

**Preparation and evaluation of anti-diabetic sustained release
tablets using grafted copolymer of fenugreek gum**

A

Thesis Submitted for the Award of Degree of

**DOCTOR OF PHILOSOPHY
in
(Pharmaceutics)**

By

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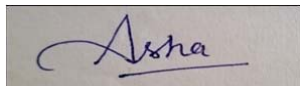


**LOVELY PROFESSIONAL UNIVERSITY
PUNJAB
2023**

DECLARATION

I, hereby declared that the presented work in the thesis entitled “**Preparation and evaluation of anti-diabetic sustained release tablets using grafted copolymer of fenugreek gum**” in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision **Dr. Narendra Kumar pandey** working as Professor, in the (Pharmaceutical Sciences (Pharmaceutics)) of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



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CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled “ **Preparation and evaluation of anti-diabetic sustained release tablets using grafted copolymer of fenugreek gum**” submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the (Pharmaceutical Sciences (Pharmaceutics), is a research work carried out by Asha Gandhi, (Registration No.)_41700191 is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



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ABSTRACT

Objective: Preparation and evaluation of SR tablet using grafted copolymers of fenugreek gum. The drug release behavior of grafted metformin HCl tablet comparatively evaluated with ungrafted fenugreek gum tablet & also compared with marketed SR formulation of metformin HCl (Glucomet SR 500mg). In vivo study were assessed for checking the antidiabetic action as sustained manner.

Purpose: Prior to the development of dosage forms, Preformulation studies were conducted for the characterization of physicochemical properties of drug as well as grafting components. The absorption maxima of Metformin HCl was observed at 233 nm in phosphate buffer (pH 6.8), at 232nm in water, at 237nm in methanol and at 233nm in 0.1N HCl (pH1.2). Melting point determination obtained was 234.18°C and FT-IR spectrum confirmed the identity, purity of Metformin HCl. Accuracy, linearity, precision, robustness, ruggedness and force degradation studies were validated by HPLC method. The partition coefficient (-2.64±0.06) indicated that the drug is freely soluble in water. Initially, the gum was extracted from fenugreek seeds. After isolation, the gum was modified in to grafted form by microwave assisted method. In this grafting method, monomer (Acrylamide) and redox initiator (ceric- ammonium nitrate, potassium- per sulfate, ammonium- per sulfate) were used. Then optimization of grafted copolymers was done by applying Taguchi OA design in which seven independent variables and three dependent variables were preferred on account of their preliminary study. The independent (formulation) variables includes monomer-concentration (X1), gum-concentration (X2), initiator- concentration (X3), irradiation-power (X4), speed (X5), time (X6) and temperature (X7). The dependent (response) variables were percent yield (Y1), percent grafting (Y2) and percent grafting efficiency (Y3). It was characterized by FTIR spectroscopy, XRD, DSC, NMR, SEM and swelling index (SI). Further, graft copolymers were used to designed the SR Metformin HCl tablet. The central composite design (CCD) was used in which two formulation variables, amount of grafted fenugreek gum (X1) and PVP K30 (X2) were used and three response variables were designed which includes % drug release in 1 hr (Y1), % drug release in 8 hr (Y2), time to 50% drug release (Y3). As stated by

ANOVA provision, statistical validity of polynomials, slope and regression coefficient were calculated and constructed 3-dimensional response surface plots along with 2-dimensional contour plots. After optimization, checkpoints analysis were carried out to further analyze the total thirteen formulation. Total ten formulations were validated in chosen experimental design and correlate with their predicted response.

Further, dissolution study of formulation (TS9, ungrafted and Glucomet SR 500mg) was performed and compared. As a consequence, it was determined that all the formulations lies within the condition of pharmacopoeia and dissolution profiles meet into distinct kinetic models. The comparison study of TS9 formulation with dissolution profile showed that the release of drug on delayed action leads to more swelling due to longer polymeric chains. Pharmacodynamics optimization studies were carried out on experimental animals which is divided into seven groups. After fasting overnight, diabetes were induced by administration of streptozotocin (45 mg/kg) intra-peritoneally which was processed in fresh citrate buffer (pH 4.5), 15 minutes after nicotinamide (230 mg/kg -NAD) intra-peritoneally. Afterwards 72 hrs administration of STZ injection, the level of FBG level was determined. The rats as FBG level greater than 200 mg/dl were involved within the study. Grafted TS9 formulation administered to the animals in mini tablet form (lower dose, medium dose and higher dose) as the interval of 21 days. Fasting blood samples which is withdrawn from tail vein of rats at 1, 7,14 and 21 days of the study and biochemical parameters (Body weight, Blood glucose level, cholesterol level, triglycerides level, HDL, LDL) and antioxidant parameters (CAT, GSH, MDA) were estimated. The results showed that grafted TS9 formulation at a medium dose was better antidiabetic action in sustained manner.

Hypothesis: After extraction, the gum was found to be $55.99 \pm 0.01\%$ w/w. The extracted gum were modified in to grafted form by microwave assisted method using acrylamide as monomer and redox initiator such as ceric ammonium nitrate

(CAN), potassium per sulfate (KPS) and ammonium per sulfate (APS). Initiator ammonium per sulfate (APS) shows the best %yield and grafting efficiency than CAN and KPS. Taguchi OA experimental designs were used for success the grafting formulation. The best optimized grafted copolymer batch was T2, in which resulted percentage grafting is 50.20 ± 4.0 with grafting efficiency 83.7 ± 0.1 , among selected concentration of acryl amide 15mg, grafted gum 0.25mg, ammonium per sulphate 0.2mg and irradiation time 60sec. Grafting of fenugreek gum confirmed on the basis of FTIR, DSC, XRD, Mass and NMR spectrum, swelling study and surface morphology analysis. Further SR tablets were formulated by using this grafted copolymers with CCD experimental designed tool. According to this designed tool, the amount of grafted fenugreek gum (X1) and PVP K30 (X2) were choosed as formulation parameters. The statistical parameters with polynomial equations were calculated which confirmed that by designing check point analysis, TS9 formulation which contains 347.98 mg of grafted gum and 74 mg of PVP K30, was best than other formulations. Besides, drug content uniformity percentage was also calculated in the range of 84.96 to 99.19%. Then selected formulation (TS9) was indicating the success of the design follows Higuchi model ($R^2=0.994$) with Fickian Diffusion ($n = 0.994$) to best fit. The consequence revealed the drug was released via grafted tablet by sustain profile. The results of animal studies were conducted for 21 days in which biochemical parameters (body weight, glucose level, Cholesterol, Triglycerides, LDL, VLDL were reduced and HDL level was increased) and antioxidant parameters (decreased CAT, GSH level and increased MDA level) were observed. The results were studied and TS9 formulation exhibited better sustained release effect of metformin HCl with grafted fenugreek gum as compare to un-grafted and marketed formulation. Overall research concludes that grafting techniques of fenugreek gum by microwave assisted method may serve as an alternative for combining properties like better sustained and anti-diabetic action of metformin HCl.

Keywords: Fenugreek gum, Acryl amide, Potassium per sulfate, Ceric ammonium nitrate, Ammonium per sulfate, Tiguchi OA design, , Central composite design, Metformin HCl, PVPK-30, MCC, Talc, Magnesium stearate, Streptozotocin, Nicotinamide, in vivo study.

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“The person who influences me most is not he, who does great deeds: but he who makes me feel that I can do great deeds.”

M.P.Follett

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CHAPTER-I

INTRODUCTION

1.1 Diabetes Mellitus (DM)

Diabetes is third metabolic disease in the world in which body is unable to utilize the sugar. It characterized the higher or lower abnormalities in carbohydrates, lipids and proteins metabolism. In case of diabetic patient, the concentration of glucose in urine is 10% than healthy individual (0.05% sugar).

1.1.1 Classification of DM

Type 1: (Insulin dependent or Junevenile onset or IDDM)

Insulin dependent means that the individual need to required insulin to live. It usually begins before age 20, which is caused by destruction of β cells in the pancreas which secretes the insulin. In this condition, insulin is totally absent & consequently glycogen amount are higher than normal levels. Due to deficiency of insulin, cells is impaired for the entry of glucose. Therefore the concentration of glucose in the blood of diabetic patients are much higher. It promotes glycogen breakdown due to high glucagon / insulin ratio in diabetic patients. Hence, in the liver an excessive amount of glucose is produced & released in to blood. When concentration of glucose in blood exceeds, the re-absorptive capacity of renal tubules also increases, leads to higher concentration of glucose excreted in urine (1).

Type2: (Non insulin dependent or maturity- onset type, NIDDM)

In this condition, insulin level in blood is normal or even higher, but they are quite unresponsive to the hormone.

Type 3: It occurs due to other hormonal disorders and drugs.

Type 4: Gestational diabetes mellitus must be treated with diet/ insulin (2).

1.1.2 Symptoms

- It increased the healing time for wound & injury.
- High blood pressure and cholesterol.

- Weakness & fatigue.
- Blurred vision and unexplained weight loss.
- Specific gravity of urine is high
- light colored urine & increase frequency of urination, especially at night.

1.1.3 Pathophysiology

Type 2 DM is characterized by pancreatic beta-cell failure, declined insulin production and insensitivity. Hence decreased glucose transportation into liver, muscle and fat cell. It also increased fat disruption due to diabetes. The defect in the function of alpha-cell has been esteemed in etiology along with disease. Due to this defect, the glucagon level and glucose in liver cell increased during eating which does not change even after meal. Hence insulin resistance increased and due to insufficient levels of insulin hyperglycemia is caused. Although in type 2 DM, abnormalities occur in activity of GIP, preserves the Glucagon like peptide insulinotropic (GLP-1) effects. Thus GLP-1 shows probably beneficial therapeutic approach which is deactivated by Di-peptidyl peptidase.

Due to central visceral adiposity, greater number of the peoples who are suffering from type 2 DM are obese. Therefore, the adipose tissue also involved in the pathogenesis of type 2 DM (3),(4),(5).

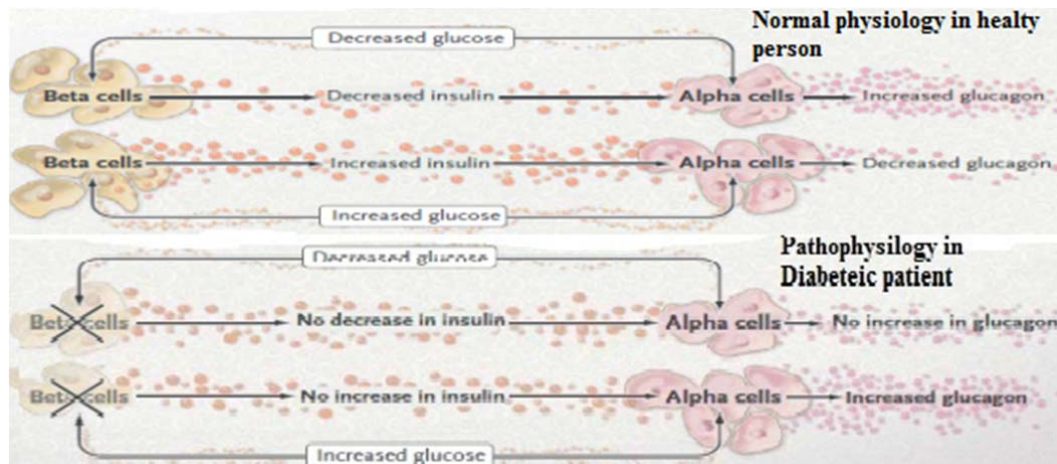


Figure 1.1: Physiology of healthy vs. diabetic person

1.2 Grafted Copolymer

Grafted copolymers includes a polymer linked side chains that comprises of a monomeric or binate unit having varied chemical nature and are attached at different sites of polymeric backbone.

In SR drug delivery systems, grafting techniques is the best manner in which various natural polysaccharides are used. Nowadays in the major area of the researches, the polymer science has great crucial role for the synthesis of grafted copolymers. The addition of functional groups in to polymer chain affects the physical, chemical and rheological properties of compound (6).

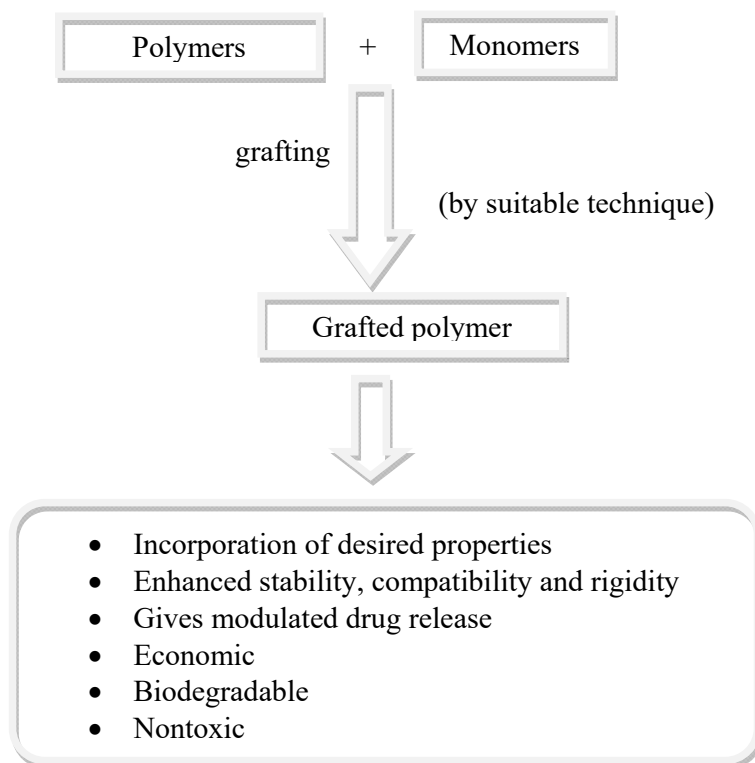


Figure1.2: Steps of grafting (7)

By insertion or implantation techniques, a unit of graft attached to another so it becomes part of it. With the help of graft techniques, chemical alteration of natural and synthetic macromolecular moiety occurs, which have achieved a great interest in novel research area. Graft polymerization is a applicable technique for modifying or

improving the polymer properties, which occurs by graft gums like gum arabic, gum acacia etc (8).

1.3 Techniques/Method of grafting

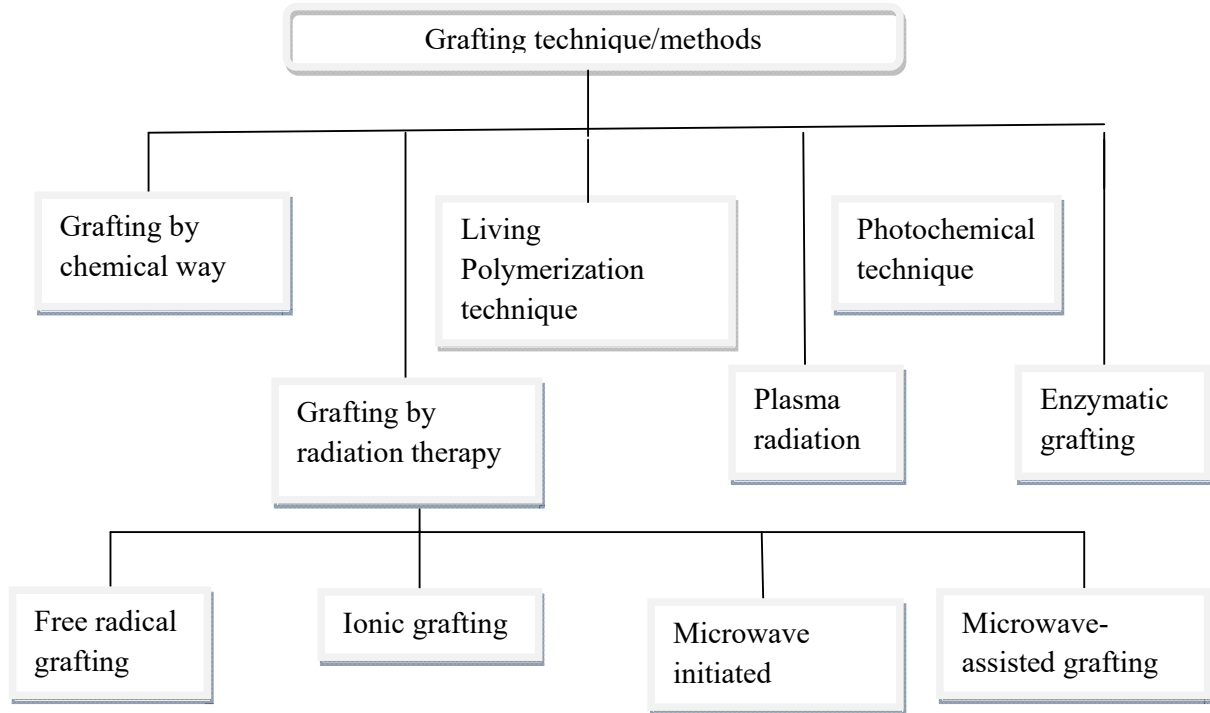


Figure 1.3:Method of grafting (7)

1.3.1 Grafting by chemical method

It includes, grafting could be initiated by synthetic way via two approaches by free radicals and in ionic mode. Initiators are used in this grafting technique, as it determined the rate of reaction (9).

1.3.2 Grafting by living polymerization

In this, the polymer possess its potential to increase the growth in uppermost size for prolong period of time, although chain transfer is however negligible. When light -absorption is not adequate, grafting could be processed using photosensitizers (10).

1.3.3 Grafting by enzymes

In this, the grafting proceeds subsequently by enzymes. e.g. tyrosine helps in converting phenol into o-quinone (reactive form), which again proceeds non-enzymatic reaction with chitosen (11).

1.3.4 Photochemical grafting

The process of photochemical grafting occurs light is absorbed on the macromolecules by chromophore and it goes to excited state in order to form free radical which are reactive. The grafting can be accomplished along photosensitizers, if light absorbed is inadequate (12).

1.3.5 Plasma- radiation induced grafting

In same potential among ionizing radiation, sluggish discharge can occur, Hence plasma conditions can be obtained (13).

1.3.6 Grafting commenced by radiation technique

1.3.6.1 Grafting by free-radical reaction

In this approach, exposure of macromolecules, polymer could be formed which give rise to homolytic fission. The duration of free radicals are subjected to constitution of the polymer's backbone. By radiation technique, the grafting take place through 3 different methods:

Pre-irradiation-In this method, polymer substrates are treated along with monomers in the liquid or vapour form or in solvent solution. Free radicals are synthesized by exposure of polymer's backbone in the vacuum or inert gas.

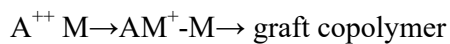
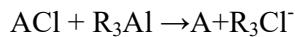
Peroxidation- After attaining irradiation condition, large amount of polymers are exposed to radiation, with oxygen to produce hydro-peroxides or di-peroxides. To start radicals grafting, peroxy products (stable) reacts with monomer and then decompose peroxides at higher temperature.

Mutual irradiation-Polymers and monomers are radiated, then free radicals are formed at same time (14).

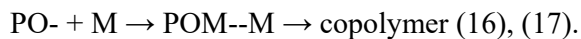
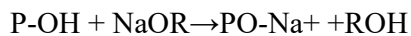
1.3.7 Ionic grafting

The grafting could occur between an ionic form employing some initiators like suspensions containing alkali metal within liquid (Lewis base), organic metallic mixture and sodium naphthalenide.

e.g: To produce copolymerization, alkyl aluminum (R_3Al) and polymer backbone in halogen form (ACl) accommodates and forms carbonium ions with polymer chain. The cationic mechanisms occurs through this reaction(15).



In grafting process, the anionic mechanism can also included. e.g sodium ammonia or methoxide of alkali metals react along monomer to form graft copolymer (alkoxide of polymer, PO^- , Na^+).



1.3.8 Microwave grafting

For graft copolymerization, microwave radiation can be used for better output, and for excessive rate of reaction. There is low degree of energy and solvent consumption, as well as they are environment friendly too (6).

Principle of microwave heating: The dielectric materials are required for inducing microwave grafting. Due to an electrical insulator, the dielectric materials become polarized by an applied electrical field which can displace electrons only slightly from their normal positions and electric dipole is generated which causes a separation of negative charges from positive charges in atomic nuclei. Hence, the material gets polarized.

The dielectric materials are of two types: polar and non polar. In polar material, the molecules have natural dipole moment where as in non polar, no dipole moment is observed. In polar substance, each molecule have dipole due to potential rotation and the material becomes polarized (18).

1.3.8.1 Microwave initiated grafting

A free radical mechanism are used in this grafting in which initiators cannot be used. In order to slow down the grafting process, inhibitor such as Hydroquinone's are used under microwave irradiation (19), (20).

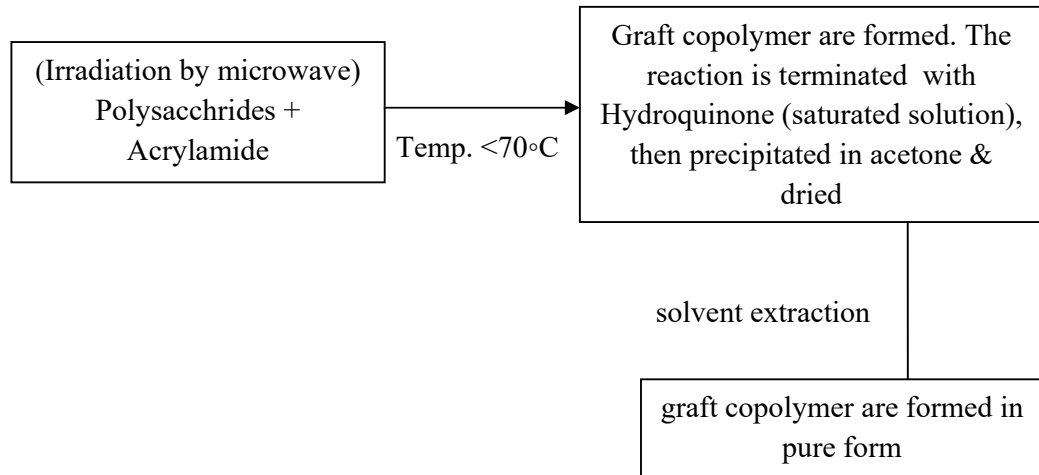


Figure 1.4: Microwave initiated grafting

1.3.8.2 Microwave assisted grafting

The redox initiator can be used for this technique. This initiators generates ions which can transfer microwave energy into heat energy to generate the free radicals under control of microwave dielectric heating (19).

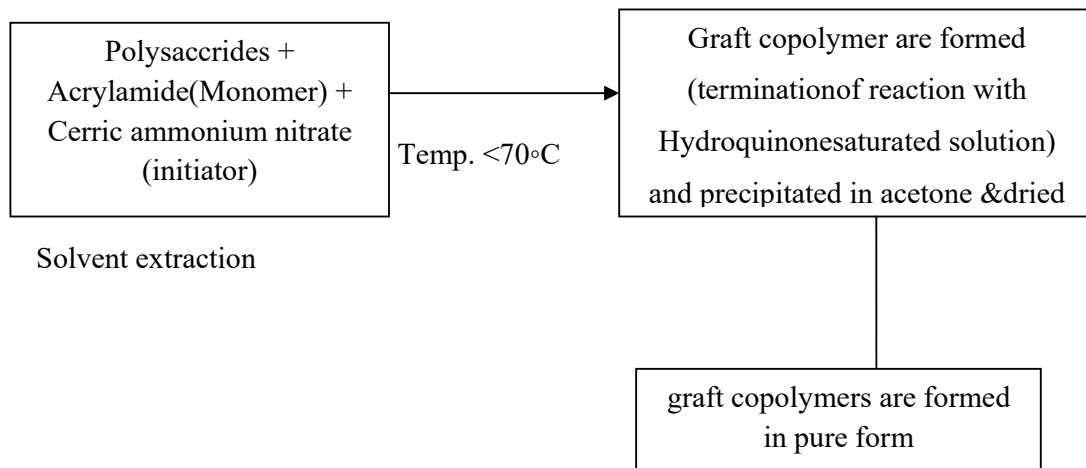


Figure 1.5: Microwave assisted grafting

1.4 Advantages of microwave grafting method

- Unlike conventional grafting technique, uniform heating occurs throughout the material.
- Process speed and efficiency is high.
- The obtained product is pure.
- Reduction in the formation of homopolymer reaction.
- Shorter reaction times.
- Higher yields.
- Reduction of side reactions.

1.5 Applications of grafted copolymers

- Sustained drug delivery
- Transdermal drug delivery
- Colon specific drug delivery
- Microspheres/hydrogel bead preparation
- Flocculation preparation
- Nanoparticles
- Buccal drug delivery system

Oral drug administration is widely used for systemic delivery of drugs. Approximately 50% oral formulations are accessible in the market (21), (22).

Tablets are most adequate for this route which is appropriate for the production on large scale with better reproducibility, stability and patient response. Conventional tablets are given with particular dose and frequency, in several times a day, to maintain plasma level (23).

The frequency of intermittent drug administration based upon its half life and therapeutic index.

In most cases, due to shorter dosing interval and half- life of drug, results in number of limitations related with conventional dosage form (24).

1. Poor patient conformity. Dose frequency is increased because there are major probability of missing the dose due to short biological half-life.
2. The unnecessary variation in drug concentration, in the process of medication or over medication.

3. It disturbs maintained steady-state condition by typical peak-valley plasma concentration time profile (25).

The modified drug delivery system such as sustained release needed to overcome this limitation. A Sustained release dosage form is that dosage form which prolongs the therapeutic activity of drug (26).

The SR drug-delivery are intended to attain the drug within therapeutic effective concentration in systemic flow with improved patients compliance, safety and efficacy. It controls the drug concentration level in plasma for prolonged time to diagnosis the acute and chronic diseases (27), (28).

The objective of this study is to synthesize the graft copolymers of fenugreek gum using acrylamide by Microwave assisted techniques and after this it is further used for the design of SR solid dosage forms using antidiabetic drug like Metformin HCl. The significance of this research work, both fenugreek gum & metformin HCl shows better antidiabetic effect as sustained manner. Therefore, there is a significant for developing such a formulation with synergistic effect as antidiabetic activity.

CHAPTER-2

LITERATURE REVIEW

2.1 Literature review based on Diabetes Mellitus (DM)

Belkis Gelvez *et. al*, 2018 itemized the different care models for those peoples who are suffering from diabetes mellitus. It actively participate in educational interposition, that is attentive on personal care, taking self support. This activity should be given by caregivers, family groups and health professionals which are trained in therapeutic education regarding diabetes. All caregivers should acquire dreadful care model to attain the intention of metabolic control, observance and standard of living. This is based on evidence for offering high quality exhaustive care in which MCC is seen to be a most essential option (29).

Piero MN *et.al*, 2014 reports the explores of DM regarding historical view, biochemical phenomenon, financial economic burden, management involvement with the future position. The blood glucose level became higher as there is insufficient pancreatic insulin secretion by target cells. It can subsequently lead to premature death. It is demonstrated by the meta-analysis that by improvement in lifestyle which includes diet and exercise, shows 63% depletion of diabetes frequency in high perilous. The positive effect of modification programs is a best complement on health in high risk factors for diabetes and initiate stability between consumption of meal, exercise and medicaments to escape this problems. The objective of dietary management is that body weight is controlled as the glucose and lipid levels are maintained (30).

Davies JM *et. al*, 2022 studied the Association of USA and European for the study of diabetes by assembled a group of professional Health care team. It provide the update of past confirmation since 2006 to last 2019 on the treatment of type2 diabetes in adults and deliberate the full details of diabetes care. New Recommendation are provided for implementation include additional focus on social factor of health and health care system, physical activity behaviours including sleep, weight management, cardio renal protection and practical tips (31).

SlivA da et.al, 2018 reports to studied the how individuals deal with chronic condition of diabetes in their health care practice. After investigations new signs were added to persons existence, which affect their habits, health care practices and quality of life. A total 16 people of diabetes mellitus were selected and included in the study and to attended the closed group meetings to rectify their own history and multiple experience deal with the disease called strategic health promotion group. The data produced with subjects like how to indentify diabetes, living with diabetes, exercising personal autonomy (32).

2.2 Literature review based on grafting components

Fenugreek gum and Acrylamide:

Brummer et. al, 2003 calculated the extraction results through chemical constitution of fenugreek gum by different techniques. It is found that when extraction is carried out by cool water, the obtained gum has small protein content a like by extraction with solvent (boiling hexane). Further, protein content reduced 0.57% using the pronase hydrolysis, which does not alter the molecular weight of FG. The sucrose, raffinose & stachyose content in ethanol containing sugar mixture were enumerated as 0.7, 0.5 and 2.84% (33).

Naser et. al, 2012 Investigated the mucilage binding effect in tablet dosage form. The mucilage of trigonella seeds were isolated & acts as binding agent in three distinct drugs (calcium acetate, theophylline & ibuprofen). In terms of solubility, calcium acetate, theophylline & ibuprofen were freely soluble, slightly soluble & experimentally insoluble drugs. As a standard, binders corn starch and polyvinylpyrrolidone (PVPK30) are well known. It extends the disintegration & dissolution rate of theophylline tablets and sustained dissolution rate of hydrophilic drug (34).

Lurian et. al, 2017 described the function of mucilage of fenugreek seed in oro-dispersible pharmaceutical lyophilisates as potential matrix forming agent. The colloidal dispersion of mucilage were prepared by freeze drying method. Their

rheological evaluations were performed in which meloxicam as model drug was used. Relation comparison studies were carried out with gelatin containing tablets which were prepared under the same conditions (35).

Zauro *et. al*, 2016 Develop the poly grafted locust-bean gum by diallyldimethylammonium chloride through microwave irradiation method. The ammonium peroxy-di-sulfate as initiator and N,N-methylene-bis-acrylamide as cross linker. The % yield calculated and is characterized by FTIR, thermo gravimetric analysis (TGA), scanning electron microscopy (SEM) as well as swelling index (SI). The maximum adsorption obtained was 35.12 mg/g and statistics were fitted into Langmuir isotherm model for the observation of adsorption data (36).

Singh *et. al*, 2011 calculated the relevance of grafted -moth bean starch by acrylamide and act as CR matrix former agent. Further, the CR tablets were prepared by different amount of grafted copolymer with model drug as lamivudine (37).

Kaity *et. al*, 2013 synthesized the copolymers of locust bean gum (LBG) with acrylamide via microwave assisted techniques to which ceric ammonium nitrate (CAN) as redox initiator was used. Further, CR matrix tablet of this grafted gum, using buflomedil hydrochloride as model drug, was prepared. The characterization of grafted gum by FT-IR, ¹³C NMR, SEM, XRD, DSC, elemental analysis, contact angle, viscosity, molecular weight, swelling and biodegradability studies. It revealed from results that grafted gum were showed biodegradable and harmless properties. By dissolution study of tablets, grafted locust bean gum (AC-LBG) exhibited rate controlling property which were the same to that of hydroxypropyl- methylcellulose (38).

Vijan *et. al*, 2012 synthesized the grafted copolymers of gellan gum using acrylamide by microwave-assisted technique, in which varying amount of monomer (acrylamide), initiator (CAN) and irradiation time were taken. Isolated gum by 20% (w/v) methanol to remove homo polymers which were produced during polymerization reaction. It was evaluated via FTIR, NMR, SEM, rheological and DSC studies. The grafting variables like %GE, % G and % C were calculated as well as subsequently

correlating along elemental analysis, DSC and viscosity effect. With use of this grafted gum, the tablets were formulated by using Meformin hydrochloride and dissolution studies were conducted which were showing release up to 8 hrs (39).

Kumar et. al, 2009 synthesized grafted copolymers of xanthan-gum with poly (acrylamide) employing microwave-assisted and ceric-induced graft copolymerization techniques. Further, by direct compression method, matrix tablets of diclofenac sodium were formulated by this grafted copolymers and FTIR, DSC, XRD and SEM studies were used for their characterization. Dissolution study of tablets were conducted in phosphate buffer (pH 6.8) at 37°C and found zero-order kinetics model for best release of drug. The result revealed that the graft copolymer matrix showed the faster drug release in contrast to xanthan gum matrix tablet. It diminishes the grafting and raises the erosion in xanthan gum (40).

Varshosazet et.al, 2006 formulated SR matrix tablets using hydrophilic drug (tramadol HCl) by xanthan as well as guar gum via direct compression method and compared with hydrophilic matrices (i.e., HPMC or CMC). The different ratios in the different mixture of polymers were applied (100:0, 80:20, 60:40, 20:80, 0:100 of G gum, X gum :HPMC, X gum: G gum and triples combination of polymers (G gum: X gum: HPMC). Afterwards, characterization of post compression parameters of tablets, dissolution study were conducted and release kinetics followed the zero-order model by swelling, diffusion and erosion mechanisms. The release rate cannot be controlled alone by guar gum whereas the blend of natural gums along HPMC exert delay release (41).

Malik et. al, 2011 synthesized copolymers of Kondagouonto gum with poly acrylamide by microwave-assisted grafting techniques with applying full factorial experimental design (2-level & 4-factor). Acute toxicity and drug-excipient compatibility studies were conducted with copolymers. Further, the tablets were formulated using this copolymers which were characterized by post compression parameters. The dissolution study revealed that optimized batch showed highest correlation (R) value with Higuchi model as well as it showed release behavior based on combination of both diffusion and erosion (42).

Odeiniyl et. al, 2017 characterized the native (natural) and modified formation of Terminaliamantaly (MTM) gum for their sustained and bioadhesive action. By using microwave irradiation technique, the natural gum was modified for 20sec, 60sec & characterized by using microscopy, FTIR, packing properties. Further, using this modified gum the tablets formulated by direct compression technique by Naproxen sodium. Beyond irradiation of Naproxen-Terminalia mantaly (NTM), the flow properties of gum were modified. By swelling studies, MTM20 and MTM60, water absorption ability were reduced. The FTIR spectra exhibit that irradiated gums distinct from native gum and they don't relate with naproxen sodium. The mechanical properties of gum were enhanced with MTM20 and MTM60, which expressed SR activity up to 12hrs (43).

Bal T et.al, 2020 Synthesized the graft copolymers of fenugreek seed mucilage (FSM)-polyvinyl alcohol (PVA) by free radical polymerization technique using APS as initiator, acrylamide as monomer and Enalapril maleate as a drug. The best group from various formulations were optimized by %grafting efficiency, viscosity factor, thermal analysis & X-rays studies. (44).

Zhou M et. al, 2019 Studied the Hydrophobically modify fenugreek properties and assessed the potential application in liver targeted drug delivery system. Stearic acid was conjugated with fenugreek (FG-C18) by simple esterification method, Further, self assembled nanomicelles (NMS) of FG-C18 in water was prepared by ultrasonic method. Then characterized on its chemical structure by FTIR & $^1\text{H-NMR}$ (45).

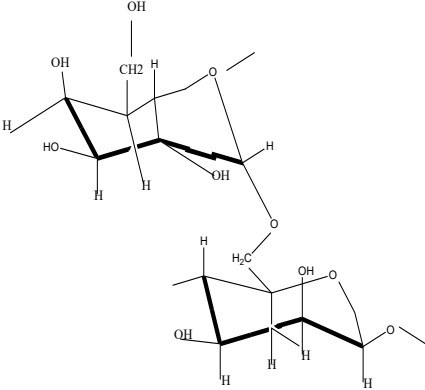
Teekanam J et.al, 2023 Developed the simple method for extracting as well as purifying galactomannan from fenugreek seeds. Then purified by centrifugation and isopropyl alcohol spirit precipitation method for stabilizing and emulsifying various products. It revealed found that presence of carbohydrates by molish test and absence of reducing sugar by Fehling's test. The pH of purified galactomannan was found 6.37 with foaming capacity 14.28%. It possesses 81% emulsifying capacity. It found from result that capacity to hold water 1480% and for oil 268% (46).

Mishra A et.al, 2006 Developed the fenugreek mucilage backbone by grafting with acrylamide using ceric ion by polymerization method under nitrogen atmosphere. A total 19 formulations were synthesized with varying concentration of AM and CAN,

reaction time and temp. The variation in reaction parameters affected the %G, %GE and intrinsic viscosity of the copolymers. The grafting formulations were characterized by FTIR, SEM, thermal gravimetric analysis, XRD& viscosity measurements (47).

2.2.1 Grafting Component I Profile:

Table 2.1: Fenugreek gum

Name	Fenugreek gum
Synonyms	Trigonellafoenumgraecum
Molecular Formula	$C_8H_{14}Cl_2N_2O_2$
Structure	
Solubility	water soluble and insoluble in organic solvents
Composition	α (1/4)- β D-mannan backbone attached to α D-galactopyranosyl group at 0-6 D-mannopyranosyl
Appearance	Fine cream colored, free flowing and odorless
Physical characteristics	powder slow hydration rate, unpleasant flavor
Uses	Anti carcinogenic, anticancer, anti cholesteromic, hypoglycemic, antioxidant, antibacterial and anti ulcer. it also used as stabilizing agent, thickening agent in food industries (48).

Extraction of Fenugreek gum:

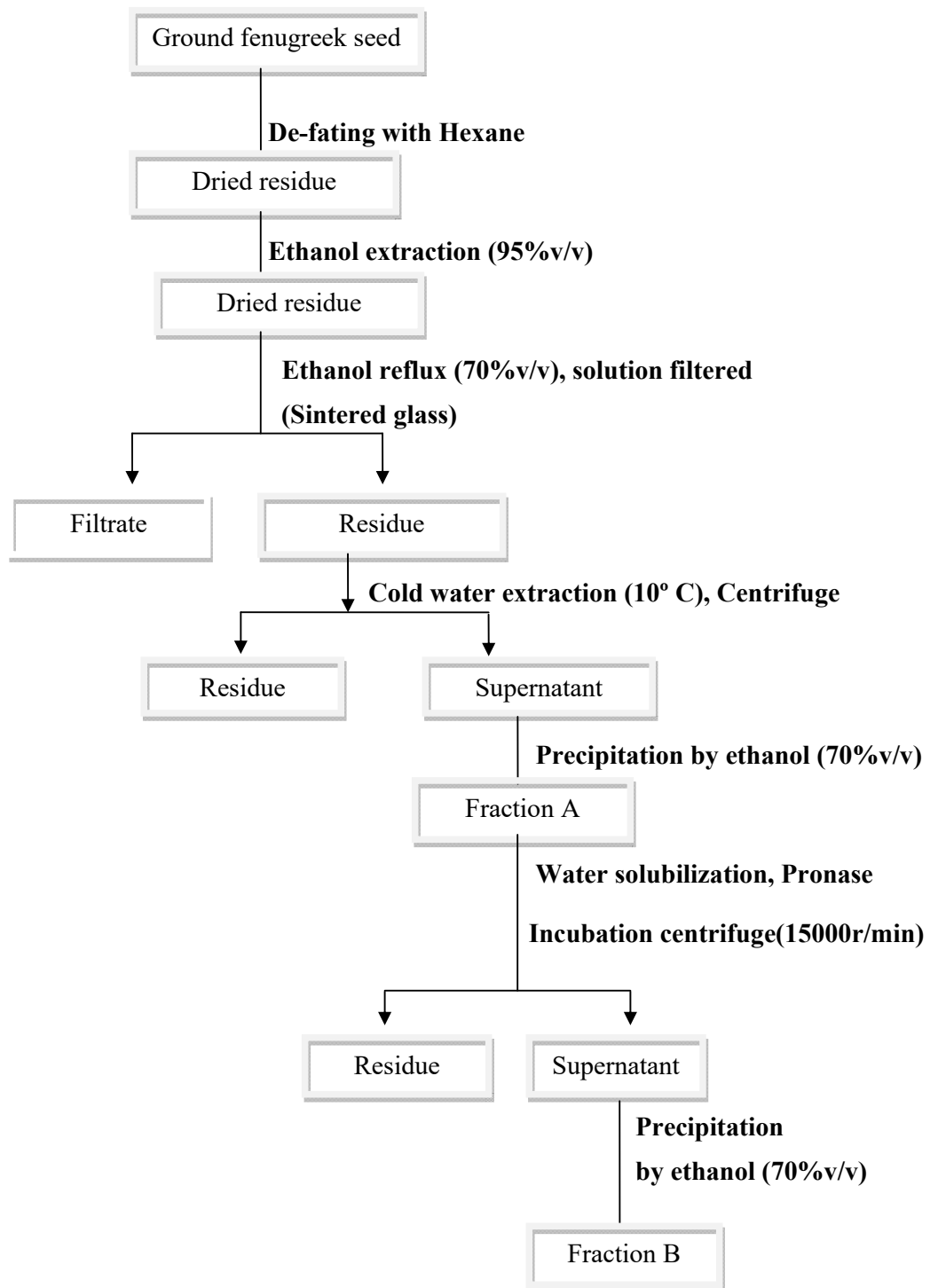
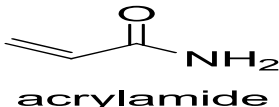


Figure 2.1: Procedure for extraction of fenugreek (33),(48)

2.2.2 Grafting component II (Monomer) profile:

Table 2.2: Acrylamide profile

Name	Acrylamide
Synonyms	Acrylamide
Chemical Name	2-Propenamide
Molecular Formula	C ₃ H ₅ NO
Structure	 acrylamide
Molecular weight	71.079g/mol
Solubility	water, methanol
Appearance Physical Properties	Colourless, odourless
Uses	Protein electrophoresis, synthesis of dyes, copolymer for contact lens (49) (50)

2.3 Literature based on Drug (Metformin HCl)

Dan et. al., 2018 synthesized the acrylamide grafting of *cassia tora* (CT) by microwave-assisted method. The tablets were prepared successfully using optimized grafted batch of AC-g-CT, in which metformin and sitagliptin acting as model drugs. According to OECD guideline, acute oral toxicity study and pharmacokinetic parameters in rats was checked by LC-MS/MS bio-analytical method (51).

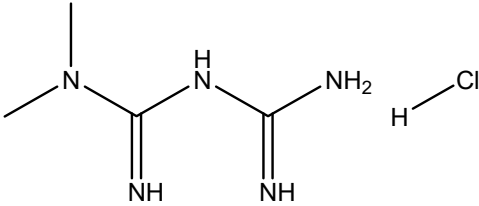
Ramteke et.al.2012 developed the calcium and aluminum ions beads with metformin HCl by ionotropic external gelation technique & optimized the best cross linking agent amongst them. The prepared beads were characterized and its release behavior was explained by kinetic model. It was found from the result, aluminium was better cross linking agent than calcium because calcium beads showed SR action of drug for 8hrs whereas aluminum showed for 10 hrs (52).

Kwabena et. al., 2015 formulated the ER matrix tablets using hydrophobic (diclofenac sodium) and hydrophilic (metformin hydrochloride) drug using direct

compression method. In this formulation, the release retardant materials (cashew gum, xanthan gum & hydroxy propyl methylcellulose) were used. To check the applicability of light grade cashew gum as excipient for a direct compression, SeDeM diagram expert system was used. In there, total 13 formulations of diclofenac sodium (100 mg) and metformin hydrochloride (200 mg) were produced along various concentration of cashew gum, xanthan gum as well HPMC. The drug release studies were carried out in phosphate buffer (diclofenac pH 7.4 metformin HCl pH 6.8) and also release kinetic profile was estimated. The drug release from diclofenac tablets showed zero order, first order or Higuchi model, whereas, drug release from metformin tablets succeeded Higuchi design (53).

2.3.1 Drug Profile

Table 2.3: Metformin HCl

Name	Metformin HCl
Molecular weight	165.62g/mol
Molecular formula	C ₄ H ₁₁ N ₅ Cl
IUPAC name	1, 1 dimethylbiguanide N,N-dimethylimidodicarbonimidicdiamide
Structure	
Synonyms	Metformin HCl
Water solubility	1.38mg/ml
Melting point	223-226°C
Log P	-2.6
pKa	12.33
Category	Oral Hypoglycemic agent

Physical state	White, crystalline powder
Absolute bioavailability	In Fasting state, absolute bioavailability of 500mg tablet about 50-60%. In clinical studies (single dose), the oral doses HCl 500-1500mg and 850-2550mg.
Plasma protein binding	Negligible bound to plasma protein
Volume of distribution	654±358L
Half-life	6.2 hrs
Clearance	Renal clearance (54)

2.4 Excipient Profile:

2.4.1 Microcrystalline cellulose

Table 2.4: Microcrystalline cellulose

Name	Microcrystalline cellulose
Synonyms	Arbocel A300,Arbocel B00
Molecular Formula	$(C_6H_{10}O_5)_n$
Molecular weight	370.35g/mol
Solubility	Experimentally insoluble (water, acetone, ethanol (anhydrous), toluene, acids (dilute), 50 g/L solution of NaOH
Appearance	white
Uses	ACCEL-101 is most widely used in direct compression and wet granulation techniques of tablet (55)

2.4.2 Binding agent profile:

Table 2.5: PVP K30

Name	Polyvinyl pyrrolidone K30
Synonyms	PVP-K25, K60,K-30, K115,
Chemical name	Polyvinylpyrrolidone
Molecular Formula	$(C_6H_{10}O_5)_n$
Molecular weight	16.04246g/mol
Solubility	Soluble in water
Density	1,69 g/cm ³
pH	3.0-5.0
Appearance	White to yellow-white powder
Uses	It used as toothpaste detergents, gelling agents, antidotes, stabilizer in skin care lotions and creams (56)

2.4.3 Lubricants-Magnesium Stearate

Table 2.6: Magnesium Stearate

Name	Magnesium Stearate
Synonyms	Magnesium Stearate
Molecular Formula	$Mg(C_{18}H_{35}O_2)_2$
Molecular weight	591.2 g/mol
Solubility	Soluble in water, slightly soluble in benzene not soluble in ether and alcohol,
Appearance	Powder, Pellets, Crystals form
Uses	Cosmetics ingredient, anti-caking agent in foods, fire extinguishers and lubricant in pharmaceutical tablets (57)

2.4.4 Lubricant-Talc

Table 2.7: Talc

Name	Talc
Synonyms	Agalite, Asbestine, trimagnesium, dioxido (oxo) silane, hydroxy-oxido-oxosilane
Chemical name	Talc
Molecular Formula	$H_2Mg_3O_{12}Si_4$
Molecular weight	379.27 g/mol
Solubility	Water insoluble and slightly soluble in mineral acids (dilute form)
Appearance	White powdered form
Uses	Talc is used as lubricating agent, abrasive agent and used in dusting powders (58)

CHAPTER-III

HYPOTHESIS OF THE STUDY

Modification of natural gums by grafting techniques enables their use for specific drug delivery by overcoming their drawbacks. The Basic rationale of this study is to develop and assessed the graft copolymer of fenugreek gum using acrylamide by microwave assisted technique and further designed SR Metformin HCl tablets by grafting copolymers of fenugreek gum to treat diabetes mellitus. A literature review revealed that SR formulation of grafted copolymers are preferable dosage form in exceptional cases where conventional dosage forms are unsuccessful for encounter the disease. Both fenugreek gum & metformin HCl shows better anti-diabetic effect as sustained manner. Therefore we can formulate SR tablets of metformin HCl by grafted copolymers of fenugreek gum for rectify the diabetic problem and increased patient conformity.

CHAPTER-IV

OBJECTIVE

1. Extraction of fenugreek gum
2. Synthesize the graft copolymer of Fenugreek gum by acryl amide using microwave assisted technique
3. Characterization & optimization of grafted copolymers by Taguchi OA design.
4. After this, Designed SR tablet of grafted copolymers using Metformin HCl as drug
5. Characterization and optimization of SR Metformin HCl tablets by Centered Composite Design (CCD).
6. The drug release behavior of SR Metformin HCl grafted tablet comparatively evaluated with ungrafted Metformin HCl fenugreek gum tablet & also compared with marketed SR formulation of Metformin HCl (glucomet SR 500mg).
7. Accelerated stability studies
8. In vivo study

CHAPTER-V

MATERIAL AND METHODS

5.1. Materials:-

5.1.1. Chemicals:- The chemicals employed in the present research work are summarized in Table 5.1 and 5.2

Table 5.1: List of API

S. No.	Name	Manufacturer
1.	<i>Metformin HCl</i>	Park Pharmaceuticals, Baddi, India

Table 5.2: List of Materials

S. No.	Material	Source
1	Potassium di-hydrogen- <i>o</i> Phosphate	Thermo Fisher Scientific, India
2	Sodium lauryl sulphate	SRL, India
3	Nylon 0.22 μm membrane filter	Pall corporation, Mumbai
4	Di-sodium hydrogen phosphate	Central Drug House Pvt. Ltd. India
5	Ethanol	ChangshuYanguan Chemical, India
6	Methanol	Fisher Chemical Ltd., India
7	AcrylamidE	Qualikams Fine ChemPvt.Ltd. Delhi.
8	Fenugreek	Local Market, Panchkula,Haryana
9	Acetone	Merc life science, India
10	PEG 400	Molychem laboratory, Mumbai
11	Ceric ammonia nitrate	Qualikams Fine ChemPvt.Ltd. Delhi
12	Ammonium persulfate	Qualikams Fine ChemPvt.Ltd. Delhi
13	Potassium persulfate	Qualikams Fine ChemPvt.Ltd. Delhi
14	HCl	Qualikams Fine ChemPvt.Ltd. Delhi
15	NaOH	Fisher scientific Pvt. Ltd., India

16	PVP K-30	BASF Corporation, Ludwigshafen
17	Talc	Central Drug House (P) Ltd. India
18	Magnesium stearate	Central Drug House (P) Ltd. India
19	Microcrystalline cellulose	Vikram Thermo (India) Limited
20	Nicotinamideadenine dinucleotide phosphate Oxidase	Sigma-Aldrich, Milwaukee, USA.
21	Streptozotocin	Sigma-Aldrich, Milwaukee, USA.
22	1,1,3,3 tetra-ethoxy propane	Keshav Int. Pvt. Ltd. India
23	2,4 dinitrophenyl-Hydrazine	Keshav Int. Pvt. Ltd. India
24	Trichloroacetate	Keshav Int. Pvt. Ltd. India
25	DTNB	Keshav Int. Pvt. Ltd. India
26	Disodium Hydrogen phosphate	Keshav Int. Pvt. Ltd. India
27	Hydrogen peroxide	Keshav Int. Pvt. Ltd. India
28	Ammonium molybdate	Keshav Int. Pvt. Ltd. India
29	Pyrogallol red	Keshav Int. Pvt. Ltd. India

5.1.2 Equipment:

The equipments used in the study are listed in Table 5.3.

Table 5.3: List of equipment

S. No.	Equipment Name	Manufacturer
1	UV spectrophotometer	Shimadzu, India
2	Mechanical stirrer	REMI equipment, India
3	Magnetic stirrer	REMI equipment, India
4	pH meter	Ohaus, USA
5	Digital balance	Shimadzu, India
6	X-ray diffractometer	X' pert Pro Malvern Analytical

		United Kingdom, Germany
7	Melting point apparatus	REMI equipment, India
8	Vortex mixer	REMI equipment, India
9	DSC	Shimadzu, zapan
10	Hot air oven	NSW, India
11	NMR &Mass	Peabody, MA, USA
12	FTIR	FTIR-8400S, Shimadzu, Japan
13	Scanning electron microscope	JSM 6100 Jeol, Ltd. Japan
14	Microwave	Samsung, India
15	Bulk density apparatus	KeshavInt.Pvt.Ltd.India
16	Vernier caliper	KeshavInt.Pvt.Ltd.India
17	Monsanto hardness tester	Keshav Int. Pvt. Ltd. India
18	Dissolution apparatus	Keshav Int. Pvt. Ltd. India
19	Rotary tablet machine	Keshav Int. Pvt. Ltd. India
20	Glucose estimation kits	Reckon Diagnostics Pvt. Ltd, India
21	Total serum cholesterol	Bayer Diagnostic kit Pvt. Ltd, India
22	Total serum cholesterol	Bayer Diagnostic kit Pvt. Ltd, India
23	Triglycerides level	Bayer Diagnostic kit Pvt. Ltd, India
24	HDL Level	Erba Diagnostics Manheim, Germany kit

5.2 Methodology

5.2.1 Pre-formulation studies

5.2.1.1 Organoleptic Properties

API were visually inspected by its physical properties like colour, odour, appearance, state and taste (59), (60).

5.2.1.2 Differential Scanning Colorimetry (DSC)

Differential scanning calorimeter (DSC2 -00347 TA Shimadzu, zapan) was used for conducting DSC study of metformin HCl at heating rate 10°C/min from 50°C to

350°C under nitrogen purge (50mL/min). Accurately weigh the sample drug (5 mg) sealed in aluminum pan as well as placed at sample stage. Empty pan was taken as a reference (61).

5.2.2 Absorption maxima of Metformin HCl

5.2.2.1. Absorption maxima of Metformin HCl in phosphate buffer pH6.8

Double beam UV Spectrophotometer was used for the determination of absorption maxima (λ_{\max}) of Metformin HCl. Standard stock solution (0.1 mg/mL) was prepared by dissolving 10mg drug in 100 ml phosphate buffer pH6.8. Then resultant solution was scanned by spectrophotometer.

Preparation of phosphate buffer pH 6.8

Weigh 2.38g disodium hydrogen phosphate, 0.19g dibasic sodium phosphate as well as 8g sodium chloride dispersed in distilled water. Then volume make up till 1000 ml along distilled water and pH was regulated up to 6.8 by 0.1N NaOH or 0.1N HCl (60).

Standard curve of Metformin HCl in phosphate buffer pH6.8

Standard curve was assembled by dissolving 10 mg drug in 100mL Phosphate buffer (pH 6.8) in order to obtain 100 μ g/mL (0.1 mg/mL) concentration. The dilutions were made within range 1-12 μ g/mL from stock solution and scanned at 233nm through UV spectrophotometer. The study was accomplished triplicate and mean data was recorded (62), (63).

5.2.2.2. Absorption maxima of *Metformin HCl* in water

Dissolved 10 mg of drug in 100 mL water to form concentration of 100 μ g/mL for the standard curve of Metformin HCl. The dilutions (1-12 μ g/mL) were formulated from stock solution and scanned at 232nm through UV spectrophotometer. The study was conducted triplicate and analyzed mean data (64).

5.2.2.3. Absorption maxima of *Metformin HCl* in methanol

Standard curve of Metformin HCl in methanol was assembled by dissolving drug (10 mg) in 100mL of water sequentially to obtain concentration of 100 μ g/mL. From the stock solution range of this dilutions (1 to 10 μ g/mL) were prepared which

were scanned at 237nm through UV spectrophotometer. The study was operated triplicate and mean data was marked.

5.2.2.4. Absorption maxima of *Metformin HCl* in 0.1N HCl pH 1.2

Preparation of 0.1N HCl: 1000 mL of clean and dried volumetric flask was taken and add 100 mL distilled water and about 8.5 mL of hydrochloric acid. About 700 mL of distilled water was put in it, mixed and finally the volume up to 1000 mL with distilled water (65).

Calibration curve of *Metformin HCl* in 0.1N HCl pH 1.2

Dissolved 10 mg drug in 100mL of water in order to produce 100µg/mL. conc. range. From this stock solution, dilutions (0.5 to 5µg/mL) were prepared, which were scanned at 233nm through UV spectrophotometer (65).

5.2.3. Solubility Studies

The drug was taken in excessive quantity in different five stopper vial (capacity 15 mL) in which 5 mL of different solvents (Phosphate buffer pH 6.8, water, methanol, 0.1 N HCl) were added. By using vortex mixer, each glass vial was mixed for 10 min. Then mixture vials placed in a bath shaker at 25±1.0 °C for 24 hrs to get equilibrium. After attaining equilibrium of sample mixture, it can be withdrawn from shaker as well as centrifuged at 15000 rpm for 30 min to get supernatant, that filtered via 0.45µm membrane filter. In each solvent, absorption maxima of *Metformin HCl* was measured by UV spectrophotometer and scanned from 200-400nm (66), (67), (68), (69), (70).

5.2.4. Partition Coefficient

Partition coefficient (o/w) is an expression of drug capability to permeable the cell membranes and also distinguish the drug nature (lipophilic/hydrophilic). The drug indicates as lipophilic, if P value is much greater than 1 where as indicate hydrophilic drug if value is less than 1. Various types of method such as HPLC, shake flask and computational method were used for determination of log P using n-octanol and water as solvent. Furthermore, Log P value of *Metformin HCl* was determined using Shake

flask method and estimated by this formula.

$$P_{o/w} = C_{n\text{-octanol}}/C_{\text{water}}$$

- **Shake flask method**

Shake flask method is used for determination of partition coefficient. In this method, take the *drug* (Metformin HCl) in excessive amount and mixed in 10 mL of two different solvents (n-octanol: water) simultaneously in ratio (1:1) as well accommodate for 24 hrs. Later, 24 hours, two layers were separated which centrifuged for 15 min. at 15,000 rpm. After suitable dilutions, the absorbance was determined in UV spectrophotometer at 233 nm (71), (72), (73),(74).

5.2.5 Validation of Metformin HCl by RP-HPLC method

5.2.5.1 Method development

Selection of wavelength

For the preparation of standard solutions of Metformin HCl, add 10mg of metformin HCl in 10mL of PBS 6.8 to obtain 100 µg/mL solution and scanned by UV/Vis spectrophotometer in range 200-400 nm. A sequence of dilutions were prepared in different concentration range (2-20 µg/mL) from working stock solution. The absorption of the drug at 232 nm wavelength was determined and the validity was checked in line along with variables in addition to system suitability, specificity, limit of quantification (LOQ), limit of detection(LOD), linearity in response, accuracy, precision (reproducibility & repeatability) and robustness.

Preparation of Standard stock solution

The drug (10 mg) was weighed exactly and put within 10mL volumetric flask containing 5 ml of Phosphate buffer 6.8. Consequently the solution was filtered via 0.22 µ millipore membrane filters and filtrate was sonicated for 10 min. Then the solutions were diluted to 100 mL along mobile phase. The volume was adjusted with diluents up to mark.

Preparation of sample solution and Diluents

The sample solution in the concentration range 2-20 $\mu\text{g/mL}$ was prepared from above stock solution (100 $\mu\text{g/mL}$) and it diluted with HPLC water. Then filtered through 0.22 μ millipore membrane filters as well as injected in HPLC system (75).

5.2.5.2 Validation

Developed HPLC method was used for validation of such parameters (Accuracy, Precision Linearity, LOD, LOQ, Robustness, and System suitability parameters and forced degradation studies) accordance with ICH guidelines

5.2.5.2.1 Accuracy

Accuracy means the % mean recovery of test sample at three distinct grade (90, 100, and 110%). It was determined by injecting about 10 μL of Metformin HCl at three different levels into the column. This procedure was repeated triplicate using peak height at each level. Its accepted limit was 90-120% and all the calculated data lies in the appropriate range that showed the sign of good recovery and accuracy range (76).

5.2.5.2.2 Precision

Precision is the closeness/compactness of agreement between quantity values. It showed numerically under specified conditions by repeated assessment of amount or randomly error in the set of individual measurements with standard deviation (SD), variance or coefficient variation (77).

5.2.6.2.3 Repeatability

It is the measurements in series of the same quantity in a rapid succession when the experimental work are conducted in same circumstances (analyst, apparatus, instruments, and day). To carry out this study, standard solution of Metformin HCl was prepared, scanned at 264 & 221nm and were six times analyzed as proposed method.

5.2.5.2.4 Intermediate Precision

It is the method in which sample was analyzed in the same amount in series during the experimental work. It was carried out in same laboratory concealed in distinct conditions like analyst, apparatus, instrument, and day. For this study standard solution of Metformin HCl (10 μ g/mL) was prepared and was analyzed.

5.2.5.2.5 Linearity

From above stock solution, dilutions of Metformin HCl from 2-20 μ g/mL concentration range were prepared and scanned in HPLC. Standard curve was composed by representing the peak area be on y-axis (vertically) against concentration (μ g/mL) be on x-axis (Horizontally) treated by least-squares linear regression analysis. Hence the method considered to be linear for Metformin HCl.

5.2.5.2.6 LOD and LOQ

The limit of detection (LOD) is single analytical method in which smallest quantity of analyte in sample was estimated but it fails to determine an accurate value. Then limit of quantitation (LOQ) is least degree of analyte within sample that could estimated quantitatively along acceptable precision and accuracy. The LOD and LOQ was measured using calibration curve:

$$\text{LOD} = 3.3\sigma/S, \quad \text{LOQ} = 10\sigma/S$$

Whereas σ =SD of response (Y intercept) , S =slope

5.2.5.2.7 Robustness

Robustness accompanied potentiality in order to prepare accurate and precise effects in distinct conditions. For assure robustness of expected analytical method, flow rate was regulated to 1.0 mL/min and 1.5 mL/min as well regulated analytical wavelength from 235 and 230nm with organic composition of mobile phase was changed to $\pm 10\%$. Then checked the changes occurred in the chromatograms after injecting the working standard solutions and sample solutions of Metformin HCl (72),(78).

5.2.5.2.8 Ruggedness

Ruggedness was indicated as % RSD which can be determined by measurement of analysis by two distinct analysts with percentage recovery.

5.2.6 Forced degradation studies

In the Force degradation studies, stability studies of developed method of Metformin HCl was determined in the presence of acid (H^+), base (OH^-), Hydrogen peroxide, temperature, ultraviolet light, and HPLC grade water.

5.2.6.1 Acid degradation

The acid degradation study was determined by mixing 1 ml standard stock solution of Metformin HCl and 1 ml 0.1M HCl then left to stand for 1, 2, 4 hour at 60 ± 2 °C. Resulted solutions diluted with 10 mL of diluent. After neutralizing procedure, samples were analyzed in triplicate and chromatogram were run.

5.2.6.2 Alkali (base) degradation

In the presence of alkaline conditions, the degradation study conducted by refluxed standard stock solution of Metformin HCl (1 mL) with 1 mL of 2M NaOH for 1, 2 and 4 hour at 60 ± 2 °C. The volume was make up to 10 mL with diluent. After neutralizing procedure, samples were analyzed in triplicate and were run in chromatogram.

5.2.6.3 Oxidative degradation

To carry out this degradation study, standard stock solution of drug (Metformin HCl) (1 mL) was refluxed along 20% v/v H_2O_2 for 1, 2 and 4 hour within 10mL of volumetric flask at 60 ± 2 °C and made up with the diluent.

5.2.6.4 Thermal degradation

For determination of thermal stability, standard stock solution of drug (Metformin HCl) was put in 10mL volumetric flask and then heated for 1, 2 and 4 hr at 60 ± 2 °C. Then resulting solution (1mL) was diluted with 10 mL of diluent.

5.2.6.5 Photolytic degradation

In this degradation study, standard stock solution of drug (Metformin HCl) was exposed to ultraviolet light in UV chamber for 7 days. The resultant mixture were analyzed in triplicate and the spectra was run.

5.2.7 FTIR Spectroscopy

FTIR analysis of pure drug (*Metformin HCl*) and physical mixture (drug, fenugreek gum and acrylamide) was done using KBr pellets. For this, about 1mg of drug or physical mixture (drug, fenugreek gum and acrylamide) mixed with KBr as well as grinded in mortar pestle. Afterwards, mixture was put in small die under pressure 5000-10000 psi in KBr press for the preparation of KBr pellets. Subsequently, the pellet was accommodate in sample holder of FTIR spectrophotometer and analyzed within 4000-400 cm^{-1} regions (78), (79), (80), (81).

5.2.8 DSC Study

DSC of physical mixture (drug, fenugreek gum and acrylamide) was obtained in the temperature range 218.79 to 234.18°C and 29.29 to 168.00°C using DSC2 - 00347 TA (Shimadzu, zapan) thermal analysis systems under nitrogen purge (50ml/min). Approximate amount of sample (5 mg) was sealed in the pan which is made up of aluminum and heated at 10°C/min. Empty pan was taken as a reference (59).

5.2.9 XRD Study

XRD diffractometry was determined for pure drug (metformin HCl) as well as physical mixture (drug, fenugreek gum & acrylamide) using K-beta filter, Cu-K α -radiation, 45 kV voltage as well as 30 mA current. The scanning was employed in range 10.00 to 50.00° at diffraction angle (2 θ) (82).

5.2.10 ^{13}C NMR & ^1H spectroscopy

The NMR spectrum of ^{13}C and ^1H used to characterized the spectra of pure drug (Metformin HCl) within ceramic rotor adjacent JEOL ECX 400 (Peabody, MA, USA) spectrometer, worked at 75MHz and 65MHz at repetition time of 0.8 sec. Before detection, the sample (300mg) was dissolved in D_2O (3ml) at 60 °C. The

chemical shifts based on sample was measured using tetramethylsilane (TMS) though an internal standard in both spectrum (83), (84).

5.3 Isolation of gum from fenugreek seed

Initially, crush the seeds of fenugreek (50gm) as well as soak them in 1000ml distilled water at room temperature for 12 hours. Later, boiled in distilled water along continual stirring to obtained slurry which was finally cooled as well as retained overnight, so that un-dissolved material settled down. Hence, the clear supernatant was obtained. After collection of supernatant, which centrifuged at 6000g for 10min and concentrated on a boiling water bath at 60°C to 1/3 of its actual amount. Then solution was cooled at room temperature and mixed thrice the volume of acetone along constant stirring to the solution. The precipitate were formed and frequently washed along with acetone. Then precipitates were further dried at 60 ± 2°C to obtain fenugreek gum, then was passed through #80 mesh and keep it in desiccators for further use (85).

5.4 Preparation of grafted copolymers

The copolymers of fenugreek gum grafting with acrylamide was synthesized by using Microwave assisted graft copolymerization reaction. Initially, the appropriate amount of monomer (Acrylamide) and required quantity of fenugreek gum was poured in a beaker. Then dispersed in milipore water (100 mL) and stirred at 50 °C under magnetic stirrer till all the polymers dissolved and then later different type of initiator was incorporated and stirred further for 15 min. Then mixture within glass beaker was micro waved. It was removed immediately and put a side in ice bath for cooling. If un-reacted initiator or any other products formed in the reaction mixture, it can be separated with acetone as solvent. Then graft copolymers were synthesized and dried in oven as well as grinded in to fine powder. The best grade grafted product was selected on the bases of their calculation of %G, %GE and % C (44), (86).

$\% \text{grafting}(\%G) = \frac{W_1 - W_0}{W_0} \times 100$
$\% \text{grafting efficiency}(\%GE) = \frac{W_1 - W_0}{W_2} \times 100$
$\% \text{Conversion}(\%C) = \frac{W_1}{W_2} \times 100$

Figure 5.1: Equation for calculation of % grafting % grafting efficiency and % conversion

Whereas, W_0 - quantity of fenugreek gum, W_1 - quantity of grafted copolymer,
 W_2 - quantity of acrylamide.

5.5 Experimental design

Taguchi OA Design was used to select levels orthogonal array and it is a specific quality indicator named signal/noise ratio. On account of the tested lower and upper values for each variable, it is used to reach the robustness of design. This OA design was particularly intended with symbol L8 (2^7) and it was used in the optimized study of grafting process, in which seven independent (formulation) variables and three dependent (response) variables were used. Independent variables are percentages of monomer (acrylamide) concentration (X1), gum- concentration (X2), initiator (X3), power (X4), stirring -speed (X5), time (X6), and temperature (X7) whereas, dependent variables are % yield (Y1), % grafting (Y2) and %grafting efficiency (Y3).

$$S/N = -10 \log [\sigma^2 / \mu^2] \dots\dots\dots (1)$$

5.5.1 Optimization and characterization of grafted fenugreek gum

The grafting process was optimized by studying the influence of reaction variable involved in synthesis of grafted fenugreek gum on the parameters, i.e. % yield, % grafting, % grafting efficiency and % conversion. For the optimization of grafted fenugreek gum- amount of monomer, amount of gum, amount of initiator, microwave irradiation exposure power and time were taken as variables (87), (88), (89), (90).

5.5.1.1 FTIR Spectroscopy

FTIR analyses of pure and grafted fenugreek gum sample was performed by KBr disc method. In this method, KBr and sample was mixed in a mortar and grinded to make the fine powder with pestle. The powdered sample was dried completely and was placed to cover bottom in pellet die of sample holder. The FTIR spectra was listed in the $4000-400 \text{ cm}^{-1}$ region using Perkin Elmer Spectrum version 10.5.2, Inc.

Waltham spectrometer. In the presence of gum, change in drug spectra was investigated that indicates the physical interaction of drug molecules with the gum (91), (92).

5.5.1.2 X-ray Powder Diffraction

X-ray diffractometer (X' pert Pro Malvern Analytical United Kingdom, Germany) patterns were used for phase (crystalline or amorphous) characterization of powder samples (pure and grafted gum). The experimental condition was involved for XRD analysis using K-beta filter, Cu-K α -radiation at 40 kV voltage as well as 35 mA current. Its scanning was employed over the range at 0-80°C with diffraction angle (2 θ) (91), (92).

5.5.1.3 Differential Scanning Calorimetry (DSC)

The DSC study of pure & grafted gum was conducted by Differential Scanning calorimeter (DSC2 -00347 TA, Shimadzu, japan) at heating rate from 20° to 168° C. Exact quantity of the sample was put in standard pan which was arranged at sample stage (92), (93).

5.5.1.4 NMR Studies

Solid state NMR studies were used for characterization of the spectra of pure and grafted gum. The appropriate sample was dissolved in D₂O at 60°C before observation and calibrated the chemical shifts of the sample by using tetramethylsilane (TMS) as an internal standard (47).

5.5.1.5 Scanning Electron Microscopy (SEM)

The samples (pure & grafted gum) were arranged on to the stubs using double-sided adhesive tape followed by coating along gold palladium alloy at 150-200 A° employed fine coat ion sputter. The samples were examined consequently under the SEM (JSM 6100 Jeol, Ltd. Japan) for external morphology (94), (95).

5.5.1.6 Swelling studies

The Swelling study of pure and grafted fenugreek gum was performed at triplicate. The dried polymer (1g) was put into petri dish containing 25 mL of 0.1N HCl (pH1.2) & phosphate buffer pH 6.8 for 24hr at 25°C. The extra water was

abolished from the polymer surface after different time (i.e., 2, 6 & 24hrs). Then polymers were re-weighted and the swelling was calculate by weighed on an analytical balance and percentage swelling (Ps) was determined by equation 2.

$$Ps (\%) = \frac{W_s - W_d}{W_d} \times 100 \dots\dots\dots (2)$$

Whereas, W_s and W_d -weights of swollen & dried polymer (96), (97), (98).

5.6 Preparation of Metformin HCl tablets by grafted copolymers of fenugreek gum using wet granulation method

Initially, the semisolid dough of grafted gum was prepared in minimum amount of hot water (50°C). Then Metformin HCl, MCC and PVP K30 were mixed in it and screening of mass was done through #18 mesh to get granules. Then it was dried in oven at 60°C for 20 min and further it was passed between # 18 mesh sieve. Finally, obtained granules were lubricated along purified talc (10mg/tablet) and magnesium stearate (10mg/tablet). After that tablets (1000 mg) were compressed in a rotary tablet machine with 13 mm single punch diameter and hardness range of 4–8 kg/m² (99). In each batch, 20 tablets were put together at a time.

5.6.1 Precompression Evaluation

5.6.1.1 Angle of repose

The maximal angle achieved among powder pile surface and the horizontal plane that can be estimated by funnel method. The accurately weighed powder was passed in funnel. The funnel height was regulated in this manner that tip of the funnel touch affected apex of powder mixture. The powder mixture was freely passed to flow between funnel upon surface.

The powder cone diameter was determined as well as the value of θ was determined using following equation.

$$\theta = \tan^{-1} (h/r)$$

Whereas θ = angle of repose, h = height, r = radius (100).

5.6.1.2 Bulk Density

Weighed the blended powder and put into 100 ml measuring cylinder. The occupied amount was recorded without interrupting the cylinder. Bulk density is measured by following equation:

$$\text{Bulk Density (BD)} = \frac{\text{Mass of blend}}{\text{Volume of blend}}$$

5.6.1.3 Tapped Density

Weighed the amount of powder blend and put within 100 mL measuring cylinder. The cylinder was put through to a fixed number of taps (~100 times) till the volume of powder blend possesses to reach the minimum level. The final volume was noted as well as tapped density was deliberate by following equation:

$$\text{Tapped Density (TD)} = \frac{\text{Mass of blend}}{\text{amount of blend after tapping}}$$

5.6.1.4 Hausner's Ratio

Hausner's ratio is determined by using following formula (101).

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

5.6.1.5 Carr's Index (CI)

CI assessed the rate at which the powder was packed down and to calculate the BD and TD of powder.

Compressibility index = $(\text{Tapped density} - \text{Bulk density} / \text{tapped density}) \times 100$
(102), (103).

5.6.1.6 Measurement of drying rate of granules

The drying rate was measured using an electronic moisture balance. The sample (4g) of wet granules was placed on sample plate. The loss of weight in course of drying was measured at 60°C, until the weight was constant for 30s. The % LOD was then recorded. LOD test sample measurements were made in triplicate (103), (104).

5.6.2 Post compression evaluations of tablets

In this parameter-weight variation, hardness, thickness, friability, drug content, swelling study and *in vitro* drug release were assessed.

5.6.2.1 Weight variation

In each batch, 20 tablets were randomly selected. Their individual weight was noted using digital balance which can be further compared along their average weight and its standard deviation was also calculated.

Acceptance Limit: Not more than two of the individual weights of tablet should vary along with the average weight by more than $\pm 5\%$.

5.6.2.2 Thickness

Thickness of selected twenty tablets were estimated by vernier caliper with mean \pm SD.

5.6.2.3 Hardness

Monsanto hardness tester was used for the measurement of hardness of six tablets. Acceptance limit: 5-8 kg/cm² (105).

5.6.2.4 Friability test

From each batch, select 20 tablets and put within Roche friabilator and which rotated at 25 rpm speed for 4 minutes. Subsequently, tablets were withdrawn, re-dusted, then reweighted. % Friability (%F) was determined using Eq. (1)

$$\%F = \frac{W_i - W_t}{W_i} \times 100$$

Whereas,

W_i = initial weight

W_t = tablets weight after test.

Acceptance Limit: <1%

5.6.2.5 Swelling study

After weighing tablet from each batch, they were transferred in wire baskets. Then the baskets was dipped into a 250 mL of beaker involving 200 ml of 0.1N HCl (pH 1.2) for a period of 4hr and phosphate buffer (pH6.8) for a period of 24hrs at 37°C. About last 24hrs, the basket was take out from the fluid and extra water on surface was wiped off using tissue paper and basket having tablets was weighed on electronic balance and calculate SI using following formula:

$$W_E = \frac{W_1 - W_0}{W_0} \times 100$$

Whereas,

W_E = swelling index (%)

W_0 = dry weight of tablet plus basket

W_1 = weight of wet tablet plus basket after removal from medium (106).

5.6.2.6 Drug content

The uniformity of content was calculated in triplicate, by powdering the tablet from experimental batch in pestle mortar. The powder equivalent to 500 mg of Metformin HCl was added in 100 mL of water and sonicated for 10 minute. Powder equivalent to 10 mg drug was mixed in methanol (100 mL). Then solution was filtered using Whatman filter paper and absorbance was determined at 232 nm against blank sample (87).

5.6.2.7 *In vitro* release study of tablet batches

The drug release study of tablet batches was carried in triplicate using three tablets from all batches in USP type I dissolution apparatus. The test was carried out in two different dissolution media-900 mL of 0.1N HCl and phosphate buffer (pH 6.8) at 37.0 ± 0.5 °C for 24hrs in 100 rpm. The release rate of tablets were noted for 2hr in dissolution medium 0.1N HCl and after that in phosphate buffer pH 6.8 for 24hrs. An aliquot of sample (5mL) were removed and substitute with another 5 mL fresh dissolution medium periodically. The %drug release within sample was measured in UV at the 232nm (107), (108).

5.7 Experimental design

As per standard protocol, design expert software- central-composite design (CCD) was employed to optimized best batch of SR grafted formulation. On account of Preformulation study, the quantity of grafted fenugreek gum (X1) and PVP K30 (X2) was preferred as independent factors, which were considered at three levels of dependent factor (% drug released within 1 hr (Y1), % drug released within 8 hr (Y2) & time to 50% drug release (Y3)). During study, an account of 13 trial experiments run with their factor combinations and coded levels (109).

5.7.1 Statistical Analysis

On account of ANOVA provision, establish the statistical data of polynomials using Design expert software. Three-dimensional (3D) response surface plots as well as two dimensional (2-D) contour plots were established which were used to found interaction effects on the response factor. Thirteen optimized checkpoints formulation was preferred to approved the selected experimental design with polynomial equations. Checkpoints batch were prepared as well as assessed their various response properties which were quantitatively correlate with predicted and observed response using linear regression plots. Obtained data were put through multiple regression analysis and result was obtained using following equations (1, 2, 3).

$$Y_1 = \beta_0 + \beta_1 A - \beta_2 B + \beta_3 AB + \beta_4 A^2 + \beta_5 B^2 \dots\dots\dots (1)$$

$$Y_2 = \beta_0 - \beta_1 A + \beta_2 B + \beta_3 AB + \beta_4 A^2 - \beta_5 B^2 \dots\dots\dots (2)$$

$$Y_3 = \beta_0 + \beta_1 A - \beta_2 B - \beta_3 AB - \beta_4 A^2 - \beta_5 B^2 \dots\dots\dots (3)$$

The optimized granules were characterized by pre- compression variables and the tablets were characterized by post compression variables.

5.7.2 Fitting the model into data

According to Design expert software, response data for all formulations was used in to quadratic model. Quadratic model was best in this software for response Y₁, Y₂ & Y₃ (% drug released within 1 hr, % drug released within 8 hrs, time to 50% drug release) with full model (FM) polynomial equation (110), (111), (112).

5.8 In vitro release study of optimized fenugreek grafted tablet (TS9), fenugreek gum tablet and Marketed formulation

The drug release study of optimized grafted tablet (TS9), ungrafted fenugreek gum tablet and marketed formulation [GLUCOMET SR 500 (500 mg)] was performed triplicate employing USP type I dissolution apparatus (113), (114), (115).

5.9 Drug release kinetics

The data from dissolution studies was analyzed and fitted within distinct kinetics model equations for calculating the % drug release (Zero order equation, First order, Higuchi's model and Korsmeyer Peppas equation) of grafted, ungrafted as well as marketed formulation of Metformin HCl tablet.

5.10 Stability studies

Stability studies were done to evaluate the expiration of formulation and to understand storage conditions of product, according to ICH guidelines. Optimized tablet batch (TS9) was kept in polypropylene bottle and evaluated for 180 days, at 30 days intervals. Then the optimized tablet batch (TS9) was stored in humidity chamber by maintaining the temperature/humidity at 40 ± 2 °C/ $75 \pm 5\%$ RH and 25 ± 2 °C/ $60 \pm 5\%$ RH for 6 months at room temperature and refrigerate (4 ± 2 °C) for 6 months. Samples were removed subsequently at the end of each month and assessed their change in physical aspect, % friability, hardness and % drug content. The storage effect of stability was determined by UV Spectrometer

5.11 Pharmacodynamics optimization of development formulation

The procedure of experimental animal protocol was authorized by Institutional animal ethical committee (BMRL/IAEC/2021-20) with registration no. 2005/PO/RcBT/S/18/CPCSEA). Animals were housed with standard rodent diet (Ashirwad industries, Chandigarh, India) and water (*ad libitum*) as per the principles for the goal of care, control and supervision of experiments on animals (CPCSEA), Ministry of Environment and Forest, Government of India. Adult male wistar rats (250-300g) were kept in standard environmental conditions at 23 ± 2 °C and %RH 50-55%. Photoperiod was 12hrs light and 12 hrs dark cycle.

Induction of Diabetes Mellitus

After overnight fasting, diabetes was induced by administration of 45 mg/kg of streptozotocin, which was processed in citrate buffer (pH 4.5), 15 minutes after nicotinamide (230 mg/kg *i.p.* NAD). After 72 hrs of STZ injection, Fasting blood glucose (FBG) level was noted. Rats having FBG level greater than 200 mg/dl were involved in further research (116),(117).

Various doses of grafted TS9 formulation were administered to rat in mini tablet (lower dose 50mg/kg, medium dose 75mg/kg, higher dose 100mg/for 21 days. Ungrafted fenugreek formulation at a dose of 100mg/kg was used. Experimental animals were divided in distinct seven groups having six rats in each group.

5.11.1 Estimation of Biochemical parameters

5.11.1.1 Body weight

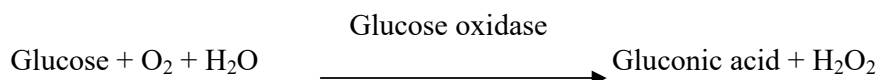
Before the induction of diabetes, body weight was measured during the treatment period. Body weight of animals was further noted for 21 days randomly.

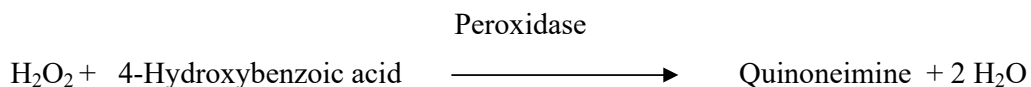
5.11.1.2 Blood Glucose Level

Fasting Blood glucose level was evaluate dafter 72 hrs of diabetes induced, at interval of 21 days. Blood glucose level was estimated colorimetrically at 530 nm along Glucose Oxidase Peroxidase (GOD-POD) method by employing commercial applicable kit which was purchased from Transasia Bio Medical Ltd., Daman.

Principle:

Quantitative estimation of glucose level was done by GOD-POD method whichis based upon principle that glucose oxidase enzyme in the presence of oxygen reacts with glucose and water to make gluconic acid and hydrogen peroxide. Further, hydrogen peroxide proceeds with 4-hydroxy benzoic acid to gives quinoneimine dye and water with enzyme peroxidase and forms pink reddish coloured end product (quinoneimine), having an absorptionn maxima at 530 nm. The intensity of pink reddish colour is proportional to glucose concentration.





Procedure:

10 µL of serum and 1000 µL of glucose working reagent was taken and mixed thoroughly. The solution was incubated for 15 minutes at 37°C. Then absorbance was measured against blank at 530 nm (119)

Table 5.4: Conc. of solution in each test tubes as Blank, standard and Test as follows:

	Blank solution	Standard solution	Test solution
Glucose colour reagent	1000 µL	1000 µL	1000 µL
Distilled water	10 µL	----	----
Standard	----	10 µL	----
Sample	----	----	10 µL

$$\text{Glucose Conc. (mg/dL)} = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times \text{Conc. of standard (mg/dL)}$$

5.11.1.3. Lipid profile assay

Total cholesterol (TC), triglycerides (TG), high density lipoprotein(HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were estimated on 1st day as well as on 21th day using commercial available enzymatic kits, from Bayer Diagnostic kit (Bayer Diagnostic India Ltd).

5.11.1.3.1 Effect of Cholesterol

Allain *et al.*, 1974 described the enzymatic method for estimation of total serum cholesterol (118).

Principle:

The total amount of cholesterol in test sample is directly proportional to the intensity of red complex (red quinone) that estimated at 505 nm.

Procedure:

Reconstituted-reagents were labeled by mixing reagent 1 and reagent 2 by gentle swirling. Three test tubes were taken, labeled as Blank (B), Standard (S) and Test solution (T). Dispensed various reagents inside three test tube as per the quantities mentioned in the Table 5.5. Incubated for 5 minutes at 37°C and intensity of the colour was measured spectrophotometrically at 505 nm. The concentration of the standard cholesterol was 200 mg/dL.

Table 5.5: Conc. of solution in each test tubes as Blank, Standard and Test are as follows:

	Blank solution	Standard solution	Test solution
Reconstituted reagent	1mL	1mL	1mL
Distilled water	10 μ L	----	----
Standard	----	10 μ L	----
Sample	----	----	10 μ L

The cholesterol conc. in the sample is directly proportional to the intensity of red complex (Red Quinone) that determined at 505 nm.

5.11.1.3.2 Effect of Triglycerides level

Estimation of triglycerides level is based on GPO-Trinder method, described by Dietz AA, 1972 (119).

Principle:

When glycerol reacts enzymatically they give a red coloured dye. The colour intensity is directly proportional to quantity of TGs within sample.

Procedure:

Mixed contents of reagent 1 with aqua 4 by gentle swirling and labeled it as reconstituted reagent. At room temperature it allowed to stand for 10 minutes. Three test tubes (blank, standard and test) were taken, label them and dispense the various reagents as per the quantities mentioned in the Table 5.6. Incubated for 10 minutes at 37°C and colour intensity was measured at 505 nm.

Table 5.6: Conc. of solution in each test tubes as blank, standard and test as follows:

	Blank solution	Standard solution	Test solution
Reconstituted reagent	1000 µL	1000 µL	1000 µL
Distilled water	10 µL	-	-
Standard	-	10 µL	-
Supernatant	-	-	10 µL

Calculation:

Absorbance of test \longrightarrow Serum triglycerides conc.= Standard absorbance x
Conc. of standard(mg/dL)

5.11.1.3.3 Effect of HDL (High Density Lipoprotein) Level

Phosphotungstate method was used as for estimation of HDL decribed by Lopes-Virella *et al.*, 1977 (120).

Principle:

In serum or plasma, the fraction of chylomicron, VLDL and LDL was isolated from HDL by precipatating along with phosphotungstic acid and magnesium chloride. After centrifugation, cholesterol remains supernatant in HDL portion and was further assayed with enzymatic cholesterol method.

Procedure:

The content of reagent 1 and reagent 1A was mixed and labeled as reconstituted reagent. The serum (0.2 mL) was taken in a centrifuge tube and precipitating reagent 2 (0.2 mL) was added in it. Then centrifugated at 1500 rpm for 10 min. Clear supernatant was separated out and it was intended for the estimation of HDLC. Three test tubes (blank, standard and test) were taken and labeled respectively and dispense the various reagents as per the quantities mentioned in the Table 5.7. Incubated for 5 minutes at 37⁰C and colour intensity was measured at 505 nm.

Table 5.7: Concentration of solution in each test tubes as blank, standard and test as follows:

	Blank solution	Standard solution	Test solution
Reconstituted reagent	1mL	1mL	1mL
Distilled water	20μL	----	----
Standard	----	20μL	----
Supernatant	----	----	20μL

5.11.1.3.4 Effect of LDL (Low Density Lipoprotein)

Iranian formula (Dietz *et al*, 1972)

$$\text{LDL} = (\text{Cholesterol, mg/dL}) - (\text{HDL Cholesterol, mg/dL}) - \text{Triglycerides} / 5$$

$$\text{TC}/1.19 + \text{TG}/1.9 - \text{HDL}/1.1 - 38 \text{ or } \text{LDL} = \text{TC} - (\text{HDL} + \text{TG}/5)$$

5.11.2 Assessment of antioxidant parameters of antioxidant enzymes like CAT, MDA, GST (Catalase, Malondialdehyde, Gluthione -S-transferase)

Oxidative stress is an variation among production and accumulation of O₂ reactive species in cells and tissues. By Increasing the level of glucose and insulin to rats suffering from diabetes, develops macro angiopathies which cause oxidative stress. Rats were scarified under chloroform anaesthia. Initially, the tissue were isolated and washed with cool solution of NaCl to discard approximately blood desirable. Liver was homogenated (5%w/v) in cool potassium phosphate buffer (50mm, PH 7.4) using Remi Homogenizer. The unbroken cell as well as cell debris

was separated using centrifugation at 3000rpm for 10min. Then supernatant so attained that was used to evaluate oxidative stress by recording the activities of the MDA, GST and CAT.

Determination of Malondialdehyde (MDA):

Preparation of stock solution: A standard of 1,1,3,3 tetra-ethoxy propane (TEP) was used. For preparation of 1M stock solution, 25 μ L TEP was dispersed in 100mL of de-ionized water. MDA was processed by hydrolysis of 1mL TEP stock solution in 50mL of 1% sulphuric acid and incubated for 2hrs at room temperature. Then the solution was reserved at 40°C and used after 4 weeks. The resulting solution of MDA standard (20n mol/mL) was further diluted with 1% sulphuric acid. Then obtained yield lies in concentration within 1-20n mol/mL.

Procedure: Take aliquot 250 μ L diluted plasma in the tube, 50 μ L of 6M aqueous NaOH was put in it. Then incubate supernatant solution at 60°C on water bath for 30min. The protein bound MDA was precipitated by adding 125 μ L of 35% (v/v) perchloric acid. Then centrifuged at 6000 rpm for 10min. The obtained supernatant put in to vial and mixed with 50 μ L of 2,4 dinitrophenyl-Hydrazine. Then incubated for 30 min at room temperature.

Determination of GSH activity: Take 1mL of supernatant and 1mL of Trichloroacetate (10% w/v H₂O). Then centrifuge it at 10000 rpm for 10min. 0.5mL supernatant was further taken, in which 2ml of disodium Hydrogen phosphate was added and then 0.25mL of DTNB (5,5 dithio-bis 2-nitro benzoic acid) (0.001M in 1.1%w/v sodium citrate) was added and mixed. Absorbance were checked at 412nm.

Determination of CAT Activity: Take 0.05 ml of sample containing catalase enzyme and also added Phosphate buffer (1.95 mL) and Hydrogen peroxide (1mL) in test and standard tube. Then test tube were vortexed and incubated at 37°C for two minutes. The aliquots from each test tube were removed and transferred to different tube containing ammonium molybdate. The test tubes were mixed well, 3mL pyrogallol red was added in each tube. The zero time valuation was noted at that point to which total amount of pyrogallol red solution mixed in test tube. Absorbance taken at 232 nm for 30 sec at 15 sec interval (121), (122), (123).

CHAPTER-VI

RESULT AND DISCUSSION

6.1 Preformulation studies

6.1.1 Organoleptic properties of drug (Metformin HCl) : As shown in Table 6.1

Table 6.1: Organoleptic properties of Metformin HCl

S. No.	Description	Observation
1.	Colour	White
2.	Odour	Odourless
3.	Appearance	Solid
4.	State	Hygroscopic

6.1.2 DSC study

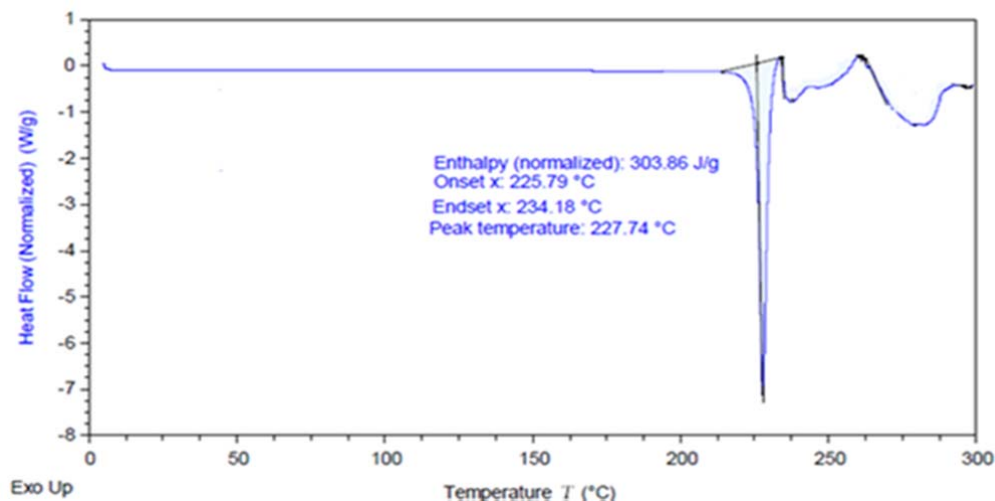


Figure 6.1: DSC of Metformin HCl

Table 6.2: Melting point of Metformin HCl by DSC

S. No.	Drug	Specification	Observation
1	Metformin HCl	225.79 to 234.77 °C	234.18°C

Discussion: The melting point of Metformin HCl observed within range 234.18°C; Thus drug sample was free from impurities.

6.1.3 Determination of Absorption maxima

6.1.3.1 Determination of absorption maxima in phosphate buffer pH 6.8

Table 6.3: Absorption maxima (λ_{\max}) of Metformin HCl in phosphate buffer pH 6.8

Absorption maxima (λ_{\max})	
Observed	Reference
233	233

The result of metformin HCl by UV spectroscopy is shown in Figure 6.2.

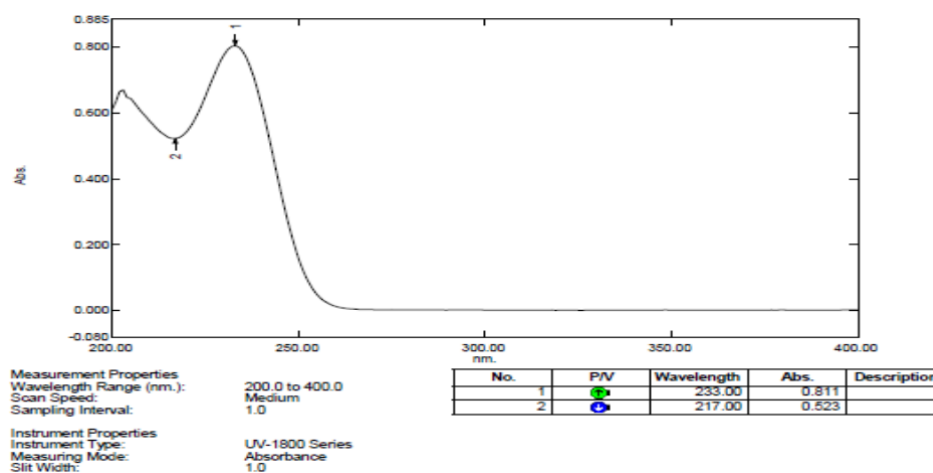


Figure 6.2: UV-visible spectrum of Metformin HCl in phosphate buffer pH 6.8

Discussion: The λ_{\max} of metformin HCl was marked to be 233 nm.

Calibration curve of Metformin HCl

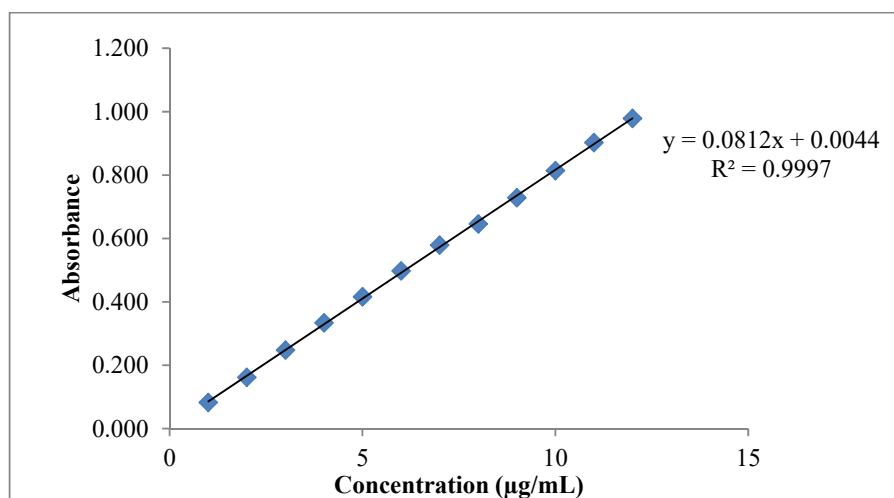


Figure 6.3: Calibration curve of Metformin HCl in phosphate buffer pH 6.8

Table 6.4: Calibration curve data of Metformin HCl in phosphate buffer pH 6.8

Concentration($\mu\text{g/mL}$)	Max. absorbance (Mean \pm SD) n=3	Statistical data
1	0.08 \pm 0.02	
2	0.16 \pm 0.02	
3	0.24 \pm 0.01	
4	0.33 \pm 0.02	
5	0.41 \pm 0.03	
6	0.49 \pm 0.02	R^2 value= 0.999
7	0.57 \pm 0.02	Regression equation
8	0.64 \pm 0.01	$y = 0.0812x - 0.004$
9	0.72 \pm 0.01	
10	0.81 \pm 0.03	
11	0.90 \pm 0.01	
12	0.97 \pm 0.03	

Discussion: The data was found to be good linear for Metformin HCl lies within range 1 to 12 $\mu\text{g/mL}$ with correlation coefficient (R^2) 0.999.

6.1.3.2 Determination of absorption maxima (λ_{max}) in water

Table 6.5: Absorption maxima (λ_{max}) of Metformin HCl in water

Absorption maxima (λ_{max})	
Observed	Reference
232	232

The result of Metformin HCl by UV shown in Figure 6.4.

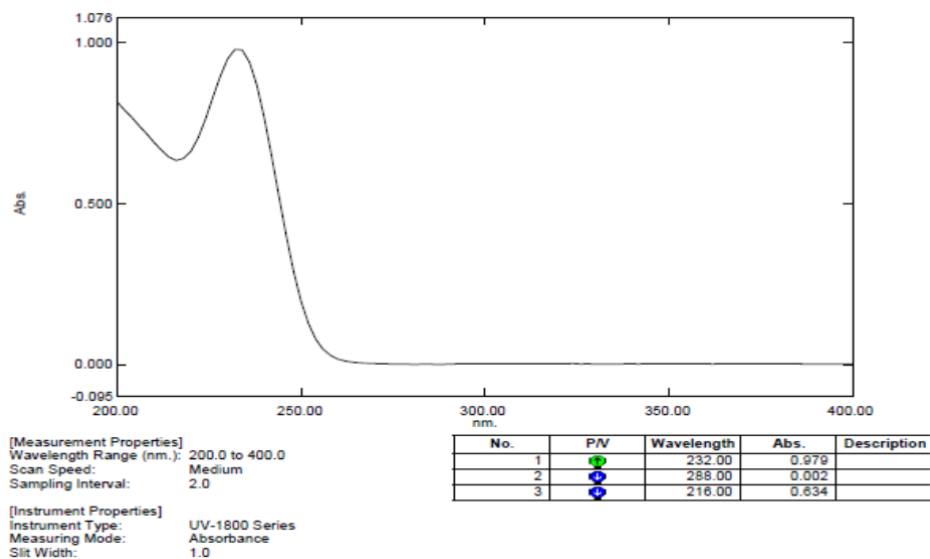


Figure 6.4: UV spectrum of Metformin HCl in water

Discussion: The λ_{\max} of Metformin HCl was observed at 232 nm.

Calibration Curve of Metformin HCl

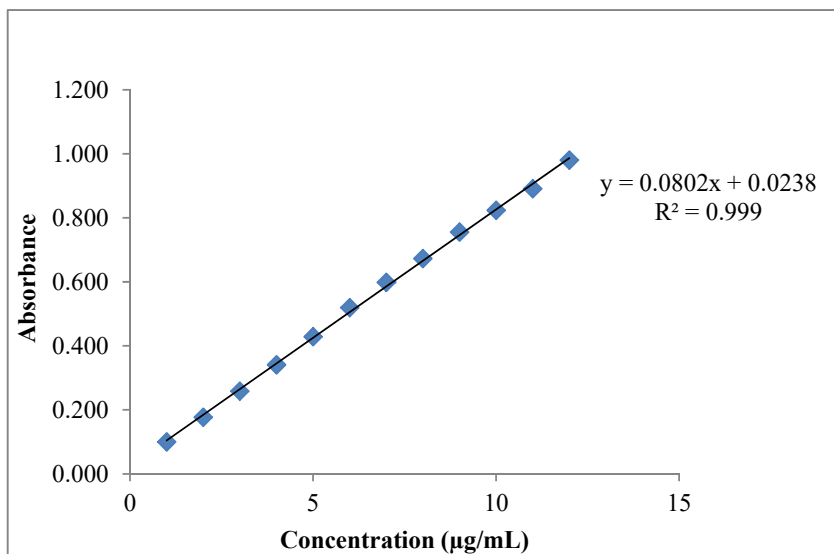


Figure 6.5: Calibration curve of Metformin HCl in water

Table 6.6: Calibration curve data of metformin HCl in water

Concentration($\mu\text{g/mL}$)	Maximum absorbance (Mean \pm SD)n=3	Statistical data
1	0.100 \pm 0.00	
2	0.177 \pm 0.00	
3	0.258 \pm 0.01	
4	0.341 \pm 0.01	
5	0.428 \pm 0.01	
6	0.519 \pm 0.01	
7	0.598 \pm 0.01	R^2 value= 0.999
8	0.673 \pm 0.01	Regression equation $y = 0.0802x - 0.0238$
9	0.755 \pm 0.01	
10	0.824 \pm 0.01	
11	0.891 \pm 0.01	
12	0.980 \pm 0.01	

Discussion: For Metformin HCl, the calibration data observed to be good linear within range 1 to 12 $\mu\text{g/mL}$ with correlation coefficient (R^2) 0.999.

6.1.3.3 Determination of absorption maxima in methanol

Table 6.7: Absorption maxima (λ_{max}) of Metformin HCl in methanol

Absorption maxima (λ_{max})	
Observed	Reference
237	237

The results of Metformin HCl in methanol by UV shown in Figure 6.6.

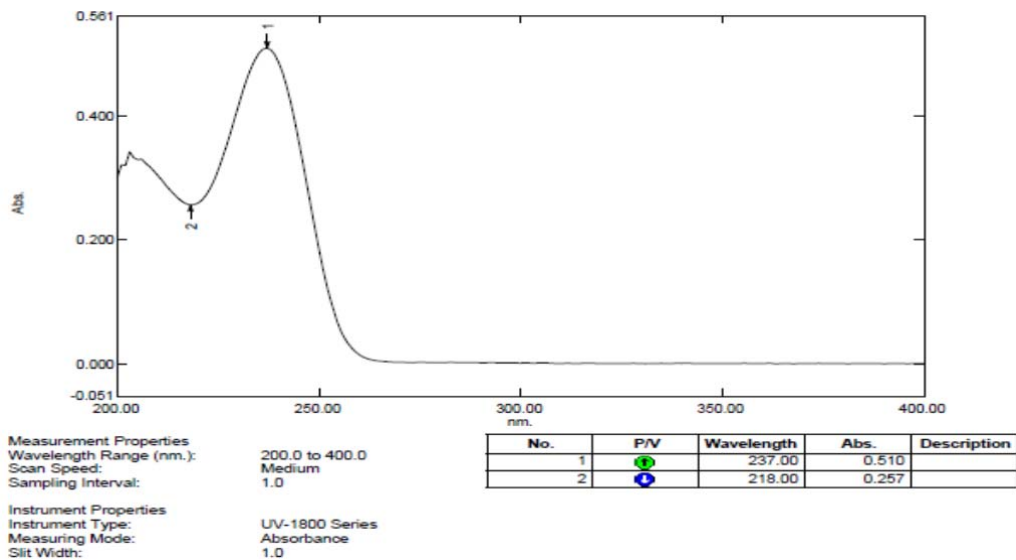


Figure 6.6: UV spectrum of Metformin HCl in methanol

Discussion: The λ_{max} of Metformin HCl noticed at 237 nm.

Calibration curve of Metformin HCl

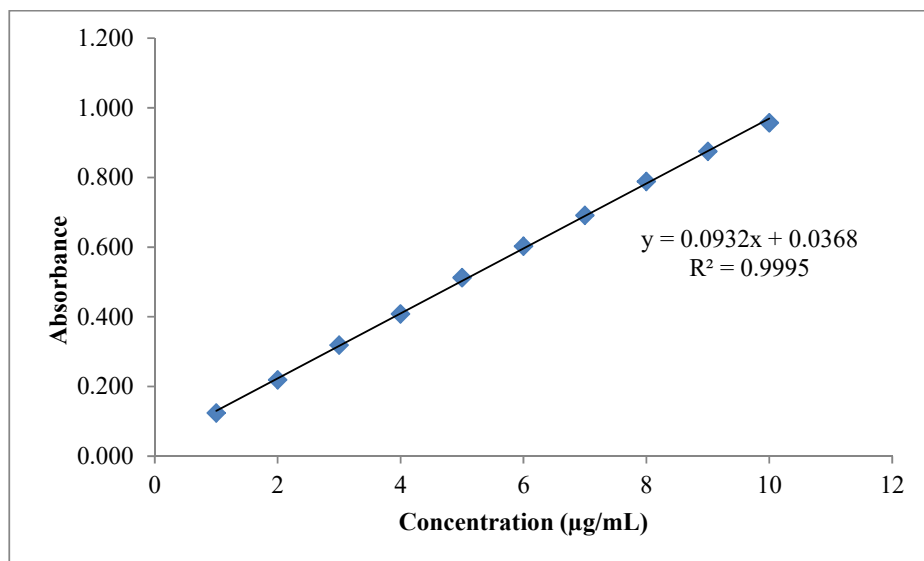


Figure 6.7: Calibration curve data of Metformin HCl in methanol

Table 6.8: Calibration curve data of metformin HCl in methanol

Concentration($\mu\text{g/mL}$)	Maximum absorbance (mean \pm SD) n=3	Statistical data
1	0.12 \pm 0.01	R ² value= 0.999 Regression equation y = 0.0932x - 0.0368
2	0.21 \pm 0.03	
3	0.31 \pm 0.01	
4	0.40 \pm 0.02	
5	0.51 \pm 0.03	
6	0.60 \pm 0.01	
7	0.69 \pm 0.02	
8	0.78 \pm 0.04	
9	0.87 \pm 0.01	
10	0.95 \pm 0.01	

Discussion: For Metformin HCl, the calibration data observed to be good linear within range 1-10 $\mu\text{g/ml}$ with correlation coefficient (R^2) 0.999.

6.1.3.4 Determination of absorption maxima in 0.1N HCl pH1.2

Table 6.9: Absorption maxima of Metformin HCl in 0.1N HCl pH1.2

Absorption maxima (λ_{max})	
Observed	Reference
233	233

The result of Metformin HCl in 0.1N HCl by UV is shown in Figure 6.8.

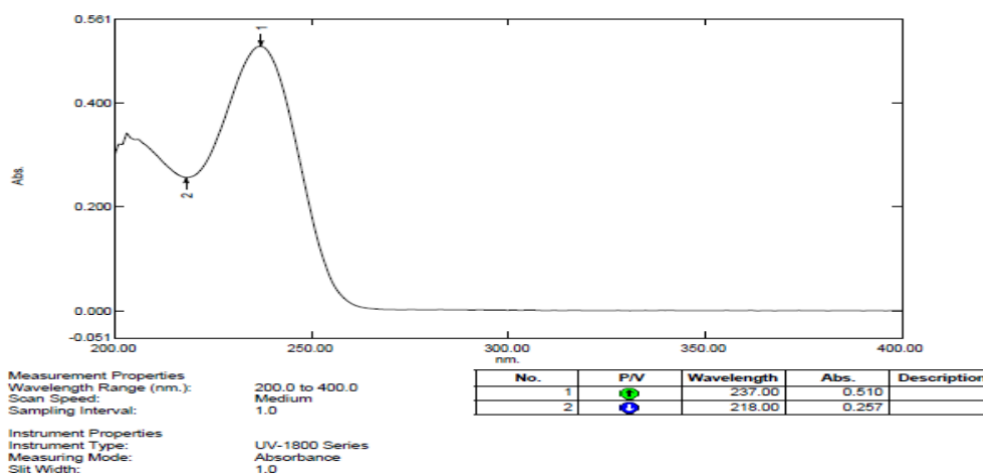


Figure 6.8: UV spectrum of Metformin HCl in 0.1N HCl

Discussion:Maximum wavelength of Metformin HCl in 0.1N HCl was observed at 233 nm.

Calibration Curve of Metformin HCl

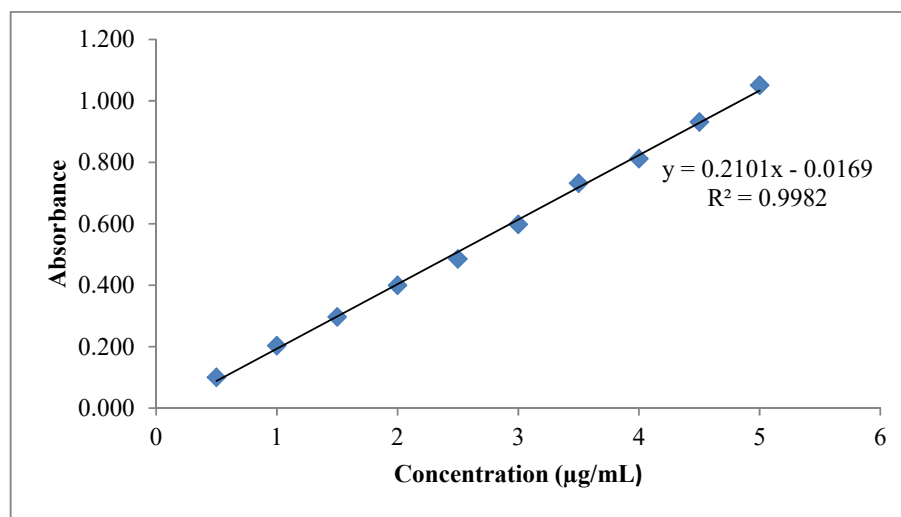


Figure 6.9: Calibration curve of Metformin HCl in 0.1N HCl pH1.2

Table 6.10: Calibration curve data of Metformin HCl in 0.1N HCl pH1.2

Concentration(µg/mL)	Maximum absorbance (Mean ±SD) n=3	Statistical data
0.5	0.10±0.01	R^2 value= 0.998 Regression equation $y = 0.210x - 0.0169$
1	0.20±0.01	
1.5	0.29±0.01	
2	0.40±0.01	
2.5	0.48±0.03	
3	0.59±0.01	
3.5	0.73±0.01	
4	0.81±0.01	
4.5	0.93±0.01	
5	1.05±0.01	

Discussion: For Metformin HCl, the data was observed to be good linear within range of 0.5 to 5 µg/mL along correlation coefficient (R^2) of 0.998.

6.1.4 Solubility studies



Figure 6.10: Solubility's Studies

Table 6.11: Solubility study profile data of Metformin HCl in distinct solvents (mean \pm SD, n=3)

Solvents	Observed solubility(mg/mL)	Descriptive term
Buffer 6.8 pH	716.70 \pm 0.68	Freely soluble
Water	102.20 \pm 0.19	Freely soluble
Methanol	47.54 \pm 0.26	Soluble
0.1 N HCl	852.91 \pm 0.72	Freely soluble

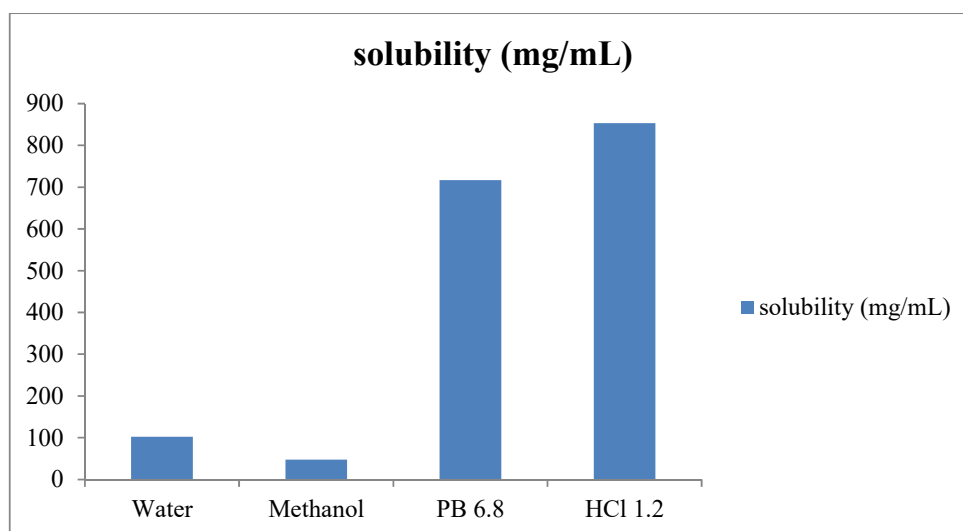


Figure 6.11: Solubility profile of *Metformin HCl* in different solvent

Discussion: From the solubility studies, the result indicates that *Metformin HCl* was soluble in methanol, freely soluble in phosphate buffer pH 6.8, 0.1NHCl and water.

6.1.5 Determination of partition coefficient of Metformin HCl

The Partition-coefficient of *Metformin HCl* is shown in Table 6.12.



Figure 6.12: Partition coefficient studies

Table 6.12: Partition coefficient of *Metformin HCl*(mean \pm SD, n=3)

Drug	Partition coefficient (log P)
<i>Metformin HCl</i>	-2.64 \pm 0.06

Discussion: The partition coefficient was recognized to be -2.64 \pm 0.06 which shows the drug is freely water soluble.

6.1.6 Validation of Metformin HCl by RP-HPLC method

Table 6.13: Chromatographic conditions

Chromatographic conditions	
Stationary Phase	C ₁₈ , 250 \times 4.6 mm, 5 μ m particle size, Nucleodur
Elution mode	Isocratic elution mode (66:34 v/v)
Absorption maxima	232nm
Column temperature	40 $^{\circ}$ C
Flow rate	1.3 mL/min
Injection volume	20 μ L
Diluent	Water
Run time	15 min

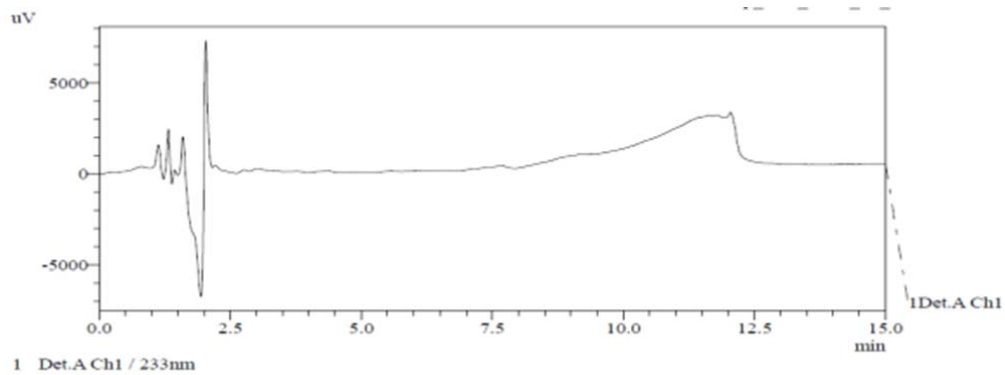


Figure 6.13: Chromatogram of Blank solution

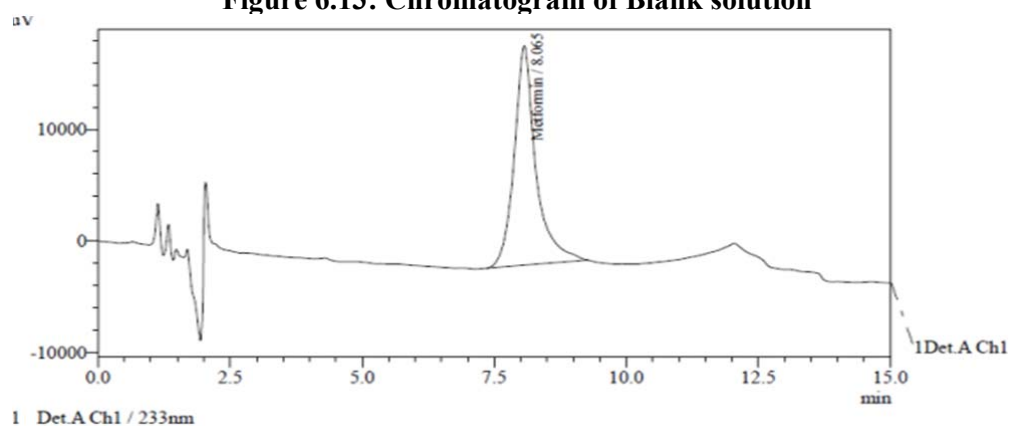


Figure 6.14: Chromatogram of standard solution (10µg/mL)

Table 6.14: Retention time of drugs (Metformin HCl)

Drug	Retention time (min)
Metformin HCl	9.406

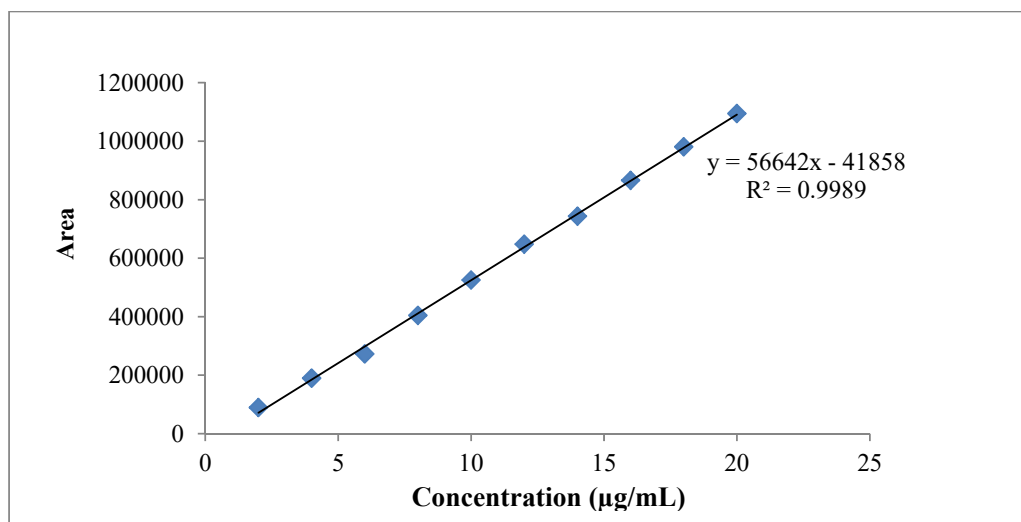
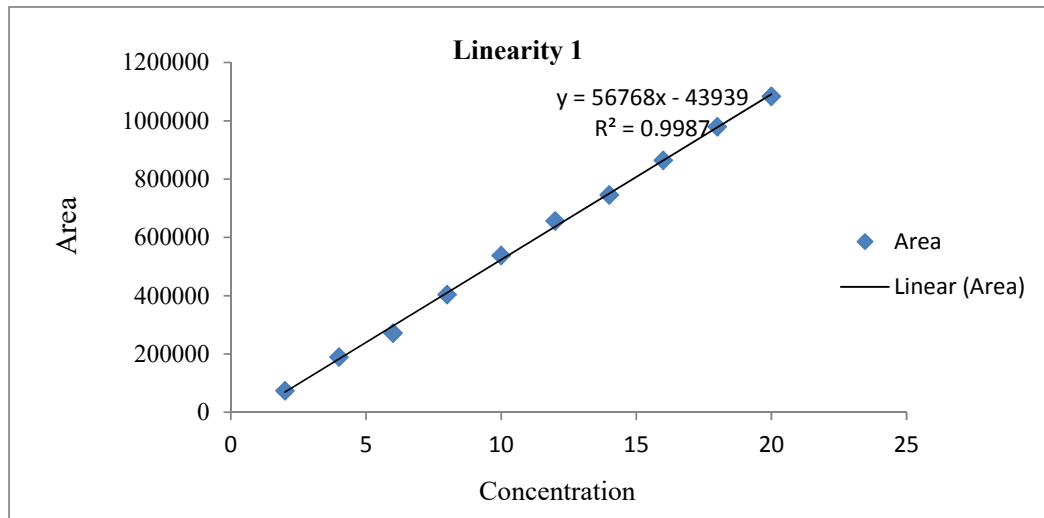
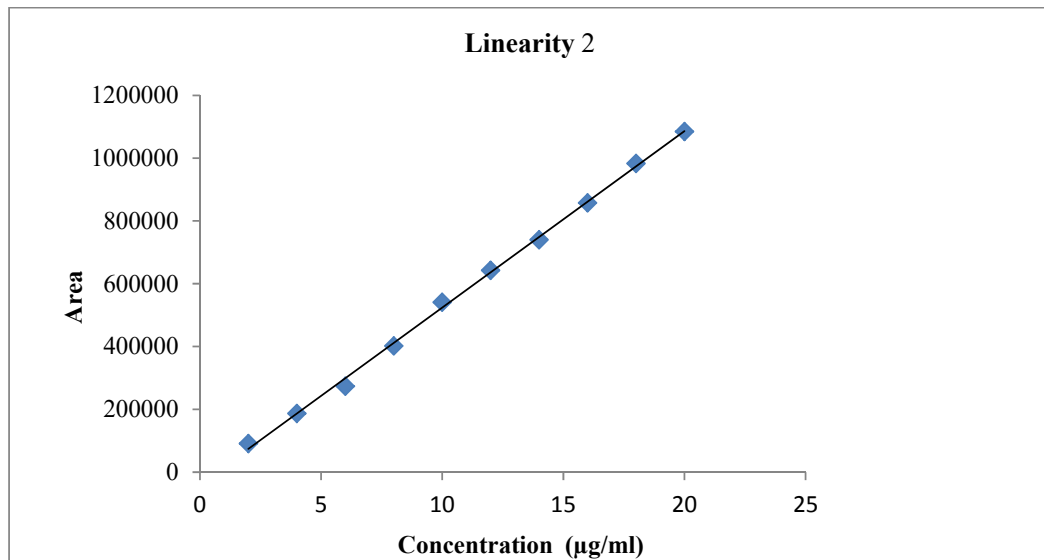


Figure 6.15: Calibration curve of Metformin HCl by RP-HPLC

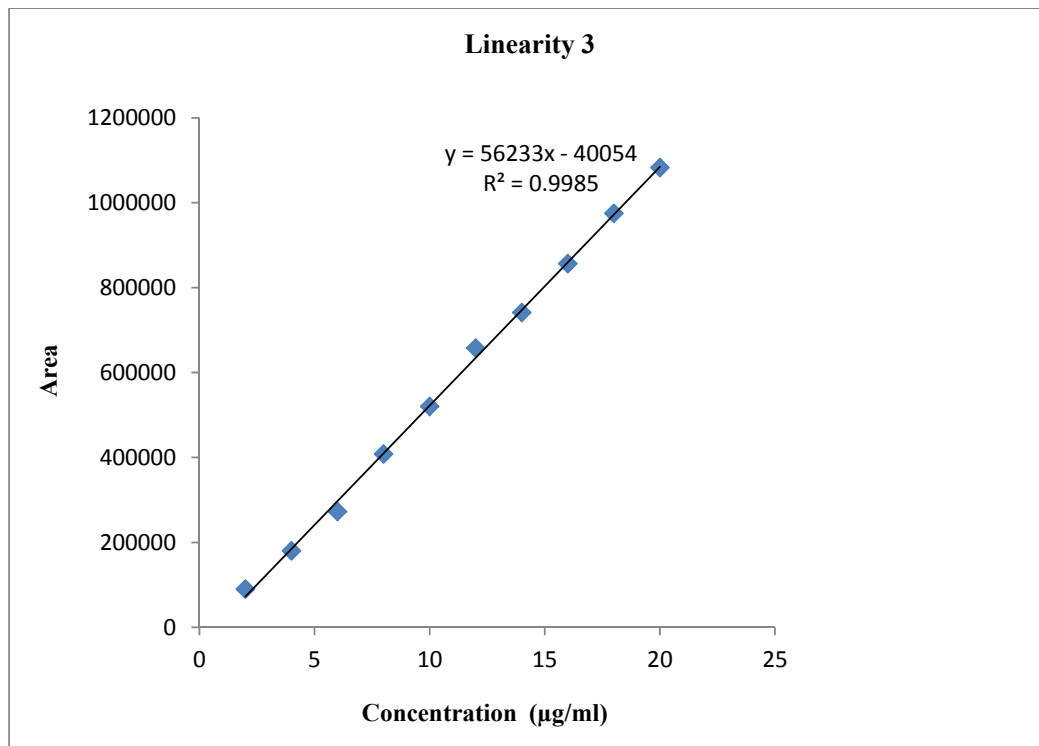
6.1.6.1 Linearity



6.16: (a) Linearity 1 curve of Metformin HCl by RP-HPLC



6.16: (b) Linearity 2 curve of Metformin HCl by RP-HPLC



6.16: (c) Linearity 3 curve of Metformin HCl by RP-HPLC

Table 6.15: Linearity table of Metformin HCl

Conc. (µg/mL)	Area 1	Area 2	Area 3	SD	Mean	%RSD	Statistical Analysis
2	73560	90942	90084	9797.2	84862.00	11.54	Intercept = 40764 Slope =56408 Straight line equation $y=56408x$ Regression coefficient $R^2=$ 0.998
4	189368	186802	180100	4785.3	185423.33	2.580	
6	271430	273528	272635	1052.8	272531.00	0.386	
8	403423	401737	408221	3364.1	404460.33	0.831	
10	537865	540878	520296	11115.	533013.00	2.08	
12	656157	642357	657694	8446.1	652069.33	1.29	
14	745678	740123	741345	2919.0	742382.00	0.393	
16	864023	857436	856745	4017.3	859401.33	0.674	
18	979633	983076	975199	3948.8	979302.66	0.403	
20	1083958	1084663	1082721	983.06	1083780.66	0.0907	

Limit of Detection (LOD)

LOD= (3.3*SD of intercepts)/Mean of slope

LOD= (3.3*2886.256226)/ 56408 = 0.168852743µg/mL

Limit of Quantitation (LOQ)

LOQ= (10*SD of intercepts)/Mean of slope

LOQ= (10*2886.256226)/ 56408 = 0.51167498µg/mL

6.1.6.2 Precision**Table 6.16: Precision of Metformin HCl**

Precision results showing repeatability	
Conc.(µg/mL)	Area
10	529166
10	537917
10	540757
10	544502
10	542886
10	538684
Mean	538985.33
SD	2465.865
%RSD	045750147
Intra-day precision	
Conc.(µg/mL)	Area
10	539603
10	547149
10	525295
10	534427
10	543178
10	541712
Mean	538560.66
SD	5636.99
%RSD	1.04
Interday precision	
Conc.(µg/mL)	Area
10	533197
10	534958
10	532395
10	532395
10	540352
10	540438
10	547149
Mean	538081.5
SD	5636.99
%RSD	1.04

6.1.6.3 Accuracy

Table 6.17: Accuracy readings of Metformin HCl

Level of addition	Area	Statistical Analysis		
		Mean	SD	%RSD
90%	979258	985173.66	10405.121.05	0.422
90%	997188			
90%	979075			
100%	1076272	1081528.33	4569.52	0.241
100%	1083758			
100%	1084555			
110%	1215892	1216276.33	2931.45	0.241
110%	1219381			
110%	1213556			

6.1.6.4 Robustness

Table 6.18: Robustness results for Metformin HCl

Change in wavelength		
235nm		
Concentration (µg/mL)	Area	Statistical analysis
10	501111	Mean = 503110.66SD =2975.395 %RSD =0.591
10	506530	
10	501691	
230 nm		
Concentration (µg/mL)	Area	Statistical analysis
10	533568	Mean = 529556.66SD =4312.914 %RSD =0.814
10	524995	
10	530107	
Change in Flow rate (1.0 mL/min.)		
Concentration (µg/mL)	Area	Statistical analysis
10	684052	Mean =686864SD =2572.0005 %RSD = 0.208
10	687082	
10	689458	
(1.5 mL/min.)		
Concentration (µg/mL)	Area	Statistical analysis
10	522528	Mean=522870.00SD =2551.250 %RSD =0.487
10	520507	
10	525575	

6.1.6.5 Ruggedness

Table 6.19: Results showing Ruggedness

Analyst 1		
Concentration (µg/mL)	Area	Statistical analysis
10	538684	Mean = 530689SD = 7295.732%RSD = 1.374
10	528991	
10	524392	
Analyst 2		
Concentration (µg/mL)	Area	Statistical analysis
10	535852	Mean =544122SD = 9421.322%RSD = 1.7314
10	542136	
10	554378	

6.1.6.6 Forced degradation

6.1.6.6.1 Acid degradation

Table 6.20: Concentration after acid degradation of Metformin HCl

Time (hrs)	Area	Corresp.conc	% Degradation
1hr	524735	10.00	2.14
1hr	518734	9.89	3.18
2hrs	501334	9.58	6.19
2hrs	507405	9.69	5.14
4hrs	468026	8.99	11.97
4hrs	472063	9.06	11.27

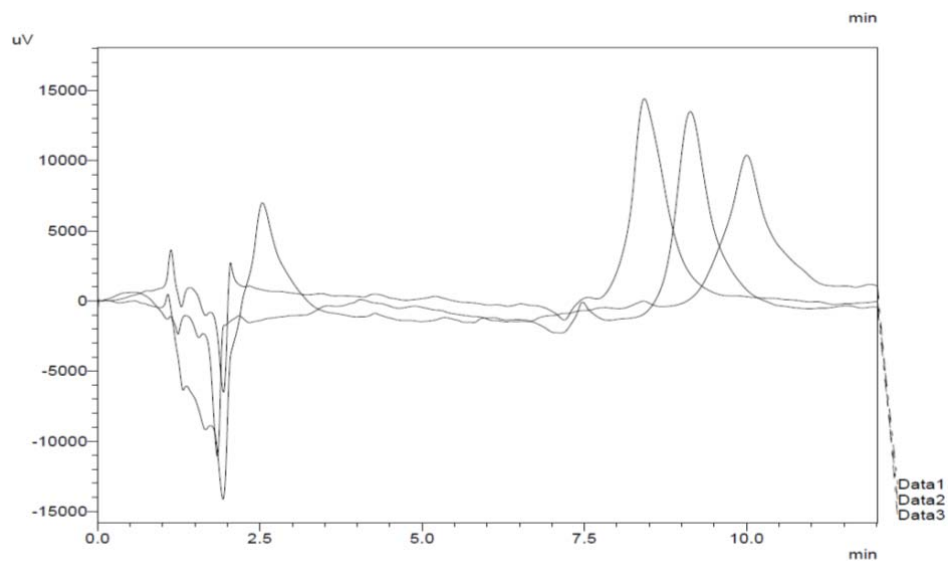


Figure 6.17: Chromatogram of Metformin HCl during acid degradation at 1hr, 2hrs and 4hrs

6.1.6.6.2 Base degradation

Table 6.21: Concentration after base degradation of Metformin HCl

Time (hrs)	Area	Corresp. Conc.	% degradation
1hr	501363	9.58	6.19
1hr	514882	9.82	3.85
2hrs	478373	9.17	10.18
2hrs	486319	9.32	8.80
4hrs	319690	6.36	37.68
4hrs	322944	6.42	37.12

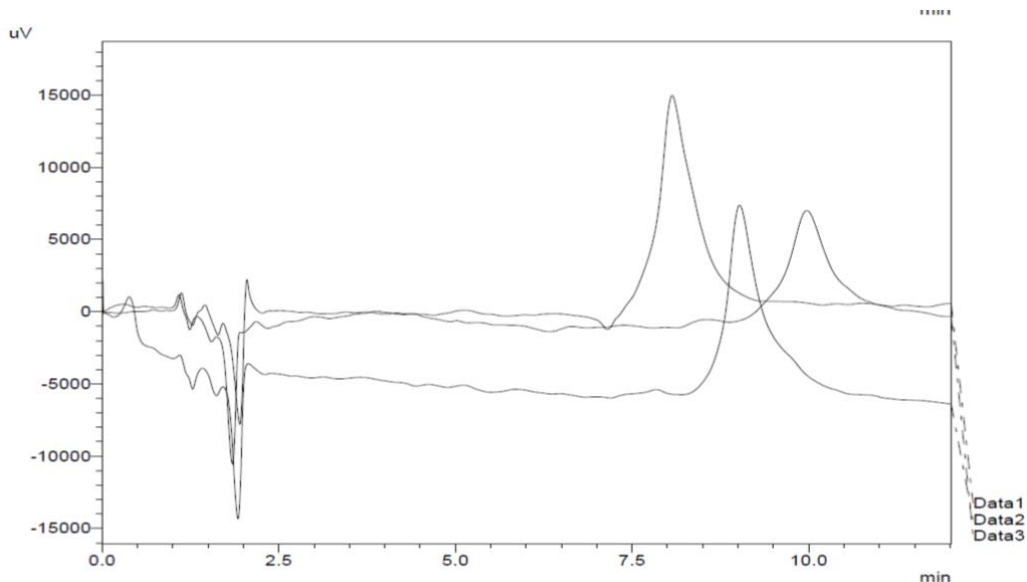


Figure 6.18: Chromatogram of Metformin HCl during base degradation 1hr, 2hrs and 4hrs

6.1.6.6.3 Photolytic degradation by UV light

Table 6.22: Concentration after UV degradation of Metformin HCl

Time (hrs)	Area	Corresp.conc.	% degradation
1hr	525915	10.02	1.93
1hr	526176	10.02	1.89
2hrs	332579	6.59	35.45
2hrs	334611	6.63	35.10
4hrs	415156	8.05	21.13
4hrs	421758	8.17	19.99

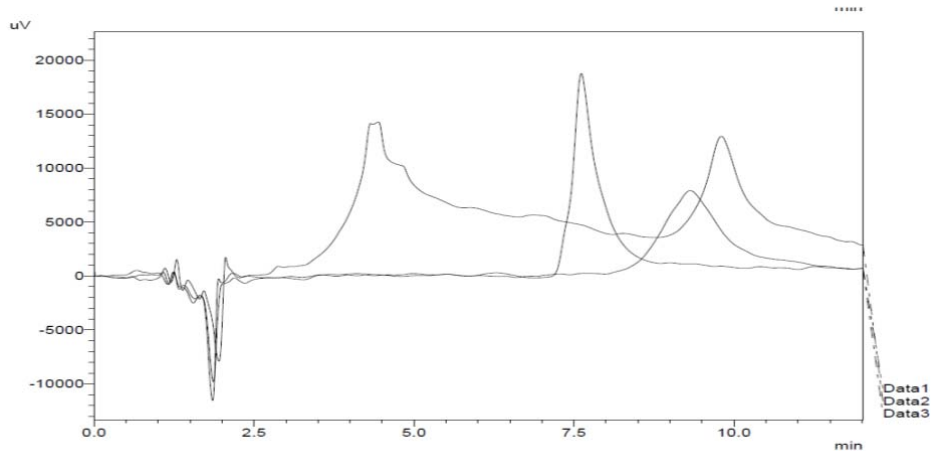


Figure 6.19: Chromatogram of Metformin HCl during photolytic degradation by UV at 1hr, 2hrs and 4hrs

6.1.6.6.4 Oxidative degradation

Table 6.23: Concentration after oxidative degradation of Metformin HCl

Time (hrs)	Area	Corresp.conc	% degradation
1hr	537044	10.21	0.009
1hr	530616	10.10	1.12
2hrs	509799	9.73	4.73
2hrs	509886	9.73	4.71
4hrs	358503	7.05	30.95
4hrs	353406	6.96	31.84

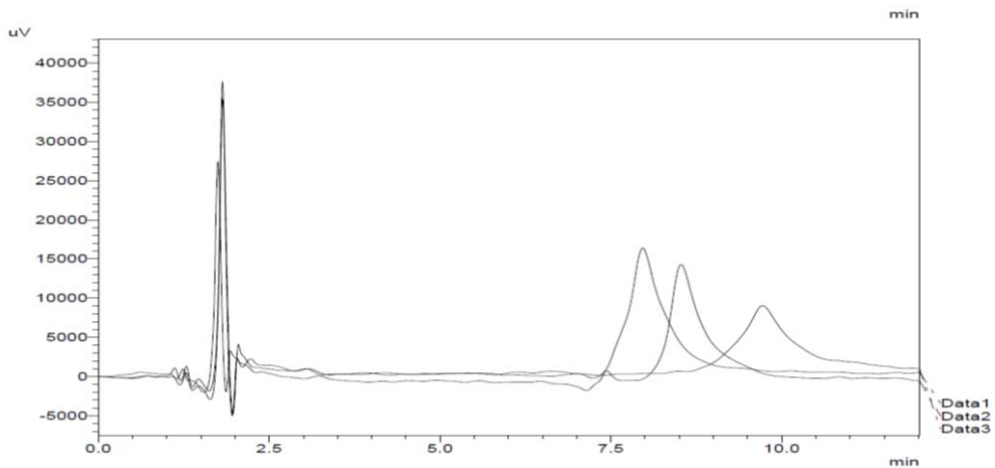


Figure 6.20: Chromatogram of Metformin HCl during oxidative degradation at 1hr, 2hrs and 4hrs

6.1.6.6.5 Thermal degradation

Table 6.24: Concentration after thermal degradation of Metformin HCl

Time (hrs)	Area	Corresp.conc	% degradation
1hr	523106	9.97	2.42
1hr	539870	10.26	0.48
2hrs	522679	9.96	2.49
2hrs	521068	9.93	2.77
4hrs	397992	7.75	24.11
4hrs	397885	7.75	24.13

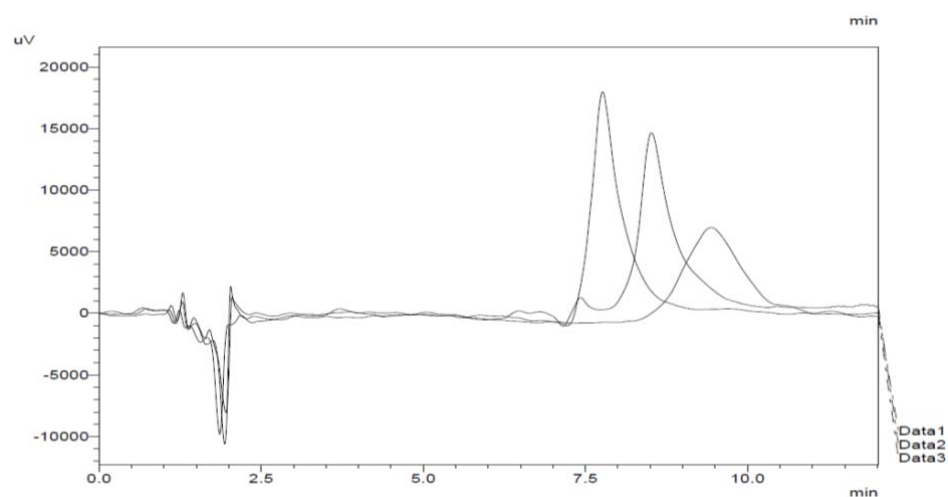


Figure 6.21: Chromatogram of Metformin HCl during thermal degradation at 1hr, 2hrs and 4hrs

Table 6.25: Percent degradation of Metformin HCl

S.No	Type of degradation	% Degradation					
		1hr	SD	2hrs	SD	4hrs	SD
1	Acid degradation	2.66	0.73	5.67	0.744	11.62	2.66
2	Base degradation	5.02	1.65	9.49	0.974	37.40	5.02
3	UV degradation	1.91	0.031	35.27	0.249	20.56	1.91
4	Oxidative degradation	0.56	0.78	4.72	0.01	31.40	0.56
5	Thermal degradation	1.83	0.82	2.63	0.197	24.12	1.83

6.1.7 FTIR analysis

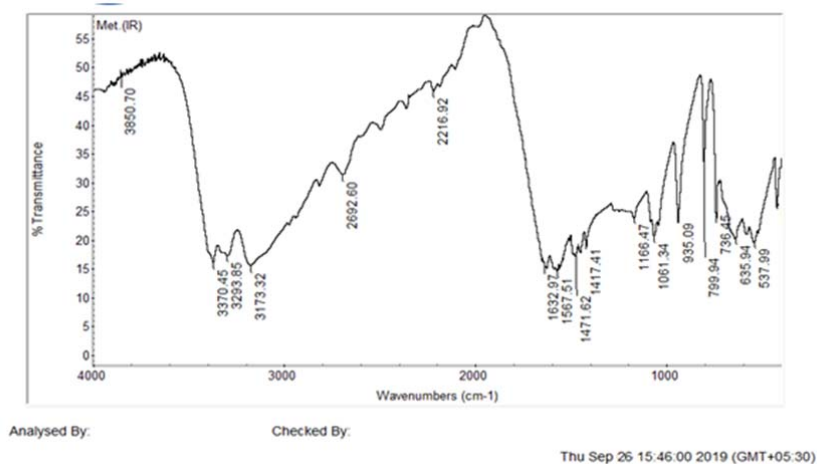


Figure 6.22: FTIR spectrum of Metformin HCl

Discussion: Pure Metformin HCl indicated the peaks on particular waves i.e. N-H extended (3173.32cm^{-1}), N-H stretching (primary amine group) -NH_2 (1632.97), asymmetric NCN stretch (1567.51cm^{-1}), CH_3 asymmetric and symmetric deformations (1471.41 , 1471.62cm^{-1}), C-N stretch (1061.34), CH rock (935.09cm^{-1}).

6.1.8 FTIR study of physical mixture

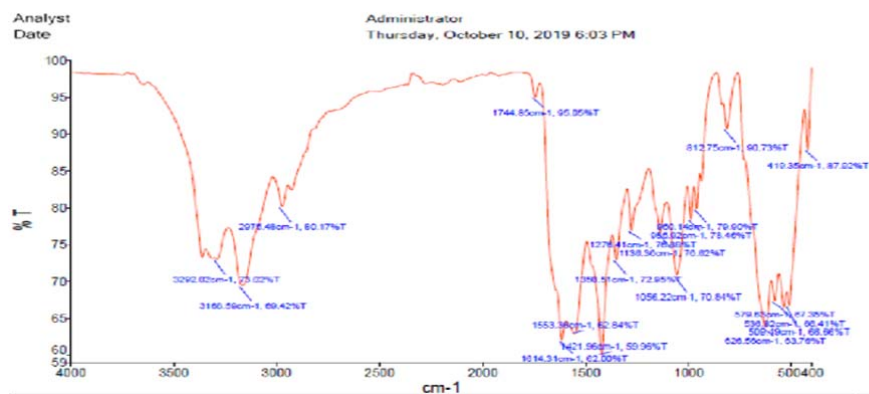


Figure 6.23: FTIR spectrum of physical mixture (Metformin HCl, fenugreek gum & Acrylamide)

Discussion: Physical mixture of Metformin HCl, fenugreek gum and acrylamide indicated the peaks at particular wave i.e. N-H stretching (3180.59cm^{-1}), N-H deformation (1614.31cm^{-1}), asymmetric NCN stretch (1553.36cm^{-1}), CH_3

asymmetric and symmetric deformations (1421.96cm^{-1}), C-N stretch (1056.22cm^{-1}) and CH rock (960.14cm^{-1}) and CN stretch 1350.51 . So, all excipients were compatible with each other, no interactions among all of them.

6.1.9 X-ray Powder Diffraction (XRPD)

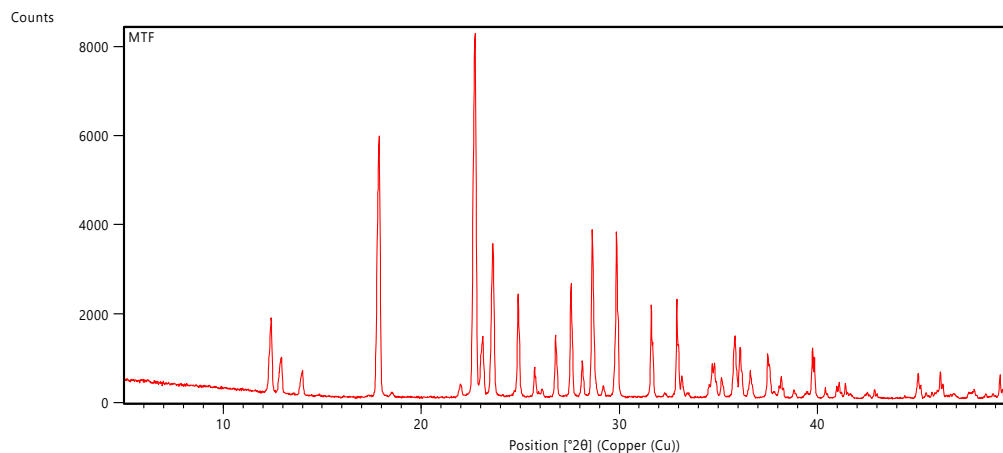


Figure 6.24: XRD study of Metformin HCl

Discussion: XRD interpretation of Metformin HCl observed at 2θ showed that 12.3896, 12.8911, 13.9563, 17.7884, 17.8762, 18.5084, 21.9645, 22.6320, 22.7198, 23.0128, 23.1034, 23.5896, 24.8857, 25.7218, 26.0904, 26.7812, 27.5561, 28.1213, 28.6304, 29.1799, 29.8521, 31.6125, 32.2921, 32.9059, 33.1516, 33.4193, 34.5329, 34.6923, 34.8135, 35.1577, 35.8094, 36.0902, 36.6028, 37.5039. Hence the drug showed crystalline nature.

6.1.10 XRD study of Physical mixture (Metformin HCl, Fenugreek gum & Acrylamide)

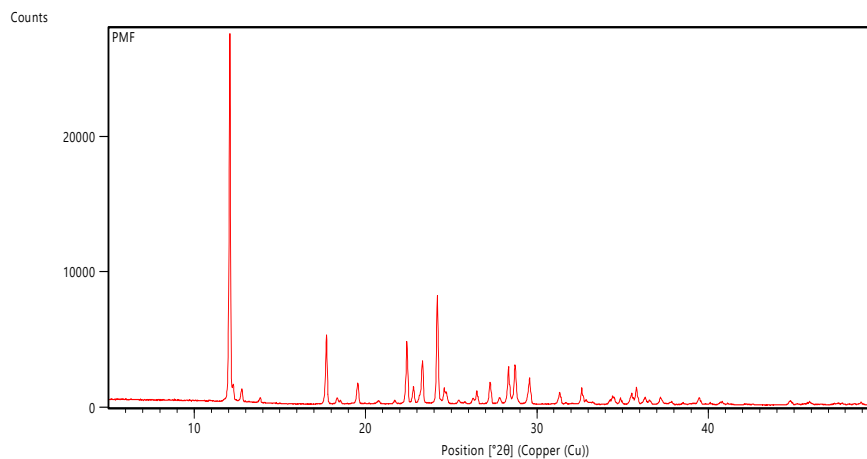


Figure 6.25: XRD study of physical mixture

Discussion: The XRD interpretation of physical mixture (Metformin HCl, fenugreek gum and acrylamide) found at 2θ showed that 12.2743, 12.7653, 13.8321, 17.7016, 18.5200, 19.5272, 20.7352, 21.6946, 22.3987, 22.7805, 23.2946, 24.7195, 25.7812, 26.2614, 26.4875, 27.2558, 27.8043, 28.3313, 28.7081, 29.5376, 31.3088, 31.9152, 32.6127, 32.8660, 33.1614, 34.2377, 34.4211, 34.8658, 35.5124, 35.7943, 36.2847, 36.5665, 37.2102, 37.791. Hence there was no incompatibility found in between drug and excipients as a physical mixture.

6.1.11 Nuclear magnetic resonance (NMR) spectroscopy

6.1.11.1 ^1H NMR of Metformin HCl

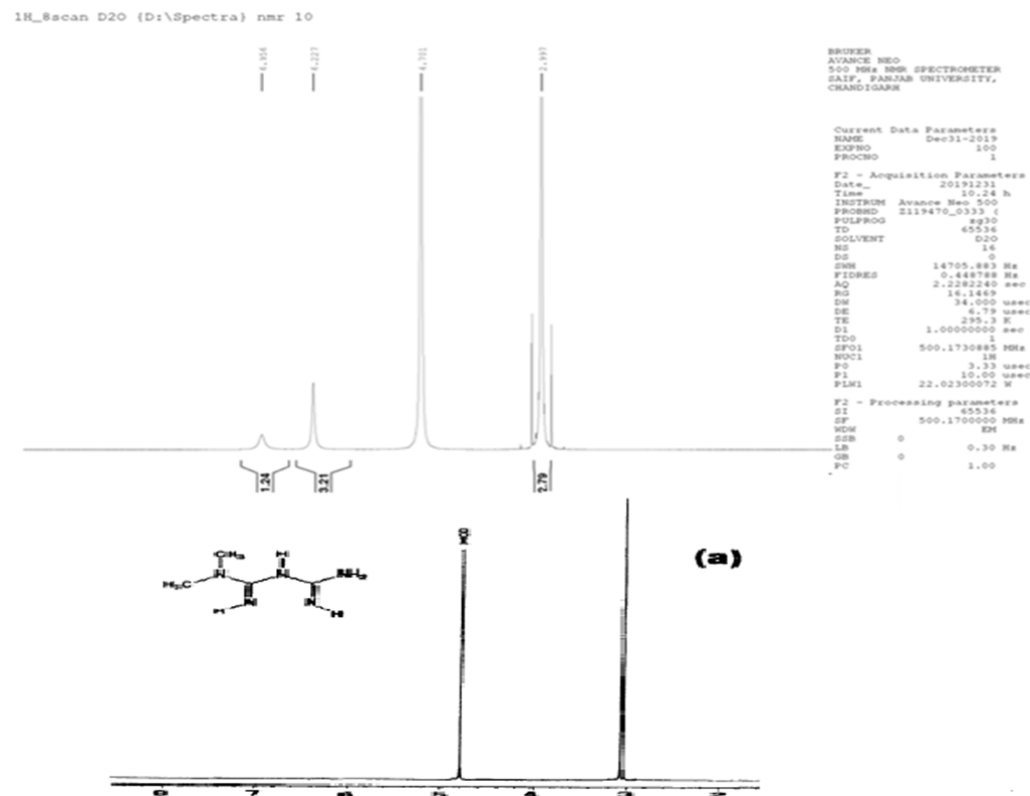


Figure 6.26: ^1H NMR of Metformin HCl

Discussion: In ^1H NMR spectroscopy of Metformin HCl, at approximately 2.997 ppm and 4.701 ppm a sharp single resonance was remarked that gives the point of signal which was delegated the signal from magnetically equivalent methyl groups.

6.1.11.2 ¹³C NMR spectroscopy of Metformin HCl

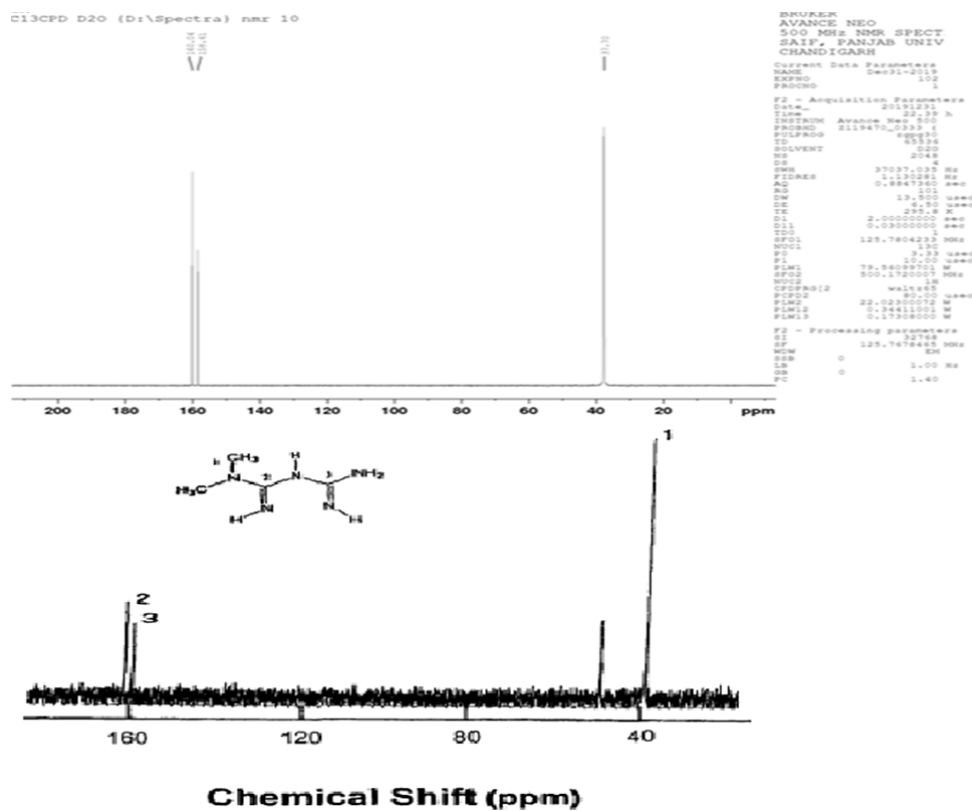
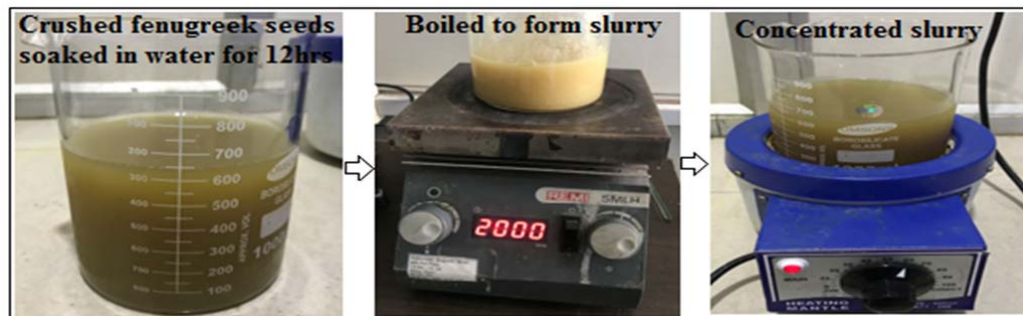


Figure 6.27: ¹³C NMR study of Metformin HCl

Discussion: In ¹³C NMR study of Metformin HCl, observed the single large aliphatic signal at 37.70 ppm that allotted resonance. Two rotationally equally methyl groups and two hetero alternated quaternary resonances at 158.41 and 160.04 ppm.

6.2 Extraction of fenugreek gum

Steps involved for extraction of fenugreek gum



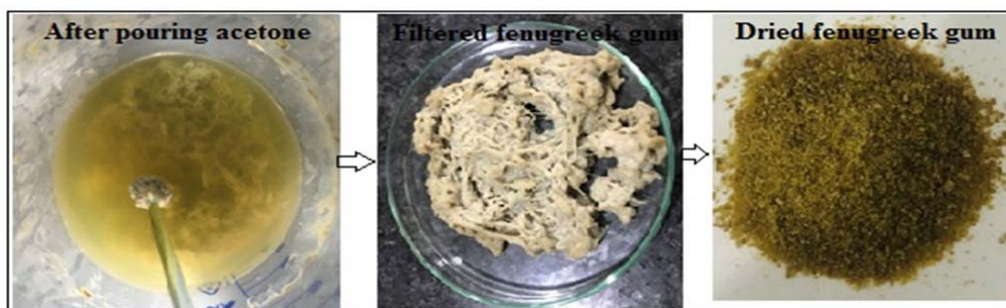


Figure 6.28: Steps involved in extraction of fenugreek gum

6.2.1 Evaluation of extracted fenugreek gum

Percentage Yield: The percentage yield was calculated (Table 6.26) for getting information about the obtained gum from the crude material by using following formula.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} * 100$$

Discussion

Table 6.26: Percentage yield of extracted gum

S. No.	Name	Amount of raw material (gm)	Amount of extract taken (gm)	% Yield
1	Fenugreek gum	50	28	55.99±0.01

The obtained yield of fenugreek gum was detect likely 55.99±0.01% w/w.

6.3 Procedure for grafting of fenugreek gum

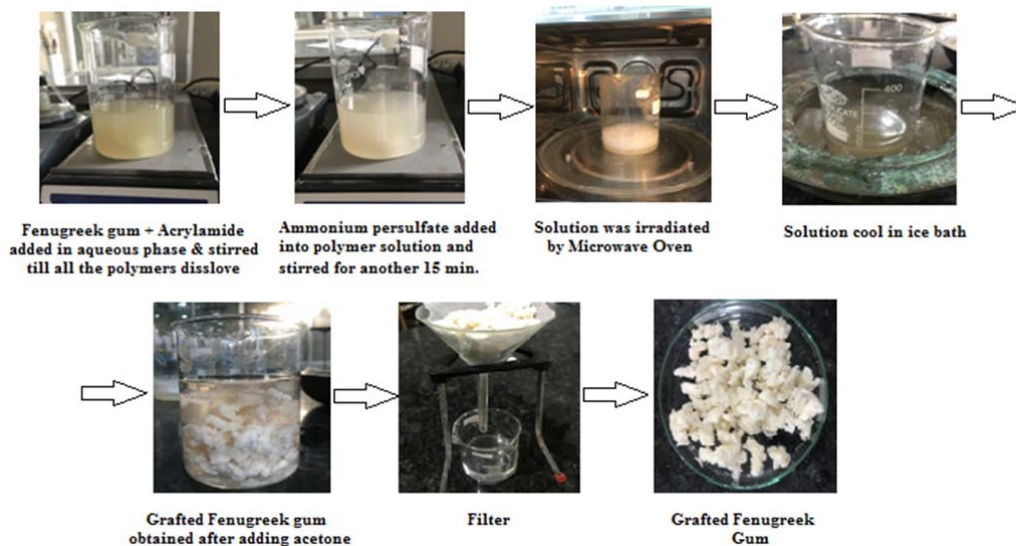


Figure 6.29: Preparation of grafted copolymers of fenugreek gum with acrylamide

Table 6.27. Composition of graft copolymers

S. No.	Formulation code	Amount of extracted Mucilage taken (%w/v)	Amount of Acrylamide (%w/v)	Initiator (% w/v)			MW irradiation Power (watt)	MW irradiation Time (sec)	%Yield	% Grafting	% Grafting Efficiency	% Conversion
				Ammonium per sulphate	Ceric ammonium nitrate	Potassium persulfate						
1	A	0.50	15	0.20	-	-	P100	60	90.33±0.07	27.36±2.08	91.21±0.07	94.5±0.07
2	B	0.50	15	-	0.20	-	P100	60	44.23±0.04	12.88±1.16	42.96±0.04	46.2±0.04
3	C	0.50	15	-	-	0.20	P100	60	No PPT formed	No PPT formed	No PPT formed	No PPT formed

6.3.1 Optimization study of grafted gum

The grafting process was optimized by studying the influence of reaction variable involved in synthesis of grafted fenugreek gum on the parameter i.e. % grafting efficiency. For the optimization of grafted fenugreek gum, the amount of monomer (acrylamide) concentration, gum concentration, initiator concentration, irradiation power and microwave exposure time was taken as reaction variables.

6.3.1.1 Effect of monomer (acrylamide) concentration

Table 6.28. Percent grafting data of grafted gum with concentration of acrylamide (Monomer)

S.No.	Formulation Code	Amount of extracted gum taken (%w/v)	Amount of Acrylamide (%w/v)	Initiator (%w/v)	MW irradiation Power (watt)	MW irradiation Time (sec)	%Yield	% Grafting	% Grafting Efficiency	% Conversion
1	A1	0.5	20	0.2	P100	60	56.33±0.05	22.32±2.00	55.8±0.05	58.3±0.05
2	A2	0.5	15	0.2	P100	60	90.33±0.07	27.36±2.08	91.21±0.07	94.54±0.07
3	A3	0.5	10	0.2	P100	60	84.17±0.05	17.01±1.15	85.07±0.06	90.07±0.06
4	A4	0.5	5	0.2	P100	60	76.2±0.27	76.86±3.06	76.87±0.31	86.87±0.31

Discussion: Table 6.28 shows that the different concentration of monomer (5-20%) in microwave assisted method. It may occur due to availability of (monomer) acrylamide at this concentration (15%) concerning polysaccharide macro radicals, noted the chances of grafting. If monomer concentration increased from 5 to 15%, the %Y, % G, % GE and %C were increased but with further increase in the concentration of acrylamide (AM) up to 20% , the % Y, % G, % GE and % C were decreased. Decreased in parameters leads to development of homopolymers which inhibit the degree of diffusion of monomer to the polysaccharide free radicals.

6.3.1.2 Effect of gum concentration

Table 6.29. Percent grafting data of grafted fenugreek gum with concentration of gum

S.No.	Formulation Code	Amount extracted gum taken (%w/v)	Amount of Acrylamide (%w/v)	of Initiator (%w/v) Ammonium per sulphate	MW irradiation Power (watt)	MW irradiation Time (sec)	% Yield	% Grafting	% Grafting Efficiency	% Conversion
1	A5	0.25	15	0.2	P100	60	80.35±0.10	48.65±6.11	81.09±0.10	82.76±0.10
2	A2	0.5	15	0.2	P100	60	90.33±0.07	27.36±2.08	91.21±0.07	94.54±0.07
3	A6	1	15	0.2	P100	60	86.54±0.06	13.02±1.00	86.80±0.07	93.47±0.07
4	A7	1.5	15	0.2	P100	60	64.51±0.07	61.82±0.77	61.82±0.08	71.82±0.08

Discussion: The (Table 6.29) shows different concentration of fenugreek gum solution were used with 15% acryl amide & 0.2% APS. As gum concentration increased, the %Y, %G, % C were decreased due to increased viscosity of reaction medium & decreased initiator ratio.

6.3.1.3 Effect of initiator concentration

Table 6.30: Percent grafting data of grafted fenugreek gum with concentration of initiator (APS)

S.No.	Formulation Code	Amount extracted gum taken (%w/v)	Amount of Acrylamide (%w/v)	of Initiator (%w/v) Ammonium per sulphate	MW irradiation Power (watt)	MW irradiation Time (sec)	% Yield	% Grafting	% Grafting Efficiency	% Conversion
1	A2	0.50	15	0.2	P100	60	90.33±0.07	27.36±2.08	91.21±0.07	94.54±0.07
2	A8	0.50	15	0.4	P100	60	94.4±0.06	29.02±2.00	96.73±0.07	100.07±0.07
3	A9	0.50	15	0.6	P100	60	95.24±0.09	29.66±3.06	98.89±0.10	102.22±0.10
4	A10	0.50	15	0.8	P100	60	54.68±0.02	16.82±0.53	56.09±0.02	59.42±0.02

Discussion: Different concentration of initiators induced graft copolymerization was applied in microwave assisted method that was shown in Table 6.30. It was clear that the amount of ammonium per sulphate (APS) as initiator has high % grafting efficiency irrespective of other initiator such as PPS & CAN. It may occur due to availability of acrylamide monomer at this concentration of initiator regarding polysaccharide macro radicals, leading to larger chances of grafting.

6.3.1.4 Effect of irradiation power

Table 6.31 Percent grafting data of grafted fenugreek gum with irradiation power

S. No.	Formulation Code	Amount of extracted gum taken (%w/v)	Amount of Acrylamide (%w/v)	Initiator (%w/v)	MW irradiation Power (watt)	MW irradiation Time (sec)	%yield	% Grafting	% Grafting Efficiency	% Conversion
1	A9	0.5	15	0.6	P100	60	95.2±0.09	29.66±3.06	98.8±0.10	102.2±0.10
2	A10	0.5	15	0.6	P80	60	87.5±0.06	27.20±2.00	90.6±0.07	94.00±0.07
3	A11	0.5	15	0.6	P50	60	75.0±0.11	23.18±3.46	77.2±0.12	80.60±0.12

Discussion: Table 6.31 depicted the % yield, % G, % GE, and % C was raised on the different reaction temperature from 50 to 100 watt. With increased temperature, the grafting parameters were also increased, consequently increased the diffusion rate of monomer & initiator. Hence increased grafting rate.

6.3.1.5 Effect of microwave exposure time

Table 6.32: Percent grafting data of grafted fenugreek gum with Microwave exposure time

S.No.	Formulation Code	Amount of extracted Mucilage taken (%w/v)	Amount of Acrylamide (%w/v)	Initiator (%w/v)	MW irradiation Power (watt)	MW irradiation Time (sec)	%yield	% Grafting	% Grafting Efficiency	% Conversion
1	A12	0.5	15	0.6	P100	120	74.93±0.09	23.12±3.06	77.09±0.10	80.42±0.10
2	A13	0.5	15	0.6	P100	90	97.04±0.04	30.24±1.15	100.82±0.04	104.16±0.04
3	A9	0.5	15	0.6	P100	60	95.24±0.09	29.66±3.06	98.89±0.10	102.22±0.10
4	A14	0.5	15	0.6	P100	30	29.04±0.03	83.50±0.95	27.84±0.03	31.17±0.03

Discussion: Table 6.32 remarked that APS initiate free radicals reaction even along room temperature. But generation of free radicals increased along increasing the microwave exposure time which can results in consequently propagation of chain reaction over fenugreek gum. Microwave-based elevated temperature improves the monomer (Acrylamide) solubility & polymer (fenugreek gum) in aqueous medium. At higher temperature, chances for the homopolymer origination were too increased, that result in reduced grafting. At raised temperature, chain transfer reaction & three dimensional polymeric configurations were further changed, specifically shows grafting separation.

6.3.1.6 FTIR Study of grafted fenugreek gum

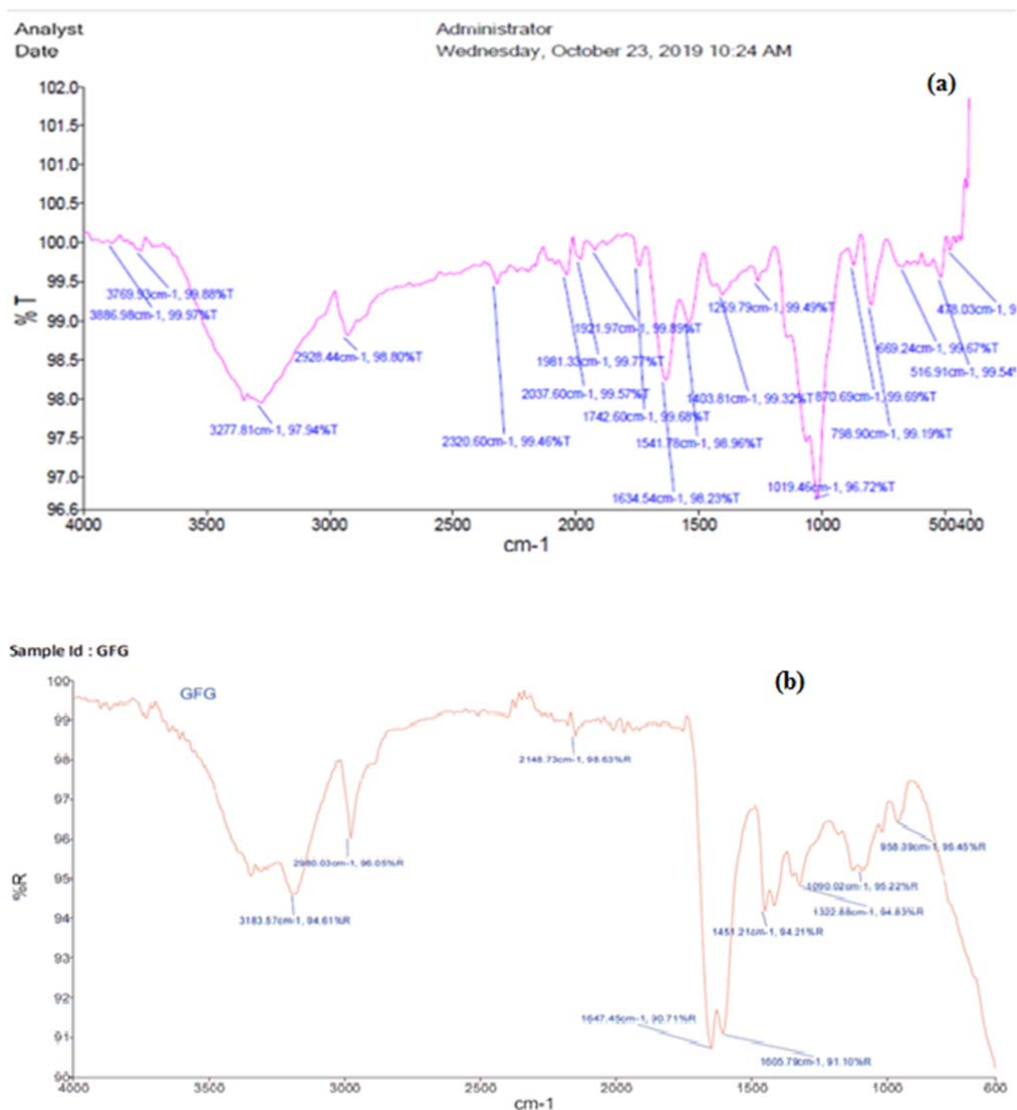


Figure 6.30: (a) FTIR spectrum of fenugreek gum (b) FTIR spectrum of grafted fenugreek gum

Discussion: The pure fenugreek gum indicate band of stretching at 3277.81cm^{-1} (O-H), 2928.44cm^{-1} (C-H), 1019.46cm^{-1} (C-O-C), 1259.79cm^{-1} (C-O), 798.90cm^{-1} (C-C), 1403.81cm^{-1} (C-H). This observation of pure gum confirmed the purity and authenticity of the fenugreek gum. In case of grafted fenugreek gum at 3187.57cm^{-1} (O-H bond stretch), 2980.03cm^{-1} (C-H bond stretch), 1090.02cm^{-1} (C-O-C bond), 1451.21cm^{-1} (C-H bond bend) was observed in the spectra of grafted fenugreek

gum. It is also noticed that FTIR spectrum of grafted gum distinct away among pure fenugreek gum by exhibiting a new peak of $-\text{CO}$ of amide at 1647.45 and 1605.79 cm^{-1} , at 1322.88 cm^{-1} of $-\text{CN}$ stretching.

6.3.1.7 DSC Study of fenugreek gum

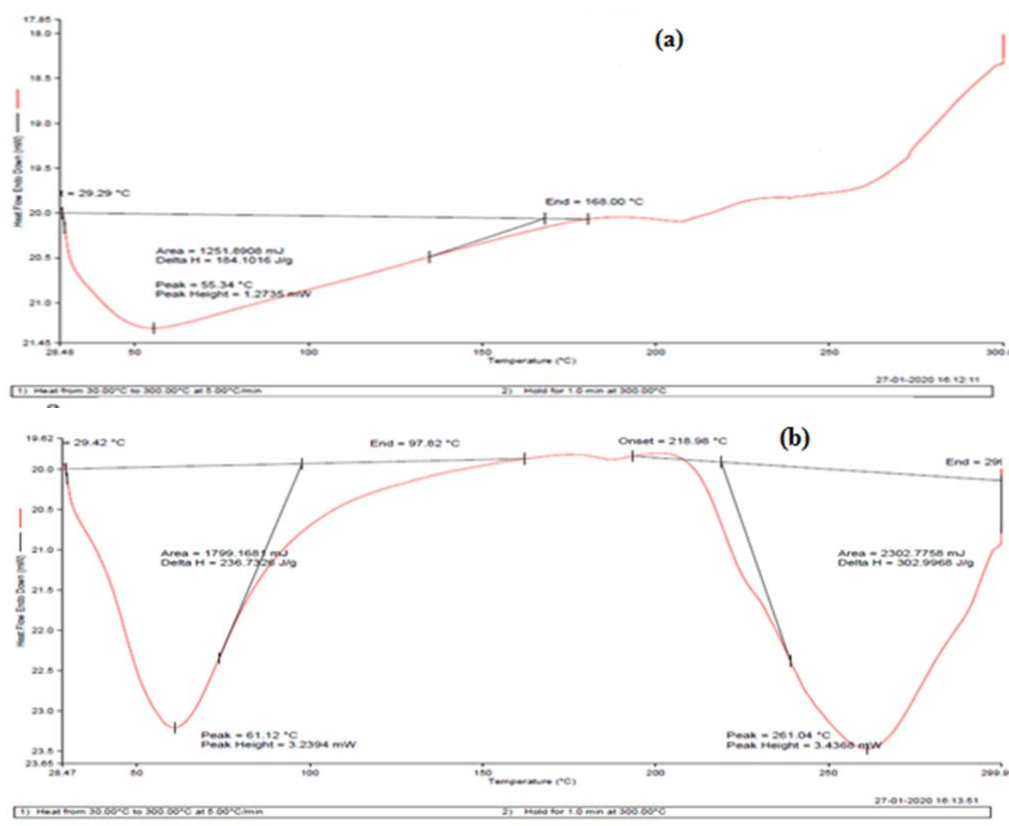


Figure 6.31: (a) DSC of fenugreek gum (b) DSC of grafted fenugreek gum

Discussion: The endothermic peak of pure fenugreek gum is noticed at 55.34°C due to appearance of trace amount moisture within the sample. The temperature range in endothermic transition was observed from 29.29 to 168.00°C . In grafted fenugreek gum, demonstrate the endothermic temperature range from 29.42 to 97.82°C and 218.96 to 298.00°C and endothermic peak is noticed at 61.12 & 261.04°C which depicts absorption of heat in appearance of moisture in given sample.

6.3.1.8 X-ray Powder Diffraction (XRPD) of Fenugreek gum

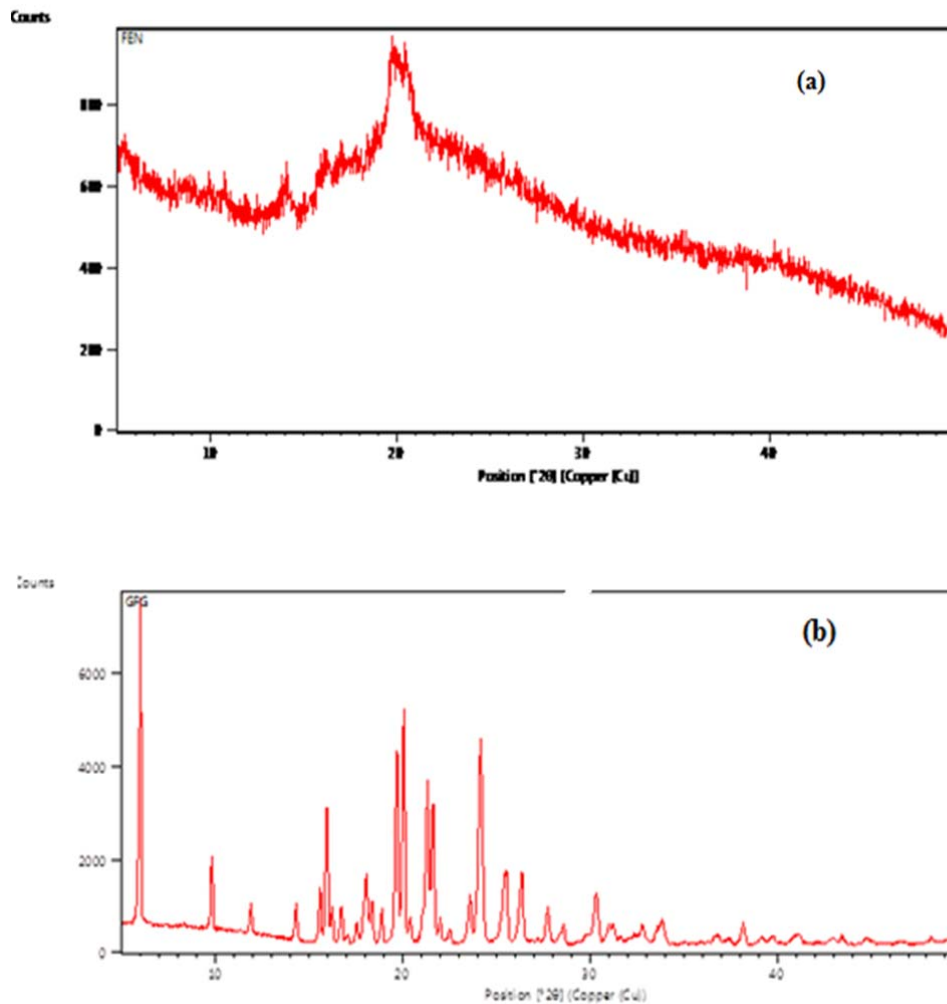
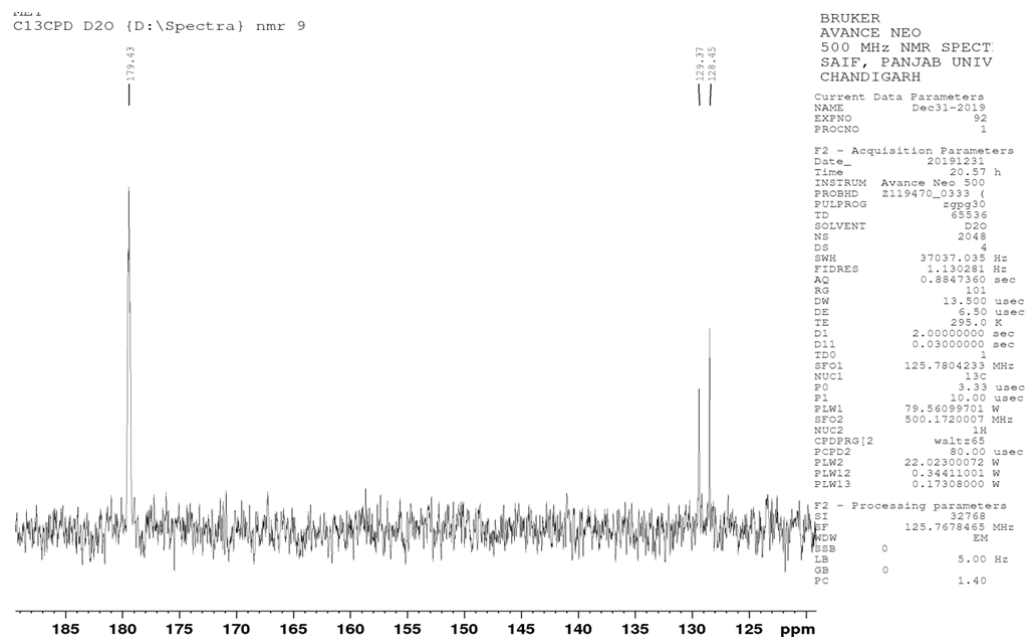
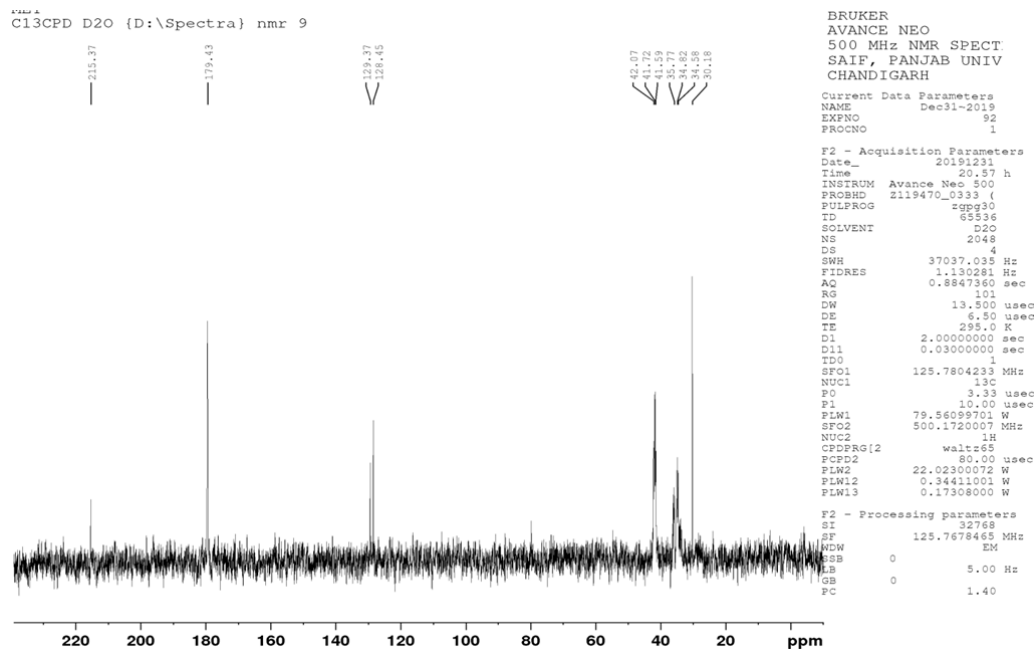


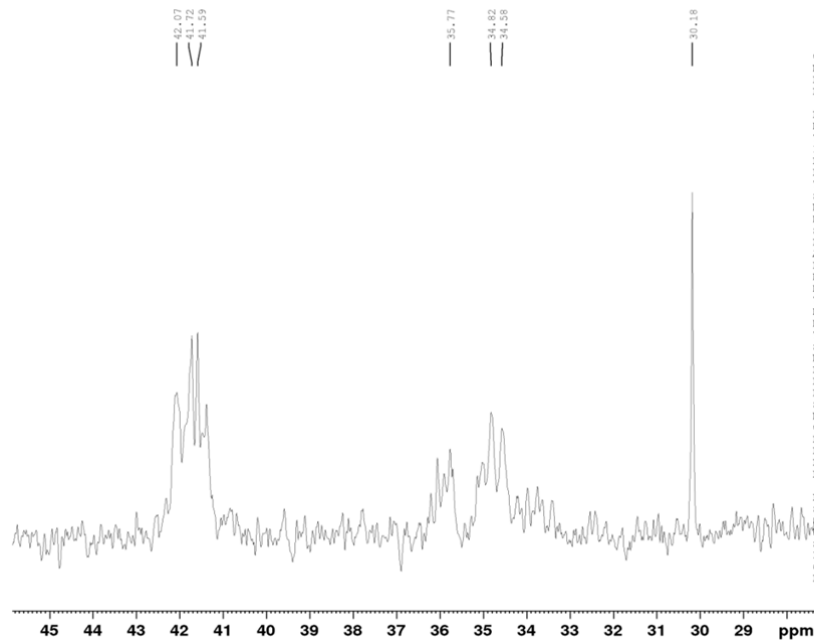
Figure 6.32: (a) XRD of fenugreek gum (b) XRD of grafted fenugreek gum

Discussion: The XRD study of fenugreek gum was showed not so prominent sharp peaks in the 2θ region equals 14.0454, 16.1137, 17.0513, 19.7532 and 20.6071 which desired its partial crystalline nature. After grafting of FG, the peaks in the 2θ region equals 12.3896, 12.8911, 13.9563, 17.7884, 17.8762, 18.5084, 21.9645 it indicate a partially crystalline nature but it was fairly distinct from pure FG. The $2Q$ values have found in each case that's were quite different in case of formation of solid phase.

6.3.1.9 NMR spectroscopy of grafted gum



13C
C13CPD D2O {D:\Spectra} nmr 9



BRUKER
AVANCE NEO
500 MHz NMR SPECT
SAIF, PANJAB UNIV
CHANDIGARH

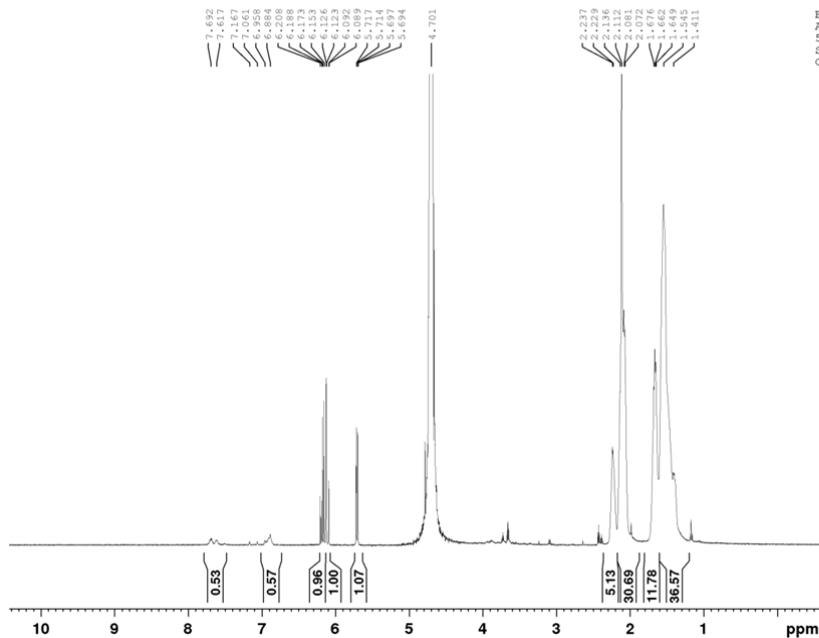
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PROCNO 1

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FIDRES 1.130281 Hz
AQ 0.8847360 sec
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DW 13.500 usec
DE 6.50 usec
TE 295.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TDO 1
SFO1 125.7804233 MHz
NUC1 13C
P0 3.33 usec
P1 10.00 usec
PLW1 79.56099701 W
SFO2 500.1720007 MHz
NUC2 1H
CPDPRG[2] waltz65
PCPD2 80.00 usec
PLW2 22.02300072 W
PLW12 0.34411001 W
PLW13 0.17308000 W

F2 - Processing parameters
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WDW EM
SSB 0
LB 5.00 Hz
GB 0
PC 1.40

Figure 6.33 : ¹³C NMR study of grafted fenugreek gum

1H_8scan D2O {D:\Spectra} nmr 9



BRUKER
AVANCE NEO
500 MHz NMR SPECTROMETER
SAIF, PANJAB UNIVERSITY,
CHANDIGARH

Current Data Parameters
NAME Dec31-2019
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PROCNO 1

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FIDRES 0.448788 Hz
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RG 52.6316
DW 34.000 usec
DE 6.79 usec
TE 295.3 K
D1 1.00000000 sec
TDO 1
SFO1 500.1730885 MHz
NUC1 1H
P0 3.33 usec
P1 10.00 usec
PLW1 22.02300072 W

F2 - Processing parameters
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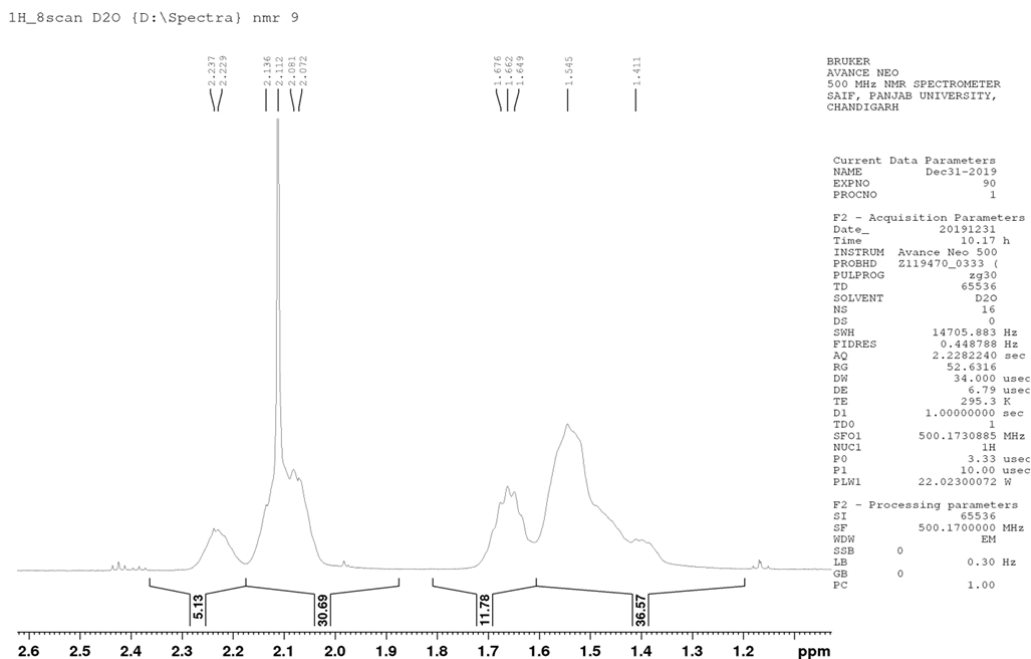
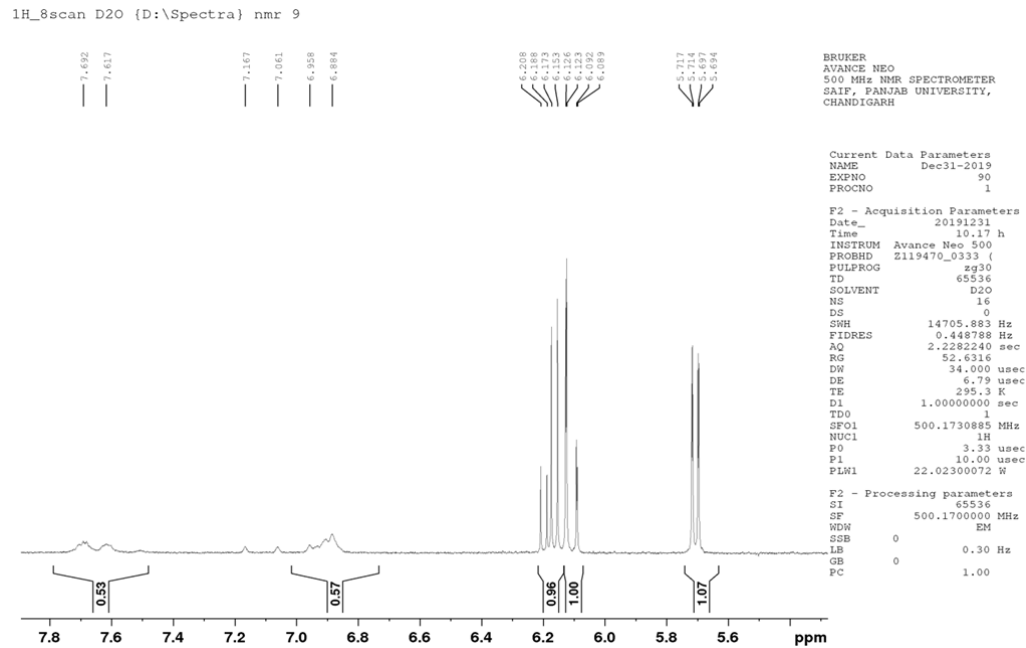


Figure 6.34 : ^1H NMR study of grafted fenugreek gum

Discussion:

NMR spectra of grafted gum is shown in Figure 6.34 & 6.35. Due to the attachment of acrylamide moiety on the polysaccharides, a little downfield shift was observed in backbone carbon of polysaccharide copolymer with their comparison to those of parent. The ^{13}C NMR spectra of grafted gum observed at 215.37, 179.43 (Amide), 128.45, 129.37, 30.18, 34.82, 35.77, 41.59, 42.07 (anomeric carbon).

The ^1H spectrum of grafted fenugreek gum showed the prominent peak at δ 1.411 ppm corresponds to hydrogen amide. The chemical shift of four anomeric signals ^1H NMR is compatible with existence of small peak at 5.697 and 5.717 ppm, which was assigned for anomeric proton of fenugreek gum.

6.3.1.10 Surface Morphology of grafted gum

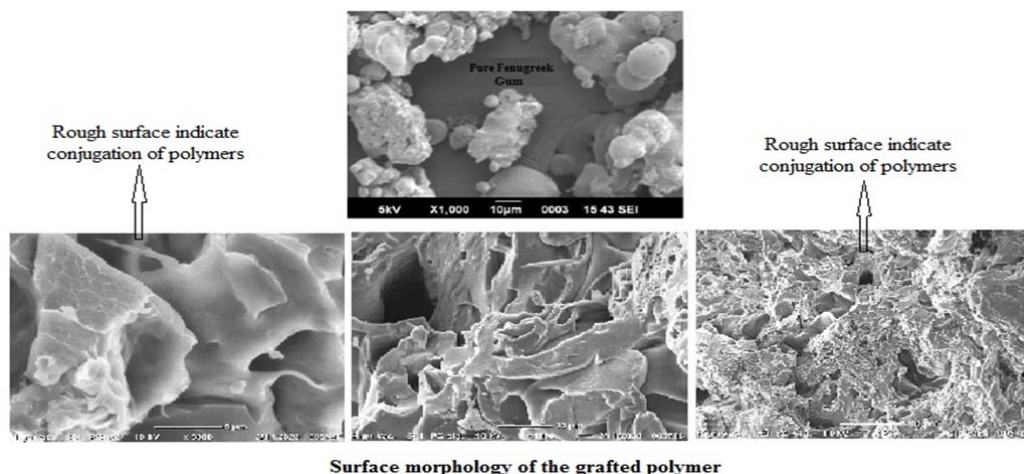


Figure 6.35: Surface Morphology of grafted gum

A significant amount of grafted polymer is accumulated which show to have a distinct structure from the pure fenugreek gum.

6.3.1.11 Swelling studies:

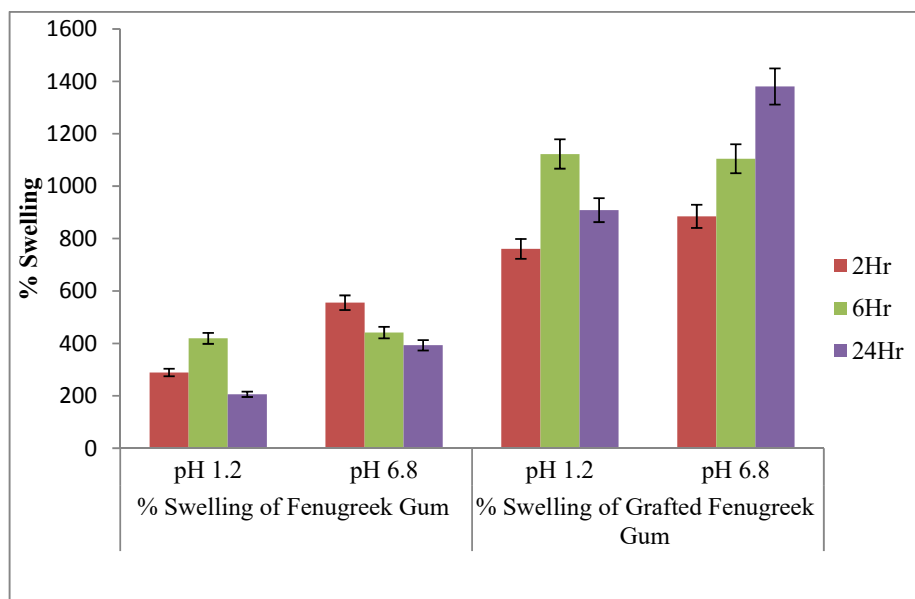


Figure 6.36: % swelling of fenugreek gum and grafted fenugreek gum at pH 1.2 & pH 6.8

Discussion: Figure 6.36, it was found that, the % swelling of grafted fenugreek gum was observed at higher level than fenugreek gum. It may due to that introduced of ionic /hydrophilic groups which penetrate water molecules inside the gum.

6.4 Experimental design

6.4.1 Factor selection with their levels

The response variables such as concentration of acrylamide, fenugreek gum, initiator (APS), Power (W), stirring speed, temperature and time were affecting factors in the grafting. Two levels for each factor were preferred which are shown in Table 6.33.

Table 6.33: Experimental control factors as well as their levels in Taguchi OA Design

Factor	Name	Unit	Levels	Level 1	Level 2
A	Acrylamide	%	2	5	15
B	Fenugreek Gum	%	2	0.25	1
C	Initiator	%	2	0.2	0.6
D	Power	Watt	2	50	100
E	Stirring speed	RPM	2	500	1000
F	Time	Second	2	60	120
G	Temperature	^o C	2	37	50

6.4.2 Selection of OA and assignment of factors

Standard tables as OA that specially composed with symbol L8 (2⁷) requires total 8 experiments in Taguchi method. The distinct values toward factor were selected through both tested lower and upper values for each variable are summarized in Table 6.34

Table 6.34: A Two-level orthogonal array (L8)

Run	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Response 1	Response 2	Response 3	Response 4
	A:A (Acrylamide)	B:B (fenugreek Gum)	C:C (Initiator)	D:D (Irradiation power)	E:E (stirring speed)	F:F (Time)	G:G (temp.)	Yield %	Grafting %	Grafting Efficiency %	Conversion %
1	5	0.25	0.2	100	1000	120	50	72.48±0.2	1480±4.0	74.24±0.02	79±0.2
2	15	1	0.2	50	1000	120	37	85.06±0.03	1278±0.4	85.02±0.03	92±0.03
3	15	1	0.2	100	500	60	50	86.05±0.2	1294±4.0	86.27±0.3	92.93±0.3
4	5	1	0.6	100	1000	60	37	64.09±0.2	323±1.0	64.6±0.2	84.6±0.2
5	15	0.25	0.6	100	500	120	37	77.60±0.1	4820±8.0	80.33±0.1	82±0.1
6	5	1	0.6	50	500	120	50	63.79±0.4	321±2.6	64.2±0.5	84.2±0.5
7	15	0.25	0.6	50	1000	60	50	78.82±0.1	4896±6.9	81.6±0.1	83.27±0.1
8	5	0.25	0.2	50	500	60	37	70.83±0.7	1444±16.0	72.2±0.8	77.2±0.8

6.4.2.1 Response 1 (% Yield)

Table 6.35: Analysis of variance for % Yield

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	510.17	2	255.08	475.93	<0.0001	Significant
A-Acrylamide	396.68	1	396.68	740.12	<0.0001	
C-Initiator	113.49	1	113.49	211.74	<0.0001	
Residual	2.68	5	0.536			
Cor Total	512.85	7				

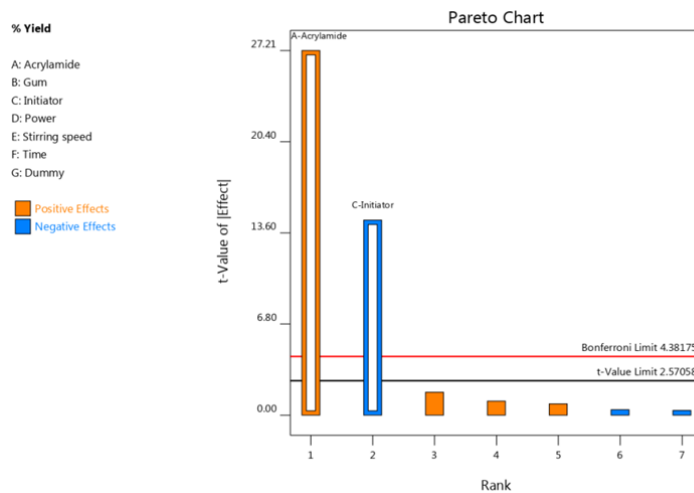


Figure 6.37: Pareto chart for % yield

Discussion: The Model F-value 475.93 mentioned the model was significant and on that point there was 0.01% possibility that F-value became large by virtue of noise. The Predicted R^2 is 0.9866 which is reasonable agreement and adjusted R^2 is 0.9927; this means distinction is lesser 0.2.

Adequate precision calculate the signal to noise ratio. The ratio 48.216 specify an adequate signal because the ratio more than 4 was acceptable. This model is common to handle the design space. P-values less than 0.0500 indicates that model terms are significant because the values more than 0.1000 designates that model terms were not significant. In such event, A and C were significant model terms.

The Pareto chart appear t values for showing the effects for entire screened factors. For factor identification (A–G), see Figure 6.37. In online figure, orange showed positive consequence as well as blue showed negative consequence.

6.4.2.2 Response 2: % Grafting

Table 6.36: Analysis of variance of % grafting

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.06E+07	2	1.03E+07	17.4	0.0056	Significant
A-Acrylamide	9.51E+06	1	9.51E+06	16.05	0.0103	
B-Gum	1.11E+07	1	1.11E+07	18.75	0.0075	
Residual	2.96E+06	5	5.92E+05			
Cor Total	2.36E+07	7				

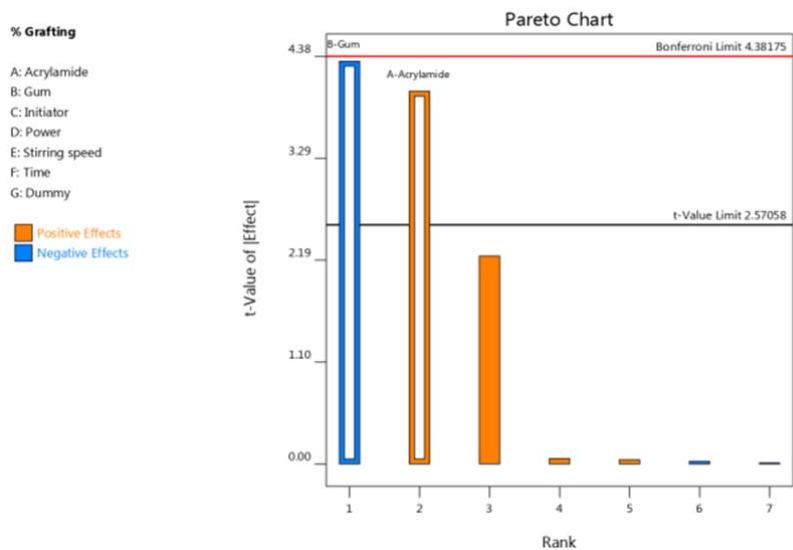


Figure 6.38: Pareto chart for % grafting

Discussion: The Model F-value 17.40 mentioned the model were significant and only 0.56% chances that F-value could appear larger due to noise. Predicted R² 0.6784 within reasonable agreement along adjusted R² 0.8241, particularly the difference is less than 0.2.

Adequate precision determine the signal to noise ratio. A ratio beyond 4 was suitable and ratio 9.626 showed an adequate signal. The model should acclimated to control design space. P-values less than 0.0500 exhibits that model terms were significant because if the values are larger than 0.1000, then model terms were not significant. In this instance A and B are significant model terms.

In Pareto chart, t values showing the effects for all over screened factors. For identification of factor (A–G), see Figure 6.38. In online figure, orange expressed positive effects as well as blue expressed negative effects.

6.4.2.3 Response 3: % Grafting efficiency

Table 6.37: Analysis of variance of % grafting efficiency

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	524.73	3	174.91	227.81	<0.0001	Significant
A-Acrylamide	426.32	1	426.32	555.26	<0.0001	
B-Gum	7.74	1	7.74	10.08	0.0337	
C-Initiator	90.68	1	90.68	118.1	0.0004	
Residual	3.07	4	0.7678			
Cor Total	527.8	7				

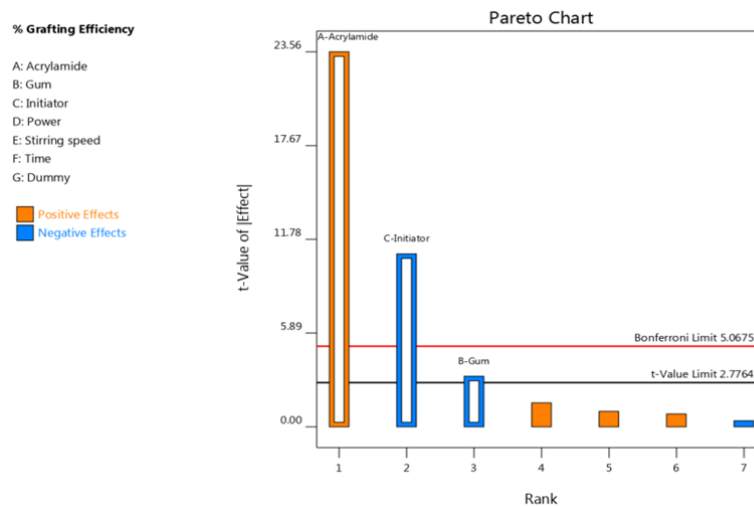


Figure 6.39: Pareto chart % grafting efficiency

Discussion: The Model F-value 227.81 mentioned the model were significant and just 0.01% possibility that an F-value could appear larger by noise. Predicted R^2 0.9767 was reasonable agreement with adjusted R^2 0.9898; i.e. the difference is lower than 0.2. The ratio 34.432 showed an adequate signal because ratio greater than 4 was appropriate. This model could be common to control the design space. P-values less than 0.0500, describes that model terms were significant. In that event A, B and C were significant model terms.

The Pareto chart showing the t values of effects for all screened factors. For factor identification (A–G), see Figure 6.39. In the online figure, orange showed positive effects as well as blue showed negative effects.

6.4.2.4 Response 4: % Conversion

Table 6.38: Analysis of variance % Conversion

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	213.62	3	71.21	92.74	0.0004	Significant
A-Acrylamide	78.54	1	78.54	102.3	0.0005	
B-Gum	129.07	1	129.07	168.11	0.0002	
C-Initiator	6.01	1	6.01	7.83	0.0489	
Residual	3.07	4	0.7678			
Cor Total	216.69	7				

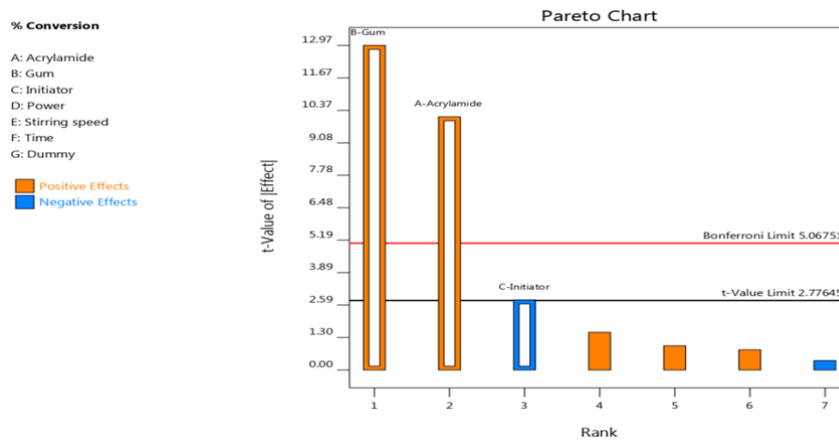


Figure 6.40: Pareto chart for % conversion

Discussion: The Model F-value 92.74 mentioned the model were significant with 0.04% chance that an F-value could appear larger by noise. The predicted R^2 0.9433 was reasonable agreement with adjusted R^2 0.9752; i.e. the difference is lesser than 0.2. Adequate precision calculated the signal to noise ratio. The ratio 23.080 showed an adequate signal because the ratio greater than 4 was appropriate. The model could be common to control design space.

P-values less than 0.0500 showed model terms were significant because the values more than 0.1000 show the model terms were not significant. In such case A, B and C were significant model terms. The Pareto chart indicates the t values of the effects for all over screened factors. For factor identification (A–G), see Figure 6.40. In the online figure, orange expressed positive effects, and blue expressed negative effects.

6.4.3 Optimized concentration of variables

Data with desirability were run again and calculated for minimum standard deviation amongst theoretical and experimental response value.

Table 6.39. Number of optimized solutions

Number	Acrylamide (%w/v)	Gum (%w/v)	Initiator	Power (watt)	Stirring speed (rpm)	Time (sec)	Temp. (°C)	% Yield	% Grafting	% Grafting Efficiency	% Conversion
1	15	0.25	0.2	100	1000	120	50	85.64	42.50	87.7	84.36
2	15	0.25	0.2	50	500	120	37	85.64	42.50	87.7	84.36
3	15	0.25	0.2	100	500	60	37	85.64	42.50	87.7	84.36
4	15	0.25	0.2	100	500	120	37	85.64	42.50	87.7	84.36
5	15	0.25	0.2	50	1000	120	37	85.64	42.50	87.7	84.36
6	15	0.25	0.2	100	1000	120	37	85.64	42.50	87.7	84.36
7	15	0.25	0.2	50	500	60	37	85.64	42.50	87.7	84.36
8	15	0.25	0.2	50	500	60	50	85.64	42.50	87.7	84.36
9	15	0.25	0.2	100	1000	60	37	85.64	42.50	87.7	84.36
10	15	0.25	0.2	100	500	60	50	85.64	42.50	87.7	84.36

Table 6.40. Predicted and observed value of responses

Number	Predicted response				Observed response				Percentage error			
	% Yield	% Grafting	% Grafting Efficiency	% Conversion	% Yield	% Grafting	% Grafting Efficiency	% Conversion	% Yield	% Grafting	% Grafting Efficiency	% Conversion
1	85.645	4250	87.7	84.367	57.9±0.3	3480±20.0	58±0.3	59.7±0.3	19.60	544.47	21.00	17.47
2	85.645	4250	87.7	84.367	79.3±0.2	4800±12.0	80±0.2	81.7±0.2	4.50	388.91	5.44	1.91
3	85.645	4250	87.7	84.367	80.5±0.1	4876±4.0	81.3±0.1	82.9±0.1	3.63	442.65	4.55	1.01
4	85.645	4250	87.7	84.367	54.4±0.1	3264±8.0	54.4±0.1	56.1±0.1	22.07	697.21	23.55	20.01
5	85.645	4250	87.7	84.367	79.9±0.2	4840±12.0	80.7±0.2	82.3±0.2	4.04	417.19	4.97	1.44
6	85.645	4250	87.7	84.367	50.2±0.1	3004±4.0	50.1±0.1	51.7±0.1	25.04	881.06	26.61	23.08
7	85.645	4250	87.7	84.367	63.8±0.2	3844±12.0	64.1±0.2	65.7±0.2	15.43	287.09	16.71	13.18
8	85.645	4250	87.7	84.367	64.5±0.2	3888±10.6	64.8±0.2	66.5±0.2	14.93	255.97	16.19	12.66
9	85.645	4250	87.7	84.367	82.8±0.1	5020±4.0	83.7±0.1	85.3±0.1	1.98	544.47	2.85	0.68
10	85.645	4250	87.7	84.367	78.4±0.1	4744±8.0	79.1±0.1	80.7±0.1	5.14	349.31	6.10	2.57

Table 6.41: Checkpoint batch

Grafting Formulation Parameter							
Number	Amount of Acrylamide (%w/v)	Amount of extracted Mucilage taken (%w/v)	Ammonium per sulphate (%w/v)	MW irradiation Power (Watt)	Stirring speed (rpm)	MW irradiation Time (sec)	Temperature (C°)
9	15	0.25	0.2	100	1000	60	37

Number	Predicted response				Observed response				Percentage error			
	% Yield	% Grafting	% Grafting Efficiency	% Conversion	% Yield	% Grafting	% Grafting Efficiency	% Conversion	% Yield	% Grafting	% Grafting Efficiency	% Conversion
9	85.645	4250	87.7	84.367	82.8±0.1	5020±4.0	83.7±0.1	85.3±0.1	1.98	544.47	2.85	0.68

Discussion: The comparisons of predicted and observed results found to be close agreement, which indicates the design success with desirable function for the optimization of grafting batch (Table 6.41). Minimum standard deviation was observed in combinatorial set-9, thus considered as optimized batch of the process.

6.5 Formulation of sustained release tablet of Metformin HCl



Figure: 6.41. Grafted fenugreek tablets of Metformin HCl

6.5.1 FTIR study

6.5.1.1 FTIR Study of polyvinylpyrrolidone K30 (PVP K30)

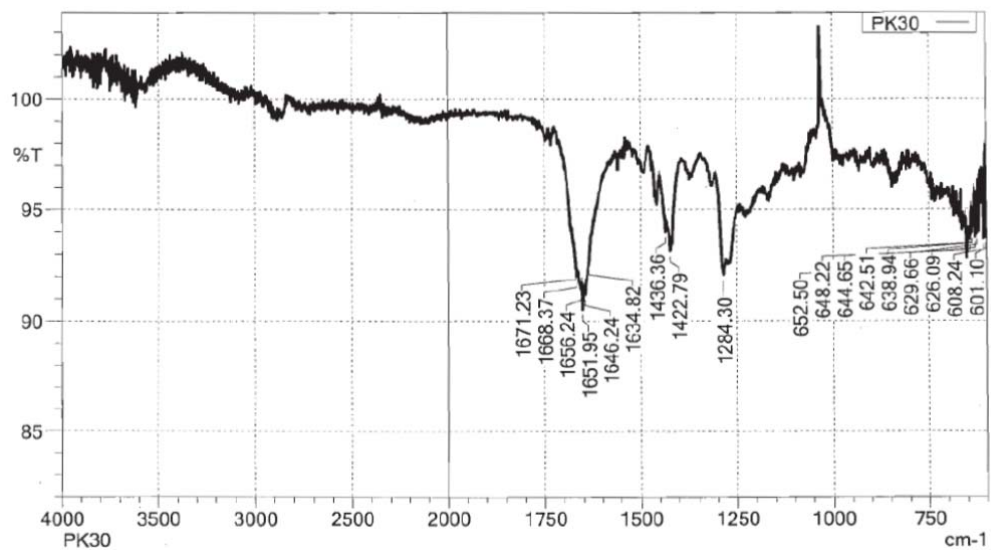


Table 6.42: FTIR spectrum of polyvinylpyrrolidone K30 (PVP K30)

Discussion: The IR absorption of polyvinylpyrrolidone K30 (PVP K30) shows peaks at 1651.95cm^{-1} (C–O stretching of the carbonyl group) and 1284.30 (N– C stretching) in PVP K30 spectra. The remarked peaks verified the purity and authenticity of polyvinylpyrrolidone K30.

6.5.1.2 FTIR Study of Microcrystalline cellulose (MCC)

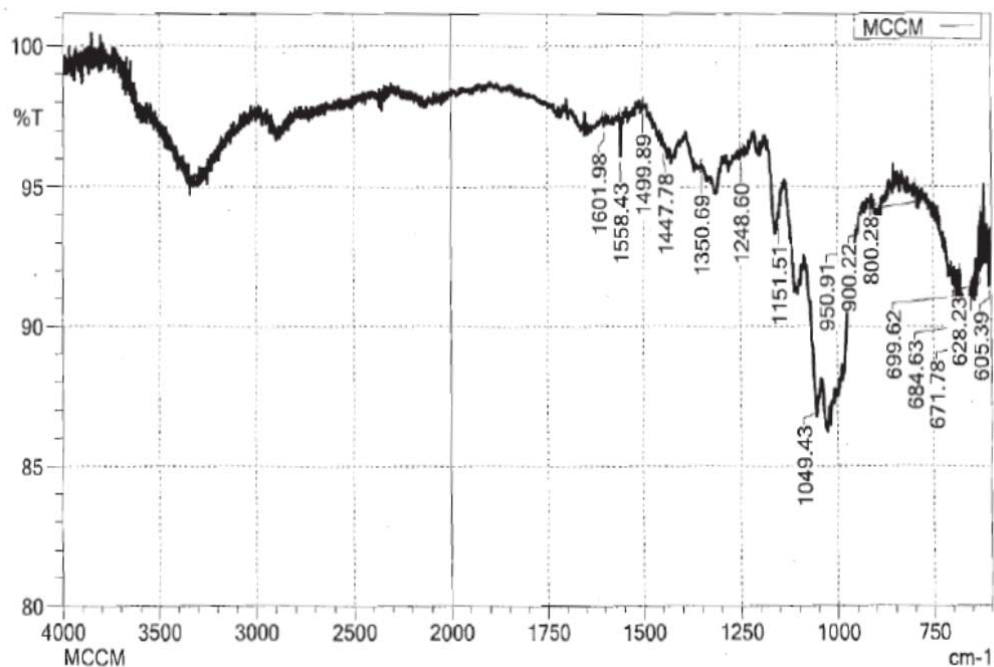


Figure 6.43: FTIR spectrum of Microcrystalline Cellulose (MCC)

Discussion: The principle IR absorption shows peaks of MCC at 1601.98cm^{-1} (C=O in the aldehyde on the terminal an hydroglucose unit), 1447.78cm^{-1} (CH_2 blending), 1350.69cm^{-1} (C–O stretching vibration of CH_2 - OH groups) and 1049.43cm^{-1} (–C–O–C– in the cellulose molecule). This observation established the purity and accuracy of Microcrystalline cellulose.

6.5.1.3 FTIR Study of Talc

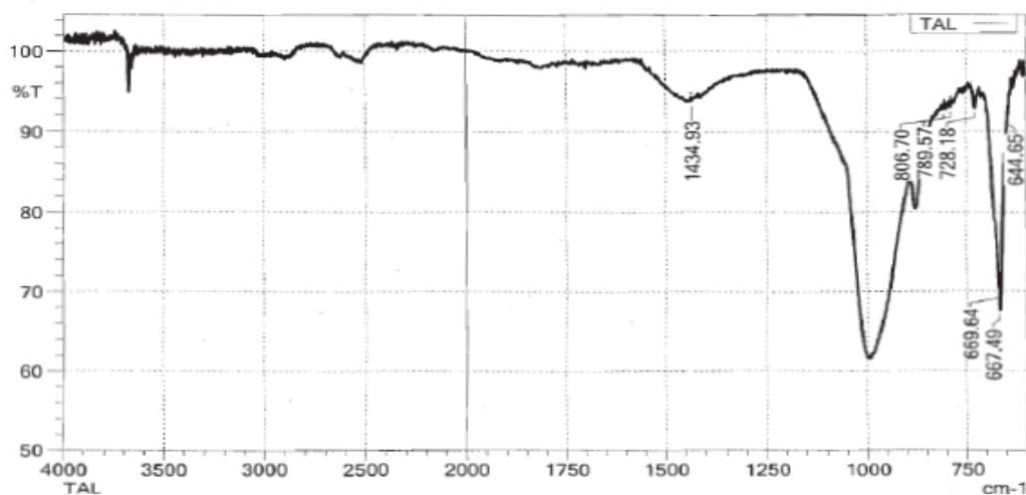


Table 6.44: FTIR spectrum of Talc

Discussion: The principle IR absorption shows peaks of talc at 669.64cm^{-1} (Sharp symmetric Si – O – Si stretching), 1000 cm^{-1} (Asymmetric Si – O – Si stretching) and 1434.93 cm^{-1} (Brucite layer hydroxyl group of chlorite) was remarked. It approved the purity and originality of the talcum Powder.

6.5.1.4 FTIR Study of Magnesium stearate

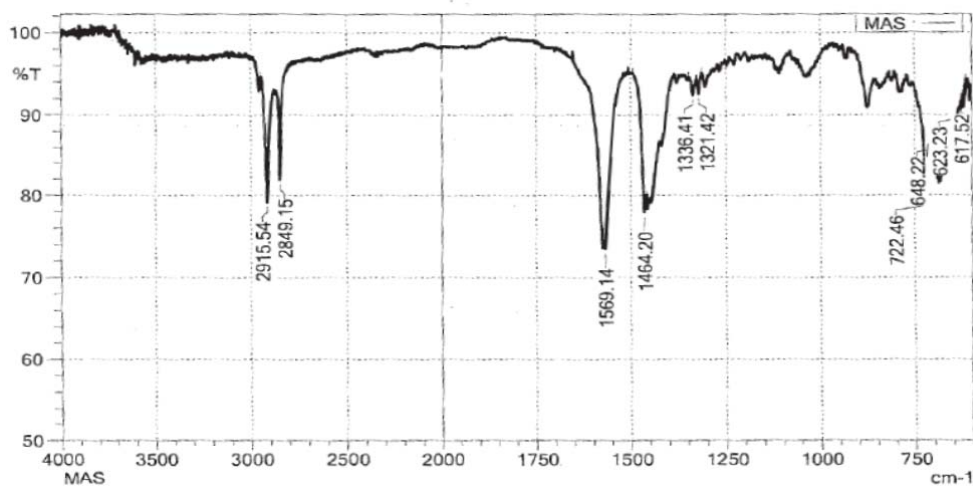


Figure 6.45: FTIR Spectrum of Magnesium stearate

Discussion: The principle IR absorption shows peaks of Magnesium stearate at 2915.54 , 2849.15cm^{-1} ($\text{CH}_2\text{-CH}_3$ vibration) and asymmetric stretch corresponding of the COO -group at 1569.14 and 1464.20cm^{-1} was observed. This observation approved the purity and originality of magnesium stearate.

6.5.1.5 FTIR Study of Physical mixture (Metformin HCl, grafted fenugreek gum, PVP K30, MCC, Magnesium stearate and Talc)

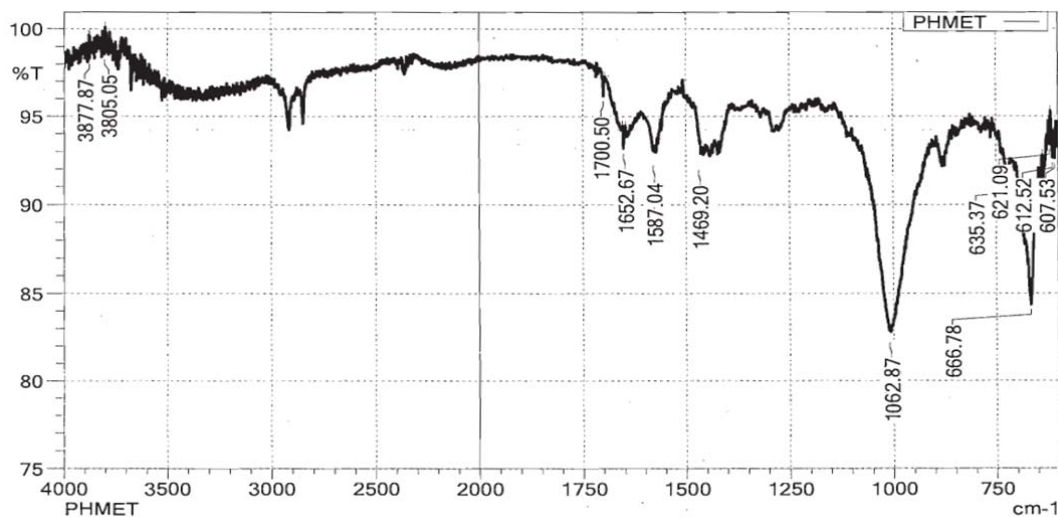


Figure 6.46: FTIR spectrum of Physical mixture

Discussion: The FTIR spectra of physical mixture (Metformin HCl, grafted fenugreek gum, PVP K30, MCC, Magnesium stearate and Talc) were studied that is shown in Figure 6.46. It was found from the result that there is no chance of interaction amongst drug and excipients.

6.5.1.6 FTIR Study of Metformin HCl grafted fenugreek tablet formulation (TS9)

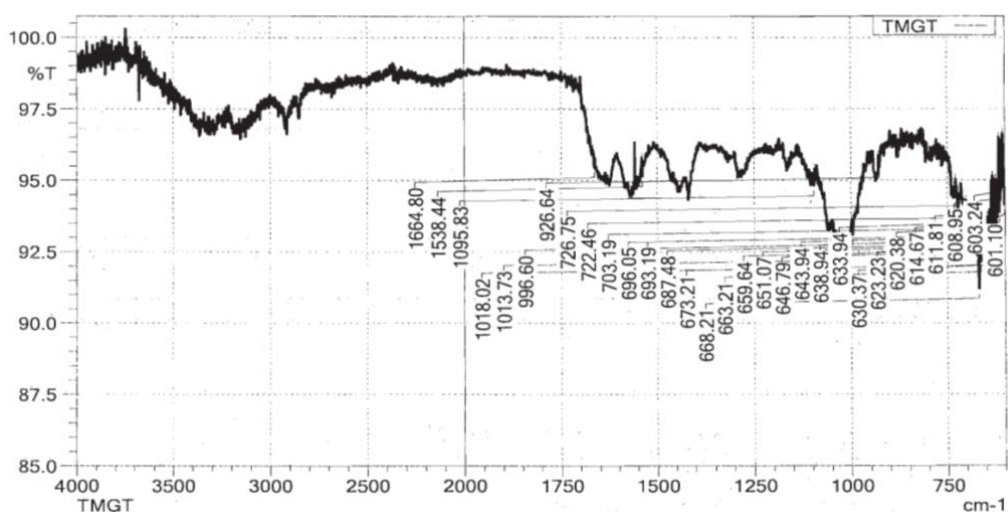


Figure 6.47: FTIR spectrum of Metformin HCl grafted fenugreek tablet formulation (TS9)

Discussion: The principle IR absorption peaks of N-H deformation at 1664.80 cm^{-1} .Asymmetric NCN stretch 1538.44 cm^{-1} , C-N stretching at 1095.83cm^{-1} , CH rock at 926.64 cm^{-1} was observed in the spectra of TS9 formulation that confirmed the purity and authenticity. Also, it do not reveal any significant polymer–drug interaction.

6.6 Optimization of Metformin HCl SR tablet using face Central Composite Design (CCD)

6.6.1 Experimental design

By using design expert software, a central composite design (CCD) was used for optimization of SR Metformin HCl grafted formulation. On account of preformulation study, quantity of grafted fenugreek gum (X1) and PVP K30 (X2) were chosen as independent factors (formulation variable) and % drug released in 1 hr (Y1), % drug released in 8 hr (Y2), time to 50% drug release (Y3) dependent factors (response variables). Experimental design of different batches of tablet as well as responses were shown in table 6.42 & 6.43. Then optimized formulation batch was preferred for further study.

Table 6.42: Summary of Formulation variables and their levels in CCD

Formulation variables	Unit	Low	High	.-alpha	+.alpha
X1=Grafted Gum	Mg	250	350	229.29	370.71
X2=PVPK 30	Mg	20	80	7.57	92.43

Table 6.43: Summary of Response variables

Response variables	Unit
Y1= % drug released in 1hr	%
Y2= % drug released in 8hr	%
Y3=Time of 50% drug release	hrs

Table 6.44: Formulation trials of Metformin HCl tablet as per experimental design

Run	Factor 1	Factor 2
	Grafted fenugreek gum (mg)	PVPK 30 (mg)
1	229.3	50.0
2	300.0	50.0
3	300.0	50.0
4	250.0	20.0
5	370.7	50.0
6	250.0	80.0
7	350.0	80.0
8	300.0	07.6
9	300.0	92.4
10	350.0	20.0
11	300.0	50.0
12	300.0	50.0
13	300.0	50.0

Discussion: All tablet batches and process parameters were kept steady all over the study. Table 6.44 examining an detail of 13 experimental trials runs along with its factor combinations as well as coded levels were translated in experimental units.

6.6.2 Statistical analysis

On account of ANOVA provision, statistical validity of polynomials was determinate for design expert software. Three-dimensional (3D) response surface plots and two dimensional (2-D) contour plots were established, which is effective to notice the interaction effects on response factors. Thirteen optimum checkpoints were choose to validate the experimental design with their evaluation. The resultant experimental data of response properties were quantitatively compared according to its predicted values and also the linear regression plots between them was constructed.

The responses of distinct batches accomplished using central composite design (CCD) are shown in Table 6.45. Obtained data were subjected to multiple regression analysis that is fitted in Eq. (1, 2, 3).

$$Y_1 = \beta_0 + \beta_1 A - \beta_2 B + \beta_3 AB + \beta_4 A^2 + \beta_5 B^2 \dots\dots\dots(1)$$

$$Y_2 = \beta_0 - \beta_1 A + \beta_2 B + \beta_3 AB + \beta_4 A^2 - \beta_5 B^2 \dots\dots\dots(2)$$

$$Y_3 = \beta_0 + \beta_1 A - \beta_2 B - \beta_3 AB - \beta_4 A^2 - \beta_5 B^2 \dots\dots\dots(3)$$

Table 6.45: Responses obtained for studied parameters from experimental batches

Experimental trial no.	Factor 1	Factor 2	Response 1	Response 2	Response 3
	Grafted Gum (mg)	PVVPK 30 (mg)	% drug released in 1hr	% drug released in 8hrs	Time of 50% drug release (hrs)
TD1	229.3	50.0	13.42	63.75	5
TD 2	300.0	50.0	11.59	56.65	8
TD 3	300.0	50.0	10.52	52.65	8
TD 4	250.0	20.0	14.04	62.01	6
TD 5	370.7	50.0	14.64	53.02	8
TD 6	250.0	80.0	12.64	60.65	6
TD 7	350.0	80.0	14.60	59.73	7
TD 8	300.0	7.6	15.21	57.21	7
TD 9	300.0	92.4	13.05	60.53	7
TD 10	350.0	20.0	14.60	52.06	8
TD 11	300.0	50.0	10.76	53.75	8
TD 12	300.0	50.0	12.01	55.97	7
TD 13	300.0	50.0	11.97	54.98	8

Table 6.46: Concentration of studied parameters from experimental batches

Experimental trial no.	Drug	Grafted Gum	PVPK 30	Magnesium Stearate	Talc	MCC	Total
TD1	500	229.29	50.00	10	10	200.0	1000
TD 2	500	300.00	50.00	10	10	130.00	1000
TD 3	500	300.00	50.00	10	10	130.00	1000
TD 4	500	250.00	20.00	10	10	210.00	1000
TD 5	500	370.71	50.00	10	10	59.29	1000
TD 6	500	250.00	80.00	10	10	150.00	1000
TD 7	500	350.00	80.00	10	10	50.00	1000
TD 8	500	300.00	7.57	10	10	172.43	1000
TD 9	500	300.00	92.43	10	10	87.57	1000
TD 10	500	350.00	20.00	10	10	110.00	1000
TD 11	500	300.00	50.00	10	10	130.00	1000
TD 12	500	300.00	50.00	10	10	130.00	1000
TD 13	500	300.00	50.00	10	10	130.00	1000

6.6.3 Precompression evaluation**Table 6.47: Characterization of different composition of experimental grafted tablet granules (Mean \pm SD)**

Formulation Code	Angle of repose (θ) (Mean \pm SD)	Bulk density (g/cm ³) (Mean \pm SD)	Tap density (g/cm ³) (Mean \pm SD)	Hausner's Ratio (Mean \pm SD)	Carr's index (%) (Mean \pm SD)	LOD (Mean \pm SD)
TD1	26.49 \pm 0.56	0.44 \pm 0.03	0.50 \pm 0.01	1.12 \pm 0.01	11.28 \pm 1.40	2.12 \pm 0.02
TD2	24.027 \pm 0.31	0.34 \pm 0.05	0.38 \pm 0.07	1.09 \pm 0.02	8.92 \pm 2.22	1.03 \pm 0.01
TD3	23.18 \pm 0.21	0.37 \pm 0.07	0.40 \pm 0.01	1.08 \pm 0.01	7.77 \pm 1.18	1.05 \pm 0.01
TD4	33.18 \pm 0.15	0.38 \pm 0.06	0.44 \pm 0.02	1.14 \pm 0.02	12.44 \pm 1.67	0.86 \pm 0.03
TD5	30.20 \pm 0.56	0.33 \pm 0.04	0.37 \pm 0.03	1.12 \pm 0.01	11.05 \pm 0.95	0.44 \pm 0.01
TD6	33.20 \pm 0.24	0.37 \pm 0.05	0.45 \pm 0.01	1.19 \pm 0.03	15.95 \pm 2.63	1.72 \pm 0.07
TD7	25.24 \pm 0.20	0.35 \pm 0.05	0.38 \pm 0.01	1.08 \pm 0.014	7.83 \pm 1.19	2.39 \pm 0.04
TD8	25.95 \pm 0.40	0.33 \pm 0.06	0.40 \pm 0.01	1.21 \pm 0.02	17.66 \pm 1.32	0.64 \pm 0.02
TD9	35.33 \pm 0.39	0.41 \pm 0.05	0.46 \pm 0.07	1.12 \pm 0.02	11.04 \pm 2.29	2.55 \pm 0.01
TD10	30.22 \pm 0.23	0.35 \pm 0.07	0.41 \pm 0.06	1.17 \pm 0.03	14.45 \pm 2.78	2.90 \pm 0.02
TD11	21.47 \pm 0.19	0.35 \pm 0.05	0.39 \pm 0.01	1.10 \pm 0.02	9.66 \pm 1.603	1.07 \pm 0.02
TD12	20.57 \pm 0.42	0.35 \pm 0.06	0.38 \pm 0.07	1.07 \pm 0.01	7.20 \pm 1.15	1.12 \pm 0.02
TD13	23.03 \pm 0.12	0.33 \pm 0.03	0.36 \pm 0.03	1.11 \pm 0.06	10.31 \pm 0.44	0.95 \pm 0.01

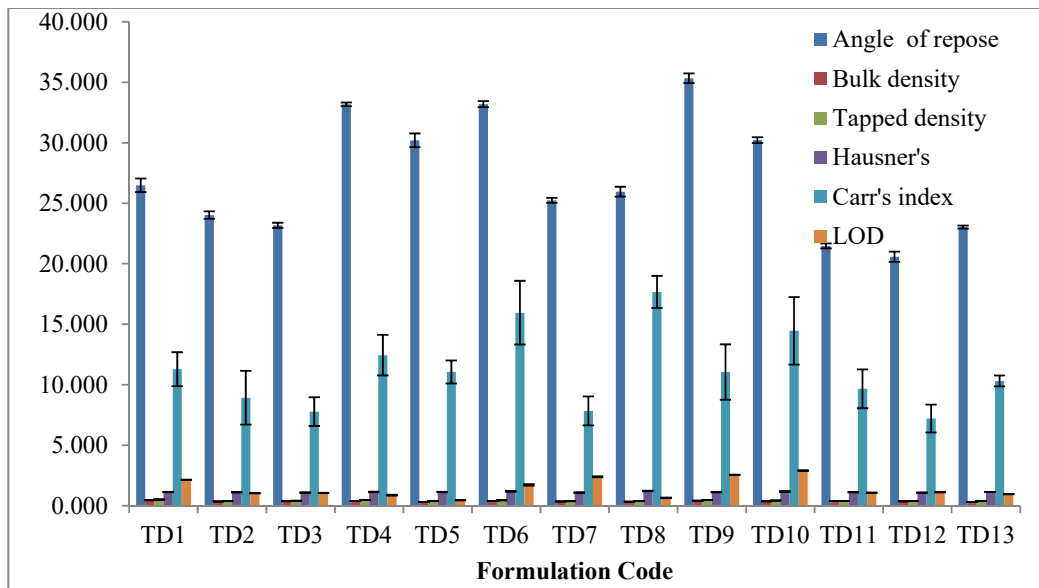


Figure 6.48: Characterization of different composition of experimental granules

Discussion: For each designed composition, granules were prepared as well as evaluated for pre-compression properties, shown in Table 6.48. Angle of repose was detected within range from 20.57 ± 0.42 to 35.33 ± 0.398 . Bulk density lies in between 0.33 ± 0.04 to $0.44 \pm 0.03 \text{ gm/cm}^3$ and tapped density between 0.36 ± 0.03 to $0.50 \pm 0.01 \text{ gm/cm}^3$ for all batches. Hausner's ratio lies within 1.07 ± 0.01 to 1.215 ± 0.02 , Carr's index was found from 7.20 ± 1.15 to 17.66 ± 1.32 and LOD was found in a range of 0.44 ± 0.01 to 2.9 ± 0.02 . All the batches shows good to excellent flow properties, Hence tablets were prepared with these granules combination by wet granulation method.

6.6.4 Post compression evaluations

6.6.4.1 Weight variation

Table 6.48: Weight variation data of experimental grafted tablets

Sr. No.	Formulation code	Weight variation (mg) Mean±SD
1	TD1	1000.6±4.9
2	TD2	995.6±3.44
3	TD3	993.5±2.07
4	TD4	1000.4±4.25
5	TD5	1002±5.72
6	TD6	993.5±3.31
7	TD7	999.8±4.37
8	TD8	1001.8±5.29
9	TD9	992.8±2.04
10	TD10	998.1±3.6
11	TD11	998.7±4.95
12	TD12	993.0±2.31
13	TD13	997.1±1.37

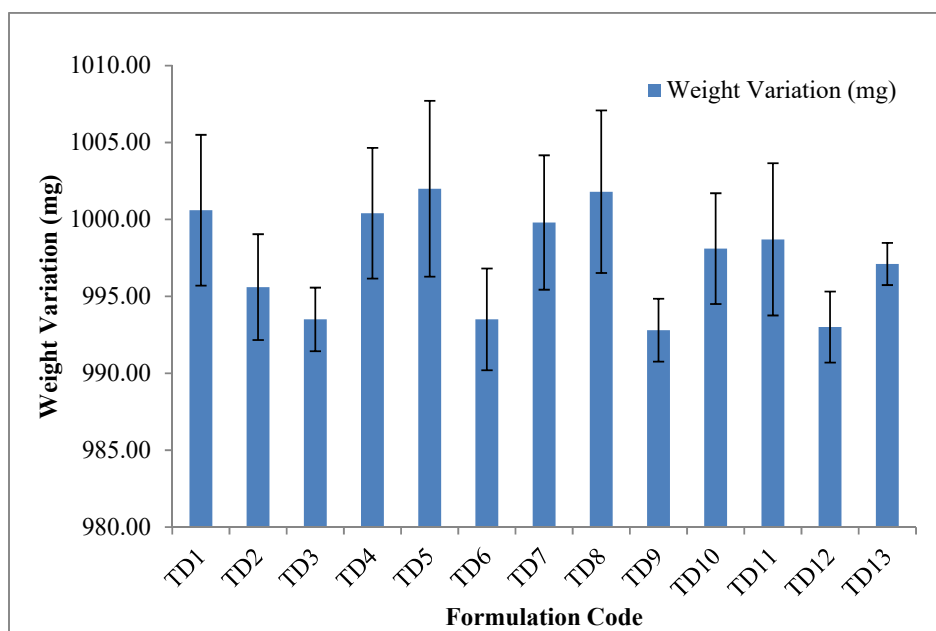


Figure 6.49: Weight variation of experimental designed tablet batches

Discussion: In all the design batches, the tablet weight was found to be in range between 993.0±2.31 to 1002±5.72mg, therefore 5% maximum difference is allowed. Weight variation for tablets are shown in the Figure 6.49& Table 6.48.

6.6.4.2 Thickness

Thickness was measured using vernier caliper and its evaluation described like mean \pm SD.

Table 6.49: Thickness of experimental designed Metformin HCl tablets

Sr. No.	Formulation code	Thickness (mm)
1	TD1	6.06 \pm 0.03
2	TD2	6.10 \pm 0.02
3	TD3	6.06 \pm 0.03
4	TD4	6.12 \pm 0.01
5	TD5	6.16 \pm 0.05
6	TD6	6.05 \pm 0.02
7	TD7	6.04 \pm 0.03
8	TD8	6.07 \pm 0.02
9	TD9	6.01 \pm 0.01
10	TD10	6.02 \pm 0.02
11	TD11	6.14 \pm 0.03
12	TD12	6.06 \pm 0.02
13	TD13	6.09 \pm 0.02

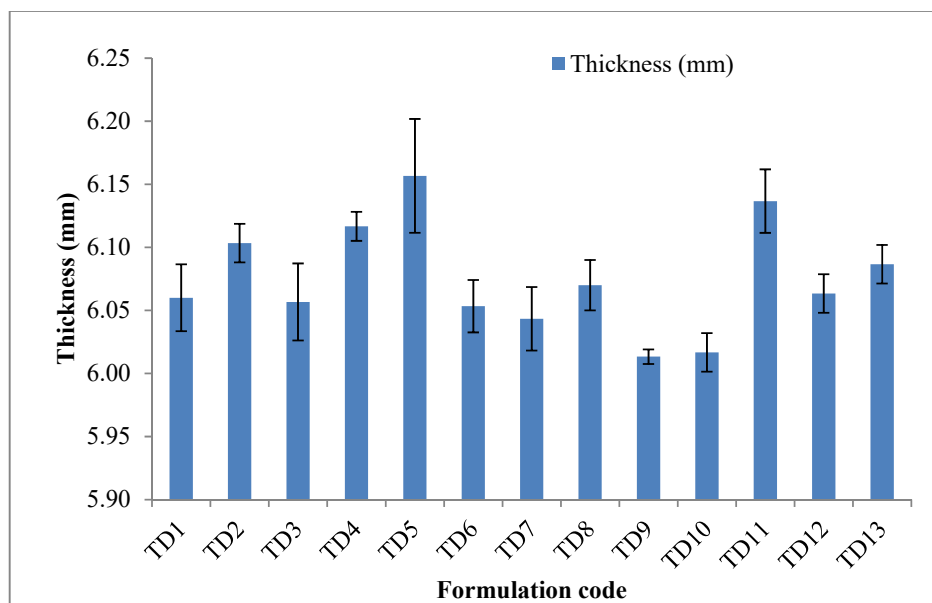


Figure 6.50: Thickness of experimental designed grafted tablet batches

Discussion: The thickness of all the batches was found to be in between 6.01 \pm 0.01mm to 6.16 \pm 0.05mm.

6.6.4.3 Hardness

The hardness was assessed by Monsanto hardness tester.

Acceptance limit: 5-8 kg/cm²

Table 6.50: Hardness of experimental designed batches

S.No.	Formulation code	Hardness (kg/cm ²)
1	TD1	5.63±0.07
2	TD2	5.76±0.13
3	TD3	5.82±0.06
4	TD4	5.79±0.09
5	TD5	6.00±0.02
6	TD6	5.85±0.04
7	TD7	6.04±0.04
8	TD8	5.93±0.07
9	TD9	5.94±0.05
10	TD10	6.03±0.02
11	TD11	5.82±0.04
12	TD12	5.76±0.12
13	TD13	5.71±0.03

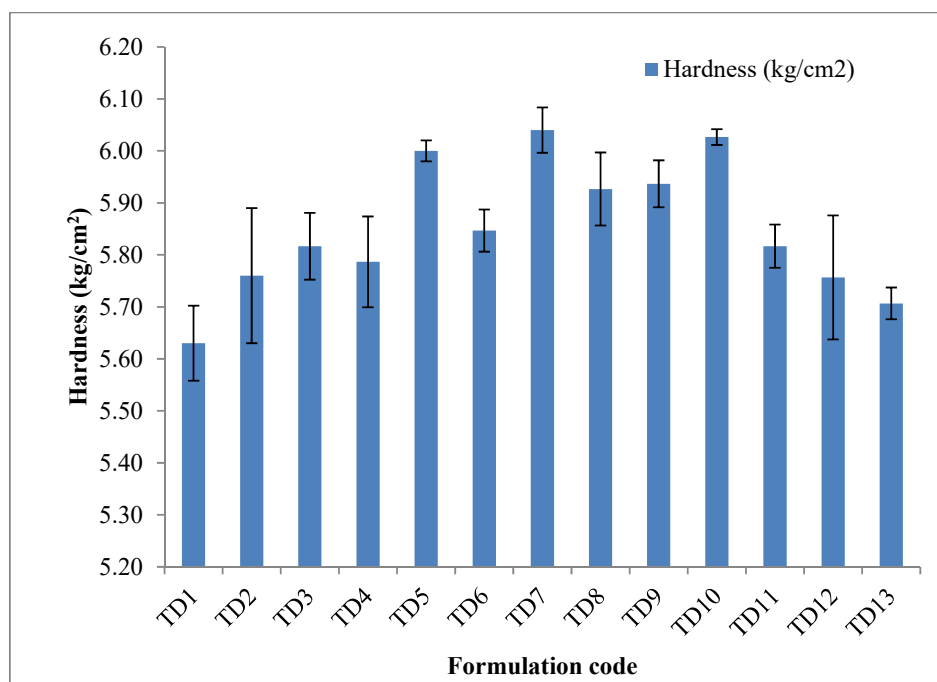


Figure 6.51: Hardness of experimental designed grafted tablet batches

Results: Table 6.50 and Figure 6.51 shows the tablet hardness within range 5.63±0.07 to 6.04±0.04.

6.6.4.4 Friability

Table 6.51: Friability data of experimental designed batches

S. No.	Formulation code	% Friability
1	TD1	0.53±0.30
2	TD2	0.65±0.13
3	TD3	0.51±0.11
4	TD4	0.33±0.11
5	TD5	0.51±0.10
6	TD6	0.87±0.41
7	TD7	0.63±0.05
8	TD8	0.31±0.10
9	TD9	0.53±0.23
10	TD10	0.30±0.17
11	TD11	0.26±0.11
12	TD12	0.53±0.23
13	TD13	0.40±0.20

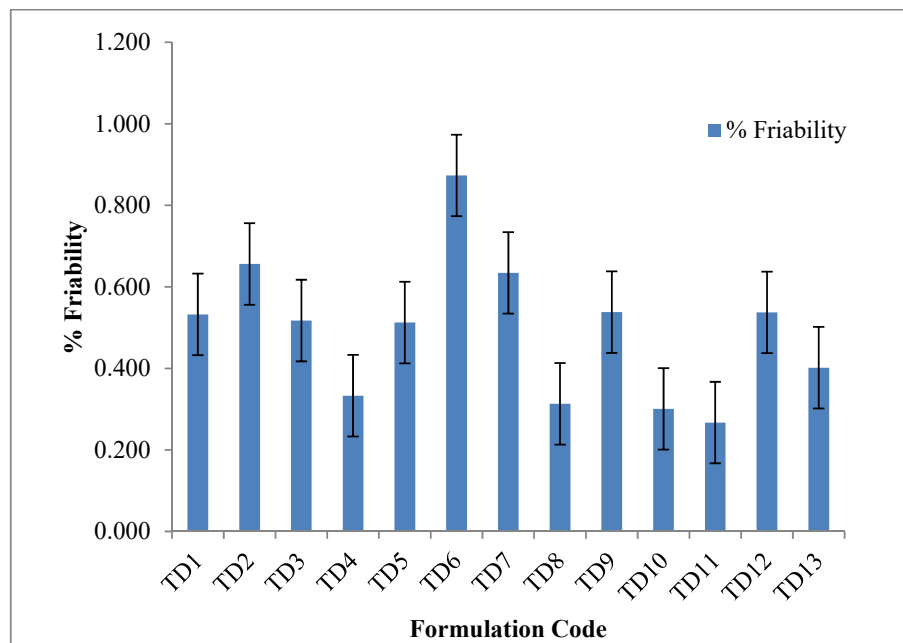


Figure 6.52: Friability of experimental designed batches

Discussion: The percent friability of the tablet batches was below 1% that lies within the prescribed limits.

6.6.4.5 Swelling study

Table 6.52:Percentage swelling study of experimental designed batches

S. No.	Formulation code	0.1NHCl (pH1.2)	Phosphate buffer (pH6.8)
		4hrs	24hrs
1	TD1	19.47±0.03	92.60±0.05
2	TD2	26.89±0.02	129.63±0.01
3	TD3	29.45±0.04	127.33±0.09
4	TD4	32.29±0.04	98.88±0.02
5	TD5	30.29±0.06	129.23±0.01
6	TD6	23.01±0.04	86.57±0.01
7	TD7	21.20±0.05	144.54±0.07
8	TD8	18.36±0.06	139.15±0.03
9	TD9	24.55±0.06	137.79±0.06
10	TD10	26.89±0.02	140.14±0.08
11	TD11	29.49±0.01	127.41±0.05
12	TD12	22.78±0.02	117.92±0.04
13	TD13	28.71±0.05	121.83±0.01

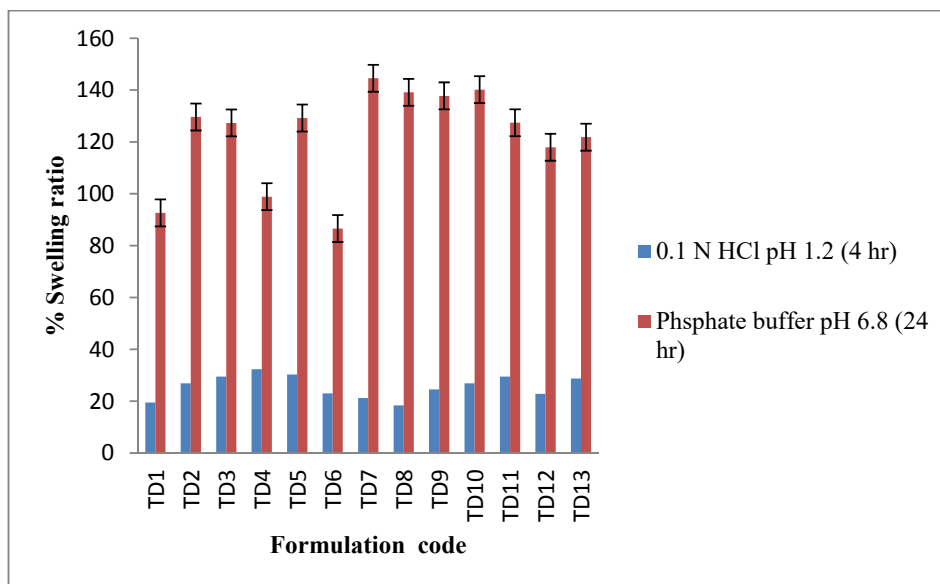


Figure 6.53:Percent swelling study of experimental designed batches

Discussion: The swelling study of grafted polymeric tablets were examined and compared their water uptake capacities. Figure 6.53 and Table 6.52 shows that grafted Metformin HCl tablet have high level of swelling, resulting from water

uptake ranges from 92.60 to 144.54% in phosphate buffer, which is increased by increasing quantity of grafted gum, that is expected to appear distinct hydrophilic groups.

6.6.4.6 Drug Content:

Table 6.53: Drug content of experimental designed grafted tablet batches

S.No.	Formulations	% Drug Content
1	TD1	98.00±0.90
2	TD2	99.75±0.25
3	TD3	98.33±0.87
4	TD4	97.91±0.80
5	TD5	99.66±0.52
6	TD6	98.75±0.50
7	TD7	96.83±1.75
8	TD8	99.66±0.52
9	TD9	97.41±0.52
10	TD10	97.83±0.57
11	TD11	99.25±0.43
12	TD12	98.58±0.38
13	TD13	98.08±0.76

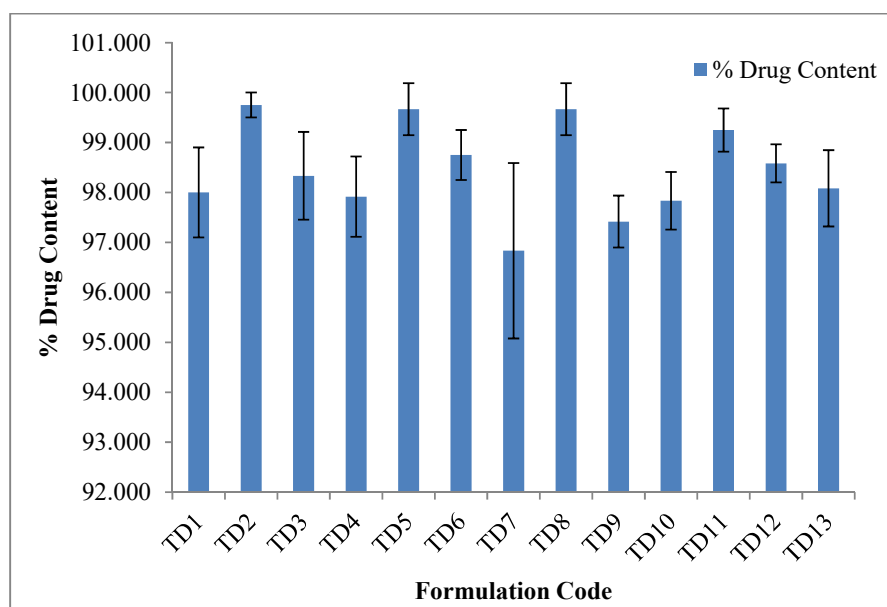


Figure 6.54: % Drug content of experimental designed grafted tablet batches

Discussion: The %drug content range lies from 96.83±1.75 to 99.75±0.25in tablet batches, which indicates the uniformity of mixing, that is shown in Table 6.53 & Figure 6.54.

6.6.4.7 In vitro drug release

Table 6.54(a): In vitro drug release of experimental designed tablet batches

Dissolution Media	Time (hr)	% drug release TD1	% drug release TD2	% drug release TD3	% drug release TD4	% drug release TD5	% drug release TD6	% drug release TD7
0.1N HCL	0.25	0.82±0.10	1.87±0.05	1.23±0.07	1.63±0.07	3.73±0.03	5.88±0.07	3.14±0.01
	0.5	5.14±0.05	8.73±0.01	5.73±0.03	6.35±0.121	9.76±0.04	10.93±0.01	7.83±0.01
	1	13.42±0.06	11.59±0.01	10.52±0.01	14.04±0.06	14.64±0.05	12.64±0.07	14.60±0.02
	1.5	21.32±0.02	14.94±0.06	15.94±0.07	21.77±0.05	18.77±0.04	18.94±0.06	18.26±0.06
	2	32.50±0.34	20.46±0.06	21.46±0.03	27.34±1.08	25.34±0.35	24.47±0.46	23.61±0.23
pH6.8 Phosphate Buffer	3	40.53±0.20	25.75±0.53	26.75±0.41	33.73±0.18	34.37±0.24	29.75±0.41	32.84±0.49
	4	45.11±0.30	36.97±0.34	32.97±0.60	39.51±0.57	37.27±0.24	36.97±0.34	39.48±0.12
	5	50.94±0.11	45.65±0.26	40.65±0.26	45.03±0.77	40.07±0.45	45.65±0.26	43.11±0.68
	6	55.24±0.28	49.15±0.11	45.15±0.26	52.76±0.68	43.51±0.34	53.15±0.65	48.53±0.31
	8	63.75±0.68	56.65±0.30	52.65±0.03	62.01±0.36	53.02±0.58	60.65±0.70	59.73±0.41
	10	69.75±0.33	60.72±0.41	59.72±0.71	66.63±0.27	57.43±0.19	64.73±0.44	64.21±0.67
	24	82.51±0.48	96.37±0.46	94.37±0.69	84.12±0.88	89.58±0.418	97.38±0.35	96.98±0.21

Table 6.54(b): In vitro drug release of experimental designed tablet batches

Dissolution Media	Time (hr)	%drug release TD8	%drug release TD9	%drug release TD10	%drug release TD11	%drug release TD12	% drug release TD13
0.1 N HCl	0.25	0.53±0.08	0.73±0.12	7.73±0.08	2.06±0.07	1.32±0.09	1.03±0.10
	0.5	6.74±0.04	6.76±0.03	10.76±0.07	9.35±0.07	6.54±0.11	4.01±0.07
	1	15.21±0.08	13.05±0.05	14.6±0.05	10.76±0.09	12.01±0.07	11.97±0.02
	1.5	21.73±0.06	16.21±0.06	22.77±0.43	17.26±0.07	16.42±0.07	15.43±0.05
	2	26.52±0.36	22.34±0.37	29.34±0.43	22.72±0.41	21.73±0.08	20.75±0.22
Phosphate Buffer pH6.8	3	35.63±0.64	31.37±0.21	38.37±0.28	30.32±0.68	29.05±0.56	24.98±0.57
	4	39.42±0.04	38.27±1.05	42.27±0.48	34.12±0.50	34.11±0.56	30.52±0.58
	5	43.82±0.31	43.07±0.49	45.07±0.12	40.18±0.40	43.85±0.50	42.53±0.54
	6	49.25±0.56	48.51±0.36	47.51±0.45	45.53±0.77	48.74±0.74	47.35±0.49
	8	57.21±0.36	60.52±0.59	55.43±0.47	53.75±0.53	55.97±0.71	54.98±0.75
	10	62.65±0.42	65.43±0.16	55.43±0.34	59.63±0.69	61.97±0.36	61.97±0.53
	24	82.62±0.51	80.58±0.42	92.58±0.30	95.12±0.68	96.01±0.28	95.84±0.69

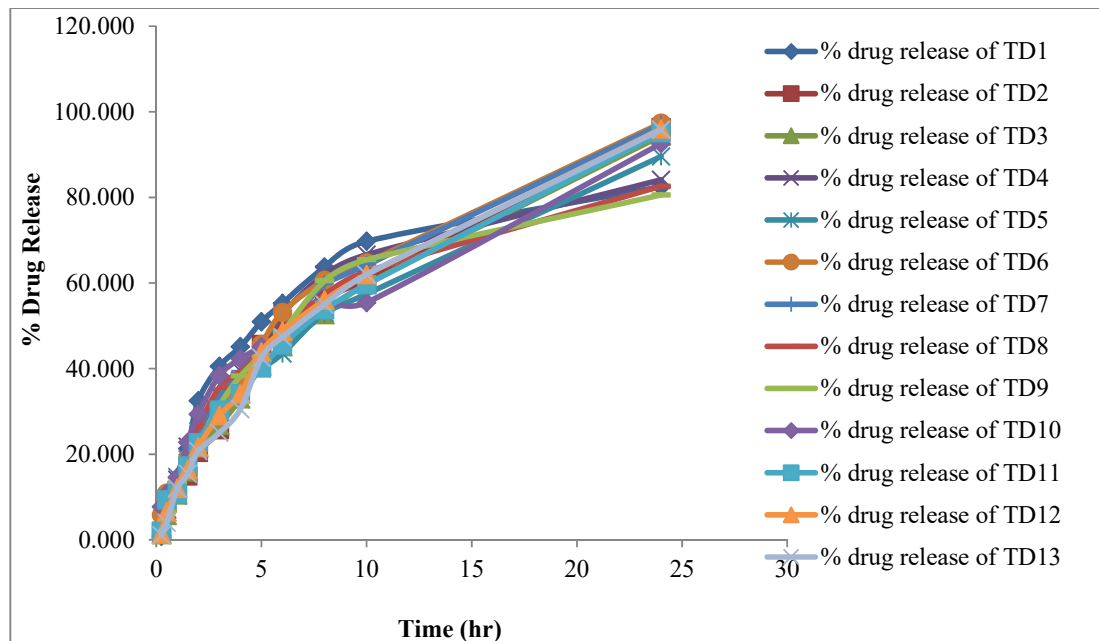


Figure 6.55: % drug release of experimental designed grafted tablet batches

In vitro drug release study of experimental designed grafted amount is demonstrated in Figure 6.55 and Table 6.54 (a,b).

6.7 Model Fitting in to data

According to Design expert software, the response data (% drug released within 1 hr, % drug released within 8 hrs, time to 50% drug release) for all the optimized formulations were fitted in to quadratic model with full model (FM) polynomial equation.

6.7.1 Coded equation

$$\% \text{ drug released in 1hr} = 11.37 + 0.530990792 * A - 0.555630555 * B + 0.351 * A * B + 1.3026875 * A^2 + 1.3534375 * B^2$$

$$\% \text{ drug released in 8hrs} = 54.8 - 3.255835387 * A + 1.374859415 * B + 2.25875 * A * B + 1.7889375 * A^2 + 2.0304375 * B^2$$

$$\text{Time of 50\% drug release} = 7.8 + 0.905330086 * A - 0.125 * B - 0.25 * A * B - 0.65 * A^2 - 0.4 * B^2$$

6.7.2 Responses- ANOVA for response surface quadratic model

6.7.2.1 Response 1- Cumulative % drug released in 1hr

Table 6.55: Analysis of variance of Cumulative % drug released in 1hr

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	26.93	5	5.38	15.98	0.0010	significant
A-Grafted gum	2.25	1	2.25	6.69	0.0361	
B-PVPK 30	2.47	1	2.47	7.33	0.0303	
AB	0.49	1	0.493	1.46	0.2658	
A ²	11.80	1	11.80	35.03	0.0006	
B ²	12.74	1	12.74	37.82	0.0005	
Residual	2.35	7	0.33			
Lack of Fit	0.44	3	0.149	0.311	0.8178	not significant

Discussion: The Model F-value 15.99 mention the model were significant, on that point it was 0.10% probability that a "Model F-Value" appeared larger by noise. If "Prob > F" is lesser than 0.0500, it specifies that model terms was significant because the values more than 0.1000 suggested the model term was not significant. In that event A, B, A², B² was significant model terms. "Lack of Fit F-value" 0.31 suggested the Lack of Fit was not significant relative to the pure error. There is 81.78% probability that a "Lack of Fit will be large due to noise. Non-significant 'lack of fit' value was suitable for the model. The "Pred R-Squared" 0.7897 was reasonable agreement along with the "Adj R-Squared" 0.8620. "Adeq Precision" compute the signal to noise ratio. The ratio 8.85 points an adequate signal as the ratio greater than 4 was recommended. These model can be comfortable with design space.

6.7.2.2 Response 2- % Cumulative drug released in 8hrs

Table 6.56: Analysis of variance Cumulative % drug released in 8hrs

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	165.45	5	33.09	17.562	0.0008	Significant
A-Grafted gum	84.80	1	84.80	45.007	0.0003	
B-PVPK 30	15.12	1	15.12	8.026	0.0253	
AB	20.40	1	20.40	10.831	0.0133	
A ²	22.26	1	22.26	11.815	0.0109	
B ²	28.67	1	28.67	15.221	0.0059	
Residual	13.19	7	1.88			
Lack of Fit	2.64	3	0.88	0.334	0.8029	not significant
Pure Error	10.54	4	2.637			
Cor Total	178.64	12				

Discussion: The Model F-value 17.56 suggested the model was significant and just 0.08% chances that "Model F-Value" could be larger by noise. Values of "Prob > F" below 0.05, recommended model terms was significant. In such case A, B, AB, A², B² was significant model terms. In that event numerous insignificant model terms may improve this model. "Lack of Fit F-value" 0.33 mentioned that it is not significant toward pure error. Indeed, there is 80.29% possibility of "Lack of Fit appeared larger due to noise. The "Pred R-Squared" 0.8026 was reasonable agreement with the "Adj R-Squared" of 0.8734. A ratio above 4 was appropriate and ratio was found to be 12.066, suggested sufficient adequate signal. This model can be common handle design space.

6.7.2.3 Response 3- Time to 50% drug release

Table 6.57: Analysis of variance : Time to 50% drug release

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	10.574	5	2.115	13.241	0.0019	significant
A-Grafted Gum	6.557	1	6.557	41.054	0.0004	
B-PVPK 30	0.125	1	0.125	0.783	0.4057	
AB	0.250	1	0.250	1.565	0.2511	
A ²	2.939	1	2.939	18.402	0.0036	
B ²	1.113	1	1.113	6.969	0.0334	
Residual	1.118	7	0.160			
Lack of Fit	0.318	3	0.106	0.530	0.6855	not significant
Pure Error	0.800	4	0.200			
Cor Total	11.692	12				

Discussion: The Model F-value 13.24 mention the model were significant and only 0.19% chances that "Model F-Value" is larger because of noise. Values "Prob > F" lesser than 0.0500 recommended the model terms were significant because the values more than 0.1000 shows the model terms was not significant. In this case A, A++2+-, B++2+- were significant model terms. "Lack of Fit 0.53 implies that it was not significant relative to pure error and only there is 68.55% probability that "Lack of Fit F-value" can occur larger by noise. "Pred R-Squared" 0.6997 was reasonable agreement along with "Adj R-Squared" 0.8361. "Adeq Precision" compute the signal to noise ratio. The ratio 10.352 indicates the signal was adequate because the ratio greater than 4 was recommendable . This model is common to handle the design space.

6.7.3 Normal plot as for residuals

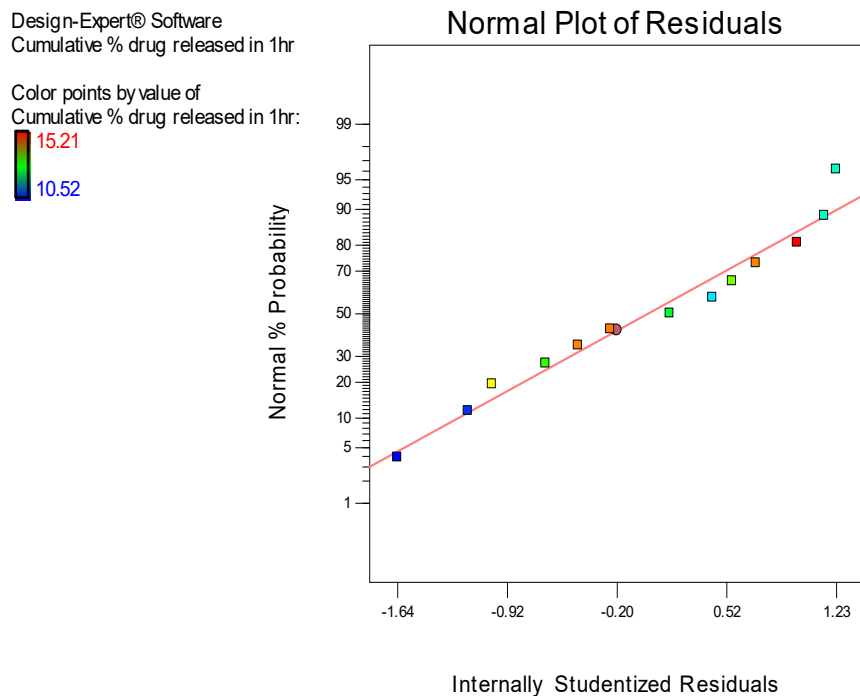


Figure 6.56 (a): Normal plot of residuals Cumulative % drug released in 1hr

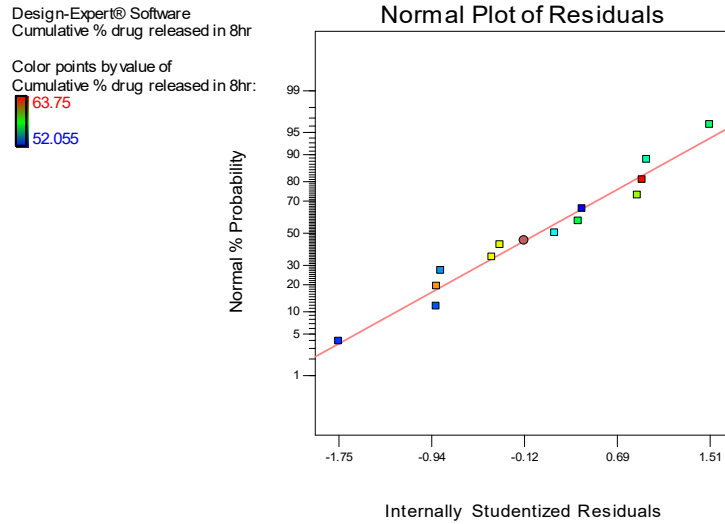


Figure 6.56 (b): Normal plot of residuals Cumulative % drug released in 8hrs

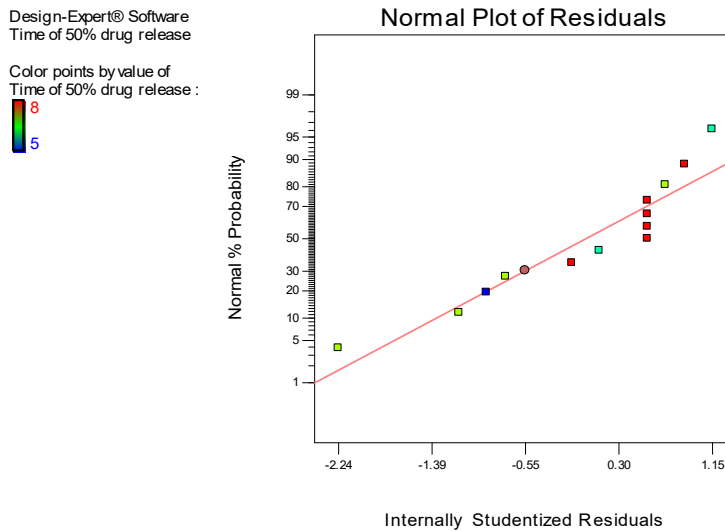


Figure 6.56 (c): Normal Plot of residuals time to 50% drug release

Discussion: The normal probability plot indicates the residuals (Figure 6.56 a, b, c) which follow a normal distribution with straight line points. With normal data, expect some moderate scatter in which assigned patterns like an "S-shaped" curve were seen particularly, indicates the variation about response which can give stronger analysis.

6.7.4 Response surface (3D) and Contour plot analysis

To analyze this result of independent factors on response, three-dimensional (3D) plots & contour plot are shown in Figure 6.57 (a, b, c) and Figure 6.58 (a,b,c). All observed response surfaces formed hillsides along with large curvatures confirms that they were impacted by effect of concentrations of dependent factors.

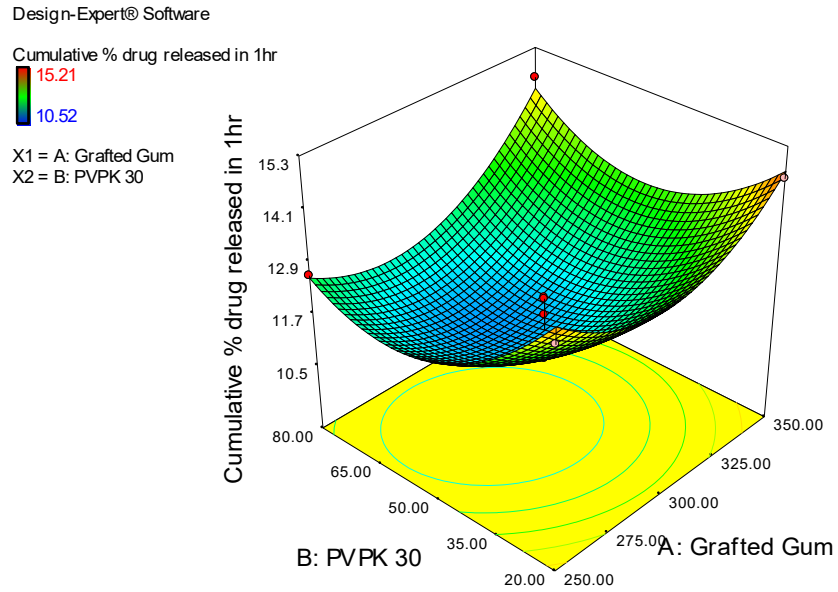


Figure 6.57 (a): 3D Response surface plot for Cumulative % drug released in 1hr

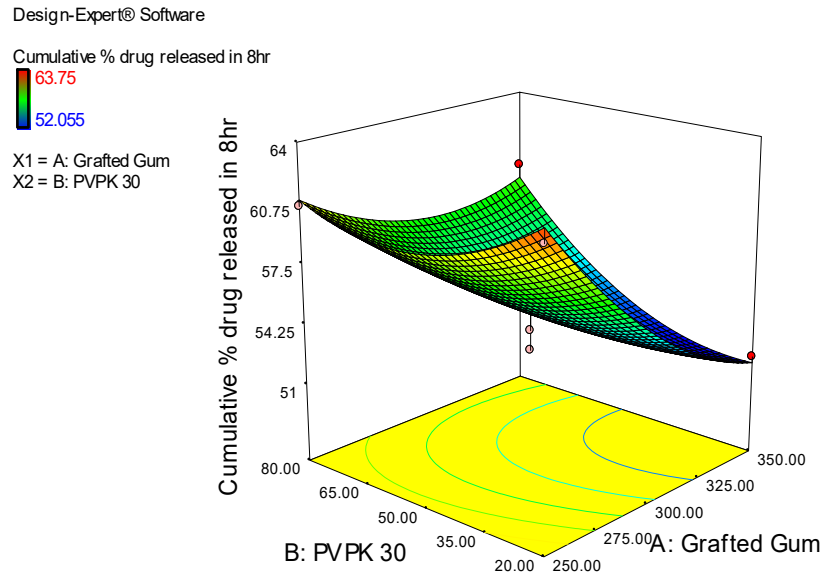


Figure 6.57 (b): 3D Response surface plot for Cumulative % drug released in 8hrs

Design-Expert® Software

Time of 50% drug release



X1 = A: Grafted Gum
X2 = B: PVPK 30

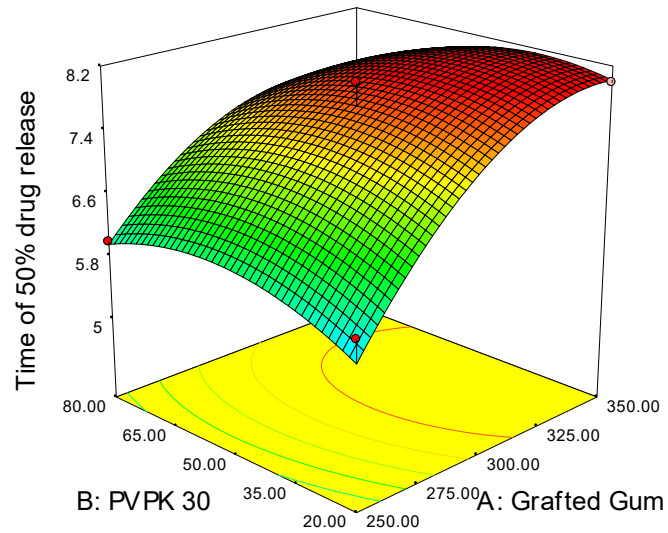


Figure 6.57 (c): 3D Response surface plot for time to 50% drug release

Design-Expert® Software

Cumulative % drug released in 1hr

● Design Points



X1 = A: Grafted Gum
X2 = B: PVPK 30

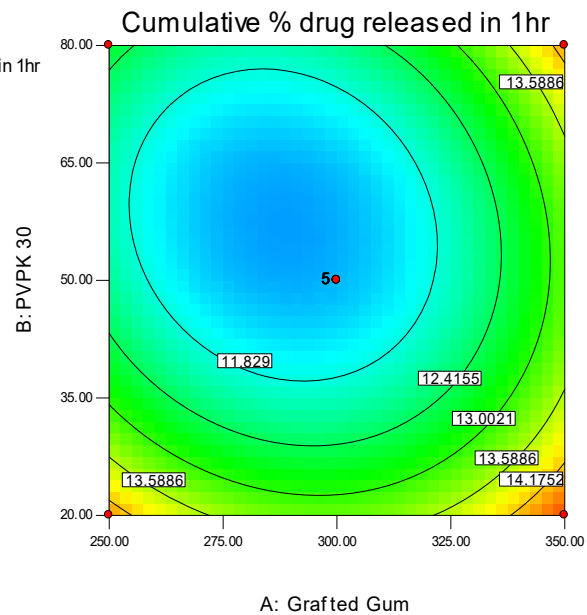


Figure 6.58 (a): Contour plot showing Cumulative % drug released in 1hr

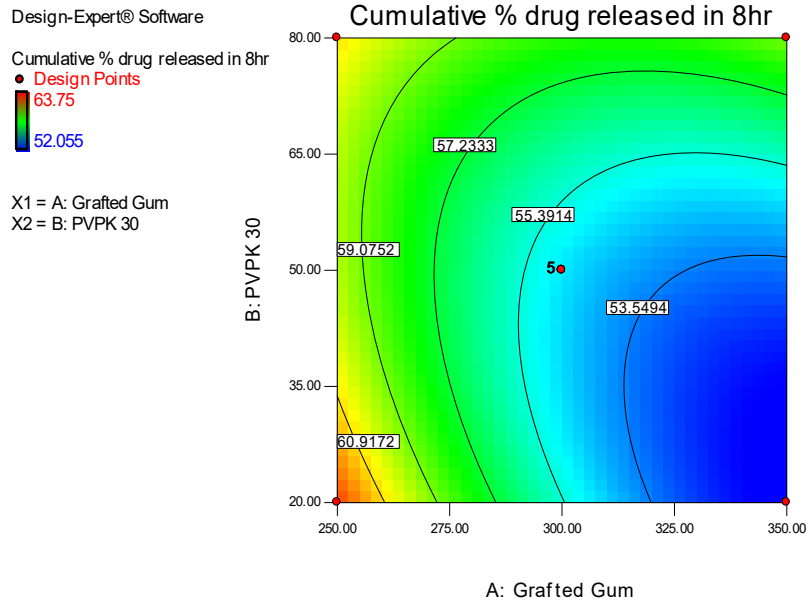


Figure 6.58 (b): Contour plot showing Cumulative % drug released in 8hrs

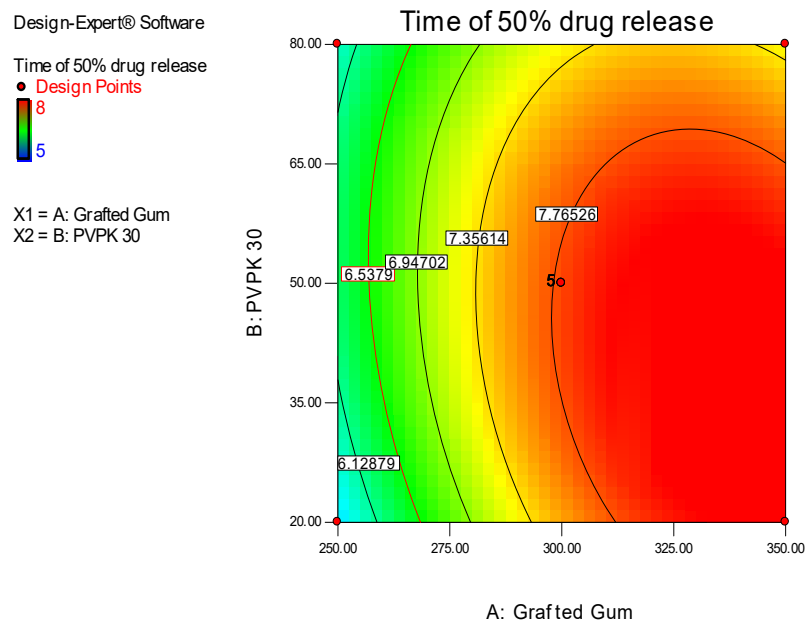


Figure 6.58 (c): Contour plot showing time to 50% drug release

6.7.5 Numerical Optimization Solution

Numerical optimization solutions found the design space and factor settings using the models which are created in the analysis, describes the objective for all response.

Then eventually, the software were bring about a list of potential factor settings which give responses, specifically assigned norms.

Table 6.58: Numerical optimization solution of experimental design batches

Number of Solutions	Grafted gum	PVPK 30	Predicted response		
			% drug released (1hr)	% drug released (8hrs)	Time to 50% drug release
TS1	273.97	40.32	11.82	57.12	7.11
TS2	264.09	33.24	12.53	58.83	6.66
TS3	271.69	55.05	11.39	57.29	7.07
TS4	302.92	20.77	13.21	55.07	7.61
TS5	283.06	55.57	11.26	56.29	7.40
TS6	299.19	76.66	11.93	57.64	7.36
TS7	251.86	41.55	12.42	59.98	6.26
TS8	307.02	78.23	12.19	57.76	7.41
TS9	347.98	73.53	13.74	57.35	7.54
TS10	262.49	58.66	11.58	58.32	6.74

6.7.6 Precompression Evaluation of optimized tablets batch (TS9)

Table 6.59: Characterization of optimized grafted tablet granules solutions(Mean \pm SD)

Sr. no.	Formulation Code	Angle of repose (θ) (Mean \pm SD)	Bulk density (g/cm ³) (Mean \pm SD)	Tap density (g/cm ³) (Mean \pm SD)	Hausner's Ratio (Mean \pm SD)	Carr's index (%) (Mean \pm SD)	LOD (Mean \pm SD)
1	TS1	26.36 \pm 0.38	0.45 \pm 0.03	0.52 \pm 0.01	1.17 \pm 0.02	14.64 \pm 1.53	0.92 \pm 0.02
2	TS2	26.24 \pm 1.09	0.35 \pm 0.03	0.38 \pm 0.05	1.08 \pm 0.02	7.53 \pm 1.79	2.25 \pm 0.02
3	TS3	23.01 \pm 0.56	0.38 \pm 0.01	0.40 \pm 0.03	1.04 \pm 0.01	4.15 \pm 0.88	0.65 \pm 0.07
4	TS4	30.74 \pm 0.85	0.40 \pm 0.04	0.44 \pm 0.03	1.11 \pm 0.07	9.87 \pm 0.60	1.13 \pm 0.02
5	TS5	33.54 \pm 0.18	0.33 \pm 0.05	0.35 \pm 0.05	1.04 \pm 0.02	4.42 \pm 2.10	1.06 \pm 0.02
6	TS6	21.65 \pm 0.25	0.38 \pm 0.02	0.45 \pm 0.01	1.18 \pm 0.04	15.28 \pm 2.82	2.35 \pm 0.03
7	TS7	30.60 \pm 0.18	0.33 \pm 0.04	0.37 \pm 0.04	1.10 \pm 0.02	9.21 \pm 1.96	0.95 \pm 0.01
8	TS8	22.55 \pm 0.42	0.33 \pm 0.03	0.38 \pm 0.01	1.13 \pm 0.01	11.74 \pm 0.92	0.35 \pm 0.02
9	TS9	23.43 \pm 0.07	0.42 \pm 0.05	0.47 \pm 0.08	1.11 \pm 0.09	10.23 \pm 0.70	1.25 \pm 0.04
10	TS10	32.56 \pm 0.53	0.42 \pm 0.06	0.44 \pm 0.08	1.06 \pm 0.01	5.71 \pm 1.52	2.42 \pm 0.02

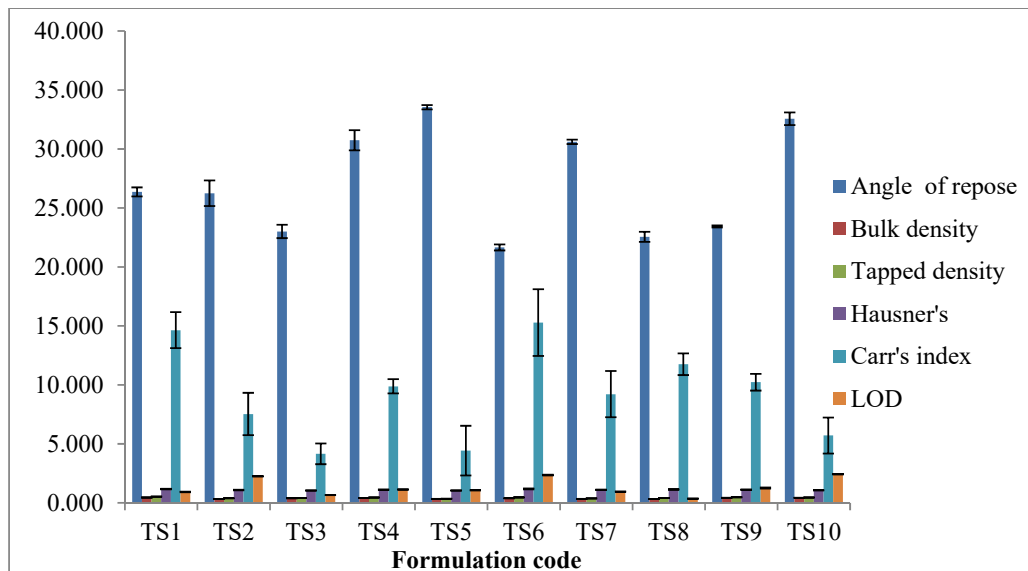


Figure 6.59: Characterization of optimized tablet solutions batches

Discussion: For each designed solution composition, granules were prepared and evaluated for pre-compression properties which are shown in Table 6.59 & Figure 6.59. Angle of repose was lies in range 21.65 ± 0.25 to 33.54 ± 0.18 . Bulk density was observed from 0.33 ± 0.05 to $0.45 \pm 0.03 \text{ gm/cm}^3$ and tapped density between 0.35 ± 0.05 to $0.52 \pm 0.01 \text{ gm/cm}^3$ for all formulations. Hausner's ratio lies between 1.04 ± 0.01 to 1.18 ± 0.04 . Carr's index lies within range 4.15 ± 0.88 to 15.28 ± 2.82 and LOD in range of 0.35 ± 0.02 to 2.42 ± 0.02 . All the batches has shown good to excellent flow properties, Hence tablets were prepared with these granules combination by wet granulation method.

6.7.7 Post compression evaluations of optimized tablets batch (TS9)

6.7.7.1 Weight variation

Table 6.60: Weight variation data of optimized grafted tablets batches

S.No.	Formulation code	Wt variation(mg) Mean \pm SD
1	TS1	1001.7 \pm 3.47
2	TS2	1001 \pm 4.06
3	TS3	998.2 \pm 4.13
4	TS4	999.5 \pm 2.22
5	TS5	998.9 \pm 3.18
6	TS6	1003.3 \pm 3.89
7	TS7	999.1 \pm 4.07
8	TS8	1001.8 \pm 4.08
9	TS9	996.5 \pm 3.10
10	TS10	997.4 \pm 2.72

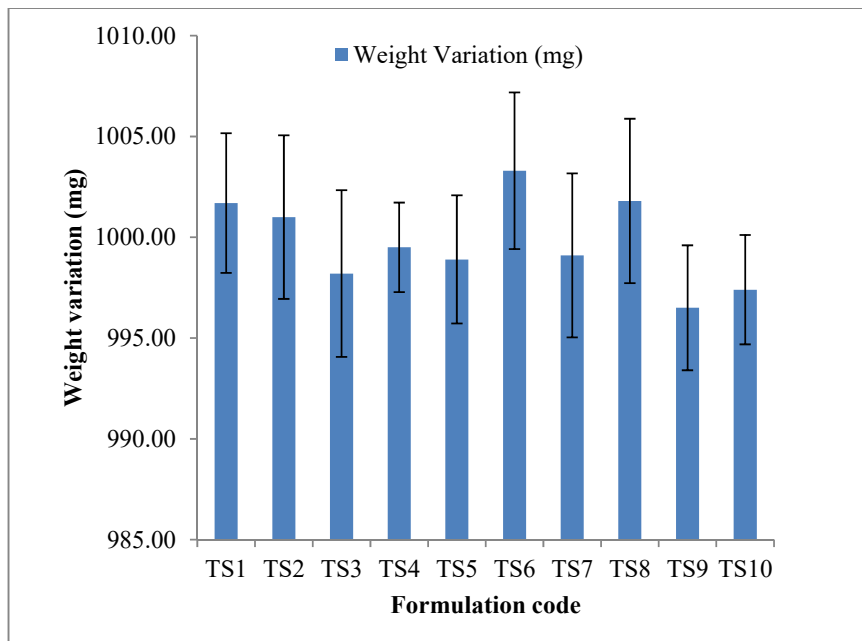


Figure 6.60: Weight variation data of optimized tablet solutions batch

Discussion: From above data, the tablet weight was found to be in range between 996.5 ± 3.10 to 1003.3 ± 3.89 mg, therefore 5% maximum difference allowed. Weight variation tolerances for tablets were shown in the Table 6.60 and Figure 6.60.

6.7.7.2 Thickness

Table 6.61: Thickness of optimized grafted tablets batches

S.No.	Formulation code	Thickness (mm)
1	TS1	6.06 ± 0.03
2	TS2	6.10 ± 0.02
3	TS3	6.06 ± 0.03
4	TS4	6.12 ± 0.01
5	TS5	6.16 ± 0.05
6	TS6	6.05 ± 0.02
7	TS7	6.04 ± 0.03
8	TS8	6.07 ± 0.02
9	TS9	6.01 ± 0.01
10	TS10	6.02 ± 0.02

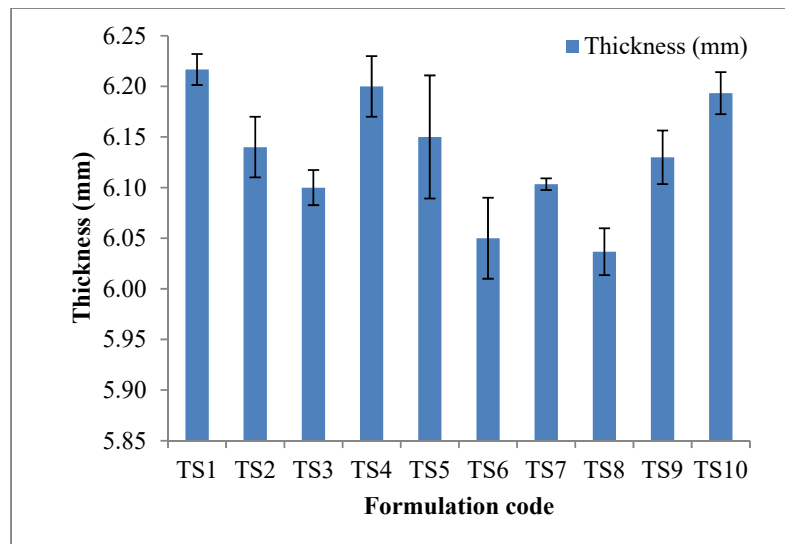


Figure 6.61: Thickness of optimized tablet solutions batches

Results: The thickness of all the batches were found to be in between of 6.01 ± 0.01 mm to 6.16 ± 0.05 mm. All formulations showed uniform thickness.

6.7.7.3 Hardness

Table 6.62: Hardness of optimized grafted tablets batches

S.No.	Formulation code	Hardness (kg/cm ²)
1	TS1	5.63±0.07
2	TS2	5.76±0.13
3	TS3	5.82±0.06
4	TS4	5.79±0.09
5	TS5	6.00±0.02
6	TS6	5.85±0.04
7	TS7	6.04±0.04
8	TS8	5.93±0.07
9	TS9	5.94±0.05
10	TS10	6.03±0.02

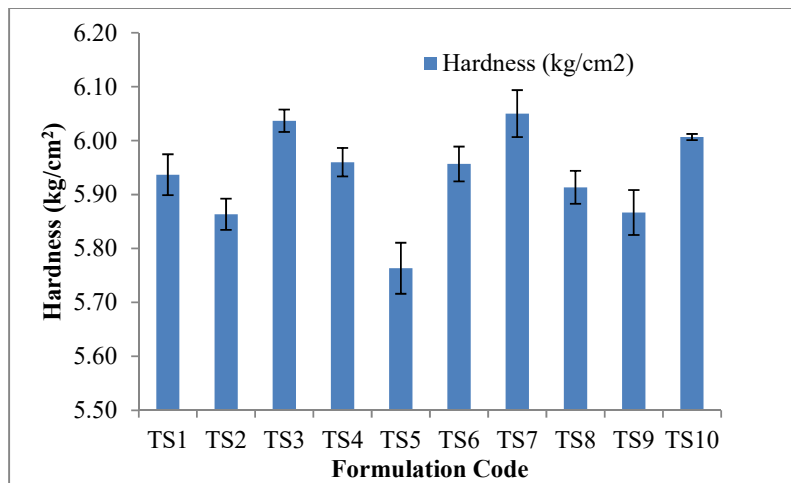


Figure 6.62:Hardness of optimized tablets solution batches

Results: From Table 6.62 and Figure 6.62,the hardness of tablets was noticed from 5.63 ± 0.07 to 6.04 ± 0.04 , which indicates the mechanical strength of tablets.

6.7.7.4 Friability

Table 6.63: Friability of optimized grafted tablets batch

S.No.	Formulation code	% Friability
1	TS1	0.62 ± 0.24
2	TS2	0.41 ± 0.41
3	TS3	0.33 ± 0.18
4	TS4	0.72 ± 0.13
5	TS5	0.68 ± 0.13
6	TS6	0.53 ± 0.35
7	TS7	0.82 ± 0.15
8	TS8	0.46 ± 0.40
9	TS9	0.60 ± 0.18
10	TS10	0.44 ± 0.22

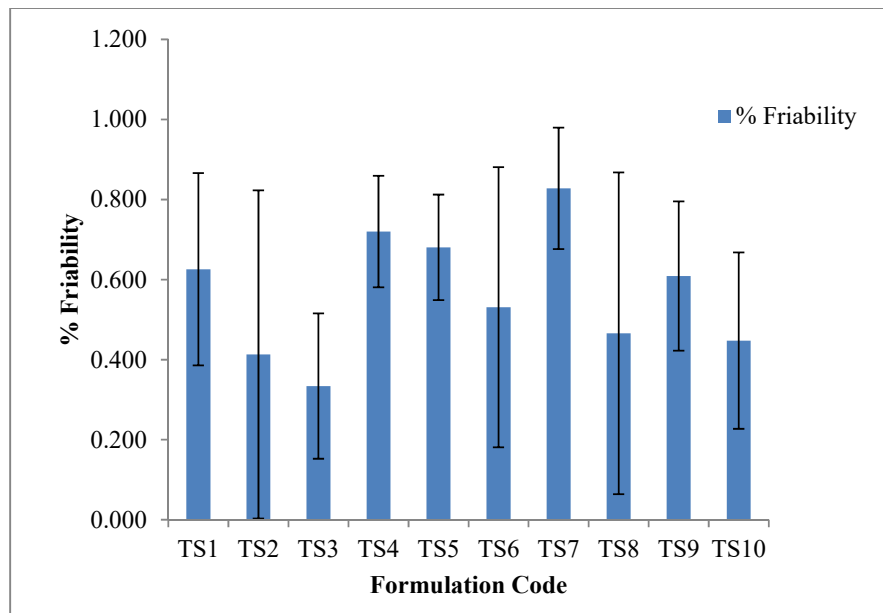


Figure 6.63: Friability of optimized tablets solution batches

Discussion: The % friability of all the formulations lies from the range 0.33 ± 0.18 to $0.82 \pm 0.15\%$, was less than 1% .

6.7.7.5 Swelling study

Table 6.64: % swelling study of experimental design grafted Metformin HCl tablet batches

S.No.	Formulation code	0.1N HCl (pH1.2)	Phosphate buffer (pH6.8)
		4 hrs	24 hrs
1	TS1	29.59±0.03	113.17±0.04
2	TS2	28.62±0.02	105.17±0.04
3	TS3	24.1±0.074	117.6±0.051
4	TS4	29.43±0.04	128.74±0.11
5	TS5	32.44±0.03	122.59±0.01
6	TS6	26.80±0.03	126.92±0.03
7	TS7	25.28±0.02	98.06±0.04
8	TS8	19.53±0.05	140.72±0.03
9	TS9	24.96±0.03	139.38±0.07
10	TS10	28.46±0.02	117.92±0.04

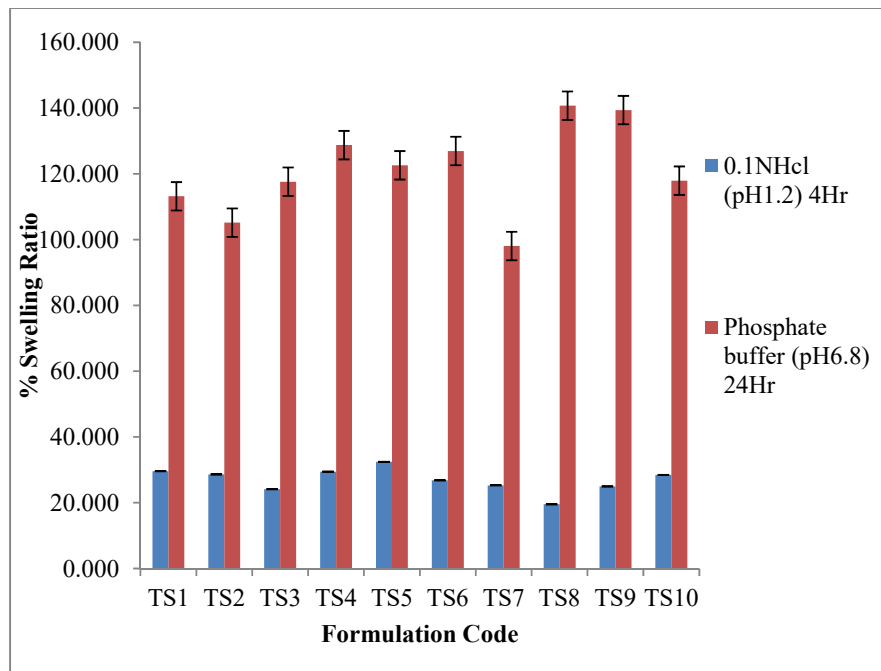


Figure 6.64: % Swelling study of optimized tablets solution batches

Discussion: The % swelling was conducted in 0.1N HCl (pH 1.2) for 4hrs and phosphate buffer (pH6.8) for 24hrs, which were represented in Table 6.64 & Figure 6.64. Due to uptake of water, grafted tablet showed the high degree of water uptake capacity, ranges from $98.06 \pm 0.04\%$ to $140.72 \pm 0.03\%$ in phosphate buffer and $19.53 \pm 0.05\%$ to $29.59 \pm 0.03\%$ in 1N HCl.

6.7.7.6 Drug content

Table 6.65: Drug content of optimized grafted tablet batches

S. No.	Formulation code	% Drug Content
1	TS1	96.50±0.50
2	TS2	98.16±1.04
3	TS3	98.66±0.38
4	TS4	99.25±0.50
5	TS5	98.83±0.94
6	TS6	97.00±0.43
7	TS7	98.25±0.43
8	TS8	96.25±1.39
9	TS9	98.25±0.43
10	TS10	97.66±0.57

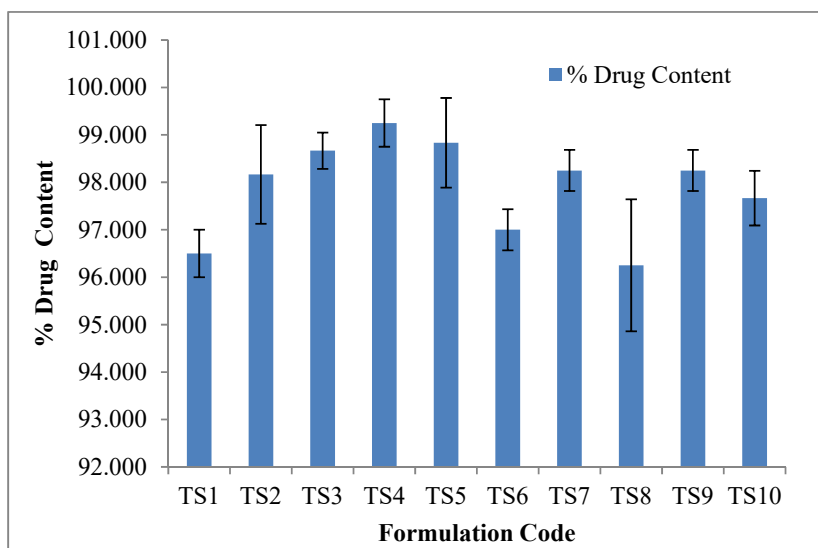


Figure 6.65: % drug content of optimized tablets solution batches

Discussion: The % drug content was constitute within the range 96.25 ± 1.39 to 99.25 ± 0.50 which indicated uniformity of mixing. The percent drug content uniformity in all formulated batches is shown in Table 6.65 (a) & Figure 6.65.

6.7.7.7 In vitro release study

Table 6.66(a): In vitro drug release of optimized tablets batches

Dissolution Media	Time (Hr)	% drug release TS1	% drug release TS2	% drug release TS3	% drug release TS4	% drug release TS5
0.1N HCl	0.25	2.18±0.08	0.56±0.01	4.64±0.03	1.65±0.05	1.34±0.09
	0.5	6.32±0.03	4.42±0.05	8.85±0.07	8.34±0.07	6.23±0.03
	1	10.56±0.03	10.78±0.02	13.76±0.08	14.32±0.09	10.02±0.08
	1.5	16.42±0.04	20.21±0.03	26.72±0.26	21.65±0.10	14.36±0.05
	2	24.63±0.11	28.94±0.78	32.75±0.36	29.94±0.22	20.34±0.50
pH6.8 Phosphate Buffer	3	32.75±0.51	35.66±0.33	38.03±0.42	32.71±0.86	26.05±0.31
	4	39.23±0.51	40.97±0.63	41.33±0.47	39.83±0.27	32.98±0.42
	5	47.31±1.11	50.67±0.16	46.11±0.47	43.99±0.41	38.11±0.88
	6	53.63±0.50	56.12±0.48	51.53±0.23	45.78±0.74	41.89±0.16
	7	58.43±0.88	61.48±0.35	55.64±0.40	52.65±0.07	47.95±0.45
	8	63.12±0.72	66.07±0.42	58.81±0.66	59.54±0.39	51.98±0.25
	10	67.43±0.41	70.75±0.55	63.23±0.63	63.72±0.91	56.98±0.34
	24	92.41±0.68	88.37±0.51	96.34±0.35	95.87±1.12	85.98±0.88

Table 6.66(b): In vitro release of optimized solution batches

Dissolution Media	Time (Hr)	% drug release TS6	% drug release TS7	% drug release TS8	% drug release TS9	% drug release TS10
0.1N HCl	0.25	4.42±0.07	6.56±0.10	5.72±0.03	2.11±0.06	3.08±0.04
	0.5	9.03±0.05	12.67±0.06	10.45±0.05	7.43±0.12	9.54±0.08
	1	15.56±0.10	16.89±0.01	14.92±0.08	13.61±0.08	12.96±0.02
	1.5	20.67±0.12	21.67±0.34	23.89±0.03	18.26±0.01	19.47±0.71
	2	24.67±0.34	24.45±0.38	29.34±0.15	23.61±0.03	26.04±0.09
pH6.8 Phosphate Buffer	3	29.54±0.25	34.53±0.29	33.89±0.15	32.04±0.27	35.54±0.18
	4	32.89±0.50	38.93±0.21	36.78±0.44	37.95±0.52	40.84±0.84
	5	39.67±0.79	42.11±0.45	42.55±0.46	40.53±0.72	46.07±0.522
	6	42.96±0.60	44.56±0.22	46.72±0.19	44.56±0.22	51.01±0.51
	7	48.42±0.87	50.41±0.02	53.55±0.19	48.92±0.08	54.32±0.65
	8	53.32±0.35	57.11±0.57	55.78±0.56	56.01±0.41	56.02±0.18
	10	62.52±0.40	66.47±0.66	58.87±0.27	60.21±0.19	59.47±0.43
	24	92.08±0.35	96.78±0.21	84.56±0.36	92.09±0.06	89.86±0.71

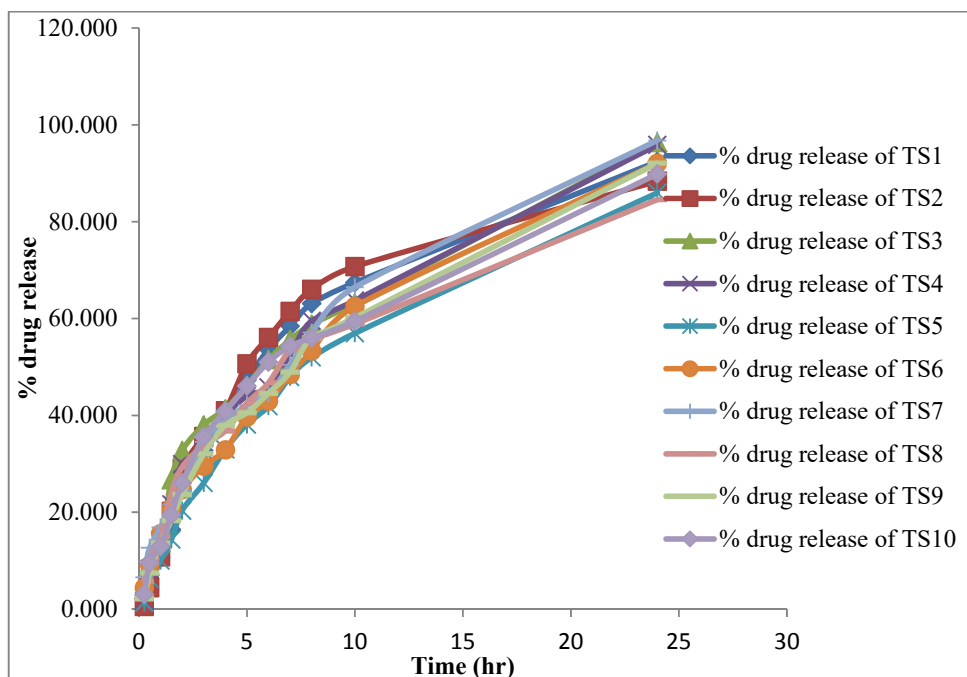


Figure 6.66: %drug release of optimized tablets solution batches

Discussion: The drug release study of different solution batches was done with 0.1 N HCl for first 2 hrs as well as in phosphate buffer (pH 6.8) for next 24 hrs. In vitro drug release for grafted tablets batches were shown in Figure 6.66 and Table 6.66 (a, b).

6.8 Numerical optimization formulation comparison

Table 6.67: Comparison data of optimized formulation, predicted and experimental data

Number of Solutions	Grafted Gum	PVPK 30	Predicted response			Observed response			Percentage error		
			% drug released in 1hr	% drug released in 8hrs	Time of 50% drug release	% drug released in 1hr	% drug released in 8hrs	Time of 50% drug release	% drug released in 1hr	% drug released in 8hrs	Time of 50% drug release
TS1	273.97	40	11.82	57.12	7	10.56	63.12	6	0.89	4.23	0.78
TS2	264.09	33	12.53	58.83	7	10.781	66.07	5	1.24	5.11	1.17
TS3	271.69	55	11.39	57.29	7	13.76	58.81	6	1.67	1.07	0.75
TS4	302.92	21	13.21	55.07	8	14.32	59.54	7	0.79	3.15	0.42
TS5	283.06	56	11.26	56.29	7	10.02	51.98	8	0.87	3.04	0.42
TS6	299.19	77	11.93	57.64	7	15.56	53.32	8	2.56	3.05	0.45
TS7	251.86	42	12.42	59.98	6	16.89	57.11	7	3.15	2.02	0.52
TS8	307.02	78	12.19	57.76	7	14.92	55.78	7	1.93	1.40	0.29
TS9	347.98	74	13.74	57.35	8	13.61	56.01	8	0.09	0.94	0.32
TS10	262.49	59	11.58	58.32	7	12.96	56.02	6	0.97	1.62	0.52

6.9 Checkpoint Analysis

The comparisons results of predicted and experimental data shows very close agreement. The designs were success with desirability function for evaluation and optimization of Metformin HCl grafted tablet formulation (TS9) (Table 6.68).

Table 6.68: Checkpoint batch with their predicted and observed value of responses

Number of Solution	Grafted Gum	PVPK 30	Predicted response			Observed response			Percentage error		
			% drug released in 1 hr	% drug released in 8 hrs	Time to 50% drug release	% drug released in 1 hr	% drug released in 8 hrs	Time to 50% drug release	% drug released in 1 hr	% drug released in 8 hrs	Time to 50% drug release
TS9	347.98	74	13.740	57.350	8	13.61±081	56.01±418	8	0.090	0.946	0.327

6.10 In vitro release study of optimized fenugreek grafted tablet (TS9), fenugreek gum tablet and marketed formulation

In vitro release study of prepared optimized Metformin HCl grafted tablet (TS9), fenugreek gum tablet and marketed formulation [GLUCOMET SR 500 mg] were operated triplicate using three tablets from each batch and compared.

Table 6.69: In vitro release study of optimized grafted tablet (TS9), marketed formulation (Glucomet SR) and fenugreek gum tablet

Dissolution Media	Time (Hr)	% drug release TS9	% drug release Marketed Formulation	% drug release Fenugreek gum tablet
0.1N HCl	0	0	0	0
	0.25	2.182±0.08	3.044±0.049	4.403±0.137
	0.5	6.321±0.038	6.158±0.072	12.908±0.047
	1	10.562±0.032	10.410±0.047	29.475±0.595
	1.5	16.421±0.046	16.928±0.091	45.525±0.687
	2	24.634±0.113	22.875±0.852	63.600±0.566
pH6.8 Phosphate Buffer	3	32.75±0.518	27.375±0.79	95.925±0.723
	4	39.231±0.516	36.525±0.79	99.825±0.468
	5	47.312±1.119	45.450±0.90	
	6	53.632±0.508	51.675±0.344	
	7	58.432±0.881	59.025±0.344	
	8	63.121±0.726	62.400±0.260	
	10	67.432±0.419	66.300±0.468	
	24	92.412±0.684	79.275±0.723	

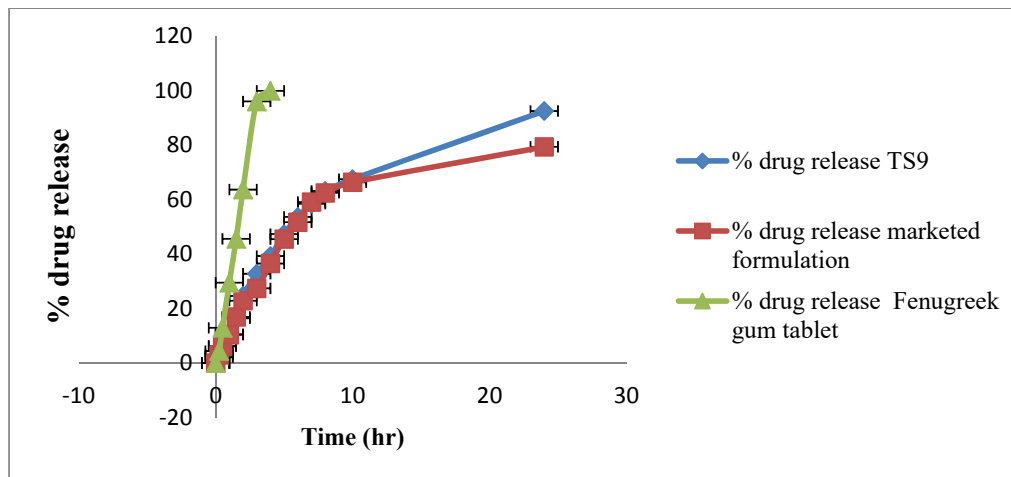


Figure 6.67: Comparative % drug release of optimized fenugreek grafted tablet (TS9), marketed formulation and fenugreek gum tablet

Discussion: The dissolution study of tablet batches conducted within 0.1 N HCl for first 2 hrs, and in phosphate buffer (pH 6.8) for next 24 hrs. Figure 6.67 shows the dissolution profile of optimized fenugreek grafted tablet (TS9), fenugreek gum tablet and marketed formulation (Glucomet SR 500 mg). It could be noticed through results that Glucomet SR released 79.27% of the drug in 24hrs; although the tablet prepared by fenugreek gum release only 99.82% drug within 4hrs and drug release from tablet formulation with grafted fenugreek gum (TS9) was sustained (92.41%). Because the graft copolymer tablets combined along with aqueous media, water disperse within the tablet subsequently and cause the matrix swelling and this swelling continues as well as developed a uniformity hydrostatic pressure in tablets. With increasing the swelling, the solubility of the grafted polymeric matrix decreased, which gets diminished slowly with slow drug diffusion from matrix. TS9 optimized batch shows the long chain of polymers which leads to increased swelling and slowed drug release.

6.11 Drug release kinetics

6.11.1 Zero order release kinetic

Its kinetic study for drug release in formulation TS9 is given below.

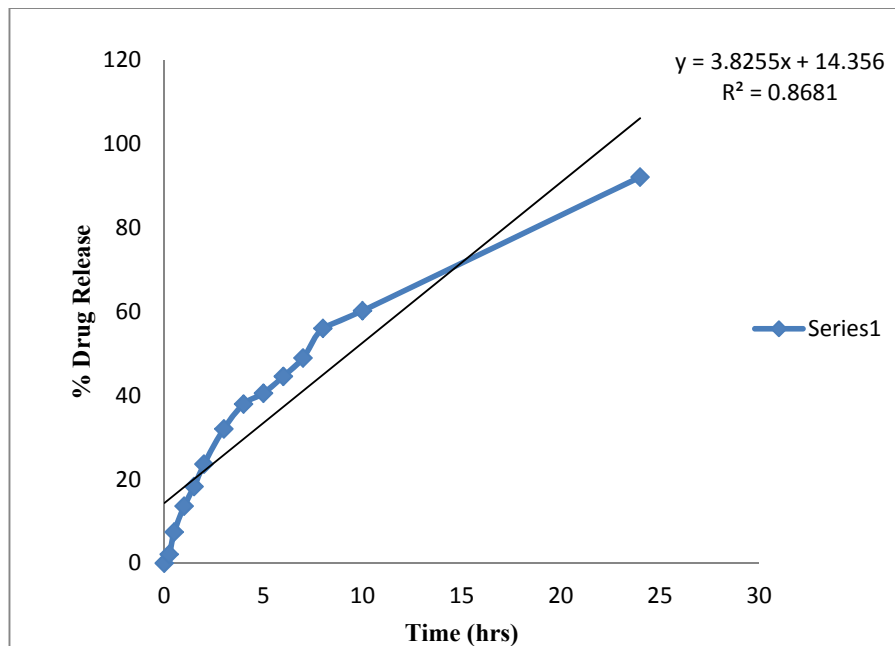


Figure 6.68(a): Zero order release kinetic

6.11.2 First Order

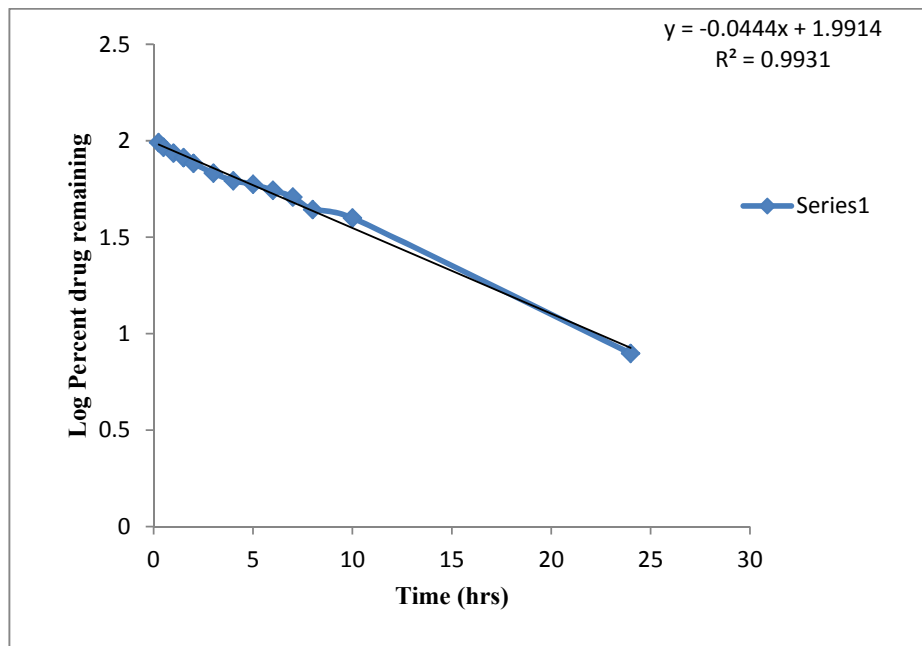


Figure 6.68 (b): First order release kinetic

6.11.3 Higuchi

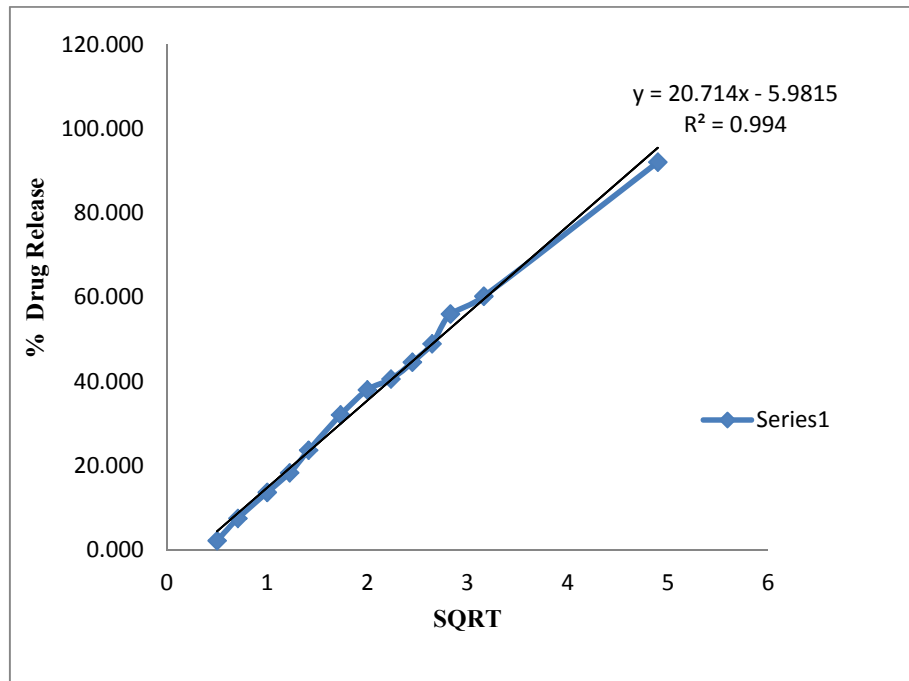


Figure 6.68(c): Higuchi order release kinetic

6.11.4 Korsmeyer peppas

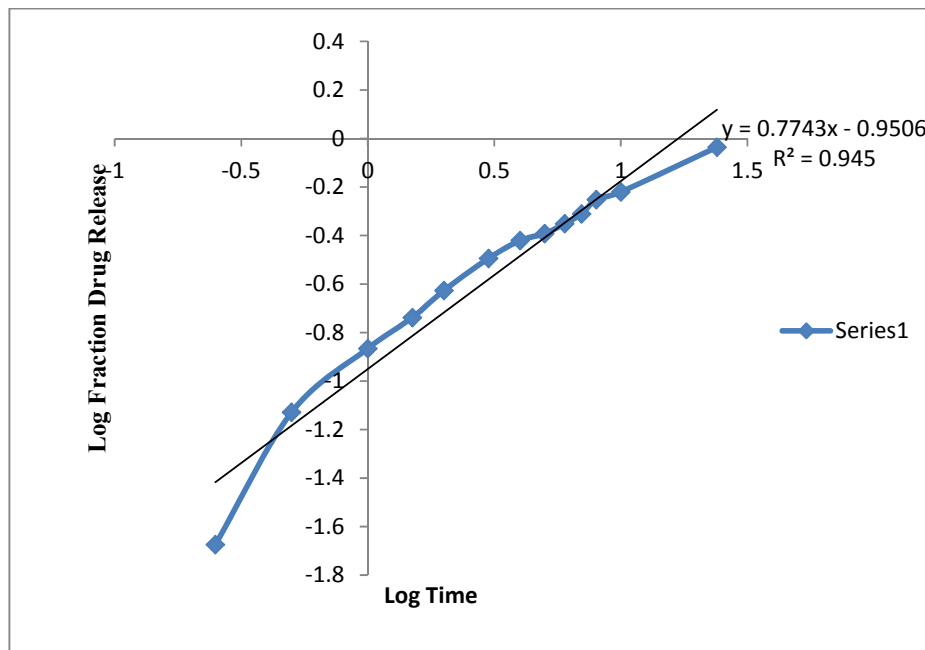


Figure 6.68 (d): Korsmeyer peppas release kinetic

Table 6.70: Kinetic parameter for TS9 formulation

Formulation name	Zero order		First order		Higuchi		Korsmeyer and Peppas	
	R ²	K ₀	R ²	K ₀	R ²	K ₀	R ²	K ₀
TS9	0.868	3.825	0.993	-0.044	0.994	20.71	0.945	0.774

Discussion: Mathematical models (% drug release vs. time (zero order), log percent drug remaining vs. time (first order), % log drug release vs. square root of time (Higuchi model), and % log drug release vs. log time (Korsmeyer and peppas exponential equation)) is commonly used to predict release mechanism. The result of optimized formulations (TS9), with R² value are reported in Table 6.70 and Figure 6.68 (a, b, c and d). The results revealed that, Higuchi model was found to be R²=0.994, that is best for release data and drug released from tablet in sustain pattern.

6.12 Stability studies

6.12.1 Physical appearance

The physical appearance like shape, color of tablets were performed by visual observations.

Table 6.71: Stability test study data of physical appearance (0-180 days)

Time (Days)	Effect of storage on visual appearance			
	Control (RT)	Refrigerator 4±2°C	25±2°C	40±2°C
0 days	Cream colour round tablet	Cream colour round tablet	Cream colour round tablet	Cream colour round tablet
30 days	Cream colour round tablet	Cream colour round tablet	Cream colour round tablet	Cream colour round tablet
60 days	Cream colour round tablet	Cream colour round tablet	Cream colour round tablet	Cream colour round tablet
90 days	Cream colour round tablet	Cream colour round tablet	Cream colour round tablet	Cream colour round tablet
180 days	Cream colour round tablet	Cream colour round tablet	Cream colour round tablet	Cream colour round tablet

6.12.2 Hardness

Table 6.72: Stability test study for Hardness (0-180 days)

Hardness (kg/cm ²)					
Temperature Condition	0 days	30 days	60 days	90 days	180 days
Control (RT)	5.86±0.04	5.81±0.07	5.78±0.04	5.80±0.03	5.79±0.06
Refrigerator 4±2°C	5.86±0.04	5.86±0.02	5.87±0.04	5.85±0.03	5.89±0.01
25±2°C	5.86±0.04	5.86±0.03	5.86±0.02	5.86±0.03	5.86±0.02
40±2°C	5.86±0.04	5.89±0.06	5.9±0.01	5.89±0.06	5.9±0.01

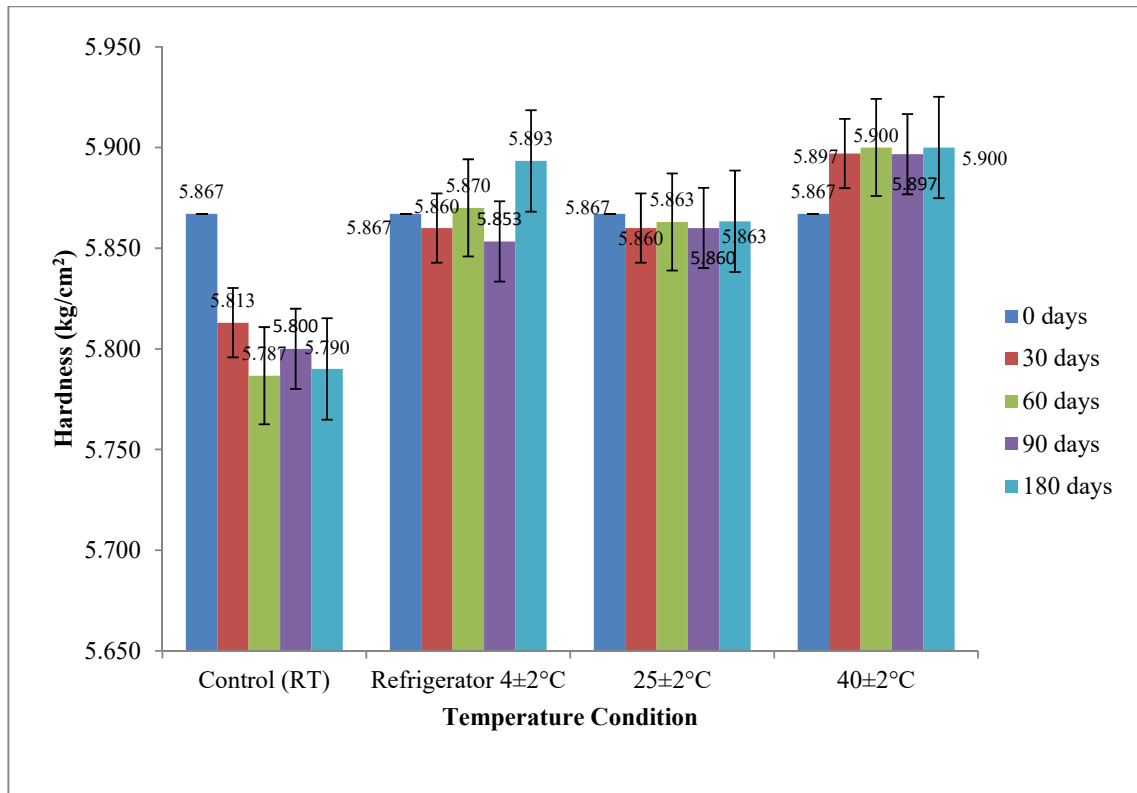


Figure 6.69: Hardness of TS9 batch at 0 to 180days

6.12.3 Friability

Table 6.73: Stability test study of Friability (0-180 days)

Friability (%)					
Temperature Condition	0 days	30 days	60 days	90 days	180 days
Control (RT)	0.60±0.18	0.63±0.34	0.60±0.18	0.67±0.23	0.60±0.34
Refrigerator 4±2°C	0.60±0.18	0.58±0.15	0.55±0.18	0.56±0.17	0.56±0.04
25±2°C	0.60±0.18	0.62±0.06	0.57±0.08	0.53±0.47	0.54±0.12
40±2°C	0.60±0.18	0.49±0.44	0.59±0.07	0.60±0.16	0.57±0.19

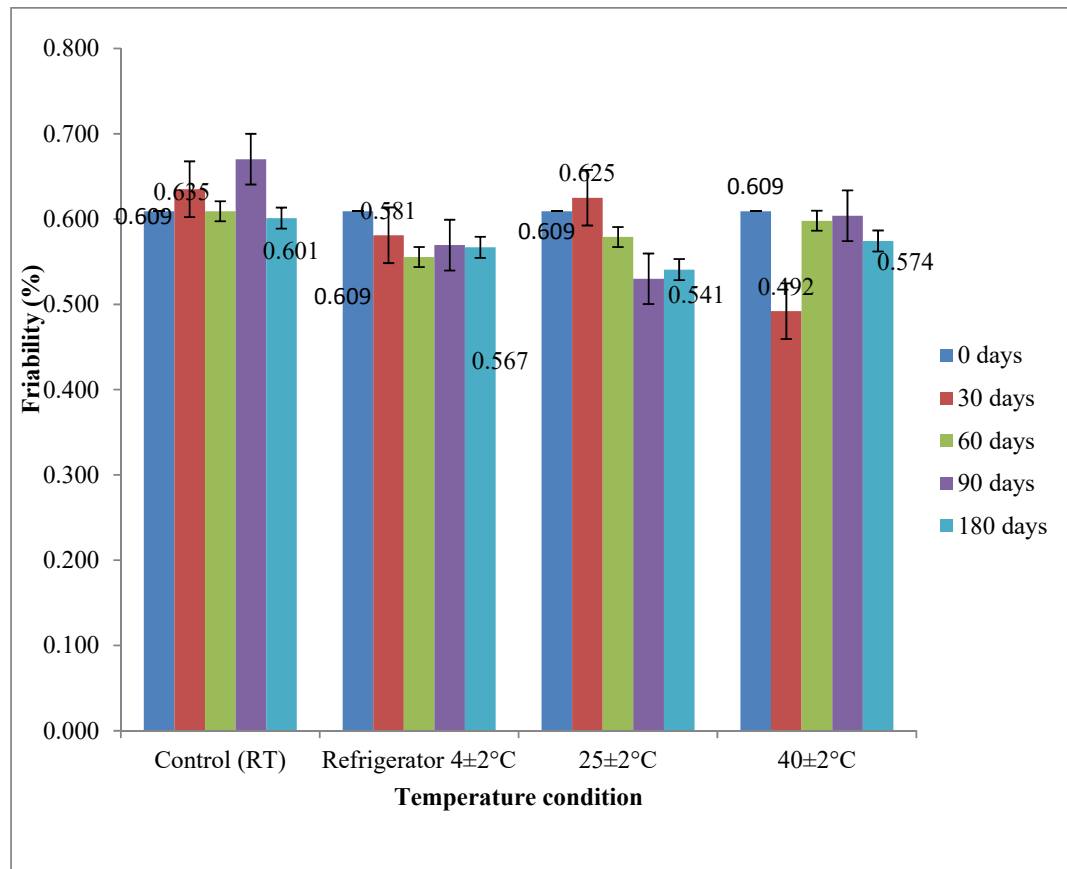


Figure 6.70: Friability of TS9 batch at 0 to 180days

6.12.4 Drug content

Table 6.74: Stability test study of drug content (0-180 days)

Drug content (%)					
Temperature Condition	0 days	30 days	60 days	90 days	180 days
Control (RT)	98.25±0.43	98.08±0.52	98.16±0.62	97.66±0.76	98.33±0.52
Refrigerator 4±2°C	98.25±0.43	98.25±0.25	97.66±0.38	98.41±0.14	98.33±0.28
25±2°C	98.25±0.43	98.16±0.52	98.58±0.38	98.00±0.66	98.12±0.57
40±2°C	98.25±0.43	98.00±0.90	97.74±0.44	98.50±0.25	97.91±0.14

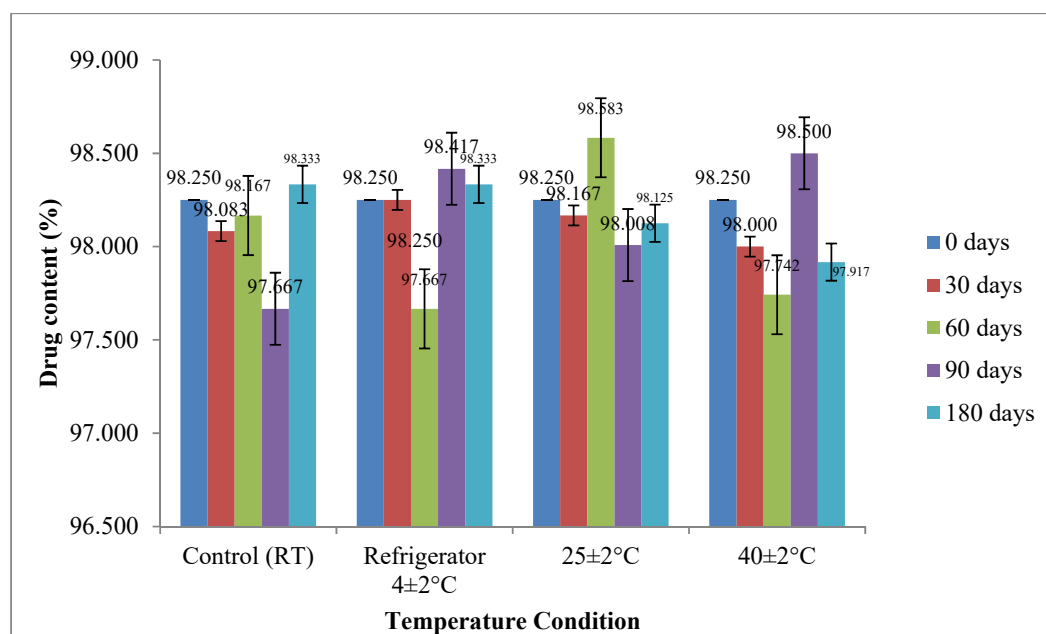


Figure 6.71: Drug content of TS9 formulation at 0 to 180days

Discussion: The stability studies of the optimized formulation were shown at control room temperature 25±2°C/60±5% RH, and refrigerator 4±2°C/75±5% RH, in which different variables like physical appearance, friability, hardness and drug content were checked for 0-180 days, that were shown in Table 6.71-6.74 and Figure 6.69-6.71. At accelerated stability conditions, no significant alteration were seen in drug content and other variables of tablets. Hence, it concluded that the formulation was stable even after 6 month of storage.

6.13 Pharmacodynamics optimization of development formulation

Pharmacodynamic studies

The pharmacodynamics studies are achieved by using six adult male albino wistar rats with 250-300 g, weight obtained from Niper, Mohali, with its initial weight 250-300g after approval from institutional animal ethical committee from (BMRL/IAEC/2021-20) with registration no. 2005/PO/RcBT/S/18/CPCSEA). The animals were maintained in the animal colony of MMU University, district Ambala and further used for the experiment. The rats were accommodate in suitable environmental conditions at $23 \pm 2^{\circ}\text{C}$ with relative humidity 50-55% and photoperiod of 12hrs light and 12hrs dark cycle. Animals were given standard rodent feed (Ashirwad industries, Chandigarh, India) and tap water.

Quarantine and Acclimatization

The rats were accredited for 7 days interval prior to pre-exposure examination (gross motor activity and physical appearance) under standard condition for the experiment. During this period, the rats were observed for physical examination and to check any clinical signs of disease and to check normal glucose level and body weight. (Garud N et.al, 2012)

Induction of hyperglycemia

Before beginning of experiment, the animals were fasted for 8-12 hrs, only water was provided till end of experiment. Hyperglycemia produced experimentally by a single intraperitoneal administration of freshly prepared streptozotocin (STZ, 45 mg/kg), prepared in fresh citrate buffer at pH 4.5, 15 minutes later nicotinamide (230 mg/kg, *i.p.*) obtained from Sigma-Aldrich, Milwaukee, USA. After 72 hrs of STZ-NAD administration, blood sugar level was calculated using glucometer. If glucose level is observed above 250 mg/dL, the rat were diabetic and included for further research.

Experimental Design

The optimized batch of metformin HCl was administered in mini tablets, orally with small amount of water. The experimental rats were subdivided in seven groups of six animals each, which is shows in Table 6.93. Group I contained normal rats, who were orally administered with 1.5 mL aqua, Normal Control (NC); Group II comprises of rats who were given STZ –Nicotinamide to induce diabetes, further orally administered with 1.5 mL aqua; Group III contain STZ induced diabetic rats, orally administered with marketed metformin HCl tablet in 1.5 mL aqua; Group IV comprised STZ effected diabetic rats, orally administered grafted fenugreek TS9 formulation (GFTS9 25 mg/kg, lower dose) in 1.5 mL aqua; Group V comprised of STZ induced diabetic rats, orally administered with grafted fenugreek TS9 formulation (GFTS9 75 mg/kg, medium dose) in 1.5 mL aqua; Group VI consisted STZ induced diabetic rats orally administered with grafted fenugreek TS9 formulation (GFTS9 100 mg/kg, higher dose) in 1.5 mL aqua; and Group VII include STZ induced diabetic rats orally administered with un-grafted fenugreek formulation 100 mg/kg in 1.5 mL aqua.

At pre-determined time periods, 0.5 mL blood was collected from tail vein of rats and put into heparinized plastic tubes. Collected blood sample were centrifuged for 10 min at 2000rpm and stored at -20°C till further use. The concentration of drug is estimated by HPLC method.

Table 6.75: Experimental Design

Group 1	Normal Control (NC)
Group 2	Diabetic Control (STZ)
Group 3	Diabetic rats + Metformin HCl + (D)
Group 4	Diabetic rats + grafted fenugreek TS9 formulation (LD+D)
Group 5	Diabetic rats + grafted fenugreek TS9 formulation (MD+D)
Group 6	Diabetic rats + grafted fenugreek TS9 formulation (HD+D)
Group 7	Diabetic rats + treated with ungrafted fenugreek formulation +(D)

Table 6.76: Accreditation and induction period

The animals were quarantine and acclimatization for one week prior to pre-exposure examination (gross motor activity and physical appearance)	
To check blood glucose level and body weight during period.	
8 th Day	Proper animal feed and water
Night	Fasting overnight
9 th Day	Administration of nicotinamide (NA) (230 mg/kg) <i>i.p.</i>) after 15 min induction of STZ 45 mg/kg <i>interperitoneal</i>
Later 72 hrs	Dose of different formulation were administered.
The rats were marked with picric acid either on head or body or tail for their proper identification and distinct label recognition in all cages.	

6.13.1 Biochemical parameters

6.13.1.1 Body weight

Table 6.77: Effect of formulations on Body weight in streptozotocin induced diabetic rat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)														
0	322.23	10.12	6	323.26	9.22	6	326.18	4.22	6	323.11	6.12	6	327.33	6.22	6	325.11	4.23	6	323.12	5.22	6
7	324.17	10.23	6	243.18	9.27	6	309.27	4.78	6	266.16	6.19	6	281.19	4.89	6	298.23	3.89	6	252.13	5.43	6
14	324.28	10.11	6	202.05	8.22	6	316.19	5.11	6	270.17	5.23	6	289.12	4.22	6	308.16	3.33	6	238.1	5.23	6
21	330.12	10	6	161.22	8.17	6	327.16	2.88	6	281.14	5.22	6	297.11	3.56	6	312.18	3.22	6	221.12	5.28	6

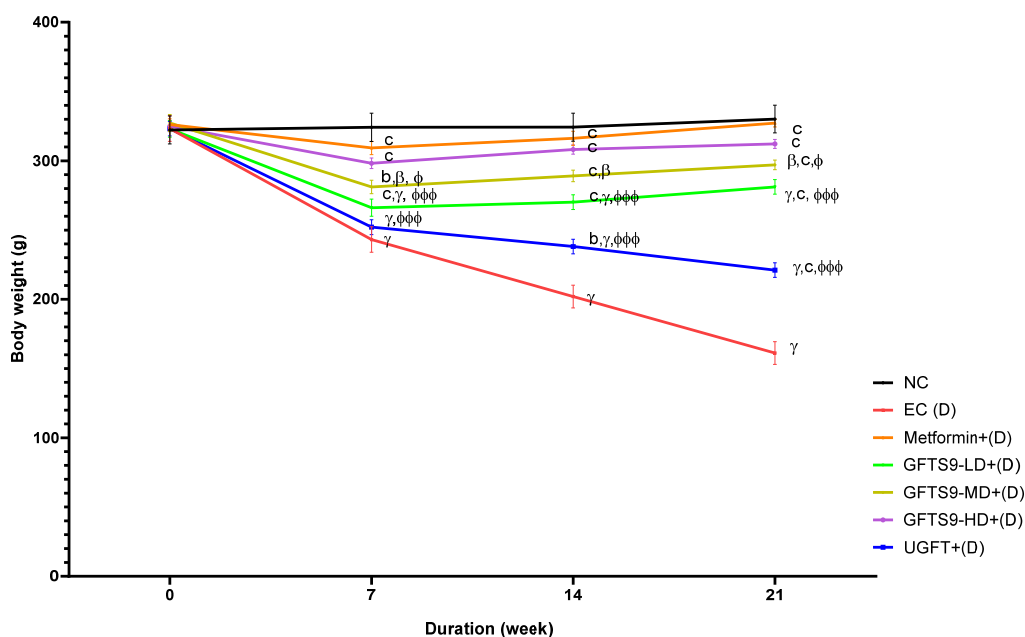


Figure 6.72: Effect on body weight

Discussion: In Body weight, all the treatment groups exhibited reduction in body weight. The groups treated with the fenugreek grafted formulation at a lower (50 mg/kg), medium (75 mg/kg) and higher dose (100 mg/kg) exhibited attenuation in body weight from day 14. This is very important to note that medium dose have provided the same level of attenuation in body weight as higher dose. Hence medium dose can be used to further research work due to equal level of body weight same as highest dose. Data are mentioned as mean±SEM (n = 6). ^a p < 0.05, ^β p < 0.01, ^γ p < 0.001 compared to the normal group (NC). ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 compared to the diabetic group [EC(D)] and ^φ p < 0.05, ^{φφ} p < 0.01, ^{φφφ} p < 0.001 compared to the Metformin-treated group [Metformin+(D)].

6.13.1.2 Blood Glucose Level

Table: 6.78 Effect of formulations on Blood glucose Level in streptozotocin induced diabetic rat

	NC		EC (D)		Metformin+(D)		GFTS9-LD+(D)		GFTS9-MD+(D)		GFTS9-HD+(D)		UGFT+(D)								
0	63	12.34	6	64.16	2.6	6	62	2.03	6	64.16	2	6	62.19	3	6	59.16	2.13	6	61.12	2.11	6
7	64.19	12.23	6	383.23	23.23	6	208.11	20	6	289.16	22.13	6	279.16	21.11	6	267.13	10.22	6	350.14	28.13	6
14	62.19	12.16	6	411.33	22.13	6	186.11	18.23	6	267.13	13.29	6	246.17	14.53	6	223.16	10.12	6	402.11	16.14	6
21	63	12.16	6	423.33	28.15	6	133.23	15.19	6	248.12	12.21	6	203.16	5	6	173.12	10.09	6	409.06	5.06	6

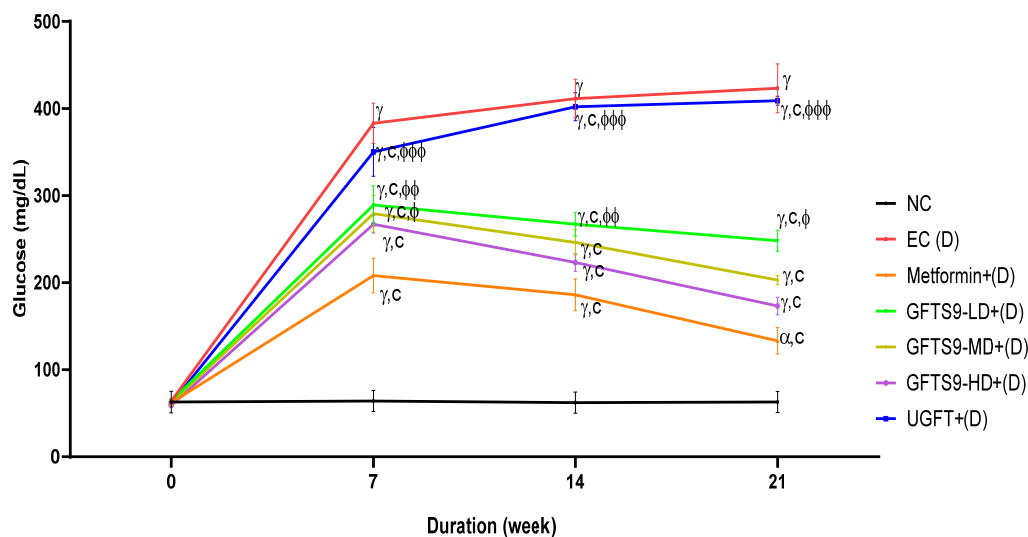


Figure 6.73: Effect on blood glucose level

Discussion: Fasting blood glucose level was increased in diabetic rats (423.33± 28.15) after interval of diabetic study. Oral intervention of different doses (lower dose 50 mg/kg, medium dose 75mg/kg and higher dose 100 mg/kg) reduced blood glucose level from 289.16± 43.13 to 248.12 ± 7.00mg/ dL in lower dose group, from 279.16± 21.11 to 203.16± 5.00 mg/ dL in medium dose group and from 267.13± 10.22 to 173.12 ± 10.11mg/dL in higher dose group in the experimental animals groups. It note that medium dose have provided the same level of attenuation in blood glucose level as higher dose. It concludes the effect of diabetic activity of grafted formulation of metformin HCl at medium dose has shown better effect as compared to higher dose or other dose.

Data are denoted as mean±SEM (n = 6). ^α p < 0.05, ^β p < 0.01, ^γ p < 0.001 compared to the normal group (NC). ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 compared to the diabetic group [EC(D)] and ^φ p < 0.05, ^{φφ} p < 0.01, ^{φφφ} p < 0.001 compared to the Metformin-treated group [Metformin+(D)].

6.13.1.3 Lipid Profile

6.13.1.3.1 Effect of Cholesterol

Table 6.79: Effect of formulations on total cholesterol in streptozotocin induced diabetic rat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
Total cholesterol	112.12	261.15	148.11	194.16	179.17	166.12	238.14

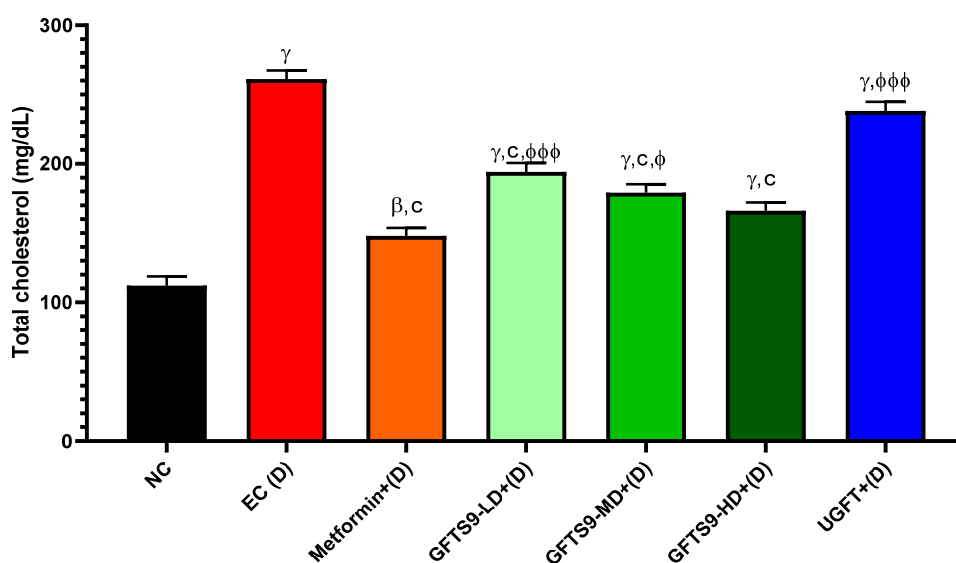


Figure 6.74: Effect on total Cholesterol

Discussion:

Cholesterol level was found to be significantly increased in experimental animals after 21 days. When treated with different grafted doses, the level of cholesterol in rats reduced. Medium dose (75mg/kg) of GFTS9 formulation produced maximum attenuating effect same as that of higher dose of formulation on cholesterol. Data are denoted as mean±SEM (n = 6). ^α p < 0.05, ^β p < 0.01, ^γ p < 0.001 compared to the normal group (NC). ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 compared to the diabetic group [EC(D)] and ^φ p < 0.05, ^{φφ} p < 0.01, ^{φφφ} p < 0.001 compared to the Metformin-treated group [Metformin+(D)].

6.13.1.3.2 Effect of Triglycerides level

Table 6.80: Effect of formulations on Triglycerides level in streptozotocin induced diabetic rat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
Serum Triglyceride	76.12	171.22	85.26	134.18	111.22	97.23	150.19
	5.22	4.27	4.16	4.11	6.11	6.96	7.12
	6	6	6	6	6	6	6

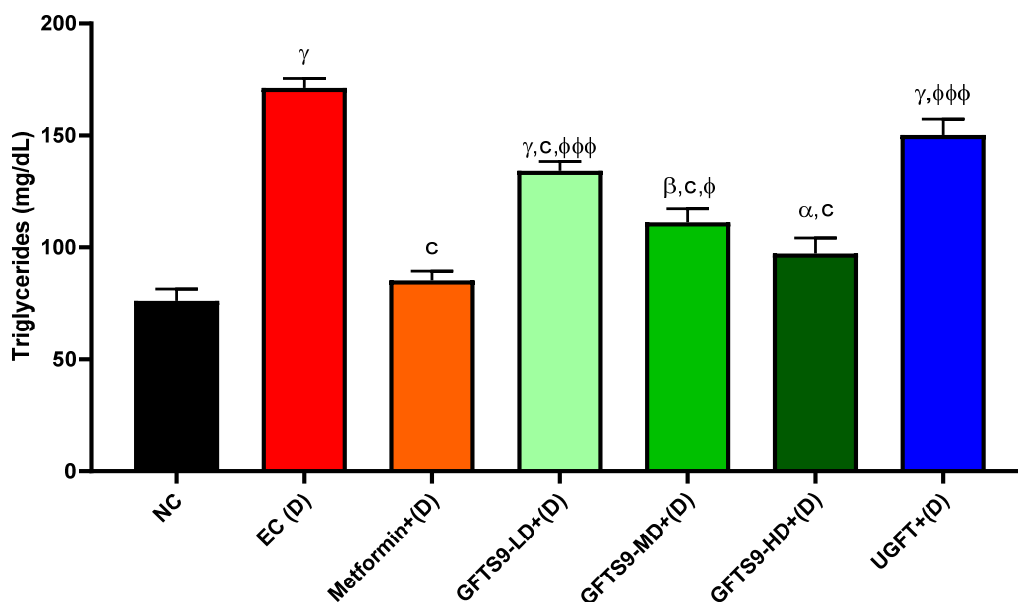


Figure 6.75: Effect on Triglycerides

Discussion:

Triglycerides level was increased in experimental animals after 21 days. when the treatment is done with different grafted doses, the level of triglycerides in rat groups decreased. The medium dose (75mg/kg) produced same attenuating effect as to higher dose. Hence, it is observed that medium dose shows best action. Data are denoted as mean±SEM (n = 6). ^a p < 0.05, ^β p < 0.01, ^γ p < 0.001 compared to the normal group (NC). ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 compared to the diabetic group [EC(D)] and ^φp < 0.05, ^{φφ} p < 0.01, ^{φφφ} p < 0.001 compared to the Metformin-treated group [Metformin+(D)].

6.13.1.3.3 Effect on HDL (High Density Lipoprotein) Level

Table 6.81: Effect of formulations on HDL in streptozotocin induced diabetic rat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
HDL (mg/dL)	46.13	23.11	41.16	32.18	36.18	39.14	28.17

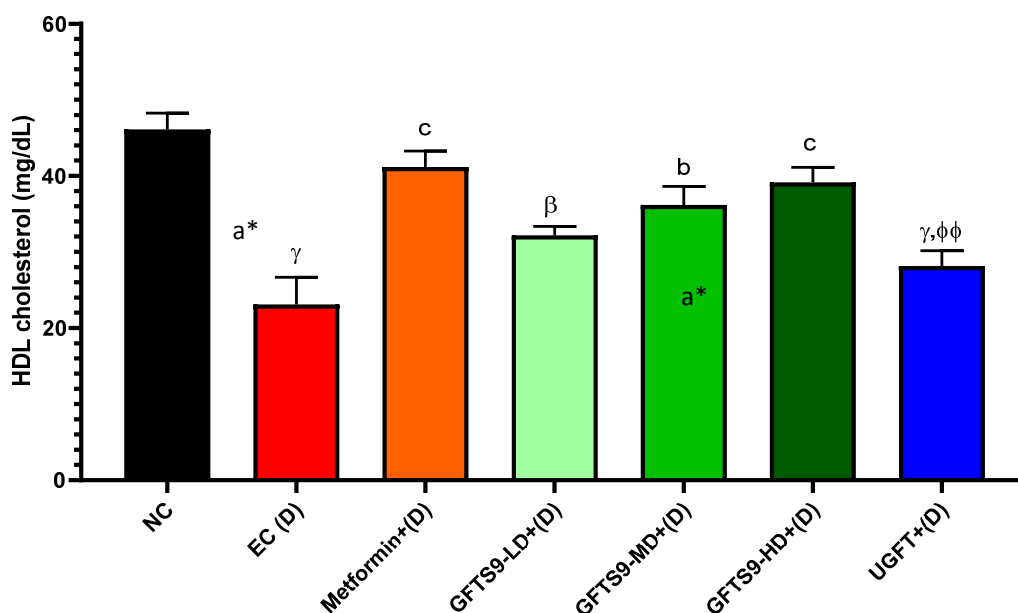


Figure 6.76: Effect on HDL level

Discussion: HDL level was decreased significantly in experimental animals after 21 days of treatment with different doses (lower, medium and higher). It significantly

attenuated the elevated level of HDL in rats. Medium dose (75 mg/kg) of GFTS9 formulation produced maximum attenuating effect same as higher dose. Hence, it is observed that medium dose shows best action. Data are denoted as mean±SEM (n = 6). ^α p < 0.05, ^β p < 0.01, ^γ p < 0.001 compared to the normal group (NC). ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 compared to the diabetic group [EC(D)] and ^φ p < 0.05, ^{φφ} p < 0.01, ^{φφφ} p < 0.001 compared to the Metformin-treated group [Metformin+(D)].

6.13.1.3.4 Effect on LDL (Low Density Lipoprotein)

Iranian formula (Dietz *et al*, 1972)

$$\text{LDL} = \text{TC}/1.19 + \text{TG}/1.9 - \text{HDL}/1.1 - 38 \text{ or } \text{LDL} = \text{TC} - (\text{HDL} + \text{TG}/5)$$

Table 6.82: Effect of formulations on LDL in streptozotocin induced diabetic rat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
LDL cholesterol (mg/dL)	54.13	164.12	66.12	98.19	81.17	71.03	154.23

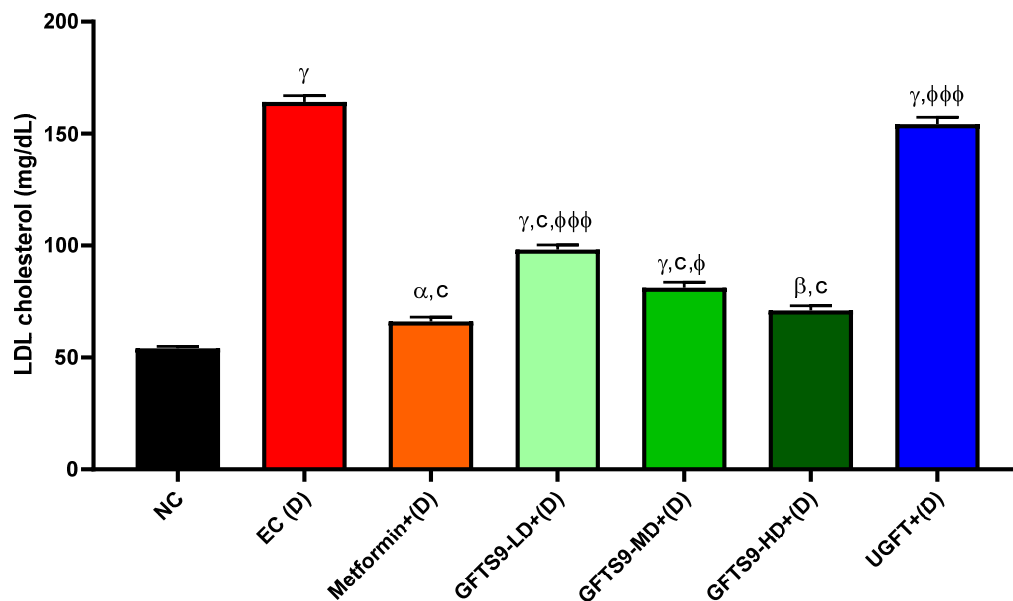


Figure6.77: Effect on LDL level

Discussion: LDL level was decreased significantly in experimental animals after 21 days on treatment with different doses (lower, medium and higher). It significantly

attenuated the elevated level of LDL in rats. 500 mg/kg of GFTS9 formulation produced maximum attenuating effect same as higher dose. Hence, it is observed that 500mg/kg dose shows best action. Data are denoted as mean±SEM (n = 6). ^α p < 0.05, ^β p < 0.01, ^γ p < 0.001 compared to the normal group (NC). ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 compared to the diabetic group [EC(D)] and ^φ p < 0.05, ^{φφ} p < 0.01, ^{φφφ} p < 0.001 compared to the Metformin-treated group [Metformin+(D)].

6.13.2 Antioxidant Parameters

6.13.2.1 Effect on CAT

Table 6.83: Effect of formulations on CAT in streptozotocin induced diabetic rat

	NC		EC (D)		Metformin+(D)		GFTS9-LD+(D)		GFTS9-MD+(D)		GFTS9-HD+(D)		UGFT+(D)								
CAT	87.06	2.81	6	29.12	2.81	6	81.14	1.93	6	42.19	2.11	6	70.14	2.61	6	74.03	2.66	6	30.1	2.03	6

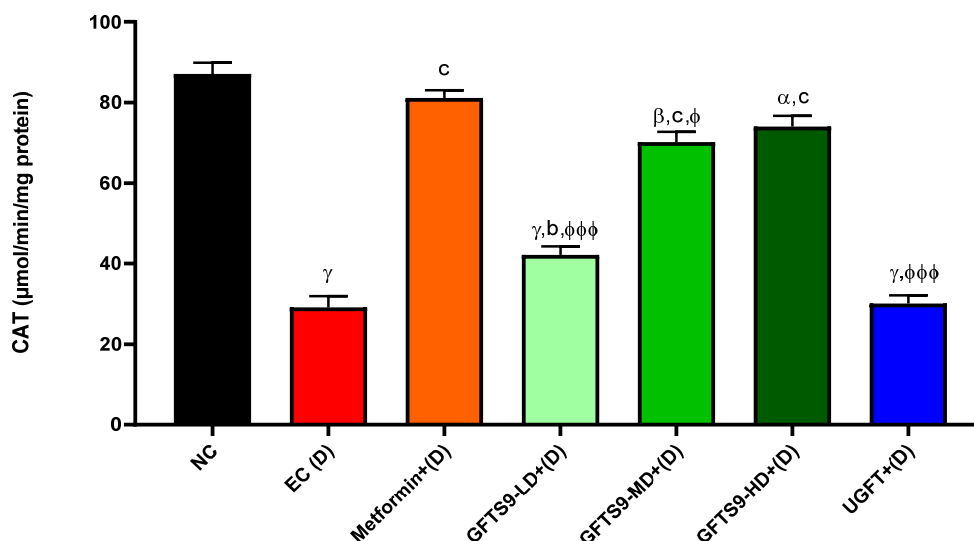


Figure 6.78: Effect on CAT level

Discussion: CAT level was decreased significantly in experimental animals after 21 days treatment with different doses (lower, medium and higher). It significantly attenuated the elevated level of CAT in rats. Medium level dose of GFTS9 formulation were produced maximum attenuating effect same as higher dose. Hence it

can be observed that medium dose were shows best action. Data are denoted as mean±SEM (n = 6). ^a p < 0.05, ^β p < 0.01, ^γ p < 0.001 compared to the normal group (NC). ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 compared to the diabetic group [EC(D)] and ^φ p < 0.05, ^{φφ} p < 0.01, ^{φφφ} p < 0.001 compared to the Metformin-treated group [Metformin+(D)].

6.13.2.2 Effect on GSH

Table 6.84: Effect of formulations on GSH in streptozotocin induced diabetic rat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
GSH (microgram/mg of protein)	54.22 ± 1.78	19.06 ± 1.32	49.23 ± 1.19	32.16 ± 1.41	43.21 ± 1.43	47.11 ± 1.1	24.23 ± 1.06

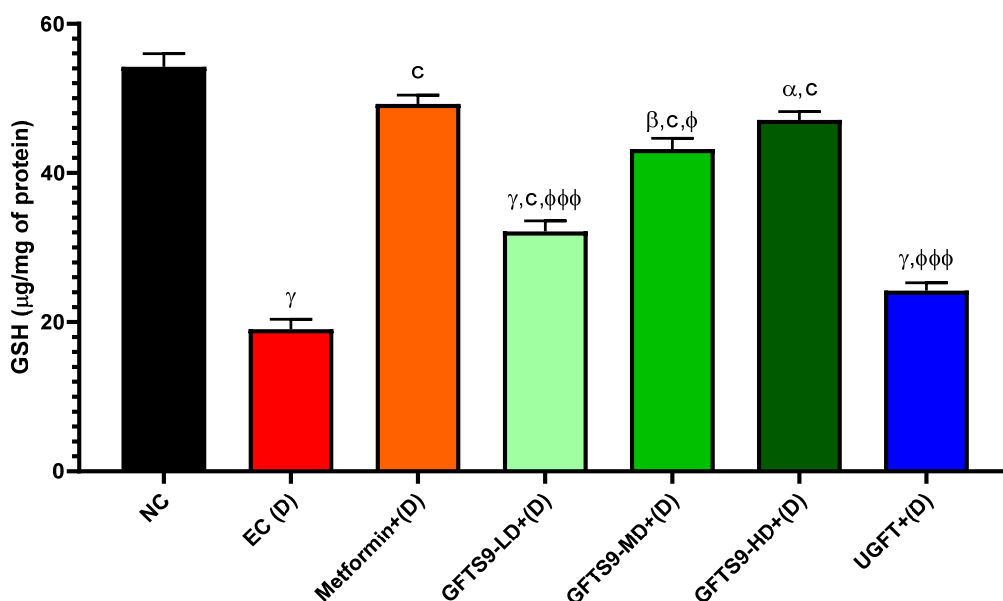


Figure 6.79: Effect on GSH level

Discussion: GSH level was decreased significantly in experimental animals after 21 days on treatment with different doses (lower, medium and higher). It significantly attenuated the elevated level in rats. Medium level dose of GFTS9 formulation were produced maximum attenuating effect same as higher dose. Hence, it is observed that

medium dose shows best action. Data are denoted as mean±SEM (n = 6). ^α p < 0.05, ^β p < 0.01, ^γ p < 0.001 compared to the normal group (NC). ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 compared to the diabetic group [EC(D)] and ^φ p < 0.05, ^{φφ} p < 0.01, ^{φφφ} p < 0.001 compared to the Metformin-treated group [Metformin+(D)].

6.13.2.3 Effect on MDA

Table 6.85: Effect of formulations on MDA in streptozotocin induced diabetic rat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
MDA (nmol/mg of protein)	54.22	19.06	49.23	32.16	43.21	47.11	24.23

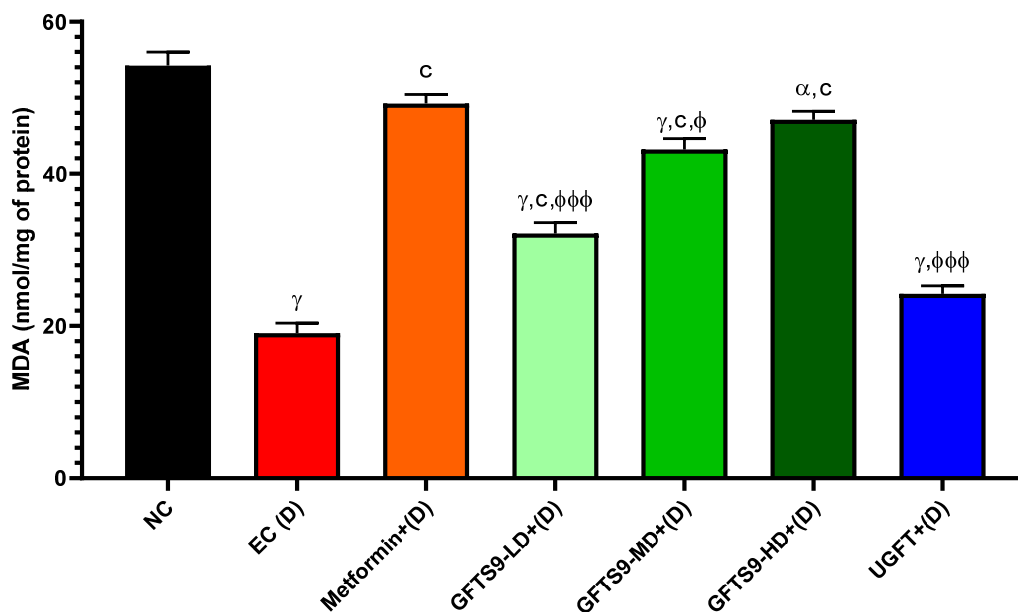


Figure 6.80: Effect on MDA level

Discussion: MDA level was increased significantly in experimental animals after 21 days of treatment with different doses (lower, medium and higher). It significantly attenuated the elevated level of MDA in rats. Medium level dose of GFTS9 formulation produced maximum attenuating effect same as higher dose. Hence, it is observed that medium dose shows best action. Data are denoted as mean±SEM (n = 6). ^α p < 0.05, ^β p < 0.01, ^γ p < 0.001 compared to the normal group (NC). ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 compared to the diabetic group [EC(D)] and ^φ p < 0.05, ^{φφ} p < 0.01, ^{φφφ} p < 0.001 compared to the Metformin-treated group [Metformin+(D)].

CHAPTER-VII

SUMMARY AND CONCLUSION

Metformin HCl is extensively used for the management of type-2 diabetes which belongs to biguanide class. It is a hydrophilic drug which is not perfectly absorbed in GI tract. Its short half-life is 1.5-4.5 hrs as well absolute bioavailability is reported to be 50-60 %. Due to short half life and low bioavailability, it is required to give two to three dose per day, so it leads to decrease patient compliance.

Hence an effort is assembled in this research work to Formulate SR tablets of metformin HCl by grafted copolymers of fenugreek gum.

1. Preformulation studies were conducted for the characterization of physicochemical properties of drug and grafting components. The absorption maxima of Metformin HCl was observed in phosphate buffer pH 6.8, water, methanol, 0.1N HCl pH1.2.
2. FT-IR spectrum confirmed the identity and purity of the drug Metformin HCl. Accuracy, linearity, precision, robustness, ruggedness and Force degradation studies were validated by HPLC method.
3. The partition coefficient (0.51 ± 0.03) indicated that the drug is freely soluble in water.
4. FTIR spectrum of physical mixture indicates that all excipients were compatible with each other and there is no interactions among all of them. XRD studies confirmed that drug showed crystalline nature and is compatible with excipients.
5. The yield obtained after extraction of fenugreek gum was $55.99 \pm 0.01\%$ w/w. The copolymers of fenugreek gum with acrylamide were synthesized by using redox initiator such as ammonium -per sulfate (APS), ceric-ammonium nitrate (CAN) potassium- per sulfate (KPS). Initiator ammonium- per sulfate (APS) shows the best %yield and grafting efficiency. After optimization of the grafting batch, Tiguchi OA design showed success with desired functions.

6. The different synthetic parameter of ammonium per sulphate (APS) induced graft copolymerization, % G, % GE and % C were calculated for the optimization of grafted fenugreek gum.
7. After that, sustained release tablet of grafting copolymers containing Metformin HCl as the API was designed. CCD design was used as part of quality by design tool to get optimized formulation containing amounts of grafted fenugreek gum (X1) and PVP K30 (X2) as formulation parameters.
8. The statistical parameters with polynomial equations were calculated that confirmed the designing check point analysis (TS9) to formulation, containing 347.98 mg of grafted gum and 74 mg of PVPK 30.
9. The comparison study of TS9 formulation with dissolution profile showed that the drug shows delayed action, due to longer polymeric chains, showing greater extent of swelling. Beside, % drug content uniformity was also calculated within range 84.96 to 99.19%.
10. Then selected formulation (TS9) indicates the success of the design which follows Higuchi model ($R^2=0.994$) with Fickian Diffusion ($n = 0.994$) to best fit. The consequence revealed the drug was released via grafted tablet by sustain manner.
11. The results of animal studies were conducted for 21 days, in which the Biochemical parameters (reduced body weight, glucose level, Cholesterol, Triglycerides, LDL, VLDL and increased HDL level) and antioxidant parameters (decreased the level of CAT and GSH and increased MDA) were observed.
12. The result exposed that the TS9 formulation exhibited a better sustained release of Metformin HCl with grafted fenugreek gum as compare to un-grafted and marketed formulation.

So, it concludes that

- The graft copolymers of fenugreek gum were synthesized.

- After optimization of the grafting batch, designed the SR Metformin HCl tablet using these grafted copolymers.
- The selected tablet formulation (TS9) were indicating the success of the design combined with a desirability function exhibited a better-sustained release action with grafted fenugreek gum as compare with un-grafted and marketed formulation by applying the animal design.
- In vivo studies indicated that the formula generated by CCD design showed better sustained release profile of the formulation.

Hence, grafting techniques of fenugreek gum by microwave assisted method may serve as an alternative for combining properties like better sustained and anti-diabetic action of metformin HCl.

CHAPTER-VIII

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Fenugreek

Article

Microwave-assisted synthesis of acrylamide grafted polymeric blend of fenugreek gum and its characterization

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Asha

Surajpal





A REVIEW ON TECHNIQUES FOR GRAFTING OF NATURAL POLYMERS AND THEIR APPLICATIONS

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Abstract

Polymer science have gained the advancement for improving the range of applications. The grafting techniques of natural gums or polymers have been modified for monomers production to transport enhanced polymeric material. These modifications are important against various challenges. It provides enhanced stability, compatibility, flexibility, and rigidity for the synthesized/modified co-polymer. This review enumerates the grafting concept, grafting modification, grafting techniques and applications of grafted natural gums in drug delivery.

Keywords: Grafting, Natural gums, co-polymer, drug delivery

Introduction

The nature is gifted with the wide variety of materials to human beings for balancing the healthiness of all living things. Polymers form the core of drug delivery system for providing the weight, consistency and volume or the administration of the drug (Bhattacharya *et al.*, 1998). Due to the complexity of polymer structure that needs to be arise for extensive understanding of the surface and bulk properties of polymers which can show the desired functionalities (Sah *et al.*, 2016). In drug delivery, both natural and synthetic polymers are used. Natural gums are derived from the seeds or tubers of plants & seaweed which consist of multiple sugar units linked together to form large molecules. Intensive research on natural polymers due to their sustainability, biodegradability & biosafety (Yvon, 2014).

Advantages of natural gum

- The gums should be biocompatible or non-toxic in nature.
- Naturally available biodegradable polymers are produced by living organisms. Gums are truly renewable source & no adverse impact or environmental health.

Applications of Natural gum

Table 1: Natural gums gaining importance for grafting.

Common Name	Botanical name	Family	Plant parts	Pharm application	Therapeutic application	Adverse effects	References
Acacia	<i>Acacia senegal</i>	Legumino-Sae	Bark	Osmotic drug delivery	Dental plaque, weight loss	Allergic reaction, respiratory problem, skin lesions	(www. Acacia gum. 2017)
Bhara gum	<i>Terminalia bellericarb</i>	Combretaceae	Plant bark	Microencapsulation	Peptic ulcer disease	Diarrhea, dizziness, Headache, anorexia	(Nayak <i>et al.</i> , 2008).
Guar gum	<i>Cyamopsis tetragonolobus</i>	Legumino-seae	Seed	Colon targeted drug delivery, micro spheres, stabilizing, thickening, disintegrants agent	Constipation, Diarrhea, Diabetes	Blockage of esophagus & Intestine, gas production, GI Obstruction	(Baveja <i>et al.</i> , 1997; Patel <i>et al.</i> , 2014; Chourassia <i>et al.</i> , 2004).

- They are low in cost & easily available. Their production cost is lowered than synthetic material.
- The collection of natural gums is easy in different season & in the large quantities due to simple production processes involved. In developing countries, governments promote the production of plants containing gums because of the wide application a variety of industries.
- As compared with synthetic gums the chances of adverse effect are fewer with natural material (Mukherjee *et al.*, 2008).

Disadvantages of natural gum

Microbial contamination- Microbial contamination accumulated natural material due to 10% or more moisture content present in the gums which can be prevented by proper handling & the use of preservatives.

Reduced viscosity on storage- Natural gums come in to contact with water then increased the viscosity of the formulations due to their complexity in the nature of gums and reduced the viscosity of formulation after storage (Mukherjee *et al.*, 2008).

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