obtained after three and six months of accelerated stability study of optimized MTG gastroretentive floating matrix tablet (G-SLD 8), showed no significant change in the physical properties and buoyancy parameters.

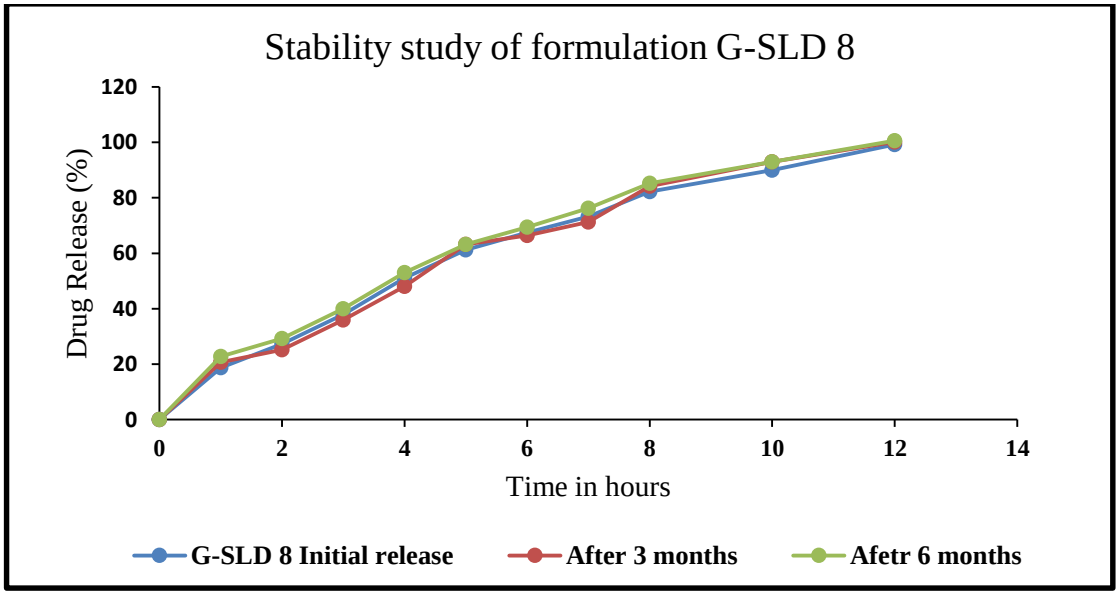


FIGURE 6A.7 Comparison of drug release from GLP optimized formulation G-SLD 8 Initially, after 3 and 6months of accelerated stability study.

The release pattern of the formulation, before and after the stability study is given in Fig. 6A.7. The release showed that the release pattern of the optimized formulation had 90% similarity (f_2) with the formulation after three and six months of stability study. The variation in the release pattern was insignificant. Hence, it can be concluded that formulation G-SLD 8 has good stability when stored at 40 ºC under 75% RH for 6 months.

6A.3.12 Radiographic Study

To determine the retention time of the optimized floating matrix tablets of GLP inside the body, radiographic studies were conducted. The barium Sulfate loaded tablets, prepared with optimized formula of matrix tablet, were given to rabbits. The X-ray photomicrographs were taken before and after administering the barium sulphate tablet to rabbits. Fig. 5A.8 shows the X-ray images taken at 0, 4 and 12 hrs, time period. The images clearly indicated that the tablets remained afloat in gastric fluid for up to 12 h in the stomach of rabbit. Hence, the study confirms the gastroretentive behavior of the developed floating matrix tablet of GLP.

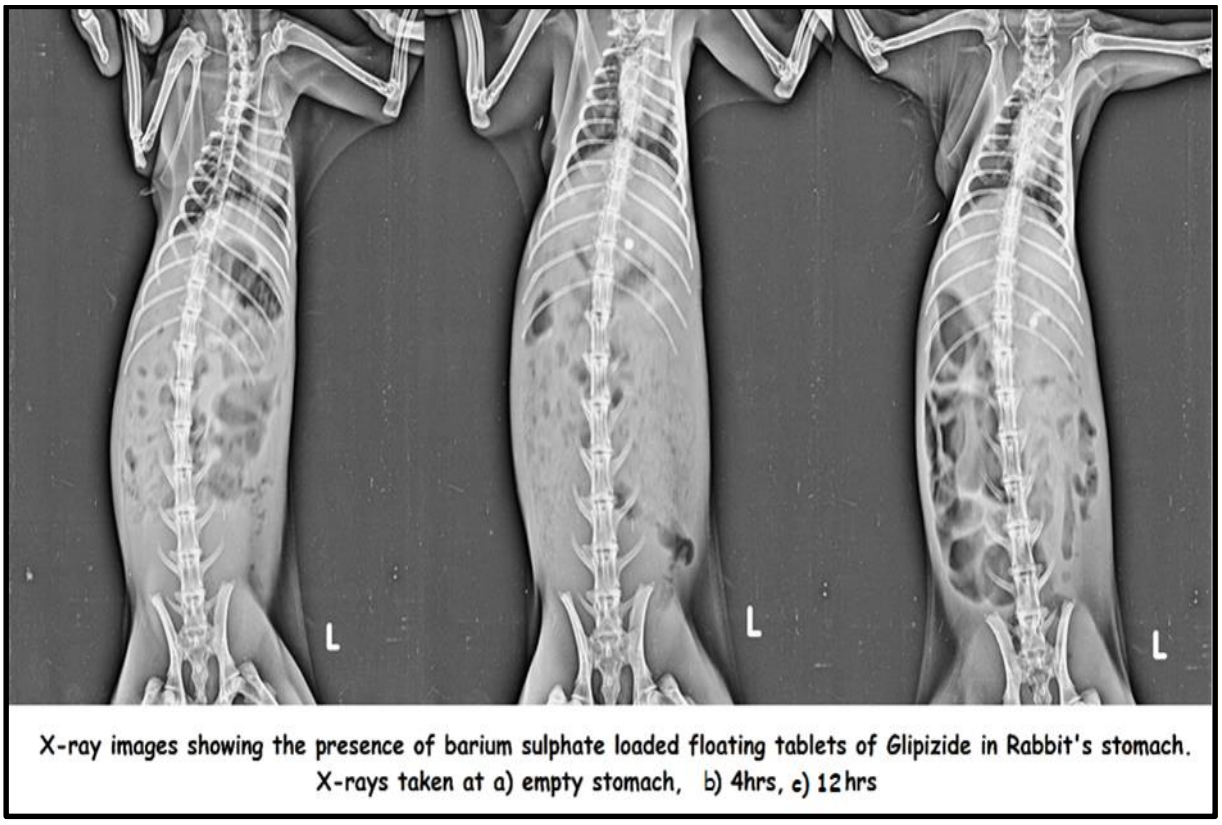


FIGURE 6A.8 X-ray images showing the presence of barium sulfate-loaded floating matrix tablet in the rabbit's stomach. a) 0 min b) 4hrs c) 12hrs

6A.4 Conclusion

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 levels of the variables was decided from preliminary studies and the tablets were prepared by wet granulation technique using PVP K30. The similarity factor ($f2$), 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 mg of X_1, 30 mg of X_2 and 10 mg of X_3, and this formulation showed highest desirability.

The obtained result is contradictory to that of the results attained with the metformin gastroretentive floating tablet, prepared with the same combination of independent variable. There it was found that the minimum quantity of k-carrageenan is required to get the desired effect.

6A.5 References

1. Foster, R.H. and Plosker, G.L., 2000. Glipizide: A Review of the Pharmacoeconomic Implications of the Extended-Release Formulation in Type 2 Diabetes Mellitus. *Pharmacoeconomics*, *18*(3), pp.289-306.

2. Chowdary, K.R., Rao, N.K. and Malathi, K., 2004. Ethyl cellulose microspheres of glipizide: Characterization, *in vitro* and *in vivo* evaluation. *Indian Journal of Pharmaceutical Sciences*, *66*(4), pp.412-416.

3. Li, V.H.K., Robinson, J.R. and Lee, V.H.L., 1987. Influence of drug properties and routes of drug administration on the design of sustained and controlled release systems. *Controlled Drug Delivery*, *29*(2), pp.4-94.

4. Patel, J.K., Patel, R.P., Amin, A.F. and Patel, M.M., 2005. Formulation and evaluation of mucoadhesive glipizide microspheres. *AAPS PharmSciTech*, *6*(1), pp.E49-E55.

5. Prabhu, P., Harish, N.M., Gulzar, A.M., Yadav, B., Narayana, C.R., Satyanarayana, D. and Subrahmanayam, E.V.S., 2008. Formulation and *in vitro* evaluation of gastric oral floating tablets of glipizide. *Indian Journal of Pharmaceutical Education and Research*, *42*(2), pp.174-183.

6. Tripathi, M., Radhika, P.R. and Sivakumar, T., 2011. Formulation and evaluation of glipizide hollow microballoons for floating drug delivery. *Bulletin of Pharmaceutical Research*, *1*(1), pp.67-74.

7. Mallikarjun, V., Ravi, P., Babu, V.R., Kiran, G. and Kumar, M.S., 2009. Design and evaluation of Glipizide floating tablets. *Journal of Pharmacy Research Vol*, *2*(4). pp.691-693.

8. Senthil, A., Suresh Kumar, P., Raju, C.H. and Mohideen, S., 2010. Formulation and evaluation of gastric oral floating tablet of Glipizide. *International Journal of Biological and Pharmaceutical Research*, *1*(2), pp.108-113.

9. Bolton, S., 1987. Statistical applications in the pharmaceutical sciences. The Theory and Practice of Industrial Pharmacy. 3rd ed. Bombay: Varghese Publishing House. Pp.243-289.

10. Prajapati, S.T., Patel, L.D. and Patel, D.M., 2009. Studies on formulation and *in vitro* evaluation of floating matrix tablets of domperidone. *Indian Journal of Pharmaceutical Sciences*, *71*(1), p.19.

11. Garg, R. and Gupta, G.D., 2009. Preparation and evaluation of gastroretentive floating tablets of silymarin. *Chemical and Pharmaceutical Bulletin*, *57*(6), pp.545-549.

12. Colombo, P., Bettini, R., Massimo, G., Catellani, P.L., Santi, P. and Peppas, N.A., 1995. Drug diffusion front movement is important in drug release control from swellable matrix tablets. *Journal of pharmaceutical sciences*, *84*(8), pp.991-997.

13. Pillay, V. and Fassihi, R., 2000. A novel approach for constant rate delivery of highly soluble bioactives from a simple monolithic system. *Journal of Controlled Release*, *67*(1), pp.67-78.

14. Bettini, R., Catellani, P.L., Santi, P., Massimo, G., Peppas, N.A. and Colombo, P., 2001. Translocation of drug particles in HPMC matrix gel layer: effect of drug solubility and influence on release rate. *Journal of Controlled Release*, *70*(3), pp.383-391.

15. Dorożyński, P., Kulinowski, P., Mendyk, A. and Jachowicz, R., 2011. Gastroretentive drug delivery systems with l-dopa based on carrageenans and hydroxypropylmethylcellulose. *International Journal of Pharmaceutics*, *404*(1), pp.169-175.

16. Pani, N.R., Nath, L.K., Acharya, S. and Bhuniya, B., 2011. Application of DSC, IST, and FTIR study in the compatibility testing of nateglinide with different pharmaceutical excipients. *Journal of Thermal Analysis and Calorimetry*, *108*(1), pp.219-226.

17. Singh, B., Chakkal, S.K. and Ahuja, N., 2006. Formulation and optimization of controlled release mucoadhesive tablets of atenolol using response surface methodology. *AAPS PharmSciTech*, *7*(1), pp.E19-E28.

18. Gaur, P.K., Mishra, S., Kumar, A. and Panda, B.P., 2014. Development and optimization of gastroretentive mucoadhesive microspheres of gabapentin by Box–Behnken design. *Artificial cells, Nanomedicine, and Biotechnology*, *42*(3), pp.167-177.

19. Jagdale, S., Kurhe, P., Kuchekar, B. and Chabukswar, A., 2013. Application of design of experiments to optimizing novel gastroretentive drug delivery of simvastatin. *Current Drug Delivery*, *10*(5), pp.527-541.

20. VenKateswarlu, K. and Chandrasekhar, K.B., 2016. Development and statistical optimization of sustained release gastro retentive floating tablets of cephalexin. *Marmara Pharmaceutical Journal*, *20*(2), pp.172-183.

21. Mandlik, S.K., Adhikari, S. and Deshpande, A.A., 2012. Application of Simplex Lattice Design in Formulation and Development of Buoyant Matrices of Dipyridamole. *Journal of Applied Pharmaceutical Science*, 2(12), pp.107-111.

22. Gohel, M.C., Parikh, R.K., Aghara, P.Y., Nagori, S.A., Delvadia, R.R. and Dabhi, M.R., 2009. Application of simplex lattice design and desirability function for the formulation development of mouth dissolving film of salbutamol sulphate. *Current Drug Delivery*, *6*(5), pp.486-494.

23. Suhesti, T.S., Fudholi, A. and Martien, R., 2016. Application of Simplex Lattice Design for the Optimization of the Piroxicam Nanosupensions Formulation using Evaporative Antisolvent Technique. *International Journal of Pharmaceutical and Clinical research*, *8*(5), pp.433-439.

24. Huisman, R., Van Kamp, H.V., Weyland, J.W., Doornbos, D.A., Bolhuis, G.K. and Lerk, C.F., 1984. Development and optimization of pharmaceutical formulations using a simplex lattice design. *Pharmacy World & Science*, *6*(5), pp.185-194.

25. Patel, D.M., Patel, N.M., Pandya, N.N. and Jogani, P.D., 2007. Gastroretentive drug delivery system of carbamazepine: formulation optimization using simplex lattice design: a technical note. *AAPS PharmSciTech*, *8*(1), pp.E82-E86.

26. Ishikawa, T., Watanabe, Y., Takayama, K., Endo, H. and Matsumoto, M., 2000. Effect of hydroxypropylmethylcellulose (HPMC) on the release profiles and bioavailability of a poorly water-soluble drug from tablets prepared using macrogol and HPMC. *International Journal of Pharmaceutics*, *202*(1), pp.173-178.

27. Timmins, P., Delargy, A.M. and Howard, J.R., 1997. Optimization and characterization of a pH-independent extended-release hydrophuic matrix tablet. *Pharmaceutical Development and Technology*, *2*(1), pp.25-31.

28. Tatavarti, A.S., Mehta, K.A., Augsburger, L.L. and Hoag, S.W., 2004. Influence of methacrylic and acrylic acid polymers on the release performance of weakly basic drugs from sustained release hydrophilic matrices. *Journal of Pharmaceutical Sciences*, *93*(9), pp.2319-2331.

29. Sankalia, J.M., Sankalia, M.G. and Mashru, R.C., 2008. Drug release and swelling kinetics of directly compressed glipizide sustained-release matrices: Establishment of level A IVIVC. *Journal of Controlled Release*, *129*(1), pp.49-58.

30. Singh, A., Sharma, P.K. and Malviya, R., 2011. Release behavior of drugs from various natural gums and polymers. *Polimery W Medycynie*, *41*(4), pp.73-80.

31. Cornell, J.A., 1979. Experiments with mixtures: an update and bibliography. *Technometrics*, *21*(1), pp.95-106.

32. Gorman, J.W. and Hinman, J.E., 1962. Simplex lattice designs for multicomponent systems. *Technometrics*, *4*(4), pp.463-487.

33. US Food and Drug Administration, 1997. Guidance for industry SUPAC-MR: Modified release solid oral dosage forms scale-up and post approval changes: Chemistry, manufacturing, and controls. *Vitro Dissolution Testing and In Vivo Bioequivalence Documentation*.

34. Moore, J.W., 1996. Mathematical comparison of dissolution profiles. *Pharmaceutical Technology*, *20*, pp.64-75.

CHAPTER 6B

Gastroretentive Floating Microsponges of Glipizide (GLP)

6B.1 Introduction

Microsponges were not explored for low density gastro retentive system until Arya et. al., developed targeted floating curcumin microsponges for improved site specific absorption for gastric cancer[1]. This study proved that microsponges have floating ability and can be used for the gastroretention of the drugs. Hence, floating microsponges are the novel means of preparing the gastroretentive formulations for antidiabetic drugs, which are needed to be present in the upper division of GIT for its better therapeutic action.

Although many gastroretentive formulations of glipizide (GLP) have been developed but no work has been reported on its floating microsponges[2]. Hence, the present research was intended for developing the floating microsponges of GLP with more loading capacity and better release of the drug in stomach. Furthermore, the formulation is supposed to provide sustained glucose lowering effect and improved diabetic condition as compared to immediate release of glipizide to achieve better treatment of the disease.

Gastroretentive floating Microsponges of GLP were prepared by quasi-emulsion solvent diffusion method. The Plackett-Burman design was employed as the screening technique to determine the most significant factors that affected the formulation of microsponges of GLP using Design-Expert® software. Plackett-Burman (PB)[3] designs are used for screening experiments because, in a PB design, main effects are, in general, heavily confounded with two-factor interactions. The PB design in 12 runs, for example, may be used for an experiment containing up to 11 factors. This designs is very useful for economically detecting large main effects, assuming all interactions are negligible when compared with the few important main effects[4,5]. Hence, these statistical tools helped in selecting the most important variables that can affect the formulation of microsponges.

The final optimization of glipizide floating microsponges was done by applying Box-Behnken design[6]. The Box-Behnken design is an independent quadratic design in that it does not contain an embedded factorial or fractional factorial design. In this design the treatment combinations are at the midpoints of edges of the process space and at the center. These designs are rotatable (or near rotatable) and require 3 levels of each factor. The designs have limited capability for orthogonal blocking compared to the central composite designs. This is a very useful for developing a formulation as it requires less experimentation and provides assessments of the relative significance of different variables[7].

6B.2 Experimental Studies

6B.2.1 Preparation of GLP Microsponges

Gastroretentive floating Microsponges were prepared by quasi-emulsion solvent diffusion method using an external phase containing distilled water and polyvinyl alcohol (PVA)[8-10]. The internal phase was prepared by adding ethyl cellulose in the organic solvent system consisting of ethanol as good solvent and Dichloromethane (DCM) as a bridging liquid[11]. To this organic phase, Glipizide was added and dissolved completely. At last, triethylcitrate (TEC) was added in organic phase to facilitate the plasticity. Then, the inner phase was poured into outer phase with stirring. After emulsification, the mixture was continuously stirred on mechanical stirrer, at a specific temperature for a specified time. The microsponges formed were filtered out immediately, washed with distilled water and dried at room temperature for 24h.

6B.2.2 Screening of Critical Factors Influencing Microsponges using Plackett and Burman Design

During the formulation of microsponges of MTG, it was found that there are many formulation and process related factors that can affect the formulation of microsponges. The literature suggested that if there are many factors that can affect the formulation of microsponges, the influence of critical formulation and process parameter can be found by applying appropriate statistical design[12]. Screening of these formulation and process related factors by trial and error technique is time consuming and can be inaccurate at times. Hence, in order to formulate the gastroretentive floating microsponges of glipizide, most significant

factors were selected by implementing the Plackett-Burman design. PB design has been earlier applied for the formulation of gastroretentive therapeutic system of atenolol[13]. Seven different factors were selected within their respective ranges for the formulation of floating microsponges, to evaluate their effect on the chosen responses, as shown in Table 6B.1. The parameter level ranges were selected based on preliminary experiments and prior knowledge about the system through literature survey.

TABLE 6B.1 Variables and their levels in Plackett–Burman screening design-of-experiments for GLP floating microsponges

Symbol Code	Factors	Experimental values	
		Low value (-1)	High Value (1)
X_1	Temperature (^oC)	30	40
X_2	Drug-Polymer Ratio	1:2	1:4
X_3	Speed of Agitation (rpm)	700	1500
X_4	Volume of TEC (%)*	10	20
X_5	PVA concentration (%)	0.5	1
X_6	DCM:Ethanol volume	1:3	1:5
X_7	Stirring time (hrs)	3	4

***TEC-triethyl citrate (% of polymer concentration); PVA-Polyvinyl alcohol**

Design-Expert® software (trial version 9.0.6, Stat-Ease) was used to apply Plackett–Burman design with 12 runs, as shown in Table 6B.2. The prepared formulations were evaluated for the morphology of the microsponges. The percentage yield and percentage entrapment efficiency were taken as dependent variable and the statistics was applied to find the most critical factors affecting the formulation of GLP microsponges.

TABLE 6B.2 Coded values of Plackett–Burman design experimental matrix for GLP

Batches	X_1 Temperature (^oC)	X_2 Drug-Polymer Ratio	X_3 Speed of Agitation (rpm)	X_4 Volume of TEC (%)	X_5 PVA concentration (%)	X_6 DCM:Ethanol concentration	X_7 Stirring time (hrs)
G-1	1	-1	1	-1	-1	-1	1
G-2	1	1	-1	1	-1	-1	-1
G-3	-1	1	1	-1	1	-1	-1
G-4	1	-1	1	1	-1	1	-1
G-5	1	1	-1	1	1	-1	1
G-6	1	1	1	-1	1	1	-1
G-7	-1	1	1	1	-1	1	1
G-8	-1	-1	1	1	1	-1	1
G-9	-1	-1	-1	1	1	1	-1
G-10	1	-1	-1	-1	1	1	1
G-11	-1	1	-1	-1	-1	1	1
G-12	-1	-1	-1	-1	-1	-1	-1

6B.2.3 Optimization of GLP Microsponges by Box–Behnken Design

Plackett–Burman (PB) screening design helped in the identification of crucial factors, affecting the formulation of GLP microsponges. Taking those factors into the consideration, a response surface method, three-factor, three-level Box–Behnken design was applied for the final optimization of floating glipizide microsponge[14].

The drug polymer ratio, stirring speed and temperature were taken as independent factors. Whereas, % entrapment efficiency, % buoyancy and % CDR12h were considered as dependent responses. The low and high levels of independent factors were directly adopted from the Plackett–Burman design and the medium levels were set as the midpoint of low and high levels (Table 6B.3). In addition, four other factors, which were evaluated in Plackett– Burman design, were set at a fixed level (PVA-0.5%, TEC-20%of polymer concentration, DCM:Ethanol-1:3 and stirring time-3hrs), in Box–Behnken design as their

effects on the response variables seemed statistically insignificant as per the results obtained from Plackett–Burman design.

TABLE 6B.3 Factors and their investigated levels in Box-Behnken Design

Independent Variables	Level		
	Upper level	Medium level	Lower level
Drug polymer ratio (X_1)	1 (1:4)	0 (1:3)	-1 (1:2)
Stirring Speed (X_2)	1 (1500 rpm)	0 (1100 rpm)	-1 (700 rpm)
Temperature (X_3)	1 (40ºC)	0 (35ºC)	-1 (30ºC)
Dependent Variables - % Entrapment efficiency, % Buoyancy, % CDR (cumulative drug release)			

Design-Expert® software (trial version 9.0.6, Stat-Ease) was used to apply the design and total 15 runs were formulated. Box–Behnken Design matrix coded values are given in Table 6B.4 (a). The actual composition of the floating microsponges of GLP prepared by applying Box–Behnken Design, is given in table 6B.4 (b).

TABLE 6B.4 (a) Coded values of variables in GLP microsponges by Box–Behnken Design

Formulation	X_1	X_2	X_3
GBB-1	-1	-1	0
GBB-2	1	1	0
GBB-3	-1	1	0
GBB-4	1	0	1
GBB-5	1	-1	0
GBB-6	0	-1	-1
GBB-7	-1	0	1
GBB-8	0	1	1
GBB-9	0	0	0
GBB-10	1	0	-1
GBB-11	0	1	-1
GBB-12	0	0	0
GBB-13	0	0	0
GBB-14	0	-1	1
GBB-15	-1	0	-1

TABLE 6B.4 (b) Actual composition of GLP floating microsponges prepared by Box–Behnken Design

Ingredients	GBB-1	GBB-2	GBB-3	GBB-4	GBB-5	GBB-6	GBB-7	GBB-8	GBB-9	GBB-10	GBB-11	GBB-12	GBB-13	GBB-14	GBB-15
GLP (mg)	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500
Ethyl cellulose (mg)	1000	2000	1000	2000	2000	1500	1000	1500	1500	2000	1500	1500	1500	1500	1000
Polyvinyl alcohol (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Triethyl citrate (%)*	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Ethanol (ml)	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Dichloro methane (ml)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Distilled water (ml)	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300
Stirring speed (rpm)	700	1500	1500	1100	700	700	1100	1500	1100	1100	1500	1100	1100	700	1100
Temperature (ºC)	35	35	35	40	35	30	40	40	35	30	30	35	35	40	30
Stirring time (hrs)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

*** % of polymer concentration**

6B.2.4 Evaluation of GLP Microsponges

Same as that of chapter 5B

6B.2.5 Validation of Experimental Design

An extra check point formulation was prepared to validate the experimental design. The values for % entrapment efficiency, % buoyancy and % CDR12h were predicted by their respective polynomial equations. The check point batch had the composition with the levels as X_1: 0.1, X_2: 0.71 and X_3: 0.99. The experimental values were determined by evaluating the dependent variables. The predicted and experimental values of the responses were compared for statistical significance using t-test at 95% confidence interval ($p < 0.05$).

6B.2.6 Physicochemical Characterization

Same as that of chapter 5B

6B.2.7 Stability Study of Optimized Formulation as per ICH Guidelines

Same as that of chapter 5B

6B.2.8 *In vivo* Studies[18]

To investigate the actual effect of the optimized GLP gastroretentive floating microsponges inside the body, various *in vivo* studies were conducted **Same as that of chapter 5B**.

6B.3 Results and Discussion

6B.3.1 Screening of Critical Factors using Plackett and Burman Design

An attempt was made to develop a gastroretentive floating microsponges of Glipizide by quasi-emulsion solvent diffusion method. Screening of the formulation and process related factors by trial and error technique is time consuming and can be inaccurate at times. QbD achieves balance between experiments, resources and time required for development of pharmaceutical formulations[19]. 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. 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).

TABLE 6B.5 Summary of ANOVA table for response parameters for Plackett and Burman design for GLP microsponges

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	p-value Prob > F
Yield (%)					
Model	1661.64	3	553.88	27.33	0.0001
Residual	162.11	8	20.26		
Corrected Total	1823.75	11			
Entrapment Efficiency (%)					
Model	861.67	3	287.22	7.93	0.0088
Residual	289.60	8	36.20		
Corrected Total	1151.27	11			

The summary of analysis of variance for response parameters is given in table no. 6B.5. The F value and p-value suggests that the model is significant for both the variables. The results of the batches prepared by applying PB design is given in table no 6B.6. The range of the % yield and % entrapment efficiency for the PB batches was found to be 53.3 to 93.75% and 52.7 to 86.34%, respectively. Such huge variation in the responses of the batches directed that the selected independent variables are extremely significant.

TABLE 6B.6 Experimental responses of Plackett–Burman design matrix

Batches	% Yield	% Entrapment Efficiency
G-1	73.53	70.36±3.8
G-2	85.87	82.7±2.4
G-3	82.24	81.32±2.3
G-4	75.68	86.34±1.8
G-5	80.4	79.21±4.2
G-6	85.2	85.8±3.8
G-7	93.73	82.25±2.6
G-8	68.8	63.81±1.9
G-9	55.34	67.2±5.1
G-10	58.1	72.4±4.3
G-11	73.8	69.7±3.5
G-12	53.3	52.7±3.5

***n=3, average of three determinations±SD**

6B.3.1.1 Effect of the Variables on Yield of the Product

The pareto chart of yield (Fig. 6B.1) and the final equation indicated that the most significant factors for yield are X_2 (drug-polymer ratio) and X_3 (stirring speed).

The R^2 value was found to be equal to 0.8485, which means model is significant. The difference between adjusted and predicted R^2 value was found to be less than 0.2 and also the F-value of 7.93 proved the significance of model.

$$Yield\ (\%) = +73.83 + 9.71X_2 + 6.03X_3 + 2.80X_4$$

The equation clearly shows that, as the drug polymer ratio increases (amount of polymer increases), the yield of micosponges increases. The outcomes were similar to that of obtaining by Arya P. et al 2014 as explained by Lee et al. (1999)[1, 20]. The high level of ethyl cellulose makes the organic phase viscous which decreases the diffusion of organic phase to aqueous phase that prolongs polymer solidification thus increases the yields.

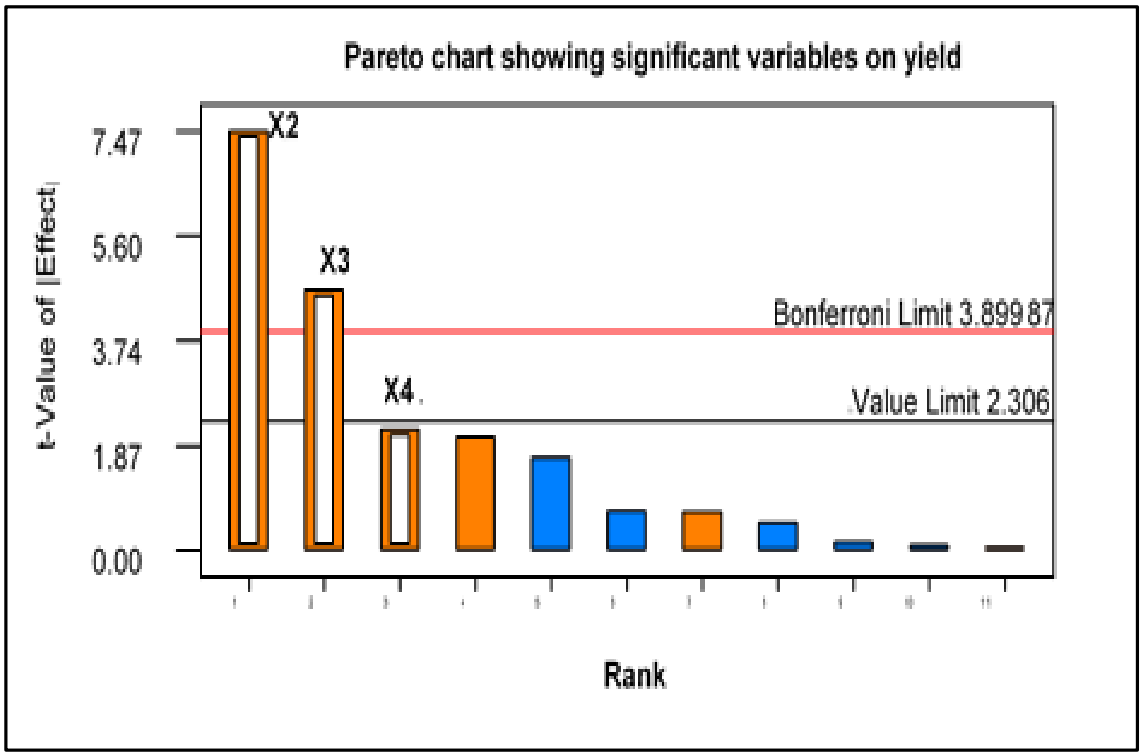


FIGURE 6B.1 Plackett–Burman design for GLP microsponges - Pareto Chart of yield

6B.3.1.2 Effect of the Variables on Entrapment Efficiency

The pareto chart of entrapment efficiency (Fig. 6B.2) and the final equation clearly indicates that the most significant factors, are X_1(temperature), X_2 (drug-polymer ratio) and X_3 (stirring speed), in terms of entrapment efficiency.

The R^2 value was found to be equal to 0.9111, which means good agreement between the dependent and independent variables. The difference between adjusted and predicted R^2 value was found to be less than 0.2 and also the F-value of 27.33 proved the significance of model.

$$Entrapment\ Efficiency\ (\%) = +74.48 + 4.99X_1 + 5.68\,X_2 + 3.83\,X_3$$

The combined effect of the selected variables on yield and entrapment efficiency indicated 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. Hence, these factors (temperature, drug-polymer ratio and

stirring speed) were further evaluated by applying Box–Behnken design to get the optimized formulation of GLP microsponges.

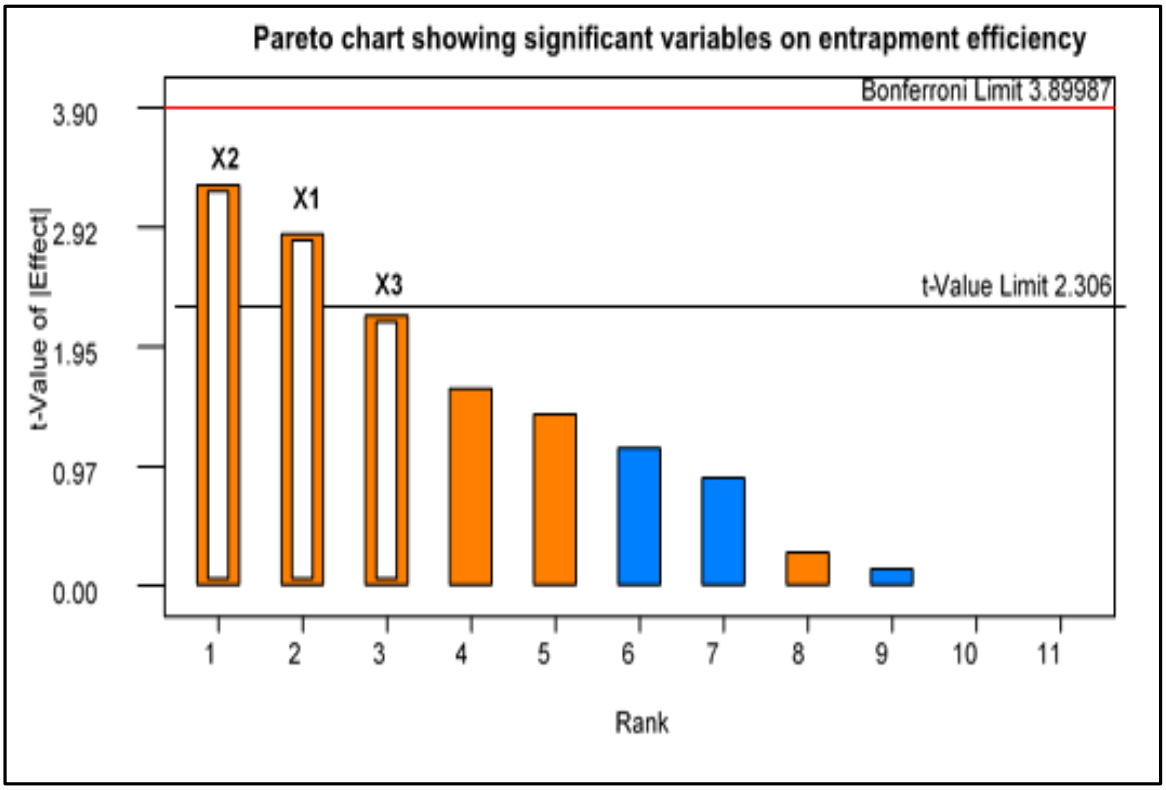


FIGURE 6B.2 Plackett–Burman design for GLP microsponges - Pareto Chart of Entrapment Efficiency

6B.3.2 Optimization of GLP Microsponges: Box–Behnken Design

The results presented in table 6B.7 shows that the percent yield for all the batches was in the range of 84.8% to 98.8%. The particle size was found to be in the orbit of 96.30 to 243.87 µm, which comes in the standard range (5-300µm) of microsponges as mentioned by Nacht and Katz[21]. General observation was that as the stirring speed increased the particle size decreased as earlier proved by Nokhodchi, et al.[22].

The polymer concentration had an optimistic effect on particle size. The high concentration of polymer makes the solution thick, hence particle size increases. This is because, as the concentration of polymer in the organic phase increases, the emulsification is hindered and big droplets of polymer are formed during the polymerization, leading to bigger particle size.

TABLE 6B.7 Result table of GLP floating microsponges prepared applying Box–Behnken Design

Formulation	% Yield	Particle size (µm)	Bulk density (g/cc)	% Entrapment Efficiency	% Buoyancy	% CDR_{12}
GBB-1	96.4	157.43±14.6	0.222	72.53±5.2	69.4±2.6	75.1±0.8
GBB-2	98.4	172.4±11.4	0.106	83.76±2.3	82.5±3.2	81±1.2
GBB-3	97.6	114.18±10.5	0.113	74.96±3.7	80.5±2.7	84.3±1.5
GBB-4	98.88	143.25±15.7	0.101	83.8±1.9	89.4±1.8	80.2±1.0
GBB-5	84.8	153.97±16.9	0.384	74.78±2.4	71.1±2.2	83.4±0.9
GBB-6	86	104.37±13.6	0.107	62.75±1.7	82.4±3.6	76.4±1.3
GBB-7	85.3	127.54±10.4	0.202	85.63±2.5	78±2.4	79.2±1.5
GBB-8	96.3	243.87±9.3	0.087	90.81±1.21	92.3±2.25	92.3±0.9
GBB-9	93.7	138.74±18.5	0.219	92.98±2.8	74±2.4	90.1±1.7
GBB-10	70.96	96.3±12.5	0.217	66.23±3.1	75.1±2.1	77.2±1.0
GBB-11	93.2	195.67±12.7	0.122	74.12±2.6	85.9±2.7	73.9±1.1
GBB-12	92.5	129.74±10.5	0.210	92.32±2.5	77.3±3.1	90.2±1.4
GBB-13	90.2	133.74±16.2	0.225	92.63±2.7	78.3±2.5	89.9±1.3
GBB-14	91.4	119.66±17.6	0.114	82.29±3.2	80.6±2.5	84.3±1.6
GBB-15	92.6	125.9±13.2	0.11	68.94±4.3	82.3±3.4	74.2±1.3

***n=3, average of three determinations±SD**

6B.3.2.1 Micromeritic Properties of GLP Microsponges

The Bulk density was found to be in the range of 0.087 to 0.384 g/cc. Particle size of all the batches was found to be less than 500µm, the range was 96.3µm to 243.87µm. The pictorial presentation of morphology of the prepared microsponges is given in figure no. 6B.3. The pictures taken by trinocular microscope, shows that the prepared microsponges are spherical in shape with different size and spongy surface.

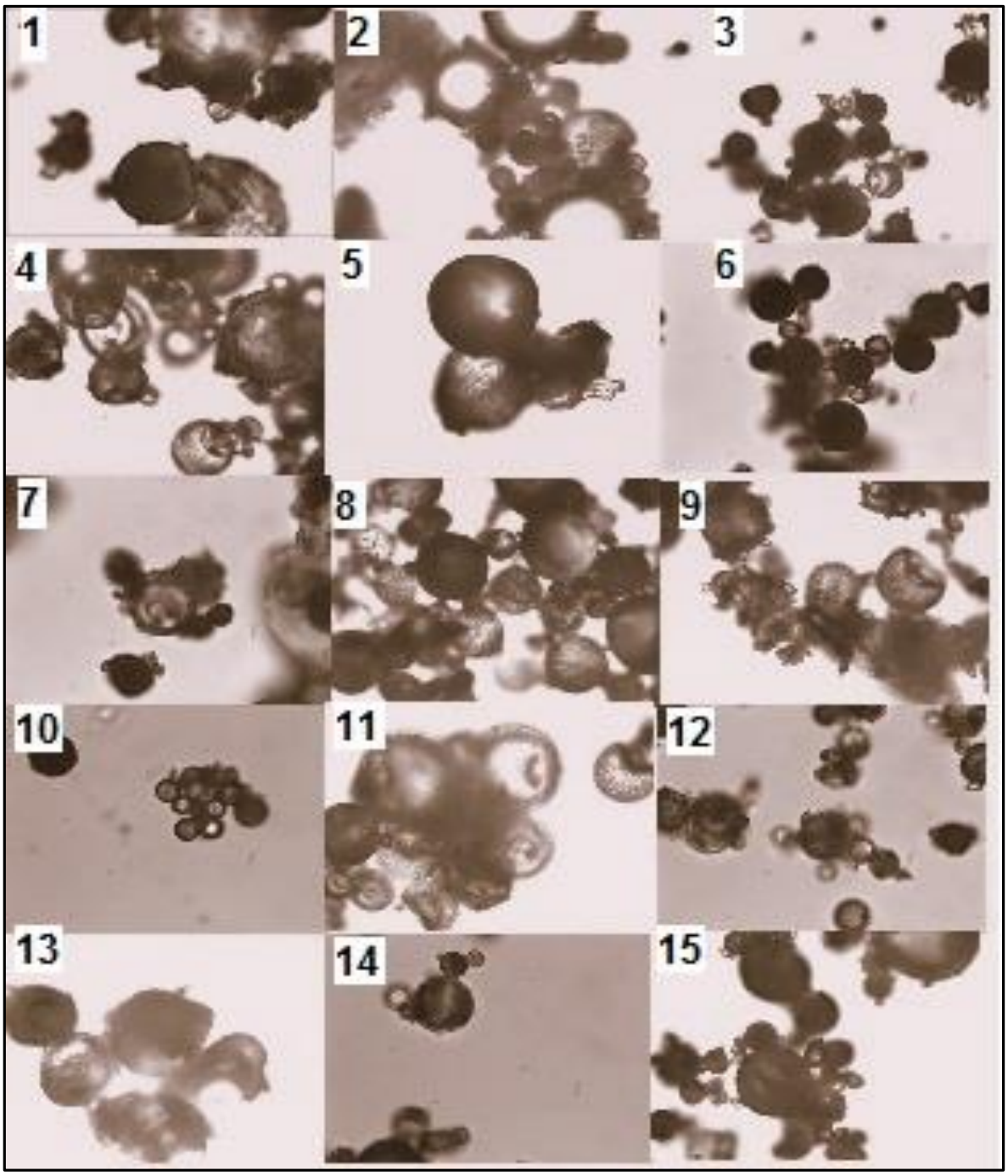


FIGURE 6B.3 Images of GLP box-behnken batches taken on trinocular microscope

6B.3.2.2 *In vitro* Drug Release

The drug release study was performed for all the batches of GLP microsponges prepared by applying design. The drug release data is given in table no. 6B.8 and the graphical representation of the same is shown in figure no. 6B.4.

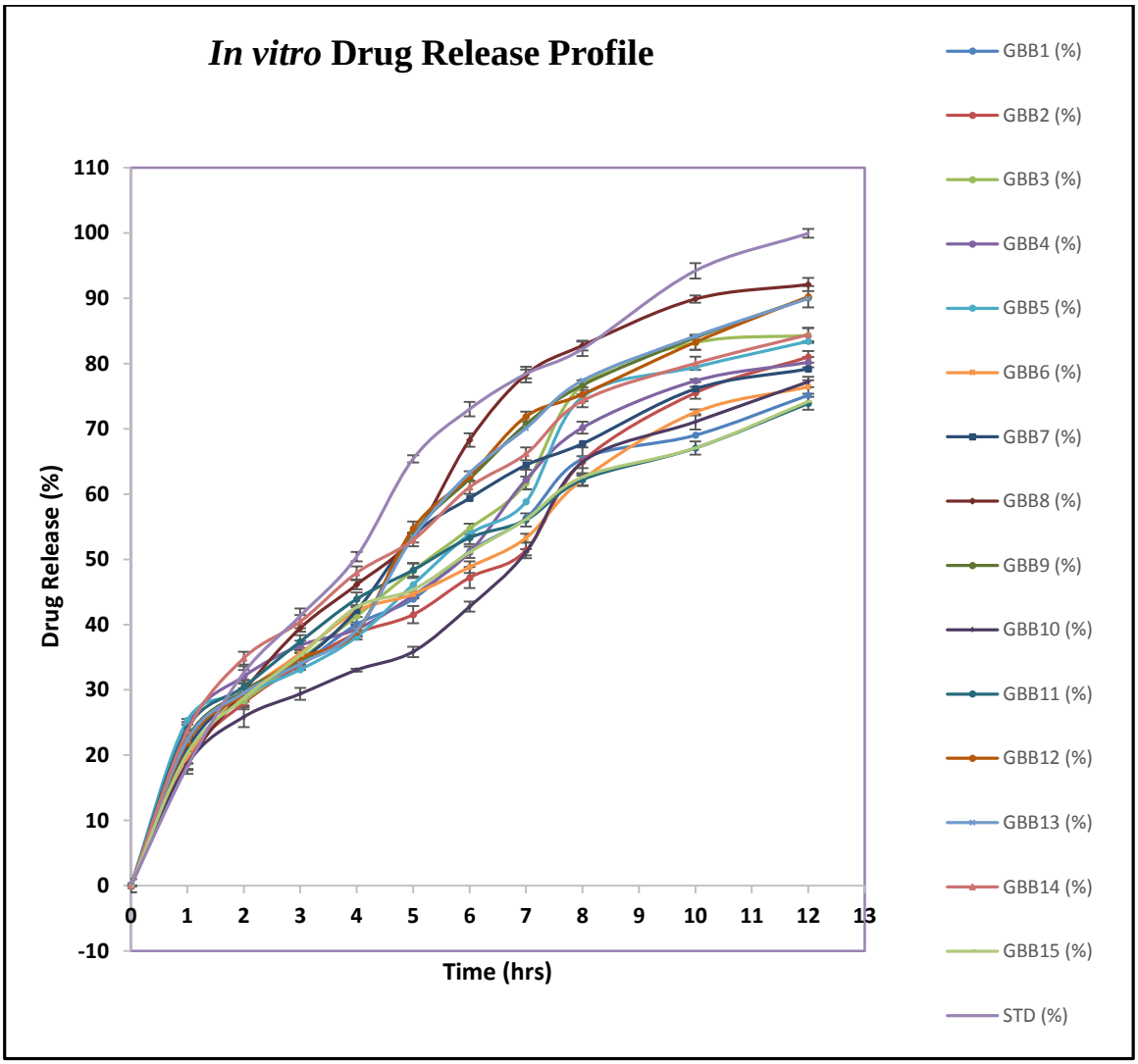


FIGURE 6B.4 *In vitro* drug release profile of GLP microsponge batches prepared using Box-Behnken design

The release profile of all the batches was compared with standard marketed (STD) sustained release formulation of GLP. A general observation was that the batches prepared with medium concentration of polymer gave better release pattern. Only these batches could pass the criteria of model independent analysis of dissolution.

The batches prepared with the high concentration of ethyl cellulose showed delayed release of the drug, which is because of the fact that more polymer concentration increases path length, which the drug molecule has to cross. Earlier finding suggest that the reason for getting delayed release with high concentration of polymer is because it decreases the amount of drug close to surface of microsponges exposed to the dissolution medium[23]. This leads to lowering in the rate of drug release from the microsponges.

TABLE 6B.8 Results table for *in vitro* drug release of GLP from floating microsponges prepared by applying box-behnken design*

Time	GBB1 (%)	GBB2 (%)	GBB3 (%)	GBB4 (%)	GBB5 (%)	GBB6 (%)	GBB7 (%)	GBB8 (%)	GBB9 (%)	GBB10 (%)	GBB11 (%)	GBB12 (%)	GBB13 (%)	GBB14 (%)	GBB15 (%)	STD (%)
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1	22.62±1.09	19.45±1.53	22.23±0.54	24.62±0.93	25.34±2.01	19.72±1.02	20.95±1.03	18.50±1.39	22.50±0.79	18.61±0.88	24.06±1.39	21.90±0.89	22.30±1.18	23.67±1.04	20.28±1.16	18.23±0.54
2	29.55±0.67	27.97±0.92	28.34±0.71	32.06±0.99	29.40±1.1	29.23±1.02	29.73±0.34	29.82±1.16	29.79±0.49	25.84±1.53	30.45±1.16	29.29±0.59	29.51±1.53	34.84±1.11	28.73±0.79	32.61±1.03
3	33.79±1.12	33.84±0.79	34.66±1.04	36.80±0.73	33.11±0.89	35.57±0.52	34.39±1.04	39.49±0.53	34.38±1.32	29.38±0.92	37.39±0.79	34.79±0.91	33.99±2.02	40.45±0.43	35.28±0.94	41.31±1.19
4	40.01±1.18	38.66±0.49	41.30±1.39	39.30±1.08	38.12±0.59	42.06±0.91	42.18±1.11	46.17±0.69	39.07±1.6	33.09±0.21	43.96±0.94	38.87±1.17	39.12±1.54	47.95±0.71	42.68±0.75	50.41±0.73
5	43.92±0.92	41.53±1.32	48.28±1.16	44.47±0.45	46.13±0.91	44.6±0.42	53.64±0.43	53.35±0.78	53.91±1.18	35.83±0.79	48.36±0.75	54.71±1.06	53.41±0.79	52.93±1.04	45.37±1.07	65.39±0.57
6	51.20±0.82	47.20±1.6	54.68±0.79	51.09±0.88	53.86±1.17	48.80±0.89	59.37±0.71	68.27±1.03	62.32±1.53	42.74±0.77	53.31±1.07	62.72±0.79	63.22±0.92	61.05±0.77	51.04±1.16	73.01±1.09
7	56.31±1.53	51.37±1.18	61.71±0.94	62.21±1.53	58.82±1.06	53.25±0.67	64.44±1.04	78.29±1.19	70.67±0.92	51.08±0.46	56.04±1.16	71.87±0.75	70.07±0.88	66.17±0.46	56.18±0.73	78.42±0.67
8	65.38±1.29	64.99±2.17	76.67±0.75	70.17±0.92	74.89±0.79	62.21±0.88	67.73±1.39	82.75±0.73	76.68±0.57	64.88±0.91	62.21±0.39	75.28±1.07	77.38±0.49	74.29±0.91	62.60±1.08	82.24±1.12
10	69.09±1.42	75.51±0.92	83.19±1.07	77.35±0.21	79.47±0.61	72.50±0.45	76.12±1.16	89.87±0.57	83.97±0.79	71.06±1.16	67.06±0.75	83.27±1.16	84.17±1.03	80.02±1.16	67.06±0.45	94.21±1.18
12	75.12±0.8	81.01±1.2	84.31±1.5	80.19±1.0	83.42±0.9	76.41±1.3	79.19±1.5	92.3±0.90	90.09±1.7	77.18±1.0	73.90±1.1	90.24±1.4	89.94±1.3	84.32±1.6	74.18±1.3	99.95±0.68

***n=3, average of three determinations±SD**

The release pattern of formulation GBB-8 had the maximum similarity factor value, 65 and minimum dissimilarity value 6, as compared marketed formulation.

6B.3.2.3 Drug Release Kinetics

The results for the analysis of model-dependent drug release kinetics and for the *in vitro* release of glipizide from floating microsponges, is given in table 6B.9.

TABLE 6B.9 Results table for *in vitro* drug release, model-dependent kinetics for GLP microsponges prepared by Box–Behnken Design

Batch code	Higuchi model (R_H)	Korsmeyer Peppas model (R_P)	Hixson Crowell model (R_{HC})	First order (R_1)	Zero order (R_0)
GBB-1	0.9881	0.9829	0.9526	0.8021	0.9000
GBB-2	0.9637	0.9969	0.9444	0.7242	0.8987
GBB-3	0.9662	0.9784	0.9735	0.7894	0.9366
GBB-4	0.9714	0.9811	0.9162	0.8065	0.8561
GBB-5	0.9580	0.9083	0.9365	0.7963	0.8953
GBB-6	0.9881	0.9946	0.9505	0.7681	0.8992
GBB-7	0.9869	0.9738	0.9798	0.8534	0.9587
GBB-8	0.9684	0.9914	0.9747	0.8632	0.9757
GBB-9	0.9691	0.9277	0.9565	0.8044	0.9481
GBB-10	0.9417	0.9840	0.9260	0.7375	0.8831
GBB-11	0.9980	0.9911	0.9623	0.7967	0.8997
GBB-12	0.9697	0.9333	0.9581	0.7965	0.9527
GBB-13	0.9677	0.9255	0.9544	0.8112	0.9509
GBB-14	0.9946	0.9949	0.9716	0.8367	0.9219
GBB-15	0.9971	0.9971	0.9677	0.7922	0.9172
STD	0.9807	0.9946	0.9908	0.8068	0.9858

The model dependent approaches evaluated for the drug release kinetics were zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas. The drug release from the floating microsponges of GLP, batches GBB-1, GBB-5, GBB-7, GBB-9, GBB-11, GBB-12, GBB-13, GBB-15, followed Higuchi diffusion model with R^2 value close to 1. This model is generated by plotting cumulative percentage drug release versus square root of time and is applicable for modified dosage forms especially to matrix drug delivery systems. All other batches, GBB-2, GBB-3, GBB-4, GBB-6, GBB-8, GBB-10, GBB-14, GBB-15 and STD followed Korsmeyer Peppas model (R_P). This data is obtained from *in vitro* drug release studies by plotting log cumulative percentage drug release versus log time.

6B.3.3 Statistical Analysis

The result of all the dependent variables is given in table 6B.7. For Box-Behnken analyses, the regression equation describes the effects of the variables on the responses in terms of linear, interactive and quadratic.

TABLE 6B.10 Summary of ANOVA table for response parameters for Box-Behnken design for GLP microsponges

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	P-value
Buoyancy (%)					
Model	537.61	9	59.73	14.59	0.0044
Residual	20.46	5	4.09		
Corrected Total	558.08	14			
Entrapment Efficiency (%)					
Model	1374.46	9	152.72	19.05	0.0023
Residual	40.07	5	8.01		
Corrected Total	1414.53	14			
CDR (%)					
Model	511.99	9	56.89	6.73	0.0246
Residual	42.24	5	8.45		
Corrected Total	554.24	14			

The polynomial equation generated by box-behnken design using Design expert software is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Where, Y is the dependent variable, b_0 is the intercept, and b_1 to b_{33} are regression coefficient. The master effects (X_1, X_2, and X_3) represent the average result of changing one element at a time from its low to high value. X_1X_2, X_1X_3 and X_2X_3 represents the interaction terms and X_1^2, X_2^2 and X_3^2 represents quadratic effect.

Using analysis of variance (ANOVA), the significance ($p < 0.05$) of the ratio of mean square variation due to the regression coefficient, and the residual error were tested. The summary of ANOVA table for response parameters is given in table 6B.10. The Quadratic model was found to be significant for all the responses. The p-value was found to be significant, which indicates that the model was significant. The high values of correlation coefficients for buoyancy (R^2 = 0.9633), Entrapment efficiency (R^2 = 0.9717) and CDR_{12} (R^2 = 0.9238) indicated a good fit (ie, good agreement between the dependent and independent variables).

6B.3.3.1 Effect of Independent Variables on Buoyancy

The percent entrapment efficiency was found to be in the range of 69.4 to 92.3%. P value was found to be 0.0044 implies the model is significant. R^2 was found to be equal to 0.9633. The difference between "Pred R-Squared" and "Adj R Squared" value was found to be less than 0.2. The established second-degree polynomial equation for entrapment efficiency was as follow:

$$Buoyancy\ (\%) = +76.53 + 0.99X_1 + 4.71X_2 + 1.82X_3 + 0.075X_1X_2 + 4.65X_1X_3 + 2.05X_2X_3 - 2.38X_1^2 + 1.72X_2^2 + 7.05X_3^2$$

The equation indicates that all the independent variables have a positive influence on the buoyancy of the microsponges. The most important element was found to be stirring speed (X_2), as the speed of stirring increased the buoyancy also increased. There was a significant positive interaction effect found with drug polymer ratio (X_1) and temperature (X_3). A quadratic effect of temperature was also noted. These effects were further illustrated in contour plots (Fig 6B.5).

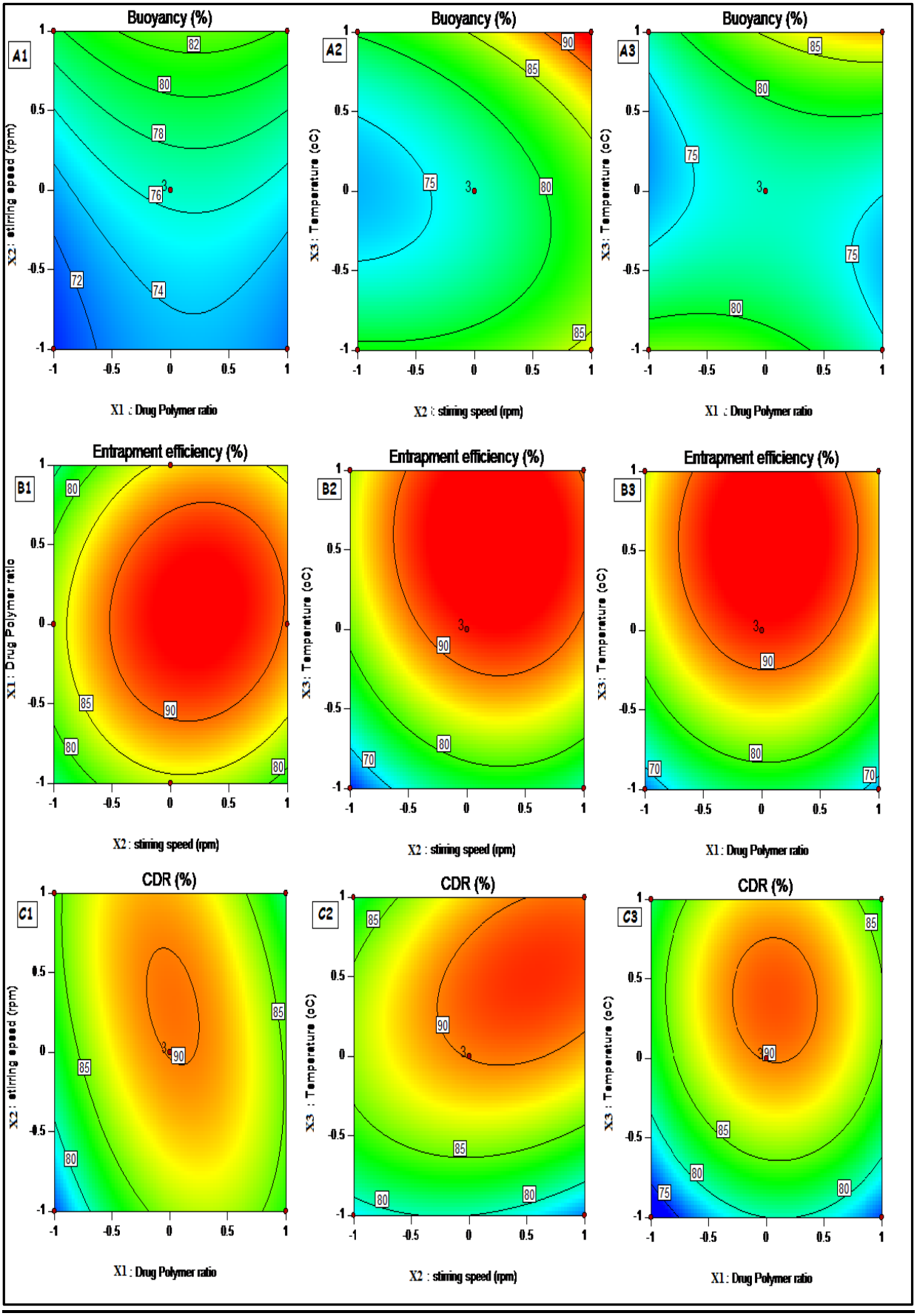


FIGURE 6B.5 GLP microsponges - Contour plot of buoyancy (A), entrapment efficiency (B) and CDR_{12} (C): 1-Plot X_1X_2 keeping X_3=0, 2-Plot X_2X_3 keeping X_1=0 and 3-X_1X_3 keeping X_2=0

The effects of X_1 and X_2 with their interaction on buoyancy at a specified level of X_3 (0) are shown in contour plot, Fig. 6B.5 (A1). The plots were found to be linear at 72% buoyancy, but above this value, the plots were found to be nonlinear indicating a nonlinear relationship between X_1 and X_2 with maximum nonlinearity found with 74% of buoyancy. It was learned from the contour plot that the maximum buoyancy 82% was noted at the highest value of X_2 and the value of X_1 maintained between the ranges 0 to 0.5. The contour plot of X_2 and X_3 showed that highest non linearity was observed at buoyancy below 85% and for obtaining the maximum buoyancy the values of X_2 and X_3 should be near 1 [Fig. 6B.5(A2)]. Contour plot of buoyancy drawn at 0 values of X_2 showed that 85% of buoyancy is achieved with highest value of both the independent variables [Fig. 6B.5(A3)]. Overall, for buoyancy, the most significant factor was stirring speed followed by temperature of the medium. This indicated that as the stirring speed increases, the porosity of microsponges increases and formulation becomes more buoyant.

6B.3.3.2 Effect of Independent Variables on Entrapment Efficiency

The percent entrapment efficiency was found to be in the range of 62.75 to 92.98%. P value was found to be 0.0023 implies the model is significant. R^2 was found to be equal to 0.9715. The difference between "Pred R-Squared" and "Adj R Squared" value was found to be less than 0.2. The established second-degree polynomial equation for entrapment efficiency was as follow:

$$Entrapment\ Efficiency\ (\%) = +92.64 + 0.81X_1 + 3.91X_2 + 8.81X_3 + 1.64X_1X_2 + 0.22X_1X_3 - 0.71X_2X_3 - 8.74X_1^2 - 7.40X_2^2 - 7.75X_3^2$$

The equation indicates that all the independent variables have a positive influence on the entrapment efficiency of the microsponges. The significant antagonistic quadratic effect of the independent factors was observed on entrapment efficiency, which was further shown by contour plots. A contour plot is a graphical technique for representing a 3-dimensional surface by plotting constant z slices, called contours, on a 2-dimensional format. This implies that the result of two variables can be pulled out by holding open the third variable constant. Here, the plots suggested that 90% of entrapment efficiency can be achieved with X_1 in the range of -0.6 to 0.75 and X_2 in the range of -0.5 to 0.9 at a fixed level of X_3 (0) are shown in

contour plot, Fig. 6B.5(B1). The contour plot of X_2 and X_3 with X_1 constant, showed that highest entrapment efficiency can be obtained when X_3 is kept in the range of -0.2 to 1 and X_2 in the range of -0.6 to 1 [Fig. 6B.5(B2)]. Contour plot of entrapment efficiency, drawn at 0 value of X_2 showed that the value of entrapment efficiency was more in the range of X_3 as -0.2 to 1 and X_1 as -0.7 to 0.7 [Fig. 6B.5(B3)]. As the quadratic effect is significant, it means that optimal levels of X are not in the extremes of the experimental region but inside it. However, as per the linear effect for entrapment efficiency, the most significant factor was the temperature of the medium. As the temperature increased, fast solidification of the dispersed phase helped in faster solidification of polymer which prevents drug diffusion across the phase boundary and hence more entrapment of the drug.

6B.3.3.3 Cumulative Drug Release (CDR 12 hrs)

Microsponges offer the controlled release of the drug as proven during earlier studies performed by Osmani R., et al[24]. The percent CDR_{12} was found to be in the range of 73.9 to 92.3%. P value was found to be 0.0246 implies the model is significant. R^2 was found to be equal to 0.9238. This model can be used to navigate the design space. The established second-degree polynomial equation for entrapment efficiency was as follow:

$$CDR_{12}(\%) = +90.07 + 1.12X_1 + 1.54X_2 + 4.29X_3 - 2.90X_1X_2 - 0.50X_1X_3 + 2.63X_2X_3 - 6.57X_1^2 - 2.55X_2^2 - 5.80X_3^2$$

The equation indicates that all the independent variables have a positive influence on the CDR_{12} of the microsponges. The significant antagonistic quadratic effect of the independent factors was observed on CDR_{12}, which was further shown by contour plots. A contour plot suggested plots suggested that 90% of CDR_{12} can be achieved with X_1 in the range of -0.3 to 0.3 and X_2 in the range of 0 to 0.5 at a fixed level of X_3 (0) are shown in contour plot, Fig. 6B.5(C1). The contour plot of X_2 and X_3 with X_1 constant, showed that highest CDR_{12} can be obtained when X_3 is kept in the range of 0 to 1 and X_2 in the range of -0.7 to 1 [Fig. 6B.5(C2)]. Contour plot of entrapment efficiency, drawn at 0 value of X_2 showed that the

value of CDR_{12} was more in the range of X_3 as 0 to 0.8 and X_1 as -0.3 to 0.4 [Fig. 6B.5(C3)]. Overall, for CDR_{12}, interaction within the independent variable was observed.

6B.3.4 Validation of Experimental Design

In order to validate the experimental design, the check point batch was prepared and evaluated for all the dependent responses. The predicted and actual values for entrapment efficiency, buoyancy and CDR_{12} were found to be 91.7606% and 91.67%, 92.3% and 93.92% and 90.0582% and 90.9% respectively. The relative error (%) between the predicted and actual values for each response was calculated and the values were found to be within 5%. The experimental values were in agreement with the predicted values confirming the validity of the model.

6B.3.5 Selection of Optimized Batch

To optimize all the responses with different targets, a numerical optimization technique by the desirability function and a graphical optimization technique by the overlay plot was used. The optimized formulation was obtained by applying constraints on dependent variable responses and independent variables. The constraints for all the dependent variables were set at 80% to 100%. The recommended concentrations of the independent variables were calculated by the Design Expert software from the overlay plots obtained which has the highest desirability near to 1.0. Using design expert software three overlay plots were obtained indicating the area of optimal process variables as applied in Figure 6B.6. Figure 6B.6 (A), represents an overlay plot obtained with variable X_1 (drug polymer ratio) and X_2 (stirring speed) by keeping X_3 (temperature) constant as 0.993937. Overlay plot 6B.6 (B), was obtained with X_1 and X_3 with X_2 constant as 0.7948 and C overlay was obtained with X_2 and X_3 after fixing the value of X_1 as 0.09930. After applying the desirability criteria and looking into overlay plots, formulation GBB-8 with medium drug- polymer ratio and maximum level of the other two variables was found to be optimum with desirability near 1. The formulation gave entrapment efficiency as 90.81%, buoyancy as 92.3% and CDR 12hrs as 92.3%.

This clearly indicates that glipizide microsponges are best formulated with 1:3 drug polymer ratio, 40ºC temperature and 1500 rpm stirring speed. Formulation GBB-8 was considered to

be the optimized formulation and it was used for physicochemical characterization of floating microsponges of GLP and also for the *in vivo* studies.

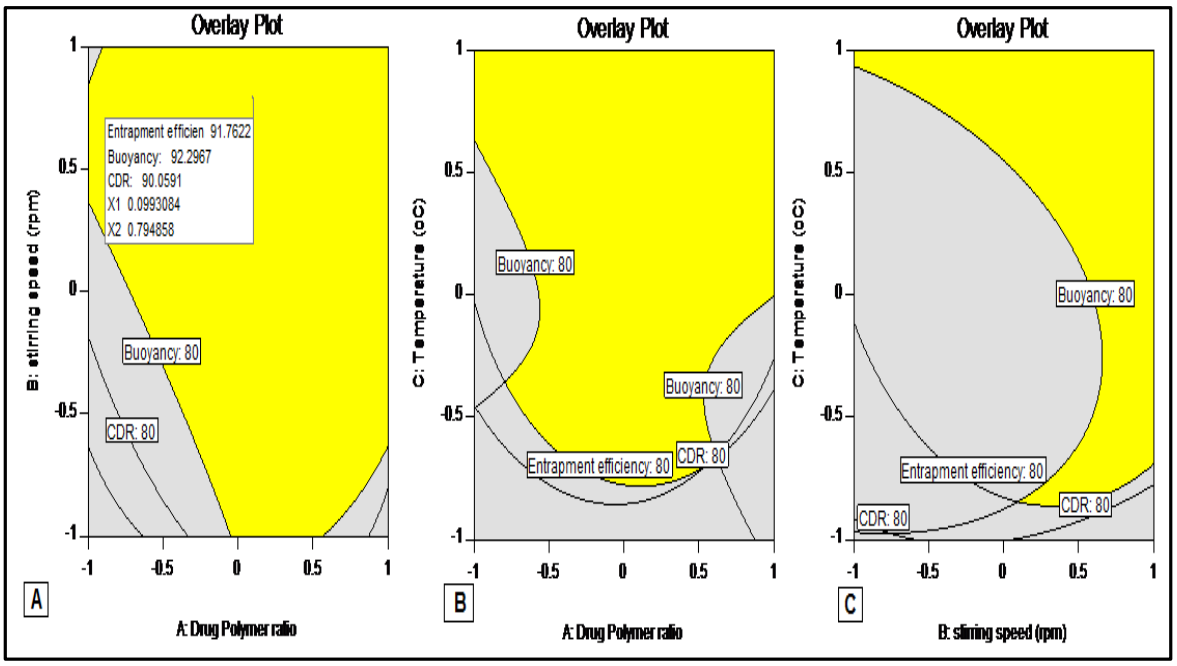


FIGURE 6B.6 Overlay Plot of GLP floating Microsponges by Box-Behnken design

6B.3.6 Physicochemical Characterization of Glipizide Microsponges

6B.3.6.1 Scanning Electron Microscopy (SEM)

SEM images of optimized batch of GLP microsponges (GBB-8) were taken to get the topographical information about the formulation. The results are shown in Figure 6B.7, which revealed that the formulated GLP microsponges are smooth surfaced spherical and highly porous in nature. Furthermore, no drug crystals were observed over the surface of formulation.

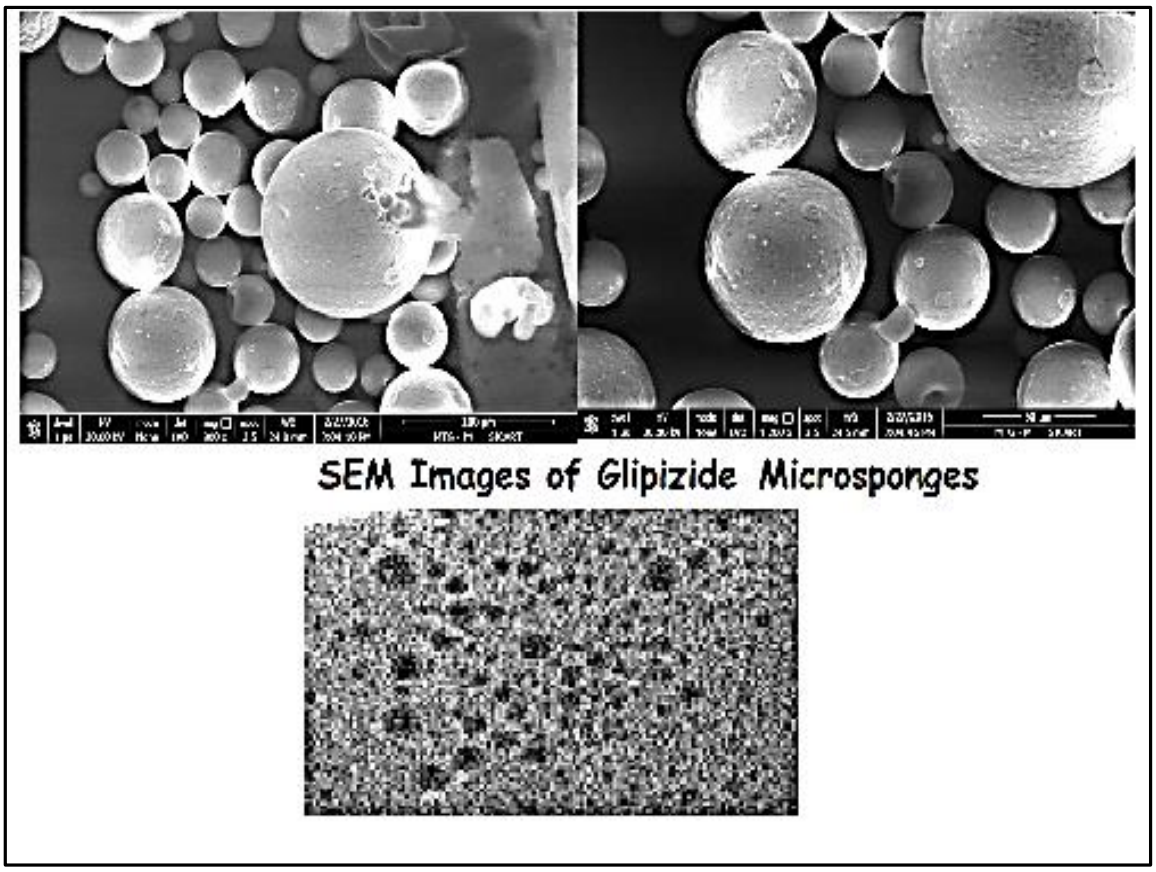


FIGURE 6B.7 SEM images of Glipizide microsponges (GBB-8)

6B.3.6.2 Differential Scanning Calorimetry (DSC)

DSC provides the information about the crystalline and amorphous form of drug and possible interaction during the polymerization and formulation of microsponges[25]. The thermogram of pure Glipizide exhibited a sharp endothermic peak at 209.62˚C corresponding to its melting point (208-209˚C), representing its crystalline nature. The thermogram of pure ethyl cellulose gives no sharp peak. The thermogram of physical mixture of polymer and drug and formulation GBB-8, gave the peaks at 212.37˚C and 212.36˚C, respectively, with decrease in the intensity of peak (Figure 6B.8). There was slight increase in the peak temperature in the both, which may be attributed to the presence of ethyl cellulose and the dispersion of drug in polymeric matrix.

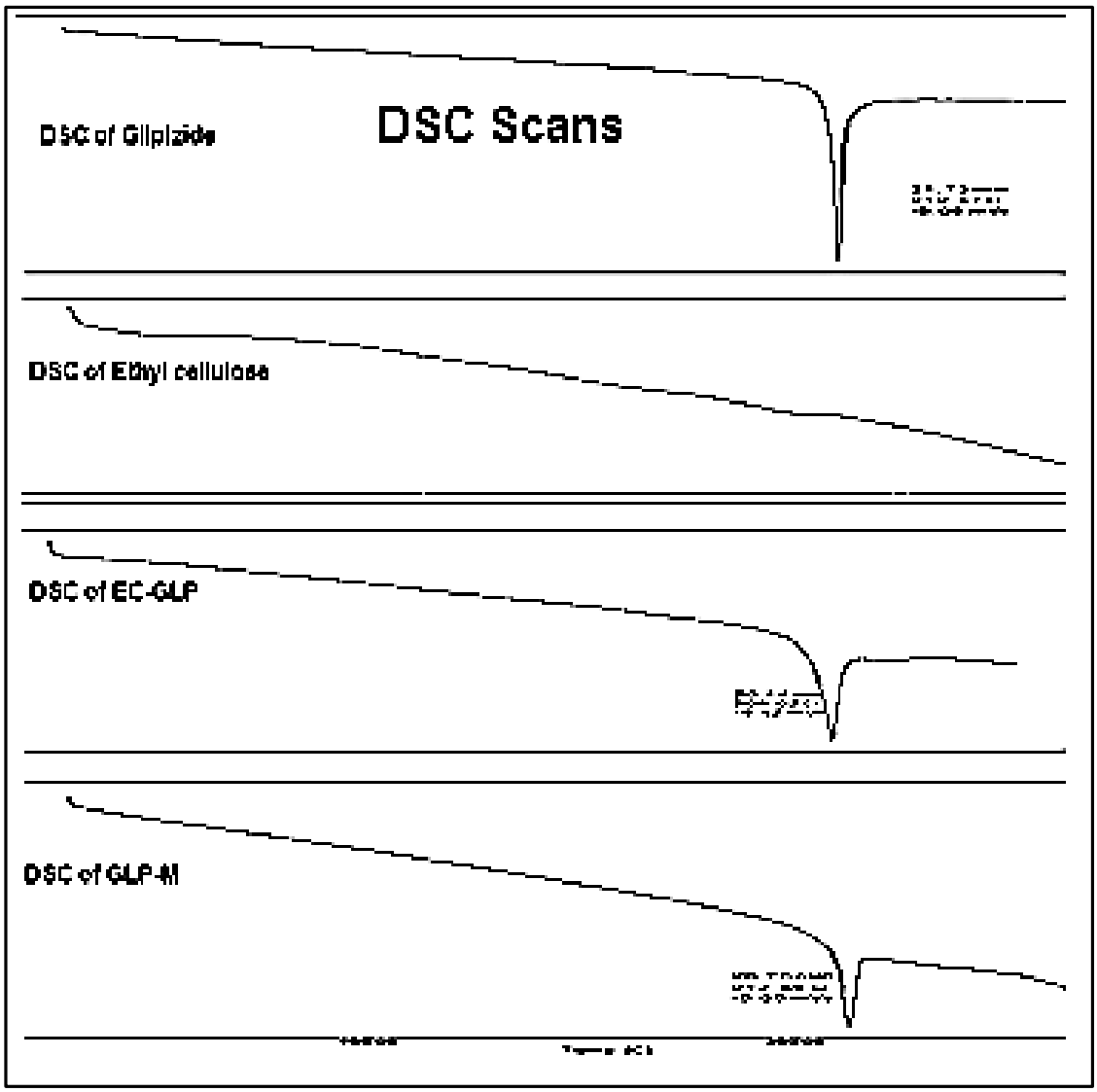


FIGURE 6B.8 DSC scan of Glipizide, Ethyl cellulose, Physical mixture of drug and polymer, GBB-8

6B.3.6.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR scan of Glipizide, ethyl cellulose, physical mixture of drug and polymer and optimized formulation of glipizide microsponges was taken (Figure 6B.9).

The peaks corresponding to the characteristics bands of the drug were found to be preserved in the spectra of the microsponges which indicates that no chemical interaction has been taken place during the preparation of the formulations. The presence of C ═ N aliphatic group, C ─ H_2 aliphatic, N ─ H stretching of NH_2, C ─ H aliphatic and C ═ O stretching was proved by the presence of peaks at 1649, 2943.22, 3325.47, 1527.57, 1688.84, respectively. The characteristic peaks confirmed the structure of glipizide.

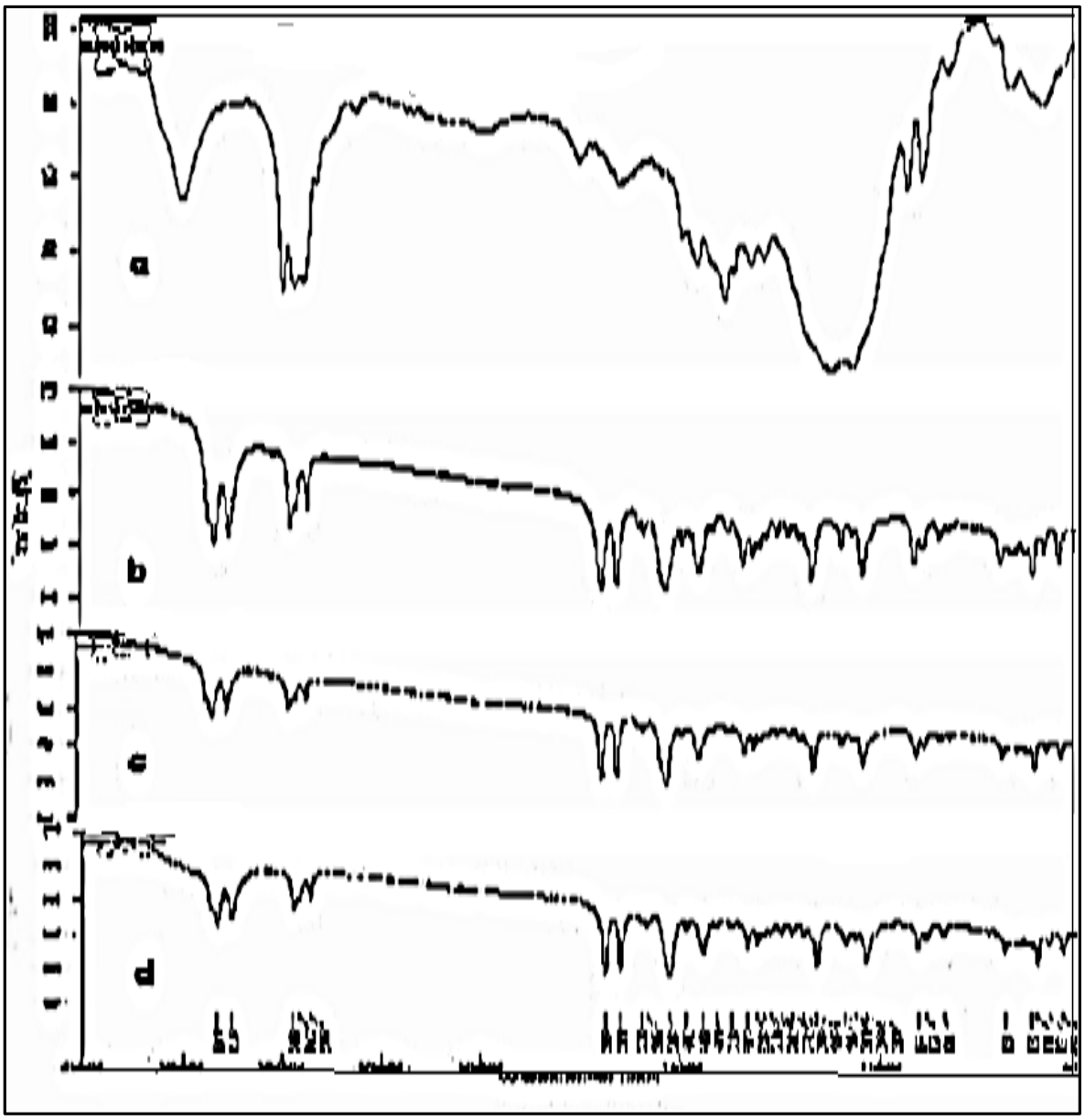


FIGURE 6B.9 FTIR scan of a) Ethyl cellulose b) pure glipizide c) Physical Mixture of Glipizide and ethyl d) GBB-8

Same peaks, with slight change in intensity, were found to be present in drug-polymer physical mixture and microsponge formulations. As there was no change and shifting of characteristic peaks of glipizide in GBB-8, it indicated no significant drug-polymer interaction. Hence, GLP is compatible in microsponge formulations.

6B.3.6.4 Powder X-ray Diffraction (XRD)

Powder X-ray diffraction (XRD) was performed for optimized microsponge formulation of Glipizide GBB-8 to investigate the effect of polymerization on crystallinity of the drug. The

XRD scan of glipizide microsponges was compared with the XRD scan of pure drug (Figure 6B.10). The distinct peaks can be observed in the XRD of pure glipizide which shows the crystalline behavior of the drug.

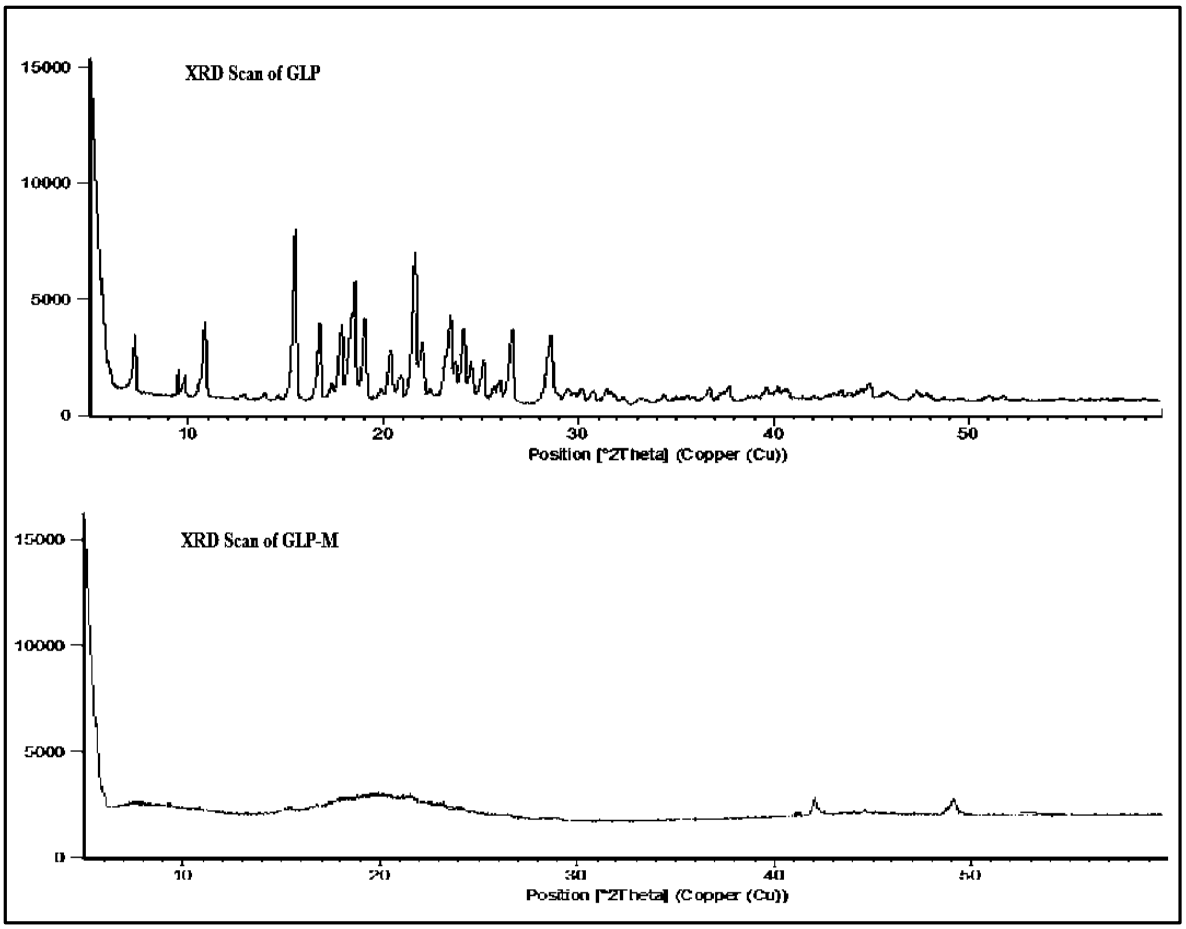


FIGURE 6B.10 XRD scan of Glipizide and GLP Microsponges

The disappearance of most of the characteristic peaks of the drug in the formulation indicates that most of the drug has been converted to the amorphous form and drug is dispersed at a molecular level in the polymeric matrix. The interpretation of the results achieved was matching with the XRD analysis obtained by Deshmukh, R[26].

6B.3.6.5 Residual Solvent Analysis

The organic solvents used for the formulation of GLP microsponges were ethanol and dichloromethane. Ethanol is considered as class 3 residual solvent which are regarded as less toxic as they have no human health hazard at levels normally accepted in pharmaceuticals prescribed by the ICH guidelines "Q3C" for the residual solvents[27,28]. Hence, it is not essential to find the residual amount of ethanol in the finished product. But, DCM belongs

to class 1 residual solvents and its amount in the finished formulation should be within limit (up to 600 ppm). The gas chromatogram of standard DCM solution in DMSO and GBB-8 dissolved in DMSO is shown in figure 6B.11. The retention time of dichloromethane was 1.181 min as observed in standard preparation of DCM. The peak of DMSO was observed at 13.4 minutes in both standard and sample preparation. But, no dichloromethane peak at 1.181 min was observed in sample chromatogram. Dichloromethane residue was within the limits, in the GLP loaded microsponges. Hence, the prepared formulation GBB-8 is considered to be safe for human use.

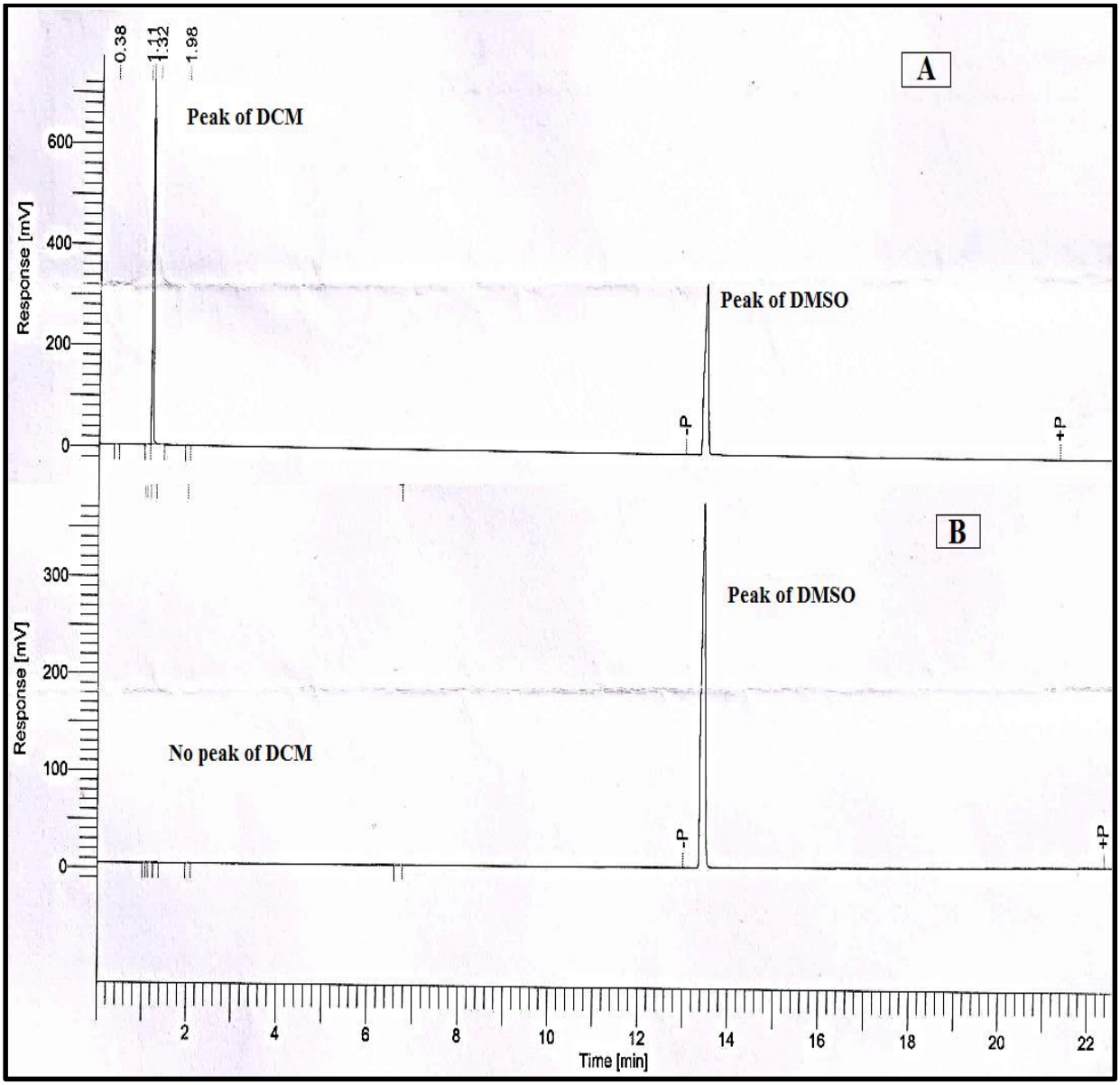


FIGURE 6B.11 GC scan for DCM residual solvent in GLP floating microsponges A) Scan of standard DCM solution; B) Scan of GBB-8 formulation, solution prepared in DMSO

6B.3.8 Stability Study of Optimized Formulation as per ICH Guidelines

Stability study conducted for the optimized glipizide microsponges indicated the physical and chemical stability of the formulation for the period six months under test condition. There was no change in the colour and shape of microsponges.

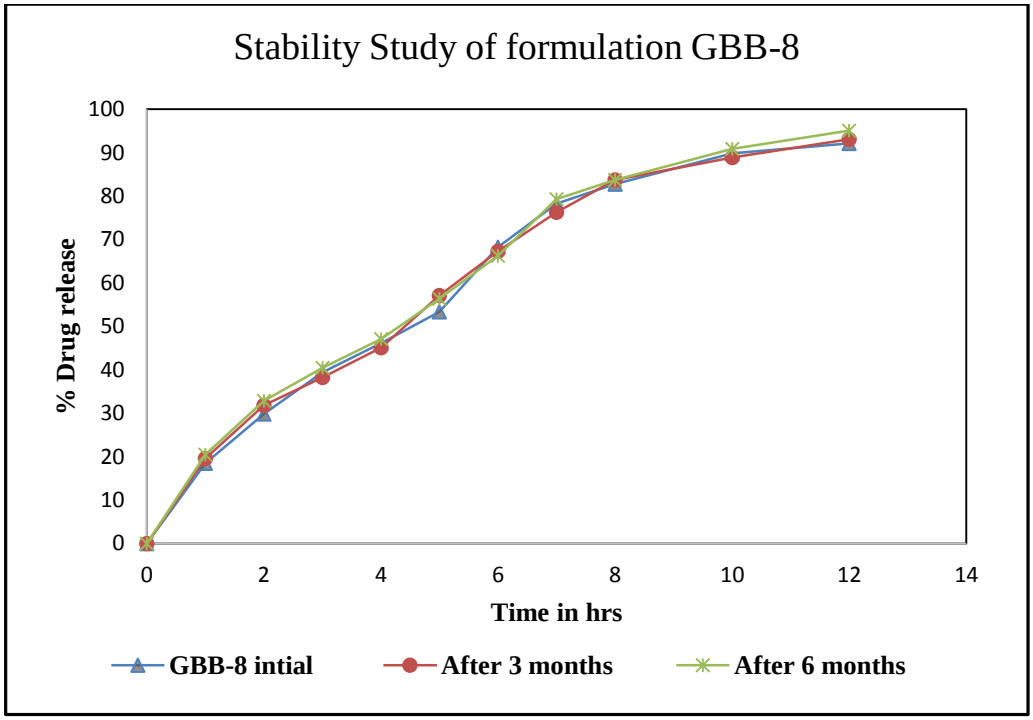


FIGURE 6B.12 Stability study of optimized floating microsponges of GLP (GBB-8)

There was no significant change observed in the drug content, drug content and drug release pattern of the drug on storage for six months. The dissolution study of the formulation was conducted after three and six months. The data indicated that there was over 90% similarity between the release data of drug during the storage period. The graphical representation of the same is given in Figure 6B.12.

6B.3.8 *In vivo* Studies

In vivo studies were executed to determine the residence of the GBB-8 in the stomach and also to determine its extended glucose lowering capacity as compared to pure GLP drug. The pharmacokinetics studies of the optimized formulation of GLP floating microsponges and pure glipizide were also undertaken to determine the extent of the drug in the body which can achieve better treatment of the disease.

6B.3.8.1 Radiographic Study

To determine the retention time of the optimized floating microsponges of GLP, in stomach, radiographic studies were conducted. The floating barium sulfate loaded microsponges were given to rabbits. Barium sulphate is a radio opaque material, which obstructs the passage of radiant energy, such as x-rays, the representative areas appearing light or white on the exposed film. This visibility provides the contrast needed to accurately locate or position the device inside the body during critical procedure. The X-ray photomicrographs were taken immediately at 0, 4 and 12 h and were recorded, as shown in Figure 6B.13.

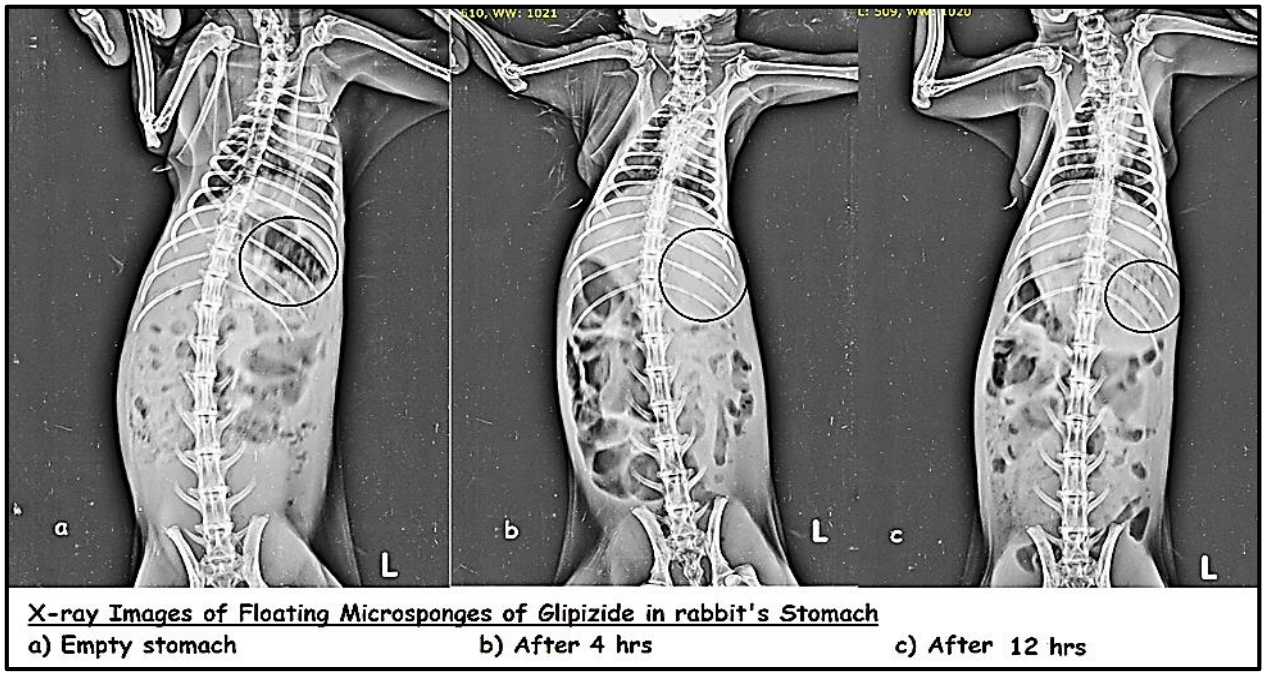


FIGURE 6B.13 X-ray images of floating Glipizide microsponges

The *in vivo* X-ray imaging study clearly indicated that before the administration of barium sulphate loaded microsponges, the stomach area didn't show any light area. However, it is evident from the picture that stomach area is appearing white on the exposure to X-rays indicating the presence of the formulation in the abdomen upto 12hours. Hence, it is believed that the developed gastroretentive microsponges of GLP will remain buoyant in the stomach of human being as well.

6B.3.8.2 Pharmacodynamic Study on Diabetic Rats

The pharmacodynamics study of optimized GLP microsponge (GBB-8) and pure GLP was performed on diabetic wistar rats. The six diabetic rats were divided into two groups with

equal number of rats (n=3) and the percent glucose reduction in blood was presented as mean ± S.E.M. The dose calculation was done based on surface area given by Dr. M.N. Ghosh in 1984, the dose given to rat was 1.8mg/kg of the body weight[29,30]. The pharmacodynamics studies showed the reduction in the blood glucose level after the single dose administration of pure GLP drug, but it could not maintain the blood glucose level for extended time. On the other hand, the formulation GBB-8 treated rats showed a significant decrease in blood glucose level over the period of 12 hrs, as shown in figure 6B.14.

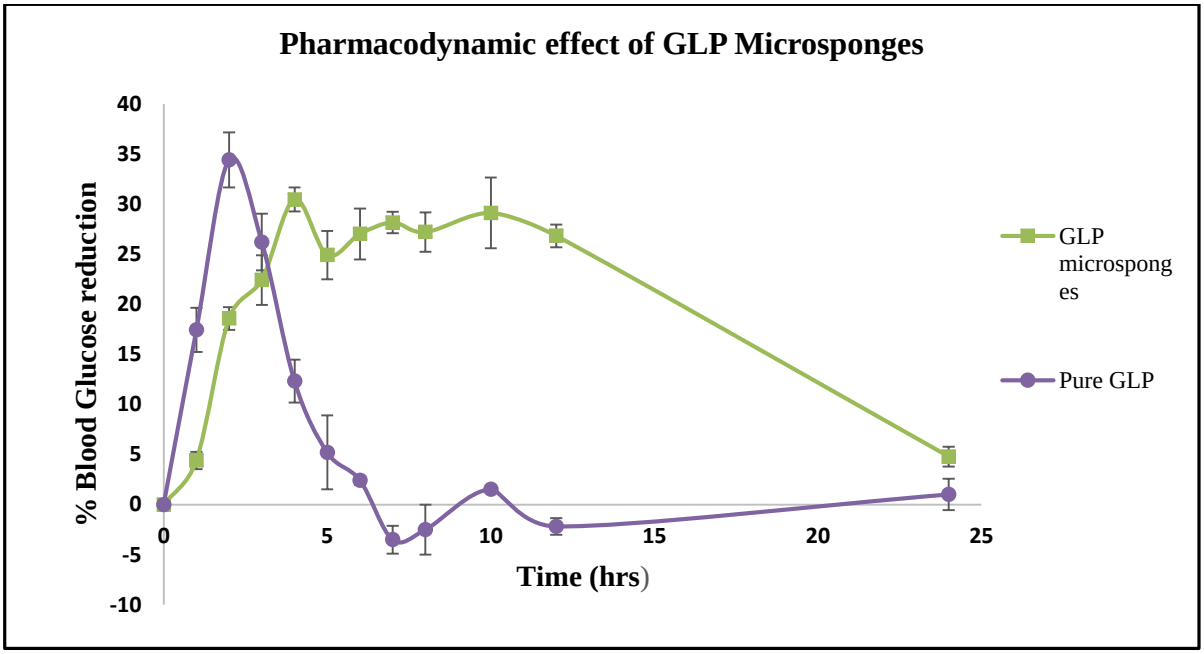


FIGURE 6B.14 Graphical representation of Pharmacodynamic study of Glipizide microsponges

This indicates the capability of floating GLP microsponges to release the drug in the body over prolonged period of time and proves the formulation to be sustained release. The single dose of pure drug could not control the blood sugar level for long period. Hence, the prepared glipizide microsponges are efficiently controlling the body glucose level as compared to pure GLP drug, which can treat type II diabetes in superior manner.

6B.3.8.3 Pharmacokinetic Study

The *in vivo* study of the optimized batch of floating gastroretentive microsponges of GLP (GBB-8) was conducted to obtain its pharmacokinetic data. The study was conducted on twelve healthy wistar rats by dividing them in two groups with equal number of rats (n=6)

and the data was expressed in terms of ± S.E.M. The blood samples collected from the rats were extracted, following the method given in chapter 3 (Preformulation studies). The analysis of the plasma samples were performed on HPLC, according to the bioanalytical method of GLP explained in chapter 3. The HPLC was carried out at room temperature and the stationary phase used was Agilent C 18 column (150 mm ×4.6 mm) with guard column. Acetonitrile:HPLC water(55:45) (pH adjusted to 3.5 with o-phosphoric acid) was used as mobile phase. Glibenclamide was used as internal standard and the flow rate was maintained as 1.2ml/min at detected wavelength 240 nm.

The graphical representation of mean plasma concentration vs time profile of, single dose of, GLP pure drug and its gastroretentive floating microsponges GBB-8 are shown in Figure 6B.13.

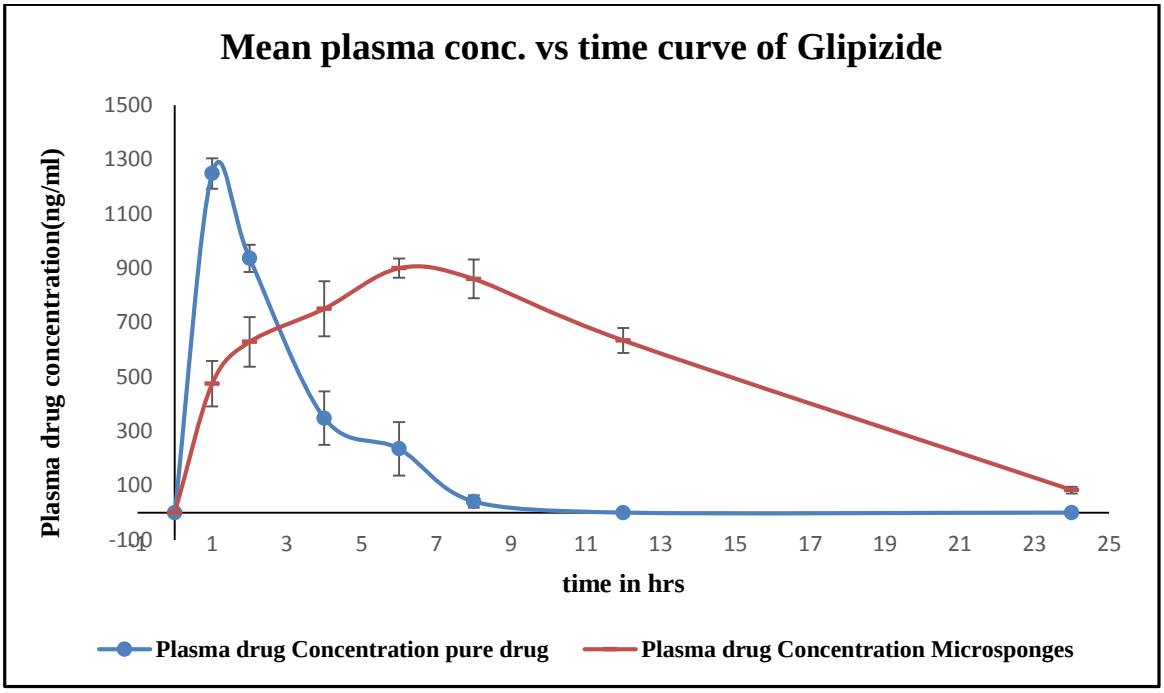


FIGURE 6B.13 Graphical representation of plasma time profile of single dose of GLP and GBB-8

The pharmacokinetic parameters such as C_{max}(µg/mL), t_{max} (hrs), $K_{el}(h^{-1})$, $t_{1/2}$(hrs), $AUC_{0-\infty}$ (µg*hr/mL) and MRT (hrs) of drug were determined from mean plasma concentration time profile, using Kinetica 5.0 software. The MRT of GBB-8 (9.14 ± 0.43hrs) was found to be increased significantly in comparison to pure GLP (2.93 ± 0.56), which indicated the increased residence time of the optimized GLP microsponges at the site of absorption. The t_{max} value of GBB-8 was found to be 6hrs, which was increased in comparison to pure drug, where t_{max} was achieved in an hour. Considerable difference was found in the elimination

half-life and elimination rate constant of GLP and GBB-8. Optimized microsponges (GBB-8) showed increased elimination half-life of the drug with diminished elimination rate, which indicates the prolonged residence of the drug in blood. A significant difference was observed in the $AUC_{0-\infty}$ of GLP and GBB-8 as, 3.79 ± 0.92 µg/ml.h and 11.09 ± 1.31 µg/ml.h, respectively, which indicates the 2.9 fold increase in the relative bioavailability of the drug in formulation GBB-8.

TABLE 6B.11 Pharmacokinetic parameters of pure GLP and optimized floating microsponges of GLP (GBB-8)

Sr. No.	Pharmacokinetic parameters	Pure drug GLP	Optimized batch GBB-8
1	Peak plasma concentration C_{max} (µg/ml)	1.25 ± 0.18	0.9 ± 0.04
2	Time to reach peak plasma concentration T_{max} (h)	1	6
3	Elimination half (h) life $t_{1/2}$	1.50 ± 0.25	4.59 ± 0.54
4	Elimination rate constant K_e (h^{-1})	0.460 ± 0.05	0.151 ± 0.02
5	Area under the curve $AUC_{0-\infty}$ (µg/ml.h)	3.79 ± 0.92	11.09 ± 1.31
6	MRT (h)	2.93 ± 0.56	9.14 ± 0.43

The conclusion of the pharmacokinetic study conducted on healthy rats is that, the developed gastroretentive floating microsponges of GLP could retains in the blood for 12 hrs, which supports the pharmacodynamics study, performed on diabetic rats. The developed formulation is successful in staying at the site of absorption for prolonged time (as proved by radiographic studies), thereby maintaining the drug concentration in blood, by getting absorbed slowly, and giving better pharmacodynamic effect as compared to pure drug.

6B.4 Conclusion

Gastroretentive floating microsponges of Glipizide were prepared successfully by quasi-emulsion solvent diffusion method, using two organic and one aqueous solvent system. The solvents were chosen based on the miscibility of the drug in individual solvents. Due to high solubility of GLP in ethanol, it was selected as the drug solubilizing solvent and dichloromethane was chosen as bridging liquid. GLP has poor solubility in water hence it was selected as non-solvent.

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. Pareto charts revealed that concentration of polymer i.e. ethyl cellulose, stirring speed and temperature were the most critical factors in the formulation of microsponges. Hence, these factors were further used for the final optimization of glipizide floating microsponges by applying Box-Behnken design. The dependent responses % entrapment efficiency, % buoyancy and % CDR12h were evaluated and results were statistically analyzed by Design expert software. The desirability function and overlay plot indicated GBB-8 (with X_1 at 0-level and X_2, X_3 at 1-level), as optimized formulation. The physicochemical characterization of optimized formulation showed no interaction between the drug and polymer and the complete dispersion of the drug in polymeric matrix and also the porous, spherical nature of the formulation. The radiographic studies performed on the albino rats proved the gastroretention of formulation in test animal for 12hrs.

In vivo studies of GBB-8 were conducted in comparison with pure GLP and the results of the pharmacokinetic study showed that the developed gastroretentive floating microsponges of GLP could retains in the blood for 12 hours in healthy rats. This supports the pharmacodynamics study, performed on diabetic rats, where the GBB-8 could reduce the blood glucose level for the period of 12 hrs. Hence, it was concluded that the developed formulation is successful in staying at the site of absorption for prolonged time (as proved by radiographic studies), thereby maintaining the drug concentration in blood, by getting absorbed slowly, and giving better pharmacodynamic effect as compared to pure drug.

6B.5 References

1. Arya, P. and Pathak, K., 2014. Assessing the viability of microsponges as gastro retentive drug delivery system of curcumin: Optimization and pharmacokinetics. *International Journal of Pharmaceutics*, *460*(1), pp.1-12.

2. Pandya, N., Pandya, M. and Bhaskar, V.H., 2011. Preparation and *in vitro* characterization of porous carrier-based glipizide floating microspheres for gastric delivery. *Journal of Young Pharmacists*, *3*(2), pp.97-104.

3. Plackett, R.L. and Burman, J.P., 1946. The design of optimum multifactorial experiments. *Biometrika*, *33*(4), pp.305-325.

4. Bolzinger, M.A., Bordes, C., Gauvrit, J.Y. and Briançon, S., 2007. Improvement of a bovine serum albumin microencapsulation process by screening design. *International Journal of Pharmaceutics*, *344*(1), pp.16-25.

5. El-Malah, Y. and Nazzal, S., 2006. Hydrophilic matrices: Application of Placket–Burman screening design to model the effect of POLYOX–carbopol blends on drug release. *International Journal of Pharmaceutics*, *309*(1), pp.163-170.

6. Box, G.E. and Behnken, D.W., 1960. Some new three level designs for the study of quantitative variables. *Technometrics*, *2*(4), pp.455-475.

7. Solanki, A.B., Parikh, J.R. and Parikh, R.H., 2007. Formulation and optimization of piroxicam proniosomes by 3-factor, 3-level Box-Behnken design. *AAPS PharmSciTech*, *8*(4), pp.43-49.

8. Çomoğlu, T., Gönül, N. and Baykara, T., 2003. Preparation and *in vitro* evaluation of modified release ketoprofen microsponges. *Il Farmaco*, *58*(2), pp.101-106.

9. Vyas, S., Trivedi, P. and Chaturvedi, S.C., 2009. Dextran-etodolac conjugates: synthesis, *in vitro* and *in vivo* evaluation. *Acta Poloneia Pharmacia.-Drug Research*, *66*(2), pp.201-206.

10. Kawashima, Y., Iwamoto, T., Niwa, T., Takeuchi, H. and Hino, T., 1993. Role of the solvent-diffusion-rate modifier in a new emulsion solvent diffusion method for

preparation of ketoprofen microspheres. *Journal of Microencapsulation*, *10*(3), pp.329-340.

11. Re, M.I. and Biscans, B., 1999. Preparation of microspheres of ketoprofen with acrylic polymers by a quasi-emulsion solvent diffusion method. *Powder Technology*, *101*(2), pp.120-133.

12. Crcarevska, M.S., Dimitrovska, A., Sibinovska, N., Mladenovska, K., Raicki, R.S. and Dodov, M.G., 2015. Implementation of quality by design principles in the development of microsponges as drug delivery carriers: Identification and optimization of critical factors using multivariate statistical analyses and design of experiments studies. *International Journal of Pharmaceutics*, *489*(1), pp.58-72.

13. Sastry, S.V. and Khan, M.A., 1998. Aqueous based polymeric dispersion: Plackett–Burman design for screening of formulation variables of atenolol gastrointestinal therapeutic system. *Pharmaceutica Acta Helvetiae*, *73*(2), pp.105-112.

14. Yerlikaya, F., Ozgen, A., Vural, I., Guven, O., Karaagaoglu, E., Khan, M.A. and Capan, Y., 2013. Development and Evaluation of Paclitaxel Nanoparticles Using a Quality-by-Design Approach. *Journal of Pharmaceutical Sciences*, *102*(10), pp.3748-3761.

15. Sharma, O.P., Shah, M.V., Parikh, D.C. and Mehta, T.A., 2015. Formulation optimization of gastroretentive drug delivery system for allopurinol using experimental design. *Expert Opinion on Drug Delivery*, *12*(4), pp.513-524.

16. Srinivasan, K., Viswanad, B., Asrat, L., Kaul, C.L. and Ramarao, P., 2005. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacological Research*, *52*(4), pp.313-320.

17. Pandit, V., Pai, R.S., Yadav, V., Devi, K., Surekha, B.B., Inamdar, M.N. and Suresh, S., 2013. Pharmacokinetic and pharmacodynamic evaluation of floating microspheres of metformin hydrochloride. *Drug Development and Industrial Pharmacy*, *39*(1), pp.117-127.

18. Lushan, Y. and Su, Z., 2006. Determination of mitiglinide in rat plasma by high-performance liquid chromatography with UV detection. *Journal of Chromatography B*, *834*(1), pp.204-207.

19. Fahmy, R., Kona, R., Dandu, R., Xie, W., Claycamp, G. and Hoag, S.W., 2012. Quality by design I: application of failure mode effect analysis (FMEA) and Plackett–Burman design of experiments in the identification of "main factors" in the formulation and process design space for roller-compacted ciprofloxacin hydrochloride immediate-release tablets. *AAPS PharmSciTech*, *13*(4), pp.1243-1254.

20. Lee, J.H., Park, T.G. and Choi, H.K., 1999. Development of oral drug delivery system using floating microspheres. *Journal of Microencapsulation*, *16*(6), pp.715-729.

21. Nacht, S. and Katz, M., 1990. The microsponge: a novel topical programmable delivery system. *Drugs and the Pharmaceutical Sciences*, *42*, pp.299-325.

22. Nokhodchi, A., Jelvehgari, M., Siahi, M.R. and Mozafari, M.R., 2007. Factors affecting the morphology of benzoyl peroxide microsponges. *Micron*, *38*(8), pp.834-840.

23. Chinna, G., Shyam, S., Vimal, M., Sleeva, R.M. and Sai, K., 2010. Formulation and Evaluation of Indomethacin Microspheres using natural and synthetic polymers as Controlled Release Dosage Forms. *International Journal of Drug Discovery*, *2*(1), pp.8-16.

24. Osmani, R.A., Aloorkar, N.H., Kulkarni, A.S., Harkare, B.R. and Bhosale, R.R., 2014. A new cornucopia in topical drug delivery: microsponge technology. *Asian Journal of Pharmaceutical Science and Technology*, *4*, pp.48-60.

25. Ford, J.L. and Timmins, P., 1989. *Pharmaceutical Thermal Analysis: Techniques and Applications*. Halsted Press.

26. Deshmukh, R.K. and Naik, J.B., 2014. Aceclofenac microspheres: quality by design approach. *Materials Science and Engineering: C*, *36*, pp.320-328.

27. Guideline, I.H.T., 2005. Impurities: Guideline for residual solvents Q3C (R5). *Current Step*, *4*, p.509.

28. Dwivedi, A.M., 2002. Residual solvent analysis in pharmaceuticals. *Pharmaceutical Technology*, *26*(11), pp.42-46.

29. Ghosh MN. Toxicity studies. Fundamental of experiment & Pharmacology. 2nd ed. Calcutta: Scientific Book Agency.

30. Pamidiboina, V., Razdan, R. and Hariprasad, M., 2010. Evaluation of the antihyperlipidemic, cardioprotective activity of a polyherbal formulation. *International Journal of Pharmacy and Pharmaceutical Science*, *10*, pp.86-91.

CHAPTER 7

Conclusion

Diabetics Mellitus type II, a metabolic disorder, is increasing across the globe at alarming rate. The treatment includes the intake of antidiabetic drugs regularly, to maintain the blood glucose level. Antidiabetic drugs which are primarily absorbed from upper part of gastrointestinal tract, fail to achieve this target, due to short residence time at the site of absorption. Gastroretentive drug delivery system is an efficient approach to retain the dosage form in the stomach, thereby achieving the better therapeutic efficacy of the drug. The current research was aimed to formulate, evaluate and optimize gastroretentive formulations for antidiabetic drugs. The antidiabetic drugs chosen for the present research work were metformin hydrochloride (MH), mitiglinide calcium dihydrate (MTG) and glipizide (GLP).

Prior to the development of the gastroretentive floating matrix tablet and floating microsponges of selected drugs, the preformulation studies were carried out. Identification of the drugs was done by doing their physical evaluation and also by performing the Fourier transform infrared spectroscopy (FTIR) study. The results verified the purity of procured drugs. The UV spectroscopy method was used for the analysis of the drugs and the λmax value of MH and GLP, in 0.1N HCl was found to be 230nm and 275nm respectively. The λmax value of MTG and GLP in methanol was found as 259nm and 274.4nm. These values were further used for the preparation of calibration curve of drug in 0.1N HCl and methanol. The UV analysis of MTG revealed that the absorptivity of the drug is very low, hence, high performance liquid chromatography (HPLC) method was developed for the analysis of drug during dissolution studies. 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. The equation, $y = 90.168x + 172.95$ was used to calculate the concentration of the drug in dissolution fluid.

The bioanalytical method for MTG and GLP was developed for pharmacokinetic estimation of drug from rat plasma. The mobile phase used for MTG was Acetonitrile:HPLC water (60:40), pH adjusted to 3.5 with o-phosphoric acid. The GLP was used as internal standard for the bioanalytical method development for MTG. The retention time of MTG and GLP was found as 5.67min and 3.70min, respectively at detection wavelength of 210 nm. The concentration range in calibration curve was 200-20000 ng/mL and the slope was found to be 0.00004 with intercept as 0.0916 ± 0.001. Correlation coefficient was found to be 0.9809 which is above 0.98 limit for bioanalytical method.

The bioanalytical method for glipizide was developed by using mobile phase, Acetonitrile:HPLC water (55:45) with pH adjusted to 3.5 using o-phosphoric acid. Glibenclamide (GLB) was used as internal standard with detected wavelength was 240 nm. The retention time GLP and GLB was found to be 3.64 min and 7.18 min, respectively. The calibration curve for plasma was constructed using six point calibration standards within the concentration range of 100-3200 ng/mL. The slope for the linear fitted graph was found to be 0.0106 and intercept was 2.87 with correlation coefficient of 0.991. These bioanalytical methods developed for MTG and GLP, were used for the estimation of respective drugs in the rat plasma and the data was used for generating pharmacokinetic data of the drugs.

After preformulation studies, the gastroretentive formulations of the selected antidiabetic drugs were developed. The first formulation, floating matrix tablets, was prepared for metformin 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 like, sodium alginate, pullulan, kappa carrageenan, xanthan gum, poloxamer 188. Prepared formulations were evaluated for swelling, floating ability, *in vitro* adhesive and drug release study. All the tablets showed acceptable physicochemical properties but, the formulation F2 (prepared with HPMC K15M and kappa carrageenan) showed excellent floating properties (floating lag time as 10.71 seconds) with extended adhesion period of 93.50 ± 3.36 minutes and sustained drug release for 8hours. The formulation had similarity factor as 92% on comparison with the theoretical release of the drug.

The basic mechanism of the gastroretention for the formulation is floatation but in case of low level of fluid in the stomach, the mechanisms like mucoadhesion and swelling can retain the formulation at the required site, which can be better achieved by formulation with kappa-

carrageenan. It has been already reported that the gastrorentive formulations prepared by using the carrageenans can modify the properties of polymeric matrices, to obtain tailor-made materials for drug delivery systems. Hence, the final optimization of floating matrix tablet of MH was done by applying a statistical mixture design, using HPMC K15M (X_1), kappa carrageenan (X_2) and sodium bicarbonate (X_3) as independent variables.

A simplex centeroid design was applied to inspect the combined effect of the three variables in the formulations.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. Results revealed that there was strongest synergistic and antagonistic effect shown by a ternary interaction of $X_1X_2X_3$ at higher level of kappa carrageenan (X_2) on amount of drug released in 1hr and t_{90}, respectively. The overlay plot displayed that (-1) coded value of gas generating agent, coded value of 0 to 1, of HPMC K15 M and 0 (coded value) of kappa carrageenan is fulfilling the desirability criteria and the formulations prepared in this region would give the desired gastroretention and sustained release of MH.

Formulation M-SCD 7 with the quantities as X_1 175mg, X_2 75mg, X_3 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 of 97.52 ± 5.42 minutes and good swelling index as 3.16, which ensures the retention of formulation in the stomach. Hence, it was concluded that the mixture of kappa carrageenan and HPMC K 15 M could give the desired release pattern of the drug by changing the polymer concentration. 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. The *in vivo* study of the formulation was not conducted because of the higher dimensions of dosage form. Moreover, due to very high dose of metformin, its microsponges were not prepared as the formulation will become bulky for oral route.

Another antidiabetic drug, for which the gastroretentive formulations were developed, was MTG. The floating matrix tablets of MTG were prepared by direct compression technique. The preliminary batches were prepared using HPMC K15M, as release retarding polymer along with other ionic and anionic polymeric substances. The results of preliminary batches revealed, that the tablets prepared using HPMC K15M and kappa-carrageenan were not able

to sustain the release of the drug. The finding were antagonistic to the results obtained for MH with the same polymer combination. The reason may be attributed to the presence of calcium ions in the drug, as the literature suggests that kappa carrageenan forms weak gels in the presence of calcium ions. The tablets prepared using HPMC K15M and sodium alginate, as release retarding polymers showed sustained release of the drug for the period of 12hrs. It also gave acceptable floatation behavior (with floating lag time of 30.43±2.87 seconds and floatation time as 12hrs), as compared to other polymers. Hence, 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. 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 and drug release study. Results showed that M-3 formulation containing maximum amount of both variables (X1; 60mg and X2; 30mg) released the MTG for the period of 12hrs and was existing in the yellow region (desired region) of overlay plot. Hence, it was considered as optimized gastroretentive floating matrix tablet of MTG. The radiographic study of the barium sulfate loaded tablets of this optimized formula, confirmed the gastroretention of formulation in the stomach of rabbit for 12hrs.

A Gastroretentive multiparticulate system of MTG was developed as floating microsponges by quasi-emulsion solvent diffusion method. The drug was dissolved in organic phase consisting of ethanol and dichloromethane, along with polymer (ethyl cellulose). Triethyl citrate (TEC) was also added to this internal phase in order to facilitate the plasticity. The external aqueous phase contained 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 of microsponges were prepared for the screening of formulation and process related factors, affecting the development of dosage form. The results provided the optimized values of stirring speed (1000rpm), temperature during formulation (40ºC) and amount of TEC (20% of polymer concentration), for preparing spherical and porous microsponges. The solubility studies of drug in presence of excipients were conducted and the results suggested that concentration of ethyl cellulose and PVA changes the solubility of

drug. Hence, the concentrations of PVA and ethyl cellulose were considered to be the most important factors, affecting the formulation of microsponges. The optimization of dosage form was done by applying 3^2 full factorial design by taking concentrations of PVA (X_1) and ethyl cellulose (X_2) as independent factors and Product yield (Y_1), % entrapment efficiency (Y_2), % buoyancy (Y_3) and % cumulative drug release (Y_4) of microsponges as dependent responses. Using design expert software, optimized batch of MTG microsponges, (F-0) was 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. Gas chromatographic studies were performed to check the limit of residual solvent, dichloromethane (DCM) in F-0. The results indicated that DCM was within the limits (less than 600ppm), in the MTG loaded microsponges. Hence, the prepared formulation F-0 is considered to be safe for human use.

The *in-vivo* radiographic study of optimized floating microsponges of MTG 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). The optimized microsponges, incorporated with barium sulphate was put in capsule and 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 remained afloat in gastric fluid up to 12 h in the stomach of rabbit. Hence, it was concluded that the optimized MTG microsponges would probably show the similar gastroretention in the stomach of human beings.

The *in vivo* pharmacodynamic and pharmacokinetic studies of F-0 were conducted on diabetic rat and healthy wistar rat, respectively. The pharmacodynamics study was conducted on two groups of diabetic rats (n=3), one group was given MTG (pure drug) and other was given formulation F-0 (optimized microsponges of MTG). 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). The study demonstrated that F-0 could significantly reduce the blood glucose level upto 50% for the period of 12hrs, which could not be achieved by pure drug.

For conducting pharmacokinetic studies, healthy wistar rats were divided into two groups (n=6). After overnight fasting one group of animals was given pure MTG and other group was given F-0 microsponges. The 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 -20ºC till further analysis. 200µl of plasma containing drug was taken in which an optimized quantity of internal standard (GLP) was added. Then, 25µl 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 4ºC. Supernatant through 0.45µm filter paper and then dried. The residue was reconstituted with 100µl of mobile phase 33. 20µl of this sample in injected in HPLC for analyzing the data.

The results were computed by Kinetics 5.0 software. The MRT of F-0 (8.847 ± 1.62hrs) was found to be increased significantly in comparison to pure MTG (4.29 ± 1.03hrs), which indicated the increased residence time of the optimized MTG microsponges at the site of absorption. The t_{max} value of F-0 was found to be 6hrs, which was increased in comparison to pure drug, where t_{max} was achieved in 0.5 hour. Considerable difference was found in the elimination half-life and elimination rate constant of MTG and F-0. Optimized microsponges (F-0) showed increased elimination half-life of the drug with diminished elimination rate, which indicated the prolonged residence of the drug in blood. Also a significant increase in $AUC_{0-\infty}$ of F-0 was observed (1.7 fold), in comparison to pure MTG drug. This indicates the presence of optimized formulation of floating microsponges of MTG in the blood plasma for about 12hrs.

This indicates the capability of floating MTG microsponges to release the drug in the body over prolonged period of time and proves the formulation to be sustained release. The single dose of pure drug could not control the blood sugar level for long period of time as indicated by pharmacodynamics studies. Hence, the prepared mitiglinide microsponges are efficient in controlling the body glucose level as compared to pure MTG drug.

Another antidiabetic drug used in present research work was glipizide (GLP). Floating matrix tablet of glipizide was prepared using the same approach, as previously applied for MH and MTG. The tablets punched by direct compression technique could not sustain the release of the drug from the formulation, hence, wet granulation method, using PVP K30, was applied for the preparation of floating matrix tablets of GLP. The final optimization of floating glipizide tablets was done by applying Simplex lattice design (SLD) using HPMC K15M (X_1), kappa carrageenan(X_2) and sodium bicarbonate(X_3) as independent variable. The similarity factor ($f2$), time to release 50% (t_{50}) of drugs and time to release 90% (t_{90}) of the 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 and HPMC K15M with maximum amount of kappa carrageenan were required to give the desired results. The optimum values of selected variables was found to be 50 mg of X_1, 30 mg of X_2 and 10 mg of X_3, and this formulation showed highest desirability. These finding were contradictory to the results attained for metformin gastroretentive floating tablet, prepared with the same combination of independent variable. The minimum amount of k-carrageenan was required for the formulation of MH tablet with highest desirability, which was antagonistic to present findings. The optimized formula of GLP matrix floating tablet showed the gastroretention for the period of 12 hrs in a radiological studiy conducted on albino rabbits.

In another approach, Gastroretentive floating microsponges of Glipizide were prepared by quasi-emulsion solvent diffusion method, using two organic and one aqueous solvent system. The solvents were chosen based on the miscibility of the drug in individual solvents. Due to high solubility of GLP in ethanol, it was selected as the drug solubilizing solvent and dichloromethane was chosen as bridging liquid. GLP has poor solubility in water hence it was selected as non-solvent. 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. Pareto charts revealed that concentration of polymer i.e. ethyl cellulose, stirring speed and temperature were the most critical factors in the formulation of microsponges. Hence, these factors were used for the final optimization

of glipizide floating microsponges by applying Box-Behnken design. The dependent responses % entrapment efficiency, % buoyancy and % CDR12h were evaluated and results were statistically analyzed by Design expert software. The desirability function and overlay plot indicated GBB-8 (with X_1 at 0-level and X_2, X_3 at 1-level), as optimized formulation. The physicochemical characterization of optimized formulation showed no interaction between the drug and polymer and the complete dispersion of the drug in polymeric matrix and also the porous, spherical nature of the formulation. There were no traces of residual solvent (DCM), found in GBB-8 formulation of GLP as shown by scans of gas chromatography study.

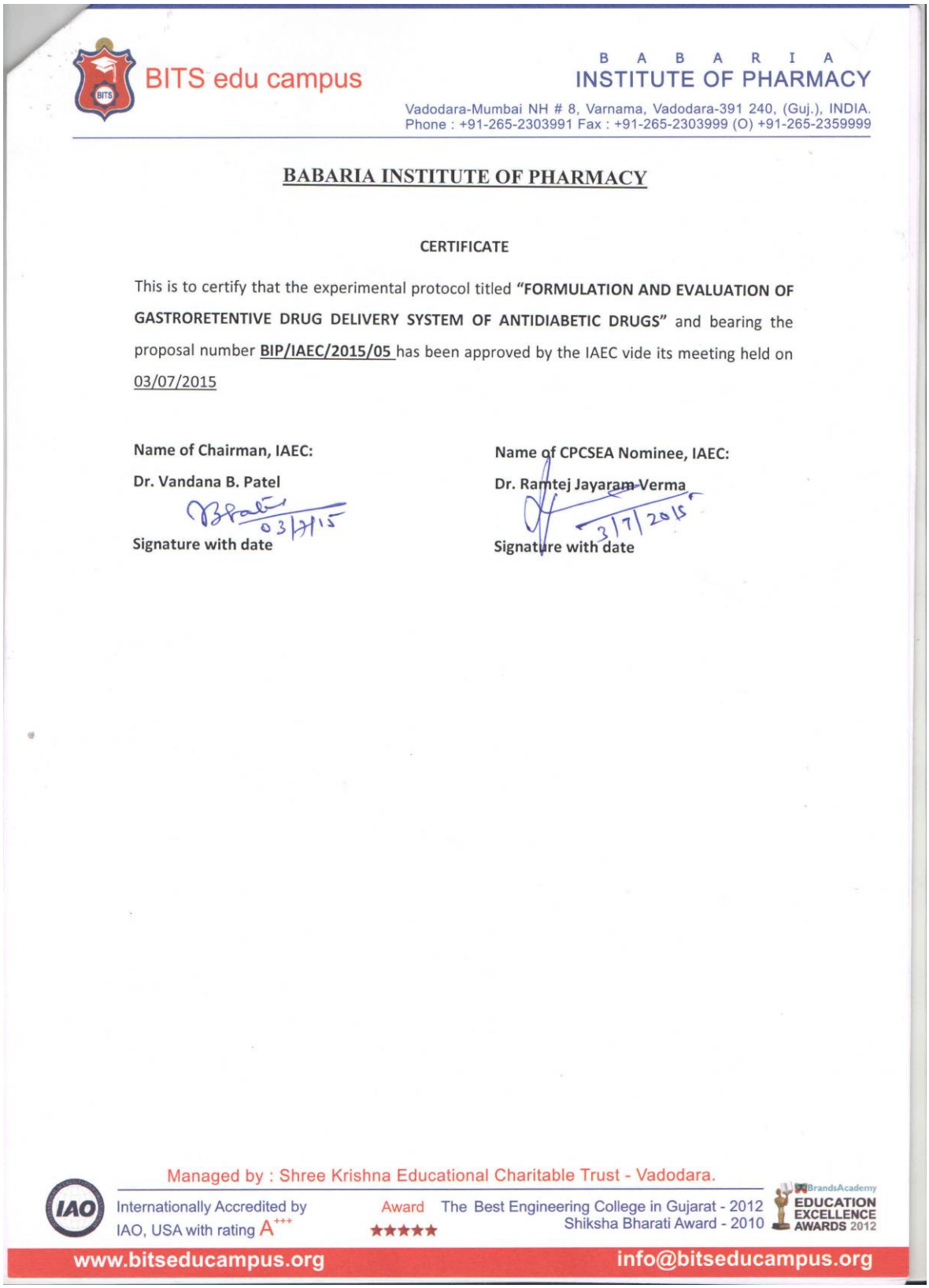
The radiological study, performed on albino rabbits, proved the presence of developed floating microsponges in the rabbit's stomach for the period of 12hrs. The *in vivo* pharmacodynamics and pharmacokinetic studies were conducted in the similar way as performed for MTG. *In vivo* studies of GBB-8 were conducted in comparison with pure GLP and the results of the pharmacokinetic study showed, that the developed gastroretentive floating microsponges of GLP was detected in the body of healthy rats for 12 hours. The MRT of GBB-8 (9.14 ± 0.43hrs) was found to be increased significantly in comparison to pure GLP (2.93 ± 0.56), which indicated the increased residence time of the optimized GLP microsponges at the site of absorption. The t_{max} value of GBB-8 was found to be 6hrs, which was increased in comparison to pure drug, where t_{max} was achieved in an hour. Considerable difference was found in the elimination half-life and elimination rate constant of GLP and GBB-8. Optimized microsponges (GBB-8) showed increased elimination half-life of the drug with diminished elimination rate, which indicates the prolonged residence of the drug in blood. A considerable increase of 2.9 fold, in $AUC_{0-\infty}$ of GBB-8 was observed, in comparison to pure GLP drug. This supports the pharmacodynamics study, performed on diabetic rats, where the GBB-8 could reduce the blood glucose level for the period of 12 hrs. Hence, it was concluded that the developed formulation is successful in staying at the site of absorption for prolonged time (as proved by radiographic studies), thereby maintaining the drug concentration in blood, by getting absorbed slowly, and giving better pharmacodynamic and pharmacokinetic effect as compared to pure drug.

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.

The studies conducted in the present research work gave promising results. The antidiabetic drugs which needs to be in the stomach for better absorption can be prepared as floating microspheres and floating tablets. It can be concluded that the drug delivery of such drugs can be improved, which results in increase in bioavailability of the drug. Also, the duration of action of drug can be extended, resulting in possible reduction in dose, less side effects, low overall cost of therapy and hence, better patient compliance. Such formulations can be developed at the industrial level to give the maximum benefit to the patient and also offer an efficient treatment of type II diabetes mellitus.

Appendix A

Approval from CPCSEA & IAEC for pharmacokinetic studies on animals



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CERTIFICATE

This is to certify that the experimental protocol titled **"FORMULATION AND EVALUATION OF GASTRORETENTIVE DRUG DELIVERY SYSTEM OF ANTIDIABETIC DRUGS"** and bearing the proposal number **BIP/IAEC/2015/05** has been approved by the IAEC vide its meeting held on 03/07/2015

Name of Chairman, IAEC:

Dr. Vandana B. Patel

03/7/15

Signature with date

Name of CPCSEA Nominee, IAEC:

Dr. Ramtej Jayaram Verma

3/7/2015

Signature with date

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Appendix B

Dose Calculation

Calculation of the Theoretical Release Profile of drug

The total dose of drug for a once-daily SR formulation was calculated by the following equation (Rawlins, 1977) using available pharmacokinetic data (Defang et al., 2005):

$D_t = \text{Dose}\ (1 + 0.693 \times t/t_{1/2})$

Where, Dt = total dose of drug;
Dose = dose of the IR part;
t = time (hr) during which the SR is desired;
and t1/2 = half-life of the drug.

For dose calculation of MH, t was considered as 8hrs and $t_{1/2}$ is 3hrs. So, the dose was calculated as follows:

$D_t = 175.6\ (1 + [0.693 \times 8]/3) \cong 500$

Hence, the formulation should release 175.6 mg in 1 hour like conventional tablets and 46.3 mg per hour up to 8 hours thereafter.

Reference:

Defang, O., Shufang, N., Wei, L., Hong, G., Hui, L. and Weisan, P., 2005. In vitro and in vivo evaluation of two extended release preparations of combination metformin and glipizide. Drug development and industrial pharmacy, 31(7), pp.677-685.

Rawlins, E. A. (1977). Bentley's text book of pharmaceutics. London, England: Cassell and Collier Macmillan.

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 published in, *Journal of Applied Pharmaceutics.* 2017; 7(04):23-30.

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. **Poster presented on**, "Microsponges of Glipizide: Formulation and Characterization", at *Controlled Release Society Annual Meeting & Exposition 2017* in Boston.