आईएफटीएम विश्वविद्यालय, मुरादाबाद, उत्तर प्रदेश

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E-Content

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FORMULATION AND OPTIMIZATION OF GASTRO RETENTIVE MULTIPARTICULATE OF GLIBENCLAMIDE AND METFORMIN HYDROCHLORIDE FOR THE TREATMENT OF TYPE II DIABETES USING GELUCIRE

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PUBLICATIONS

Prashant Upadhyay, Jayanta Kumar Pandit and Arun Kumar Wahi, 2014. Studies on biological macromolecules lipid-gelucire based gastro retentive multiparticulate**,** ***International Journal of Biological macromolecules, Vol. 67,*** pp. 463-477.

Prashant Upadhyay, Jayanta Kumar Pandit and Arun Kumar Wahi, 2013. Gelucire: An alternative formulation technological tool for both sustained and fast release of drugs in treating diabetes mellitus type II disease**,** ***Journal of Scientific and Industrial Research, Vol.72, pp.776-780.***

Prashant Upadhyay, Jayanta Kumar Pandit and Arun Kumar Wahi, 2013. Effect of Gelucire based Gastroretentive multiparticulates of Metformin hydrochloride and Glibenclamide on histology of Diabetic rat pancreas, ***Pharmacologyonline,*** Vol. 2, pp.110 – 119.

Prashant Upadhyay and Jayanta Kumar Pandit, 2012. Fast Release Gastro Retentive Multi-Particulates of Glibenclamide for The Treatment Of Type II Diabetes Mellitus using Gelucire 50/13, ***Tropical Journal of Pharmaceutical Research, Vol. 13, No.3, pp.361-369.***

Prashant Upadhyay and Jayanta Kumar Pandit, 2011. Dissolution, HSPM, PXRD, DSC studies on Gastro Retentive Multi-Particulates of Metformin Hydrochloride for the Treatment of Diabetes using Gelucire, ***Latin American Journal of Pharmacy (formerly Acta Farmaceutica Bonaerense), Vol.30, No. 9,*** pp.1675-81.

INTRODUCTION

- Diabetes type II affects over 150 million people worldwide and this number is expected to double by 2025 (King et al 1998).
- Glibenclamide is sulfonylurea class II drug with less solubility and high permeability nature and Metformin hydrochloride is biguanide class III drug with high solubility and low permeability with bioavailability 50 -60 % from conventional dosage form and is absorbed from upper part of GIT. It has contradictory report in single unit gastro- retentive dosage form due to its faster release and high toxicity.
- Gelucire is mixtures of mono-di-and tri glycerides with PEG esters of fatty acids. Grades 39/01, 43/01 has extreme hydrophobicity and low density. Recent attention focus on as carriers in gastroretension, using it as density control delivery system. Gelucire 50/13 act as fast delivery system due to higher HLBs (Chauhan et al 2004 & Shimpi et al 2004).
- Biological macromolecules lipids have property of low density and insolubility in water. Unlike other classes of biological macromolecules, lipids do not form large polymers. Two or three fatty acids are usually polymerized with glycerol to create a triglyceride, but other lipids, such as steroids do not form polymers (Hauss et al 2007).
- In this study, a simple, rapid (short elution time) and adequate sensitive isocratic reversed-phase HPLC method with UV detection has been adopted for determination of glibenclamide (Li et al 2012) and metformin (Wanjari et al 2008) in rat plasma and finally, *in vitro–in vivo* correlation (IVIVC) study will be performed (Schamp et al 2006).

OBJECTIVE OF THE STUDY

To increase bioavailability and reduce toxicity due to high dose dumping, it was decided to formulate novel gastro retentive multiparticulate of Glibenclamide and Metformin hydrochloride and evaluated with respect to:-

1. Preformulation studies
2. Preparation method of Fast release gastro retentive multiparticulate of Glibenclamide using Gelucire 50/13.
3. Preparation method of Sustained release gastro retentive multiparticulate of Metformin Hydrochloride using Gelucire 39/01 and 43/01.
4. Evaluation of Fast release gastro retentive multiparticulate of Glibenclamide using Gelucire 50/13.
   - 4.1 Drug contents by UV spectrophotometry.
   - 4.2 *In vitro* floating time.
   - 4.3 Drug release *in vitro*.
   - 4.4 Mathematical modeling of *in vitro* release data.
   - 4.5 Statistical Optimization of formulation by considering dissolution data, so as to formulate stable, safe and efficacious formulation.
   - 4.6 Physical Characterization
   - 4.6.1 Surface topography using scanning electron microscopy (SEM) and Hot Stage Polarized Microscopy (HSPM)
   - 4.6.2 Drug carrier interaction using FTIR
   - 4.6.3 Powder X Ray Diffraction (PXRD)
   - 4.6.4 Differential Scanning Calorimetry (DSC)
5. Evaluation of Sustained release gastro retentive multiparticulate of Metformin Hydrochloride using Gelucire 39/01 and 43/01.
   - 5.1 Drug entrapment efficiency
   - 5.2 *In vitro* floating time.
   - 5.3 Drug release *in vitro*
   - 5.4 Mathematical modeling of *in vitro* release data.
   - 5.5 Statistical Optimization of formulation by considering dissolution data, so as to formulate stable, safe and efficacious formulation.
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   - 5.6.2 Drug carrier interaction using FTIR
   - 5.6.3 Powder X Ray Diffraction (PXRD)
   - 5.6.4 Differential Scanning Calorimetry (DSC)
6. Stability studies.
7. Animal study showing hypoglycemic activity in streptozotocin induced diabetic rats.
8. Validation of HPLC method for *in vivo* studies.
9. Determine the pharmacokinetic profile of prepared formulation.
10. Simultaneously administration of gastro retentive multiparticulate of Glibenclamide and Metformin Hydrochloride and its rationale.
11. *in vitro- in vivo* correlation.
12. Histopathological studies of pancreas.

METHODOLOGY

- **Preparation procedure:** various batches of Floating multiparticulate system containing different ratios of Glibenclamide and Metformin HCl, Gelucires and release modifier will be prepared by Hot Melt technology.
- **A) Fast release Gastro retentive multiparticulate of Glibenclamide Using Gelucire 50/13:** The Glibenclamide (GLB) was dispersing in melted Gelucire 50/13 at various ratio 1:1, 1:2, 1:4, 1:6, 1:8 and 1:10 by using hot melt technique (**Fini et al 2005 and Barmpalexis et al 2011**). After cooling on aluminium foils and keeping in refrigerator, solid lump was passed through fine mesh (150µm) to obtain multiparticulates and were desiccated for 48h. The saturation solubility and melting point was determined further of above prepared multiparticulates of GLB **(Varma et al 2005**). Each experiment was performed in triplicate. On basis of saturation solubility and melting point, the 1:10 ratio was optimized to prepare physical mixtures (PMs) and solid dispersions (SDs) multiparticulates of GLB and hydrophilic additives PEG 200, 400, 4000, 6000 and Gelucire 50/13 as following batches. Physical mixtures of drug and Gelucire were prepared by triturating them on mortar and pestle for 15 min, followed by sieving (150µm).
- **B) Sustained release Gastro retentive multiparticulate of Metformin hydrochloride Using Gelucire 39/01 and 41/01:** Metformin Hydrochloride (MH) loaded Multiparticulates were prepared by dispersing MH in melted Gelucire 39/01 and 43/01. After cooling on aluminium foil in refrigerator, solid lump was passed through sieves to obtain multiparticulates and kept in desiccator. All the samples were passed through fine mesh (150um) and stored in desiccated environment until further study. By using hot melt technique **(Fukuda et al 2006**); a number of formulations were prepared using different drug: lipid ratio. Lipid was melted at 50°C and the drug or drug additive mixture was added to the melt. The system was mixed well and cooled to room temp. The mass so prepared was passed through sieve of appropriate size (20-40) to obtain uniform size multiparticulates system. As shown in table further, the following batches of MH using Gelucire 39/01 and 43/01 were prepared according to methodology adopted by **Chauhan et al 2004 and Shimpi et al 2004.**

CHARACTERIZATION OF DRUG

Physical characterization of drugs

Glibenclamide and Metformin hydrochloride was evaluated for its physical properties and it was observed that drugs physical properties were found as similar as reported in literature that proves the identity of drug.

Characteristics	Glibenclamide	Metformin Hydrochloride
1. Physical form	Amorphous	Crystalline powder
2. Color	White	Off White
3. Odor	Odorless	Odorless
4. Taste	Tasteless	Tasteless

Melting point determination

Melting point of Glibenclamide and Metformin hydrochloride were found to compile with the literature value of 170°C to 174°C and 222 °C to 226°C respectively, indicating the identity and purity of drug sample (IP 1996).

	Glibenclamide	Metformin hydrochloride
Melting point (°C)	171±1	224±1

GLIBENCLAMIDE FORMULATIONS

INGED	FI	FII	FIII	FIV
RATIO	1:1	1:2	1:5	1:10
GLB	5	5	5	5
Gel 50/13	5	10	25	50
Total	10	15	30	55

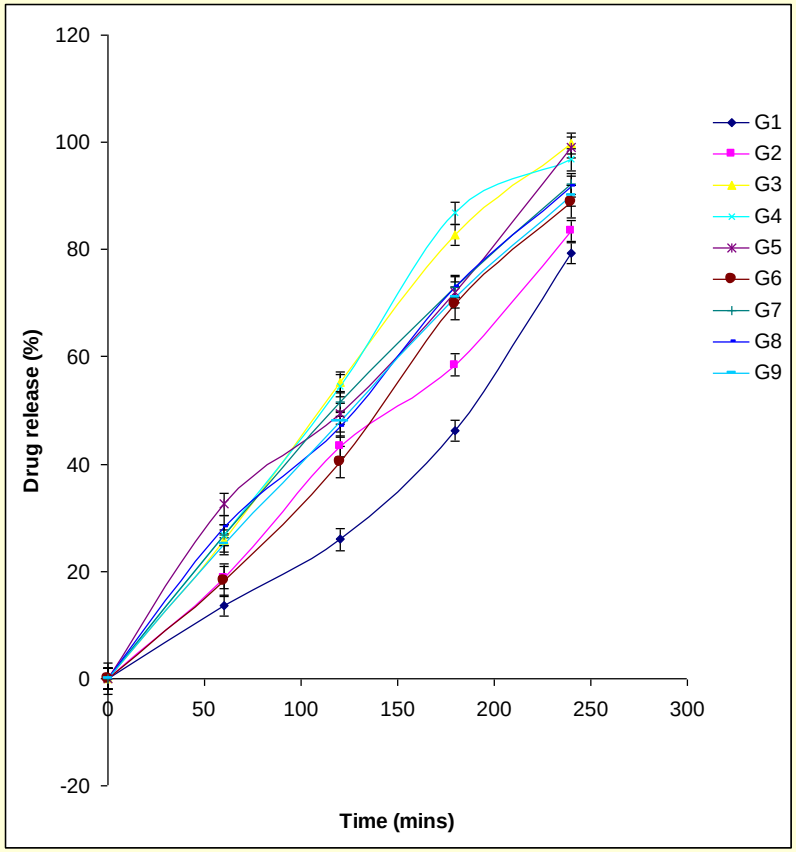
INGED	GI	G II	G III	G IV	G V	G VI	G VII	G VIII	G IX
GLB	5	5	5	5	5	5	5	5	5
PEG 200	-	5	-	-	-	10	-	-	-
PEG 400	-	-	5	-	-	-	10	-	-
PEG 4000	-	-	-	5	-	-	-	10	-
PEG 6000	-	-	-	-	5	-	-	-	10
Gel 50/13	50	50	50	50	50	50	50	50	50
Total	55	60	60	60	60	65	65	65	65

DRUG RELEASE KINETIC MODELS
(Glibenclamide formulations)

Formulation code	First order		Higuchi's model		Zero order		Korsmeyer and Peppas model		
	r2	k	r2	k	r2	k	r2	k	n
GI	0.6893	0.3602	0.9874	22.726	0.9531	11.04	0.9116	1.49142	0.8378
G II	0.6673	0.3441	0.9707	19.934	0.9152	9.57	0.8939	1.521248	0.8054
G III	0.72	0.366	0.9463	23.166	0.9722	11.61	0.9266	1.431858	0.8398
G IV	0.8005	0.3763	0.9265	21.575	0.9888	11.02	0.9714	1.231119	0.8384
G V	0.7238	0.359	0.975	20.88	0.9611	10.25	0.9334	1.390593	0.8246
G VI	0.7506	0.4061	0.921	32.501	0.971	16.5	0.9462	1.388353	0.9221
G VII	0.6927	0.3953	0.9732	32.788	0.9742	16.22	0.9131	1.544543	0.918
G VIII	0.6959	0.3557	0.9915	21.414	0.9585	10.41	0.9162	1.466898	0.8258
G IX	0.7072	0.3532	0.899	20.953	0.9288	10.53	0.9135	1.44544	0.8119

Influence of *in vitro* release

- *In vitro* fast release profiles of different SDs formulation of GLB that lasted for 4 h in 0.1N HCl (pH 1.2) and showed *in vitro* buoyancy for 11 h and drug entrapment efficiency up to 99.8 %.
- The Kinetic model that best fitted the release data was evaluated from regression coefficient values (r2) of all formulated batches.
- As indicated in Figure and Table the formulations followed zero-order equation, with regression values near to one. To confirm the diffusion mechanism, further the data were fitted into Korsmeyer-Peppas equation and from the n values it was evident that the drug release mechanism followed non-fickian case II diffusion controlled mechanism (n values ranges from 0.80 to 0.9221) and the K value indicated interaction between the drug and polymeric materials as previously established by FTIR data.
- The fast release gastro retentive solid dispersion GIV formulation shows maximum comply for optimized formulation on the basis of its r2 value close to one for zero order release profile and floating reaching upto 11 h.



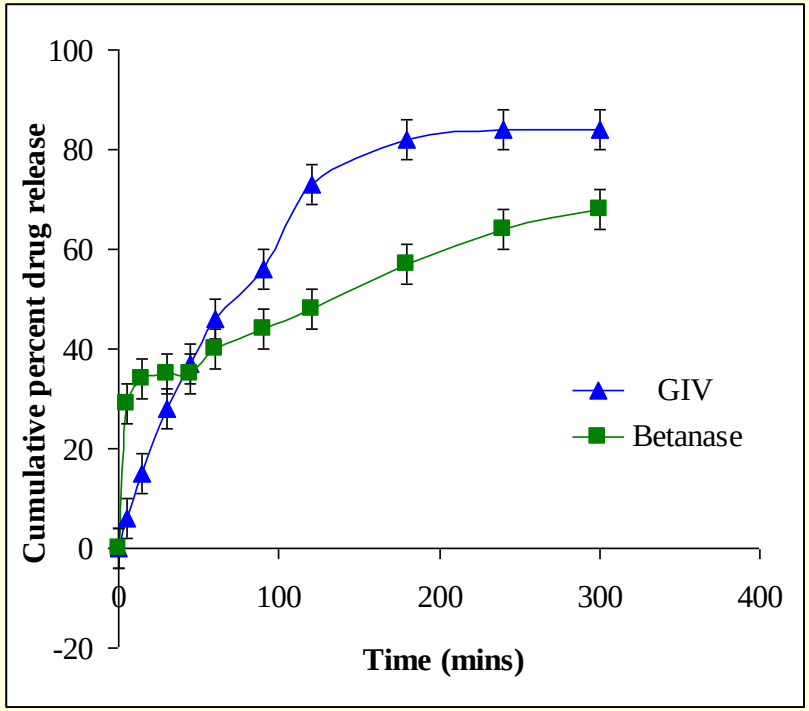
Entrapment and floating parameters of GLB loaded multiparticulate:

Formulation code	Entrapment efficiency (%)	Floating Time (h)
GI	98.4±0.12	10.5±0.20
G II	99.1±0.14	10.2±0.35
G III	98.3±0.20	9.8±0.10
G IV	99.8±0.11	11.0±0.20
G V	99.2±0.15	8.8±0.50
G VI	99.1±0.16	9.2±0.30
G VII	99.2±0.12	8.4±0.20
G VIII	99.3±0.17	7.4±0.80
G IX	99.67±0.18	10.4±0.22

Kinetics parameters of GLB loaded multiparticulate:

Formulation code	T 50%(h) ±SD	MDT (h) ±SD
G I	3.12±0.01	2.89±0.01
G II	2.53±0.01	2.75±0.01
G III	1.84±0.01	2.67±0.01
G IV	2.01±0.01	2.73±0.01
G V	2.15±0.01	2.68±0.01
G VI	2.48±0.01	2.81±0.01
G VII	1.92±0.01	2.69±0.01
G VIII	2.14±0.01	2.52±0.01
G IX	2.05±0.01	2.40±0.01

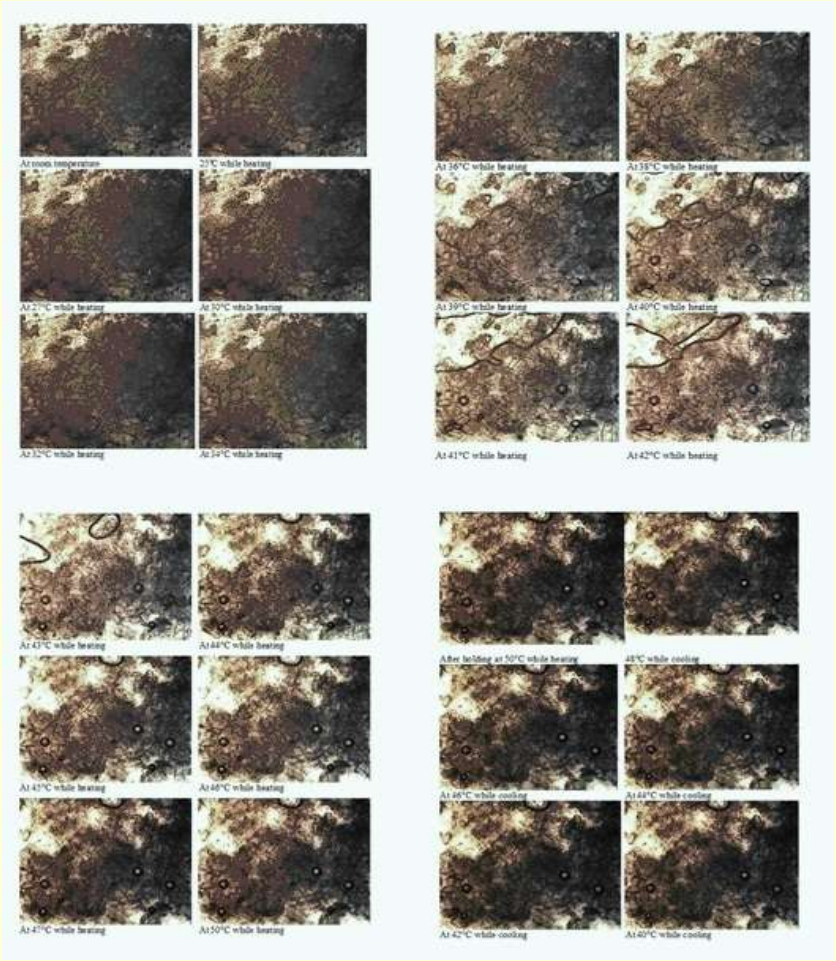
Comparative *in vitro* drug release of Solid Dispersions of glibenclamide (GIV) (▲) and a commercial brand, Betanase (□).



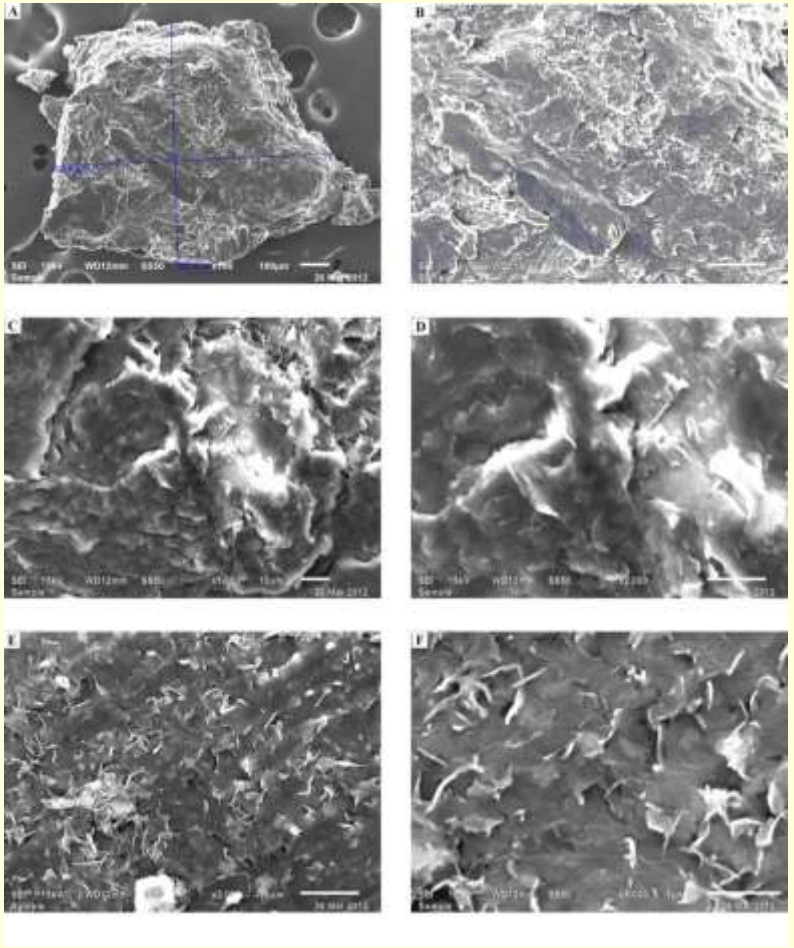
•The *in vitro* release data from fast release gastro retentive solid dispersion (GIV) were further compared based on model independent study with *in vitro* release data existing marketed fast release **Betanase** tablet and similarity factor (f2) was found to be 40 while difference factor (f1) was found to be 33 at 60mins.

Influence of Physical Characterization

HSPM of SDs containing GLB and Gelucire 50/13 shows continuous melting from room temperature to 50 ºC and cooling back to 40 ºC. Change in physical form was observed at different temp and photomicrographs were recorded. Large crystals of pure GLB reduced to small particle size might be due to close contact between hydrophilic carrier and drug.

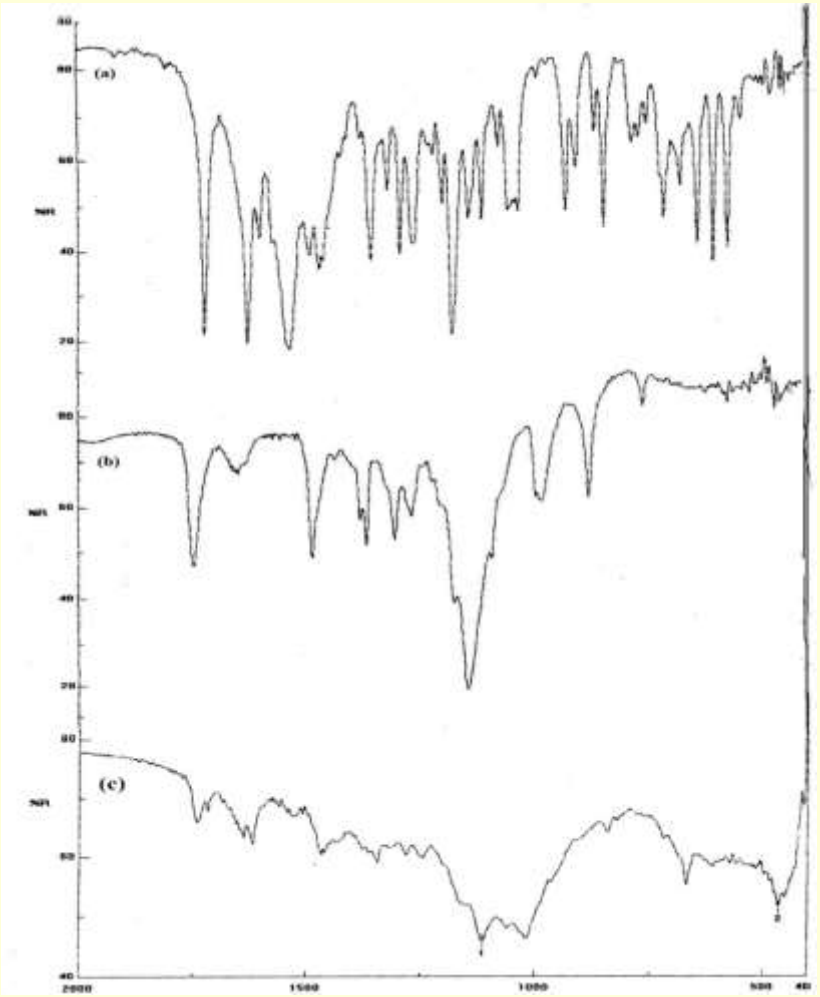


SEM photomicrograph showing surface topography. Solid dispersion matrices of glibenclamide show clear flakes like structures at 1000, 2000 & 5000X magnification.



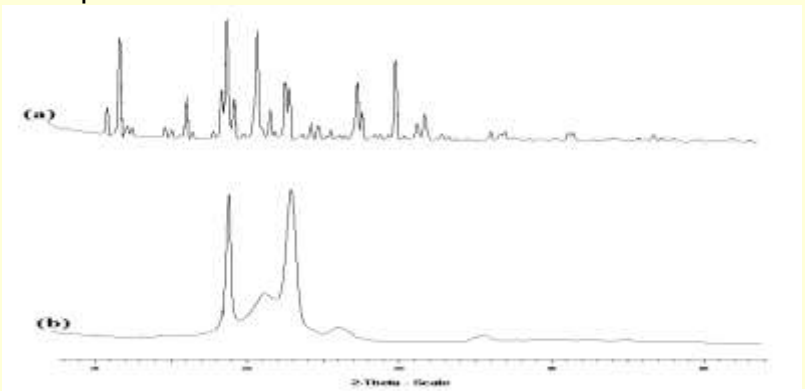
FTIR spectra

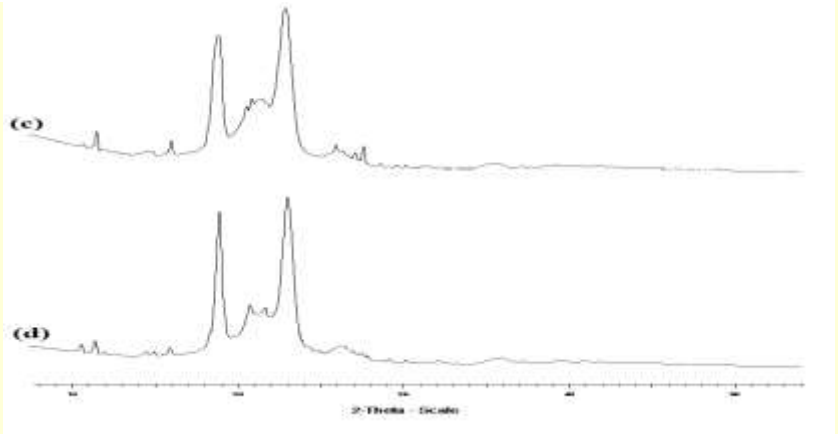
- FTIR spectra of pure GLB showed characteristic **amide peaks** at 1715.37 cm-1, **urea carbonyl** stretching (urea NH stretching) vibration at 1617.98 and 1523.49 cm-1, **SO2** stretching vibration at 1342.21 and 1159.01 cm-1.
- Whereas FTIR spectra of SDs showed almost complete disappearance of amide peaks at 1716.53 cm-1 and the concomitant shift to higher frequencies of urea carbonyl stretching vibration at 1618.2 and 1521.73 to 1635.5 and 1558.38 cm-1 respectively. It might be as a consequence of intermolecular interaction such as hydrogen bonding.
- In fact, the possible interaction would occur between the **amide of GLB and Oxygen of polyglycol chain (Gelucire**), inducing a shift of N-H vibration with an extent that depends on strength of interaction.
- However the site of the interaction of Gelucire would be expected in the C=O group, also affecting the N-H vibration. This observation was in accordance with the data generated from PXRD studies.



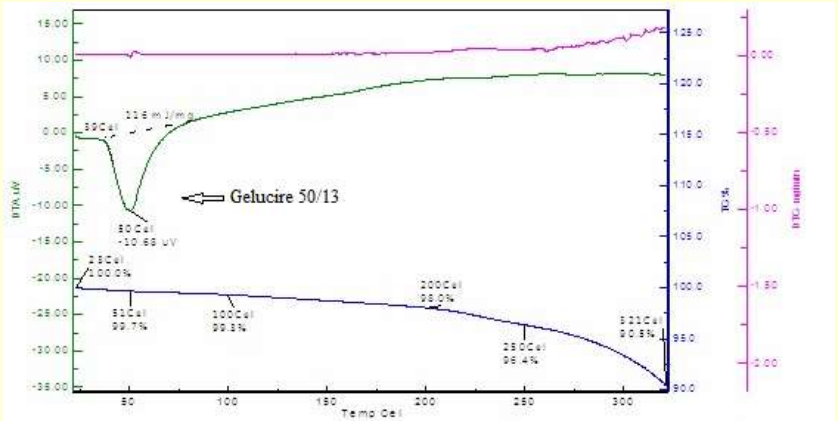
PXRD and DSC studies

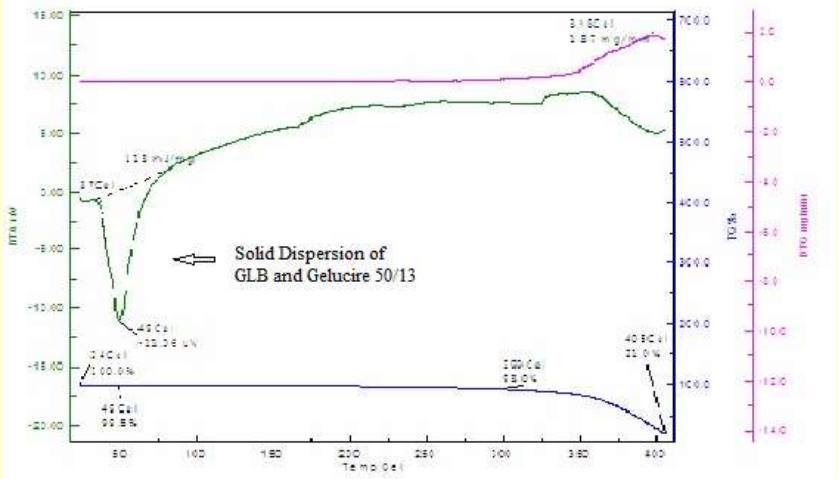
PXRD spectrum of pure GLB have prominent **diffraction peaks (d**) 8.035, 7.464, 4.649, 4.20, 3.860, 3.2, 2.933, 2.14, 1.89 and 1.687 at 11.0 , 11.84, 19.07, 21.11, 23.02, 27.85, 30.44, 42.12, 47.86, 54.33º **on 2θ scale** which indicates its crystallinity nature, further the peaks were found decreased in PMs and SDs that confirm its conversion to amorphous state while Gelucire 50/13 showed two prominent diffraction peaks(d) 4.61 and 3.81.with the highest intensity at 19.22 and 23.32º on 2θ scale. All the principles peaks of Gelucire 50/13 were present in their PMs and SDs. Diffractogram of SDs showed absence of any traces of crystallinity, which indicating the existence of Amorphous state of GLB.





DSC Thermograms obtained for pure GLB, pure Gelucire and Solid dispersion, **endothermic curve** starts from 37 to 49º showing melting point of Gelucire 50/13 with heat of fusion 113 J /mg. Thermal profiles of SDs exhibited a single endothermic peak corresponding to the fusion of the carrier. No peak was present representing the melting of the drug in solid dispersion which pretends to complete melting of amorphous drug. The results were consisted with the previous data of XRD patterns.





METFORMIN FORMULATIONS

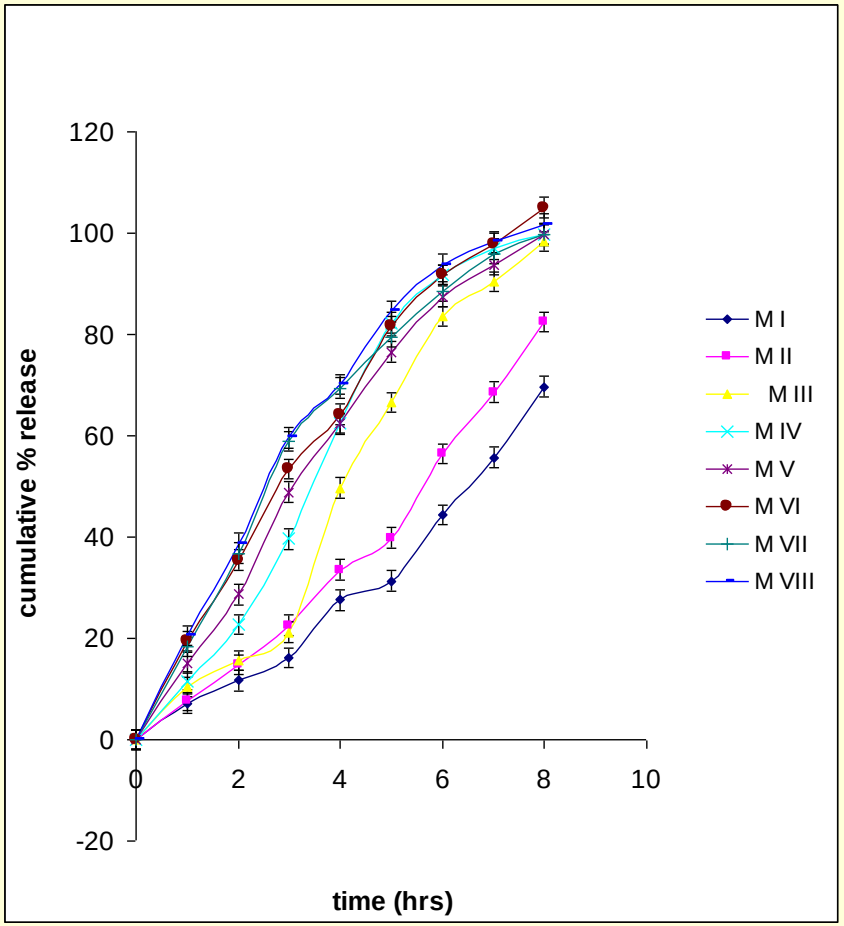
INGED	M I	M II	M III	M IV	M V	M VI	M VII	M VIII
MH	400	400	400	400	400	400	400	400
EC	-		100	100				
MC					100	100		
MCC							100	100
Gel 39/01	400		400	-	400		400	
Gel 43/01	-	400	-	400		400		400
Total	800	800	900	900	900	900	900	900

DRUG RELEASE KINETIC MODELS
(Metformin formulations)

Formulation code	First order		Higuchi's model		Zero order		Korsmeyer and Peppas model		
	r2	k	r2	k	r2	k	r2	k	n
M I	0.8461	0.1915	0.818	23.601	0.9671	8.4183	0.9853	1.2936	0.63
M II	0.821	0.1984	0.8416	28.84	0.9807	10.213	0.98	1.385798	0.6605
M III	0.8096	0.2124	0.8464	38.72	0.9678	13.583	0.9676	1.425608	0.7078
M IV	0.7361	0.2052	0.9053	41.173	0.9629	13.93	0.9406	1.728622	0.7071
M V	0.6773	0.1922	0.947	39.033	0.9766	13.003	0.9079	2.080176	0.6782
M VI	0.6383	0.1866	0.9622	40.185	0.9749	13.27	0.8815	2.361022	0.6686
M VII	0.6202	0.1839	0.9694	38.773	0.9502	12.593	0.87	2.413237	0.664
M VIII	0.6104	0.1832	0.97	39.878	0.9465	12.923	0.8628	2.506686	0.6641

Influence of *in vitro* release

- The formulations showed extended release profiles that lasted between 6-8 h. Ethyl cellulose and methylcellulose were evaluated as release rate modifiers. The dissolution data (from the values of 1 to 8 h drug release) of all formulated batches were **fitted to first-order, Higuchi, zero-order and Korsmeyer-Peppas models.** To describe the kinetics of drug release from mini matrices, release data was analyzed according to different kinetic equations.
- The model that best fitted the release data was evaluated from the regression coefficient values (r2) of all formulated batches. The formulations did not follow first-order release kinetics. When the data were plotted according to a zero-order equation, the prepared batches of formulations were found to show a fair linearity, with regression values near to one.
- To confirm the diffusion mechanism, further the data were fitted into Korsmeyer-Peppas equation and from the n values it was evident that the drug release mechanism followed anomalous diffusion controlled mechanism (n values range from 0.63 to 0.70).
- Release of a drug from hydrophobic mini matrices generally involved both pore diffusion and matrix erosion. M II formulation was optimized formulation on the basis of zero order release profile as its r2 value was close to one for zero order kinetics.



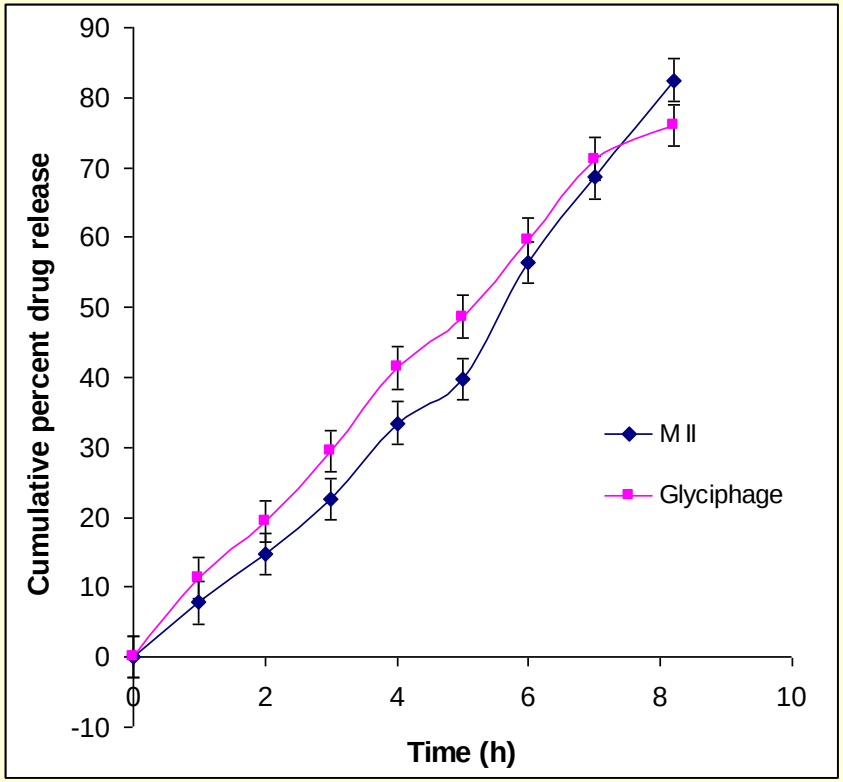
Entrapment and floating parameters of MH loaded multiparticulate:

Formulation code	Entrapment efficiency (%)	Floating Time (h)
M I	98.9±0.22	11.45±0.28
M II	99.6±0.35	11.30±0.47
M III	98.6±0.20	10.35±0.18
M IV	99.3±0.18	10.45±0.26
M V	99.7±0.19	10.50±0.56
M VI	99.6±0.24	10.40±0.31
M VII	99.6±0.31	9.55±0.36
M VIII	99.8±0.27	9.50±0.45

Kinetics parameters of MH loaded multiparticulate:

Formulation code	T50%(h) ±SD	MDT(h) ±SD
M I	6.51±0.01	5.59±0.01
M II	5.82±0.01	5.57±0.01
M III	4.12±0.01	5.59±0.01
M IV	3.63±0.01	5.44±0.01
M V	3.13±0.01	7.17±0.01
M VI	2.94±0.01	5.18±0.01
M VII	2.72±0.01	5.12±0.01
M VIII	2.83±0.01	5.10±0.01

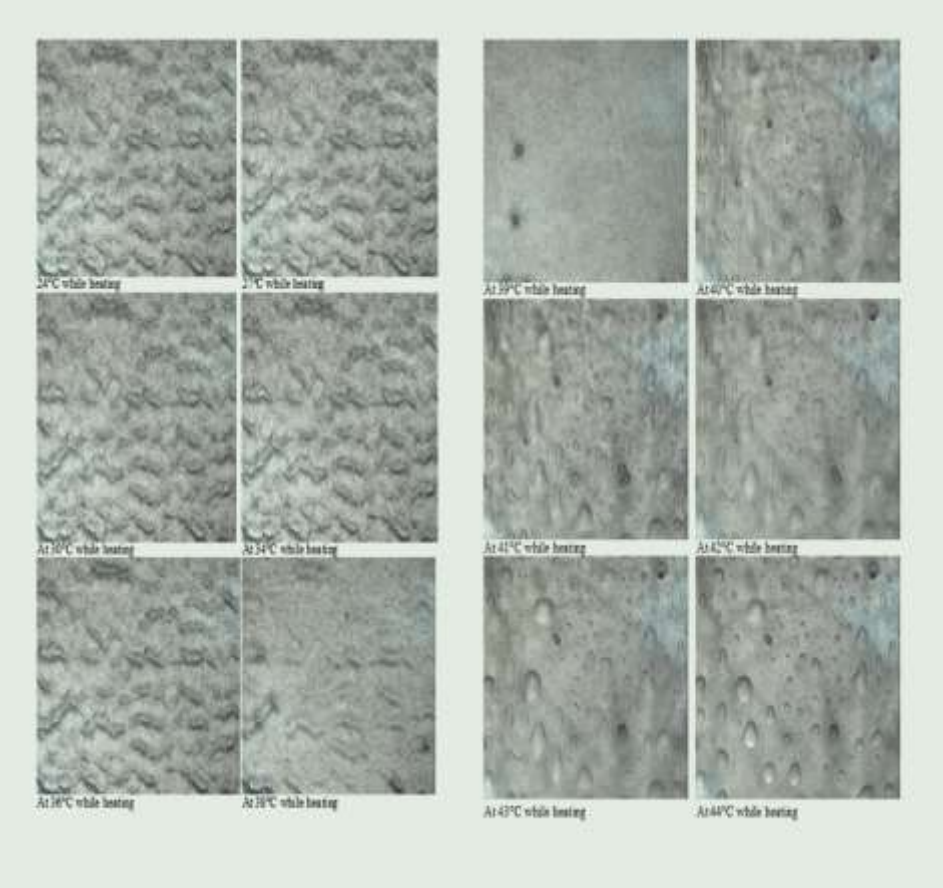
Comparative *in vitro* drug release from mini matrices of M II with Glyciphage tablet:



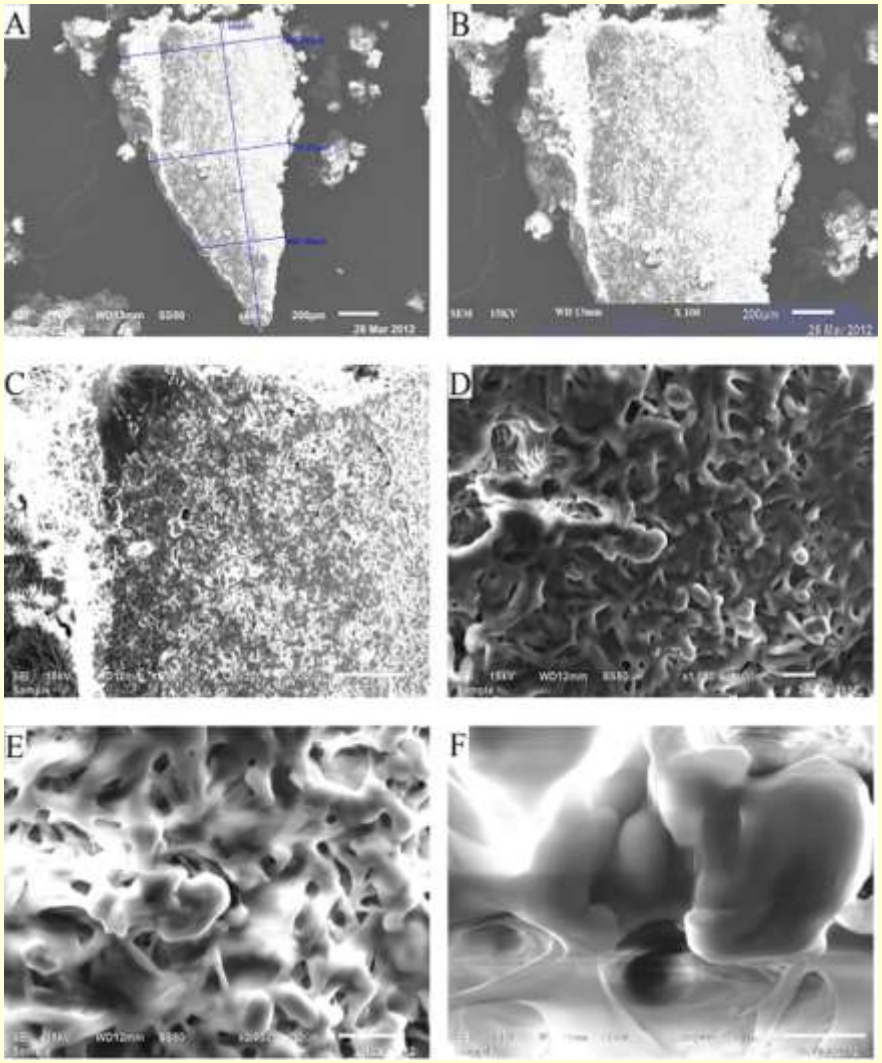
- The release profile of optimized formulation was further compared based on model independent study with **Glyciphage** tablet with similarity factor (f2) found to be 54 and difference factor (f1) to be 12 at 8 h.

Influence of Physical Characterization

HSPM of optimized formulation containing MH and Gelucire 43/01 shows continuous melting from room temperature to 44°C. HSPM photomicrograph showed presence of some un-melted portion even at 42 °C and completely melt at 43 °C. The energy required for melting increased, which might be attributed to **phase transformation** due to crystallization of glycerides. Change in physical form was observed at different temperatures and photomicrographs were recorded by HSPM. Large crystals of pure MH could not reduced to small particle size, might be due to crystalline nature of drug.

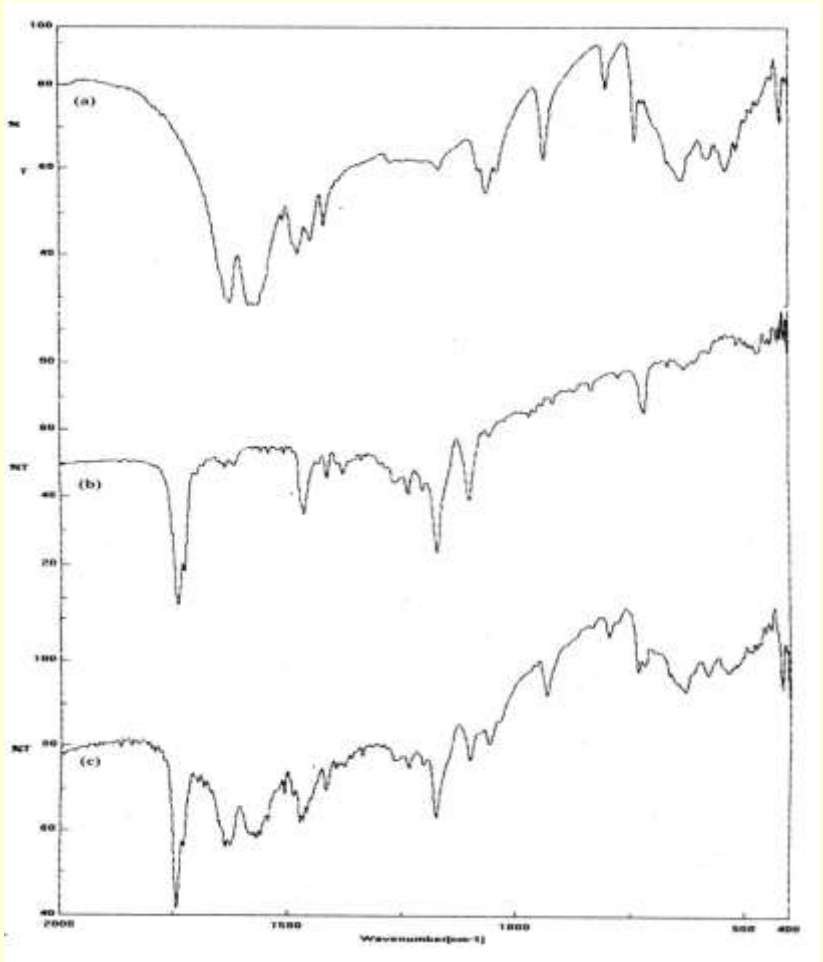


SEM photomicrographs are showing different morphology of metformin matrices at different magnification. The porous and layering nature was clearly visible at 1000X and 5000X magnification which confirms the anomalous diffusion controlled mechanism.

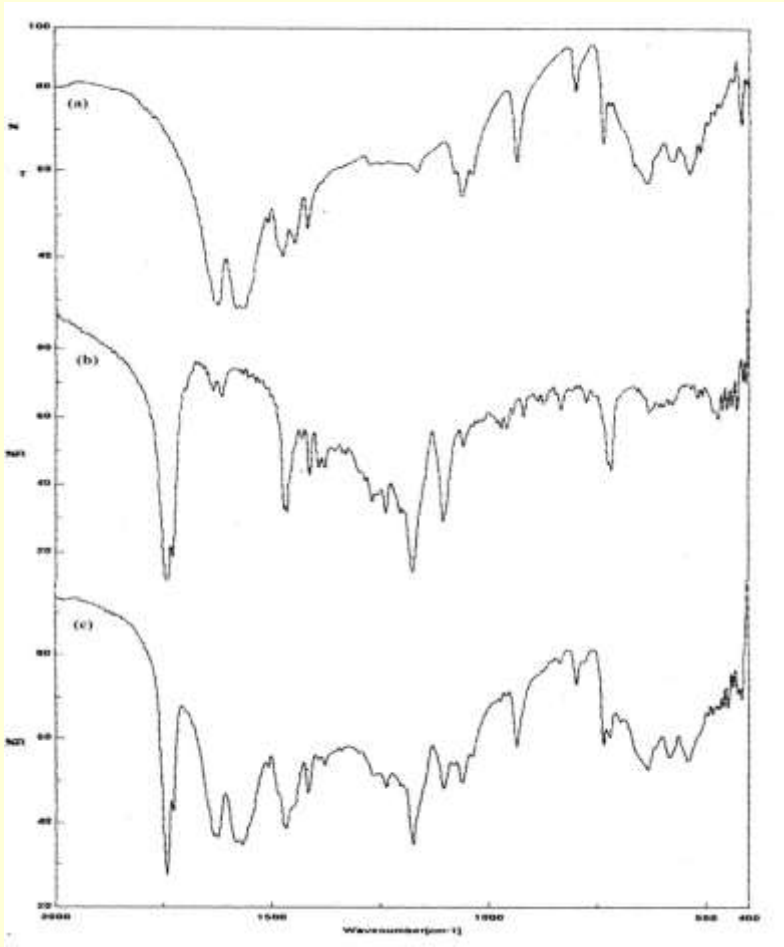


FTIR spectra

FTIR spectra scanned from 400 cm-1 to 2000 cm-1 wave numbers of pure MH (a) showed **characteristic peaks at 1567.84, 1061.62, 936.31 and 636.39 cm-1**. Whereas FTIR spectra of **Gelucire 39/01(b) showed peaks at 1742.37, 1465.63, 1174.44, 1106.01, 720.28 cm-1** and FTIR spectra of multiparticulates of MH and Gelucire 39/01 (c) showed the peak at 1742.37,1636.30,1472.38, 1174.44,633.50, 417.51 cm-1. This observation was in accordance with the data generated from PXRD studies and concluded that there was no interaction found between metformin and gelucire 39/01 when formulated.

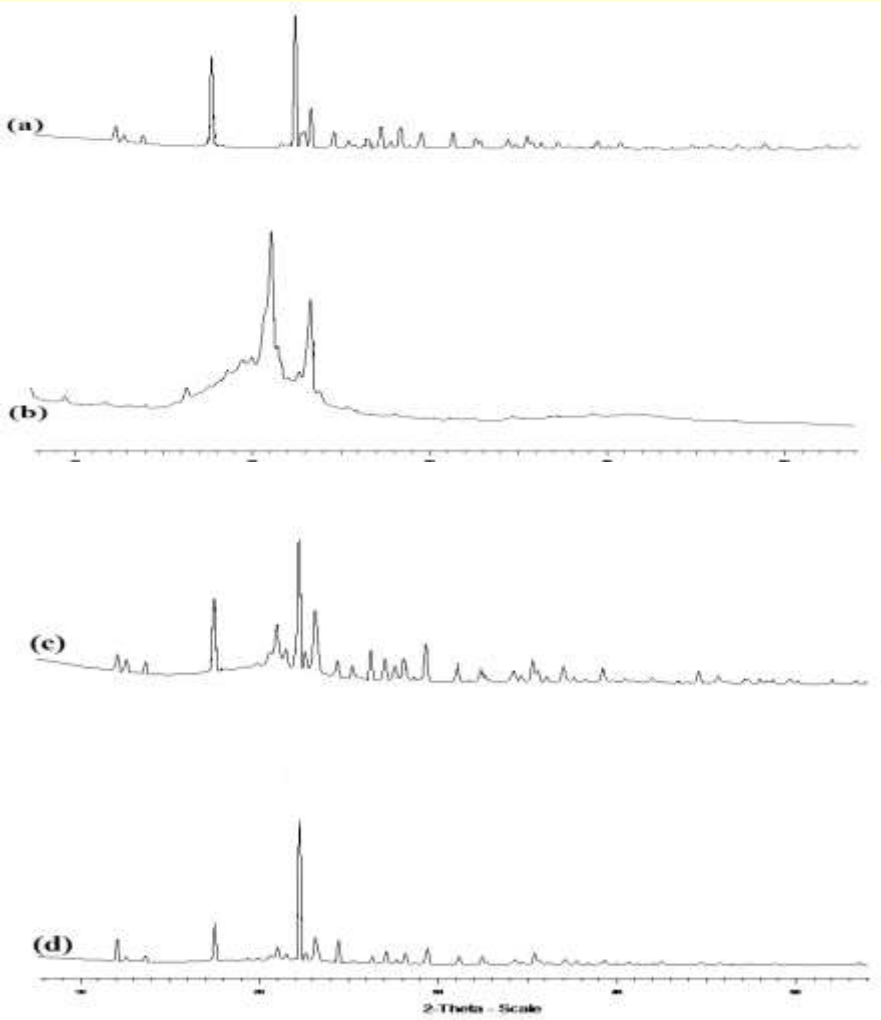


FTIR spectra scanned from 400 cm-1 to 2000 cm-1 wave numbers of pure MH (a) showed characteristic peaks at 1567.84, 1061.62, 936.31 and 636.39 cm-1.Whereas FTIR spectra of **Gelucire 43/01 showed peaks at 1742.37, 1465.63, 1413.57, 1175.40, 1105.01, 720.28, 470.55 cm-1** and FTIR spectra of multiparticulates of MH and Gelucire 43/01 showed the peaks at 1742.37, 1624.73, 1568.81, 1466.60, 1174.44, 1105.01, 936.27, 634.47 cm-1. This observation was in accordance with the data generated from PXRD studies and concluded that there was no interaction found between metformin and gelucire 43/01 when formulated.

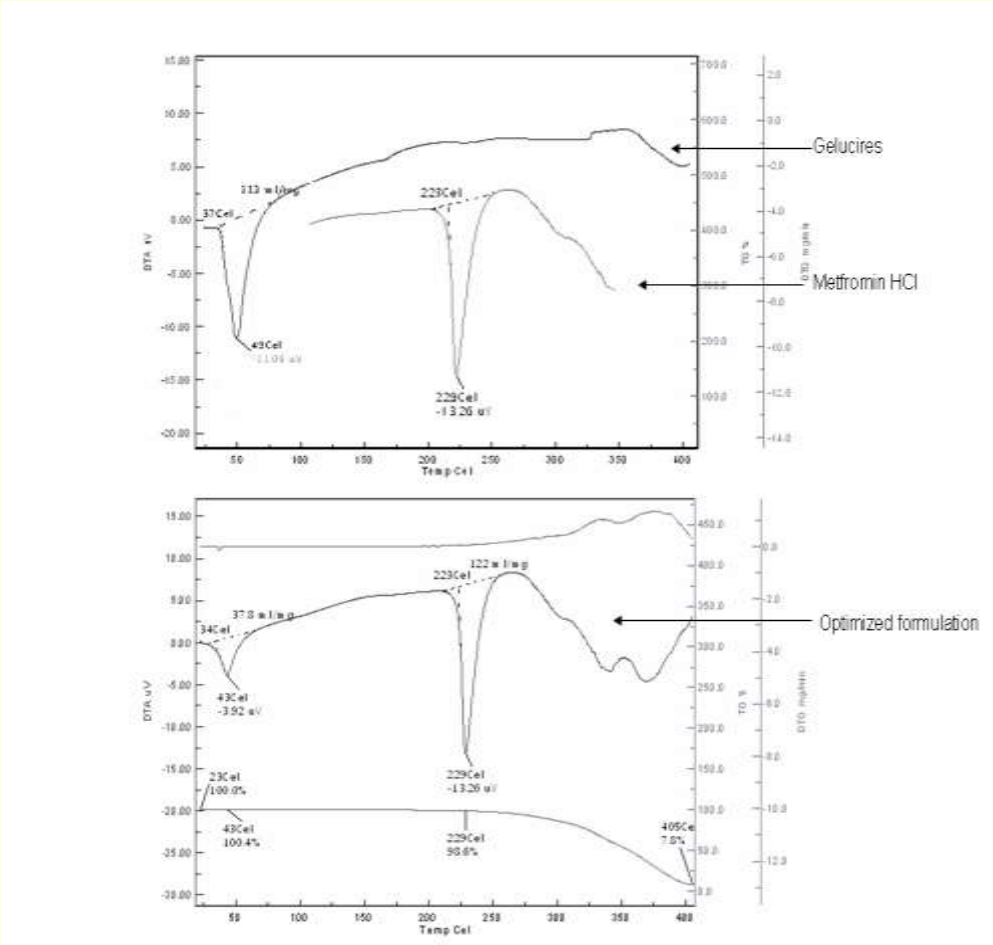


PXRD spectrum and Thermal analysis

PXRD spectrum of pure MH (a) have two major prominent diffraction peaks d 5.03, and 3.97 with 17.60 and 22.34 ° respectively 2θ scale, which indicates its crystallinity nature, further these peaks were found in physical mixtures (c) and multiparticulates (d) that confirm its crystalline state while Gelucire 43/01(b) showed prominent peaks d 12.87, 4.22 and 3.82 with 6.86, 21.01 and 23.25 ° respectively 2θ scale, which was found further loose intensity in physical mixture and Solid dispersion formulation too.



DSC Thermal analysis graph of optimized formulation, showed that the **endothermic curves** showing melting point of drug and Gelucire 43/01 in the above mixture was found to be slightly shifted towards higher value in case of Gelucire that is from 39 to 44 º that might be due to consistency in the final formulation in presence of polymers used. More over the melting point of pure drug MH was found to be slightly decreased (229 º C) in the formulation which shows decreased crystallinity. The results were consisted with the previous data of PXRD patterns.



Stability studies according to ICH guidelines

- Stability studies were carried out according to ICH guidelines.
- A graph of Log percentage of drug remaining was plotted against time to know the slope which is equal to -K/2.303.
- Stability studies of MII and GIV multiparticulates were performed at temperature 30ºC±0.2°C and 65 % Relative Humidity (R.H.) for 3 months
- It was found that **degradation rate constant (K)** for GIV multiparticulates was 11.52 X 10-4 whereas for M II multiparticulates was 6.909 X 10 -4.
- Moreover, no change was observed over the period of 3 months in the consistency of multiparticulates and drug degradation was also nominal through out the period of storage.

Animal Studies

As per CPCSEA Guidelines of Good Laboratory Practice and housed in polypropylene cages with free access to std lab diet and water. The research protocol (Registration no: 837/ac/04/CPCSEA; Resolution no: 20/PhD/2008-2009; Dated 08/03/2010) approved by 'Institutional Animal Ethical Committee' of College of Pharmacy, IFTM, Moradabad.

***In vivo* Blood glucose level (BGL) lowering studies**: of optimized formulation were determined in comparison with pure drugs in experimentally induced diabetic wistar rats of either sex weighing 150-200 g by streptozotocin,35mg/kg body weight /i.p in citrate buffer (pH 4.4) to naive Wistar rat. Total 48 Wistar rats were divided in the following groups and Glucose Tolerance Test will be done i.e. feeding of glucose solution after dosing with formulation and determination of blood glucose level (BGL) periodically.

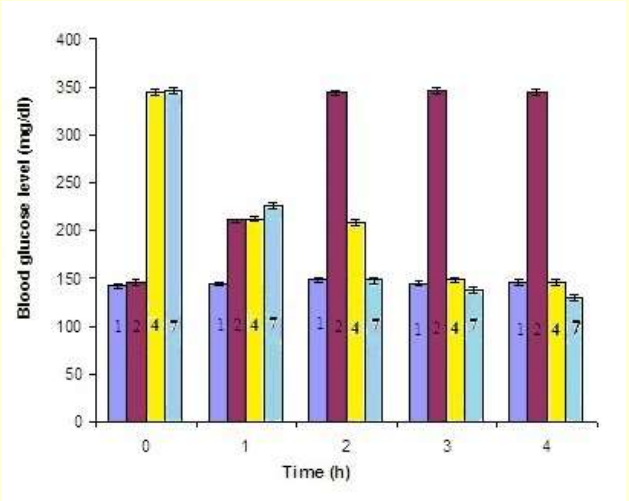
Bio - Chemical Analysis: Blood was collected without any anticoagulant and serum glucose level was found using Ascensia Entrust Glucometer (Bayer HealthCare, USA).

Animal Group Dosing:

(**The dosage of drugs administered orally was decided upon human therapeutic dose extended to animals)**

1. Sham or Control Group I (n=6): were given 1ml saline p.o.
2. Diabetic Control Group II (n=6) were given streptozotocin 35mg/kg body weight of rat /i.p injection.
3. Diabetic standard control Group III (n=6) were given GLB (0.25mg/Kg) pure drug in aqueous solution p.o through oral cannula.
4. Diabetic standard control Group IV (n=6) were given MH (50 mg/Kg) pure drug in aqueous solution p.o through oral cannula.
5. Diabetic standard control Group V (n=6) were given GLB (0.25mg/Kg) and MH (50 mg/ Kg) pure drug in aqueous solution p.o through oral cannula.
6. Diabetic treated control Group VI (n=6) were given GIV formulation optimized batch containing GLB reduced to equivalent of drug dosage of 0.25mg/Kg body wt. of rat /p.o with 1ml of 1% Sodium CMC through oral cannula.
7. Diabetic treated control Group VII (n=6) were given M II formulation optimized batch containing MH reduced to equivalent of drug dosage of 50mg /Kg body wt. of rat /p.o with 1ml of 1% Sodium CMC through oral cannula.
8. Diabetic treated control Group VIII (n=6) were given combination of GIV and M II formulation optimized batch containing both GLB and MH reduced to equivalent of drug dosage of GLB (0.25mg/Kg) and MH (50mg /Kg) body wt. of rat /p.o with 1ml of 1% Sodium CMC through oral cannula.

Influence of *in vivo* BGL lowering in animal study



- Based on ageing studies, SDs of optimized batch (GIV) were selected for *in vivo* BGL lowering studies in streptozotocin induced Diabetic Wistar rats .
- The **hypoglycemia** produced in diabetic treated (SDs) **control group VI** was significantly higher ($P < 0.01$) than the diabetic standard (GLB) control group III.
- The BGL in diabetic treated control group VI in 4 h were almost the same with sham group I as compared with diabetic control group II and diabetic standard control group III.

- Similarly based on ageing studies, optimized (MII) formulation were selected for *in vivo* BGL lowering studies in streptozotocin induced diabetic Albino Wistar rats.
- The hypoglycemia produced in diabetic treated (optimized formulation) **control group VII** was significantly higher ($P < 0.01$) than the diabetic standard (MH) control group IV.
- The BGL in diabetic treated control group VII in 4 h were almost the same with Control group I as compared with diabetic control group II and diabetic standard control group.

HPLC Chromatographic conditions

- The chromatographic system consisted of a HPLC equipment i.e. Shimadzu, SPD-20A UV visible Detector and Shimadzu LC-20 AT solvent delivery system. The equipment was controlled by a Personal computer workstation for data collection and processing. DS-200 software (USA) was used for instrument control. Compounds were separated on a 250 X 4.6 mm i.d, 5 µm particle, Phenomenex Luna C-18 (2) column under reverse phase partition chromatographic conditions.
- The mobile phase was an acetonitrile: potassium dihydrogen phosphate buffer pH=4.0 (55:45 % v/v) adjusted to pH=3.0 ± 0.1 with ortho-phosphoric acid (5%) at flow rate 1mL min-1 and at pressure 140-150 kg/cm2 (Chaturvedi and Sharma 2008, Porta et al 2008). The run time was 10 min. Before analysis both mobile phase and sample solution were degassed and filtered through 0.2 µm cellulose nitrate filter paper. The analytes were monitored at 230 nm. Both drugs compound were identified by comparison of retention times obtained from sample and standard solutions. The work was performed in an air- conditioned room maintained at 25°C ± 2 °C.
- **Validation of HPLC Method for *in vivo* studies** for linearity range, accuracy (recovery) and precision (repeatability) of drugs in plasma samples as per **ICH Q2 (R1) Harmonized Tripartite Guidelines.**

Standard solutions and Plasma standard curve

- Blank plasma was prepared from heparinized whole-blood samples collected from healthy wistar rat and stored at −20 ºC (Li et al 2012 and Wanjari et al 2008). Stock solutions (2 mg mL−1) of Glibenclamide were prepared in mobile phase. After thawing, stock solution of glibenclamide was added to plasma to yield final concentrations ranging from 50, 100, 150, 200, 250, 300 µg mL-1 working standards and internal standard Metformin (50 µg mL-1) was added to each of these samples and stored at +4 ºC . Stock solutions (2 mg mL−1) of Metformin hydrochloride were prepared in mobile phase. Then 200, 400, 600, 800, 1000 µg mL-1 working standards were prepared in plasma from the stock solution and internal standard Glibenclamide (50 µg mL-1) was added to each of these samples and stored at +4 ºC (Ranetti et al 2009).

Pharmacokinetic Study:

- Blood samples (0.2ml) were taken at 0.5h, 1h, 2h, 4h, 6h and 8h interval into heparinized eppendorf tubes from tip of Wistar rat tail after fine cut using fresh blade. After each sampling, loss of blood volume was supplemented with an equal volume of saline containing 100U/mL heparin (**Li et al 2012 and Wanjari et al 2008**). Above blood sample were centrifuged at 10,000rpm on cooling centrifuge (Remi, India) for 5min and then plasma (0.1mL) was withdrawn kept frozen at -20°C in deep freezer until analyzed.

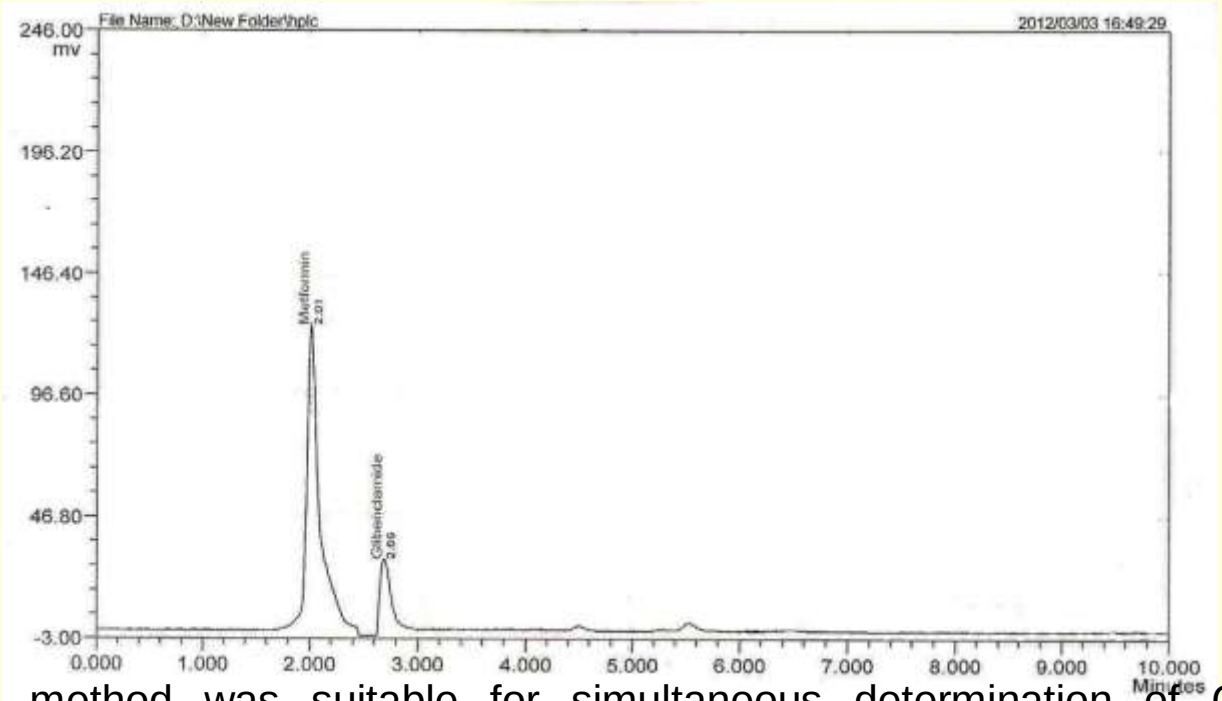
Extraction of MH and GLB from plasma

- The plasma (0.1mL) was vortex-mixed with 0.2ml acetonitrile for 30s to deproteinate, and then centrifuged at 10,000rpm for 5min (**Li et al 2012 and Wanjari et al 2008**). The supernatant 50µl was collected using micro-pipette and was again diluted with 50µl of 1:1 ratio of acetonitrile and mobile phase, vortexed for 30s and then centrifuged at 10,000 rpm for 5 min. A 20 µl aliquot was injected into the HPLC system using Rheodyne 7725 I universal loop injector.

Determination of pharmacokinetic parameters

- Individual and Simultaneous *in vivo* pharmacokinetic estimation of pure and optimized multiparticulates formulation of glibenclamide and metformin hydrochloride in Wistar rats was done using RP-HPLC system (**Chaturvedi and Sharma 2008; Porta et al 2008**) with adaptation. Pharmacokinetic parameters of metformin hydrochloride and glibenclamide were determined by non-compartmental methods. Estimation of pharmacokinetic parameters was obtained from the plasma concentration vs. time data.

RP- HPLC Assay performance of drugs in rat plasma



•The proposed method was suitable for simultaneous determination of Glibenclamide and metformin quantification in plasma samples. It showed specificity, since Glibenclamide and Metformin were well resolved and with no interfering peaks from endogenous components were observed.

•The chromatographic method for simultaneous analysis of glibenclamide and metformin hydrochloride in rat plasma was performed on simple, rapid and precise RP-HPLC. The Metformin hydrochloride and Glibenclamide were eluted with retention times of 2.01 min and 2.62 min, respectively.

•A good separation of metformin was achieved with the retention time of 2.01 min without the interference of any endogenous comp in plasma. Each sample was injected five times and same retention times were observed in all the cases. In addition, the total run time for each injection per sample was only 5 min.

Pharmacokinetic parameters for individual dosage form:

- Metformin HCl and Glibenclamide levels in rat plasma were determined by HPLC according to Sharma et al (2008) and Porta et al (2008) with adaptations.
- The pharmacokinetic parameters, namely the extent of absorption (AUC0-t), maximum plasma concentration (Cmax) and time to reach maximum concentration (Tmax) calculated from the individual plasma-drug concentration data using non-compartmental methods.
- Pharmacokinetic parameters reveals Statistically significant differences ($p < 0.05$) observed among the Tmax values of MII and PM and Cmax values for MII and PM resp.
- The area under the drug plasma concentration-time curve (AUC0-t) for metformin drug in MII and PM in µghmL-1, which were statically different with each other and clearly showed the **peroral bioavailability** of metformin-loaded gastro retentive multiparticulates, and glibenclamide drug in GIV multiparticulates and PG were estimated respectively.

Pharmacokinetic Parameters	Group III PG	Group IV PM	Group VI GIV	Group VII MII
Tmax(h)	2±0.3	2±0.2	2±0.4	2±0.5
Cmax(µg mL^{-1})	78.84±1.4	73.2±1.3	20.2±0.8	31.28±0.5
AUC_{0-t}(µg hmL^{-1})	431.71±1.0	462.83±1.2	147.49±1.7	170.33±1.8
$AUMC_{0-t}$(µg h^2mL^{-1})	1761.42±1.2	1900.24±1.3	630.35±1.5	684.20±1.6
t1/2(h)	5.53±0.51	11.77±0.53	78.62±0.53	3.81±0.54
Kel (h^{-1})	0.1253±0.01	0.0588±0.05	0.0088±0.03	0.1818±0.06
MRT_{0-t}(h)	4.080±0.15	4.105±0.16	4.273±0.14	4.017±0.12

- MRT0-t value for MII and PM as well as GIV and PG was estimated to be hours.
- The **relative bioavailability** of gastro retentive multiparticulate (MII) with pure drug was found to be 18.36% and for gastro retentive multiparticulate (GIV) with pure drug was found to be 31.58%.

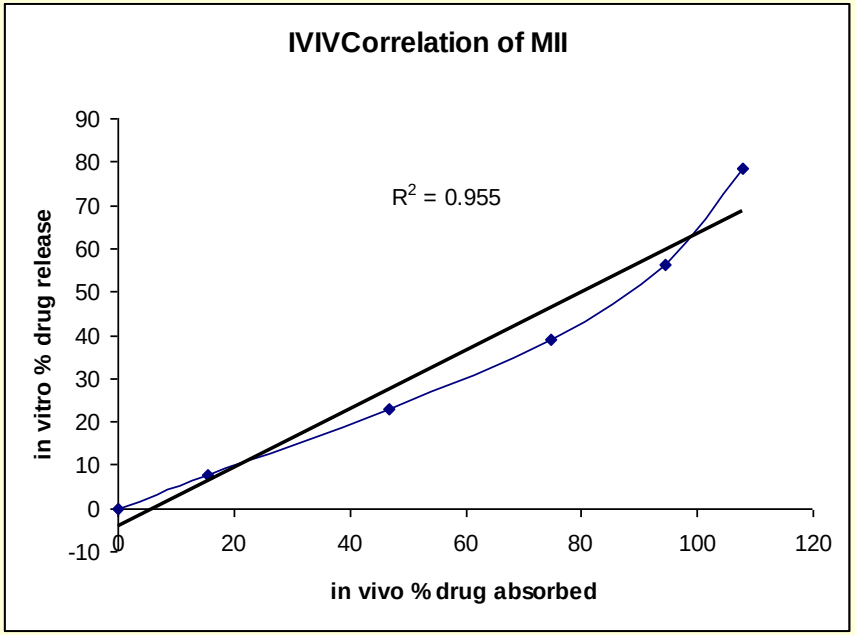
Pharmacokinetic parameters for combination dosage form

- Pharmacokinetic parameters reveals that Statistically significant differences ($p < 0.05$) observed among Tmax values of MII and GIV multiparticulates in combination which were 1±0.8 and 6±0.1h respectively and for the pure metformin and glibenclamide in combination were 2±0.6 and 2±0.4h in the same order.
- The Cmax values for MII and GIV multiparticulates in combination were 11.45±0.8 and 48.53±1.3 µgmL-1 and for pure metformin and glibenclamide in combination were 95.35±0.9 and 56.05±1.2 µgmL-1 respectively.
- The **peroral bioavailability**, area under the drug plasma concentration-time curve (AUC0-t) for MII and GIV multiparticulates in combination were estimated to be 430.65±1.1 and 320.78±1.4 µghmL-1, whereas for pure metformin and glibenclamide in combination were 402.47±1.8 and 294.95±1.5 µghmL-1 respectively.
- MRT0-t values for MII and GIV multiparticulates in combination were estimated to be 3.02±0.11 and 3.97±0.13 h respectively, whereas for pure metformin and glibenclamide in combination was 3.71±0.15 and 3.83±0.17 h respectively.

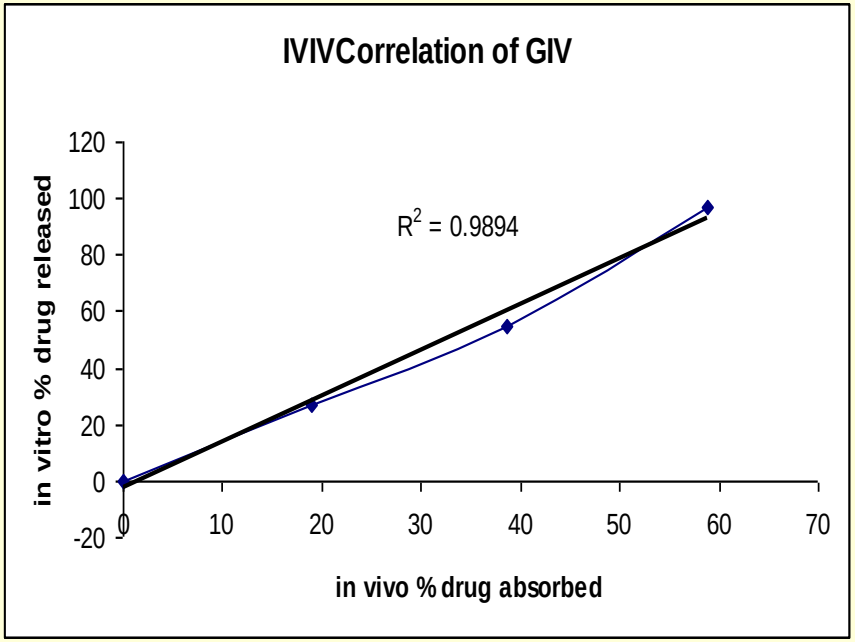
Pharmacokinetic	Group V		Group VIII	
Parameters	PG	PM	GIV	MII
Tmax(h)	2±0.4	2±0.6	6±0.1	1±0.8
Cmax(µg mL^{-1})	56.05±1.2	95.34±0.9	48.53±1.3	11.45±0.8
AUC_{0-t}(µg hmL^{-1})	294.95±1.5	402.47±1.8	320.78±1.4	430.65±1.1
$AUMC_{0-t}$(µg h^2mL^{-1})	1128.76±1.2	1491.61±1.3	1274.89±1.5	1302.18±1.9
t1/2(h)	4.75±0.53	3.37±0.54	5.56±0.51	3.35±0.53
Kel(h^{-1})	0.1458±0.02	0.2056±0.05	0.1245±0.03	0.2071±0.04
MRT_{0-t}(h)	3.83±0.17	3.71±0.15	3.97±0.13	3.02±0.11

- **The relative bioavailability** of MII multiparticulates with PM drug was found to be 45.99%; whereas for GIV multiparticulates with PG drug was found to be 34.16%.
- The **relative bioavailability** of drug in MII and GIV multiparticulates **in combination** with that of pure drugs in combination was found to be 134.35%.
- This shows that MII and GIV multiparticulates in combination have much better relative bioavailability as compared to MII and GIV multiparticulates administered individually to the Wistar rats.

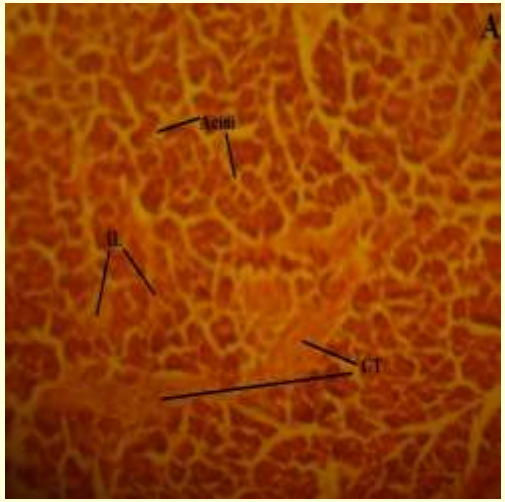
***In vitro in vivo* correlation (IVIVC)** methods shows that level A correlation has given the best correlation coefficient compared to level B and level C. This type of correlation represents a point to point relationship between *in vitro* % dissolution and *in vivo* % drug absorbed (input rate). Level A correlation is considered to be the most informative of all levels of correlation. The present study is able to achieve the required level A correlation.



In vivo Pharmacokinetic data	*In vitro* dissolution rate parameters	MII	GIV
MRT_{0-t} (h)		4.017±0.12	4.273±0.14
	MDT_{0-t} (h)	5.57±0.01	2.73±0.01
C_{max} (μgmL^{-1})		31.28±0.5	20.2±0.8
t_{max}(h)		2±0.5	2±0.4
AUC_{0-t} ($\mu g\ hmL^{-1}$)		170.33±1.8	147.49±1.7
	$T_{50\%}$(h)	5.82±0.01	2.01±0.01

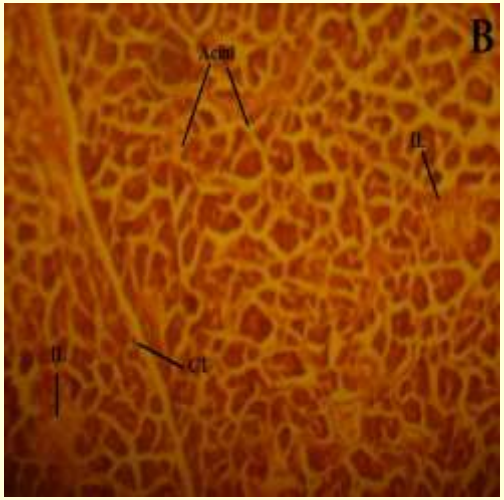


Photomicrographs showing Histopathological observations

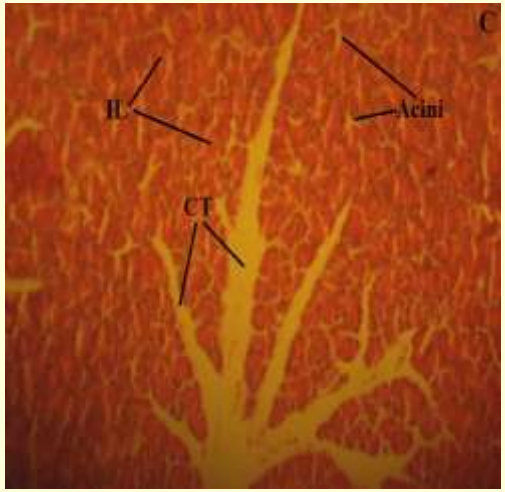


Slide A: Islet of Langerhans from normal rat pancreas

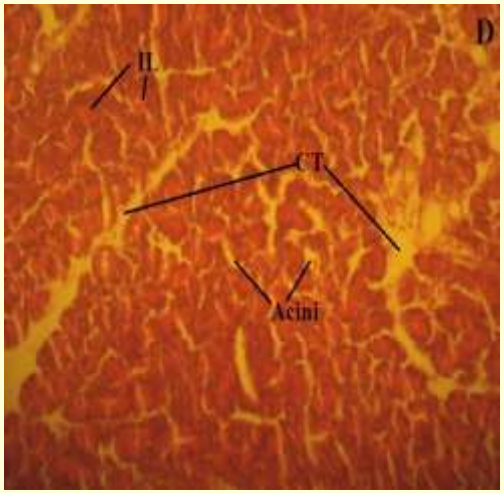
- Photomicrographs of pancreas in slide A showed **normal acini, and normal cellular population in the islets of Langerhans (IL) in** pancreas of vehicle-treated rats. The histological appearance of the pancreatic islet cells of the control was normal.
- In slide B, **extensive damage** to the islets of Langerhans and reduced dimensions of islets.
- Microscopic examination of the pancreatic sections of the untreated **STZ induced** diabetic group revealed a **breakdown of micro-anatomical features including degenerative and necrotic changes**, **and shrunken** in the pancreatic islets of Langerhans, **decreased islet cellular density** though ductal and connective tissues (CT) appeared normal.
- On Comparison of Slide A and B, the **reduction in the number of pancreatic islets and β-cells** was found in the diabetic rats. As it is evident from slide B, the islets are **irregularly shaped and atrophic**. Most cells of the islets are **small and dark with scanty cytoplasm**. **Severe vacuolation and degranulation** are present in the β-cells of a number of islets.
- In slide C and D **restoration of normal cellular population** size of islets **with hyperplasia** by standard pure drug MH and GLB 50 mg/kg b.w and 0.25mg/kg b.w respectively.



Slide B: Islet of Langerhans from STZ induced diabetic rat pancreas.

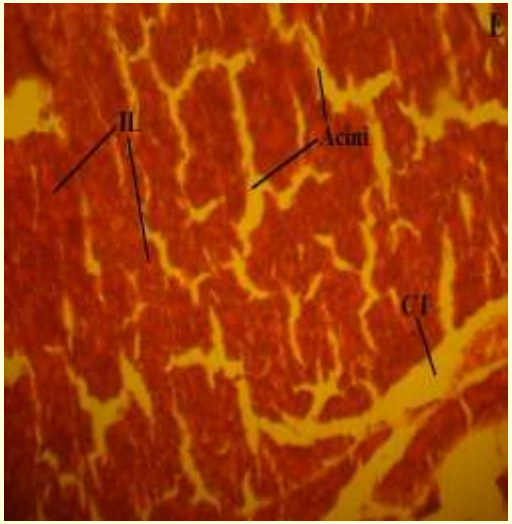


Slide C: Islets of Langerhans from standard MH treated diabetic rat pancreas

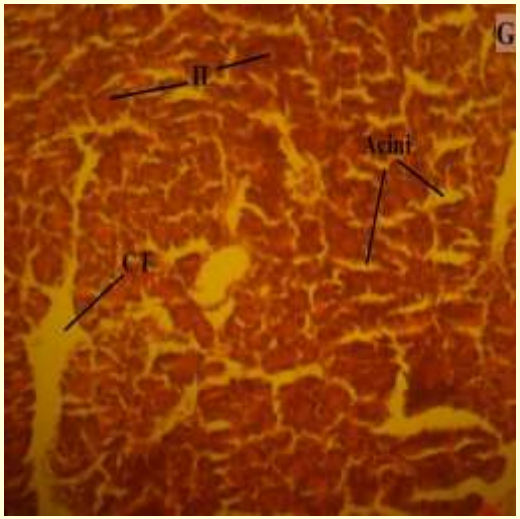


Slide D: Islets of Langerhans from standard GLB treated diabetic rat pancreas

Where, IL= Islets of Langerhans and CT= Connective Tissues.

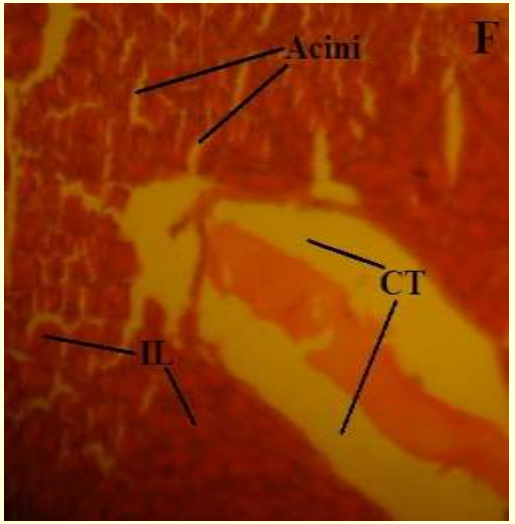


Slide E: Islets of Langerhans from Optimized MII treated diabetic rat pancreas

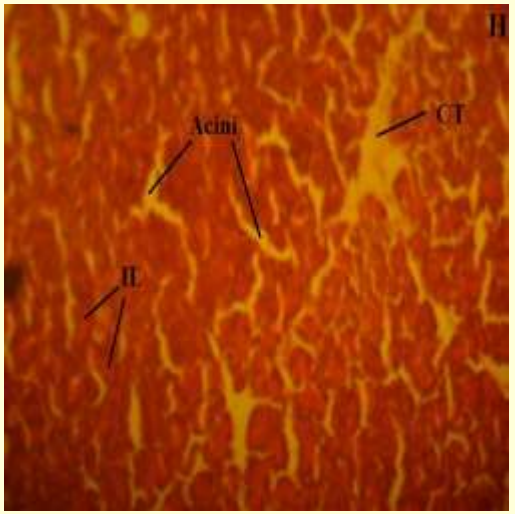


Slide G: Islets of Langerhans from Standard MH-GLB combination formulation treated diabetic rat pancreas

- The morphology of the pancreas of **Standard and formulation treated** diabetic rats as shown in **Slide E and F revealed remarkable improvement in the islet of Langerhans.** There was an increase in the islet cellular density, with an increase in granulation, but vacuolation was reduced or absent in many islets.
- **Slide G and H** were also shown the **partial restoration of normal cellular population** and enlarged size of β-cells **with hyperplasia** by **treatment with combination of standard drugs and optimized drug formulation.**
- Histopathology of pancreas in control animals showed normal pancreatic parenchyma and islet cells. In diabetic control, pancreas sections showed moderate hyperplasia of islet cells, severe congestion in pancreatic parenchyma, and mild infiltration of inflammatory cells.
- In diabetic animals, treated with GLB and MH, pancreas show **mild hyperplasia** of islet cells and congestion of pancreatic parenchyma. In normal animals pancreas section showed normal pancreatic parenchyma structure.
- However, compared to the untreated diabetic rats, histopathological examination of the GIV formulation and MII and GIV combination treated diabetic rats revealed an increase in the number of pancreatic islets and the number of β-cells in slide F and in slide H.
- In conclusion to our histopathological investigation suggests the possibility of the islets regeneration upon formulation treatment without any side effect and without toxicity.



Slide F: Islets of Langerhans from Optimized GIV treated diabetic rat pancreas.



Slide H: Islets of Langerhans from Optimized MII-GIV combination formulation treated diabetic rat pancreas.

STATISTICAL ANALYSIS

- The experimental data of formulations were carried out by Student's t-test and one way analysis of variance (ANOVA) at a significance level of probability ($p < 0.05$) using SPSS 12.0 software (IBM).
- Numerical values in tables & figures were expressed as mean ±standard deviation. Means were assumed to be statistically significant when $P<0.05$.
- Further the Pharmacokinetic data of the formulations results were compared with standard drugs by post test such as Tukey multiple comparision test (in SPSS).

CONCLUSION

•The study showed the successful application of hot melt granulation technique.

• Metformin hydrochloride and Glibenclamide are simultaneously used in type II Diabetes and Gelucire 39/01, 43/01 and Gelucire 50/13 may be considered an appropriate carrier for designing gastro retentive multi-particulates by dispersing above two drugs in melted Gelucire based on HLBs.

• Solid dispersion system of Glibenclamide using Gelucire 50/13 and PEG 200, 400, 4000, 6000 were prepared at different ratios using hot melt. The combination effect of ethylcellulose, methylcellulose and microcrystalline cellulose with Gelucire 39/01 and 43/01 were noted for sustained release of Metformin, floatability and consistency for optimized formulation.

• Glibenclamide formulations didn't follow First -order release kinetics. When the data were plotted according to a zero -order equation and Higuchi's model, the prepared batches of formulations were found to show a fair linearity, with regression values near to one. To confirm the diffusion mechanism further the data were fitted into Korsmeyer- Peppas equation and from the n values it was evident that the drug release mechanism followed non- fickian case II diffusion controlled mechanism (n values ranges from 0.80 to 0.9221) and the k value indicated mid type of interaction. Release of a drug from hydrophobic mini matrices generally involved both pore diffusion and matrix erosion.

• Metformin formulations didn't follow First -order release kinetics. When the data were plotted according to a zero -order equation, the prepared batches of formulations were found to show a fair linearity, with regression values near to one. To confirm the diffusion mechanism, further the data were fitted into Korsmeyer- Peppas equation and from the n values it was evident that the drug release mechanism followed anomalous diffusion controlled mechanism (n values ranges from 0.63 to 0.70).

•Gelucire 50/13 was used successfully to enhance the solubility of poorly soluble drug Glibenclamide by showing promising results as fast release gastro retentive solid dispersions
•Gelucire 39/01 and 43/01 was used successfully to retard the release the highly soluble drug Metformin hydrochloride as sustained release gastro retentive multiparticulates.
•Prepared formulation fulfill the criteria for Gastro retention as well as Drug delivery system for both poorly soluble and highly soluble drug.
• Evaluated for percent drug entrapment, in vitro floating ability, and in vitro drug release data analysis. Prepared formulations followed zero order kinetics and drug release mechanism was anomalous diffusion controlled. Physical Characterization follows SEM, HSPM, DSC, PXRD analysis, FTIR analysis studies revealed upon comparison of peak graphs that no physical interaction was found between them.
•The X- ray analysis and DSC were conducted to test state of GLB and found that drug was in amorphous state and was uniformly distributed through out the SDs matrices and not showed any melting endotherm of GBM, whereas The DSC and X- ray analysis were also conducted to test crystallinity of metformin and found significant decease in crystallinity.
•Animal Studies comprise successful completion of *in vivo* BGL lowering studies and the *in-vivo* pharmacokinetic studies using RP-HPLC and Histopathology of Pancreas.
•Pharmacokinetic results are in accordance to Histopathology of pancrease results with no toxic effects.
•Level A correlation was obtained for in vitro in vivo correlation.
•The developed formulations showed better bioavailability and no toxicity as confirmed by animal pharmacokinetic and histopathology studies.
•Thus, it was concluded that matrices of Gelucire served as an effective carrier for both highly water-soluble drugs and less water soluble drug based on their HLBs for the controlled delivery.

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My sincere condolence to my Co Supervisor Prof A.K. Wahi Sir……..

THANK YOU

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