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Original Article

BI-LAYER TABLETS OF SITAGLIPTIN PHOSPHATE & METFORMIN HYDROCHLORIDE - PREPARATION AND EVALUATION

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ABSTRACT

Bi-layer tablets with sitagliptin phosphate as a Immediate Release (IR) layer and metformin HCl as a Sustained Release (SR) layer were developed for hypoglycemic control. The IR formulations were formulated using *Aegle marmelos* gum (natural antidiabetic agent and disintegrant) by direct compression method and was found to have good pre- and post-compression properties. Disintegeration time for SP3 (2.5 mg of gum) was found to be 128 seconds. Among the IR formulations, SP3 drug release was found to be 99.31% within 10 minutes, aligning with the marketed product Januvia. For sustained release layer optimization, 3^2 full factorial design was used to study the effects of sustained release polymers X1 - calcium starch (matrix forming polymer) and X2 - HPMC K100 on drug release of metformin at 1, 6, and 12 hours. Metformin SR layer was evaluated for weight variation, hardness, friability and dissolution. MF7 was selected as optimized formulation by factorial design and it achieved 98.52% release over 12 hours similar to marketed product Glycomet. Sustained drug release followed first-order kinetics (R^2 = 0.9832) with non-Fickian diffusion (n = 0.765). Further bi-layer tablets were punched and characterized. Bi-layer tablet having MF7 and SP3 as sustained and immediate release layer was considered as optimized and evaluated. Immediate IR layer from the bi-layer tablet had shown 96.15% of sitagliptin release in 10 min and Metformin SR layer have sustained drug release 97.16% up to 12 similar to Innovator bilayer tablet (Istamet). Compatibility studies (FT-IR) showed no component interactions. Bi-layer tablet formulation had shown promising release profiles, offering effective glycemic control and improved patient compliance.

KEYWORDS: Bi-layer tablet, immediate release, sustained release, factorial design

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1. Introduction

Formulation scientists are focused on developing drug delivery systems that enhance efficacy, safety, and patient compliance by reducing dosing frequency. Solid oral dosage forms are recommended method for many medications because of their ease of use, patient compliance and ease of production [1]. But problem with single-layer tablets is they often require frequent dosing and can result in unpredictable plasma drug levels for drugs with shorter half-life [2]. Many diseases require immediate drug release to address acute conditions, followed by sustained drug levels for long-term management. To address this need, the multilayered tablet concept has been developed [3]. Two layers make up bi-layer tablets, which enable combination therapy to be administered in a single dose form [4]. These layers frequently include distinct active chemicals or formulations [5].

Bi-layer tablet is the fixed dose combination therapy containing Immediate Release layer (IR) and Sustained Release layer (SR) where Immediate Release layer acts as an initial dose and Sustained Release layer acts as a maintenance dose [6]. Diabetes is a widespread and rapidly growing condition, affecting approximately 6.6% of the global population. Managing Type 2 Diabetes Mellitus (T2DM) often involves a combination of medications to control blood glucose effectively. Metformin HCl and sitagliptin are commonly used for this purpose [7] as metformin decreases the amount of glucose absorbed from food and the glucose made by the liver and also increases the body's response to insulin, whereas sitagliptin helps to control blood sugar levels by increasing substances (incretins) in the body that make the pancreas release more insulin. Metformin hydrochloride has low permeability, requires frequent dosing (250–500 mg, three to four times daily) due to its low bioavailability (50–60%) and a biological half-life of 6.2 h.

A sustained release formulation of metformin is needed to reduce dosing frequency. Sitagliptin has a long half-life of 12.4 h and is given (100 mg) once daily. Sitagliptin IR formulation is required for rapid absorption for immediate DPP-4 inhibition for effective glycemic control.

There is a growing global interest in the use of natural excipients in pharmaceutical formulations, driven by their eco-friendly and cost-effective properties. Natural disintegrants such as *Aegle marmelos* gum, locust bean gum, *Mangifera indica* gum, *Hibiscus rosa-sinensis* mucilage, and dehydrated banana powder are increasingly being utilized to achieve immediate release in tablet formulations. Additionally, starch, a biodegradable polymer, is widely employed as a filler, disintegrant, and dry binder in various pharmaceutical applications. Calcium starch, a novel modified starch, has shown great promise in controlling the release rate of drugs like diclofenac, gliclazide, and diltiazem HCl from matrix tablets.

In view of the above advancements, the current research focuses on developing a bi-layer tablet combining sitagliptin IR with *Aegle marmelos* gum as the disintegrant for immediate release, and metformin HCl SR, utilizing calcium starch, a laboratory-made polymer, along with HPMC K100M as the matrix-forming polymers. This formulation leverages natural excipients to optimize drug release profiles while maintaining sustainability and patient safety.

2. Materials and Methods

2.1. Materials

Metformin HCl and Sitagliptin Phosphate was purchased from Livmore Life sciences Pvt. Ltd, Gujarat., India. *Aegle Marmelos* Gum as a complimentary sample was provided by Zeus Hygia life sciences Pvt. Ltd, Hyderabad. Calcium starch was prepared in laboratory. All ingredients were of Pharmacopoeial grade.

2.2. Methods

2.2.1. Preparation of calcium starch polymer

Calcium starch was made by taking 5 g of potato starch dispersed in 50 mL of water to form a slurry. 3 g of sodium hydroxide dissolved in 30 mL of water was added to the slurry and mixed for 30 minutes, forming a viscous gel. The gel was added to 300 mL of 20% (w/v) calcium chloride solution and agitated at 1000 rpm for 1 hour. The precipitate was collected via vacuum filtration, washed with water, dried at 80°C, and sieved through a 100-mesh to obtain the powdered polymer [8].

2.2.2. Determination of λ_{max} of sitagliptin phosphate and metformin HCl

The λ_{max} of sitagliptin and metformin in 0.1 M HCl and pH 6.8 buffer was determined using UV-Visible spectrophotometer. A primary stock solution (1000 µg/ml) was prepared by dissolving 10 mg of the drug in 10 ml of the respective medium. From this, 1 ml was diluted to 10 ml to form a secondary stock solution (100 µg/ml), and 1 ml of this was further diluted to 10 ml to obtain a working solution (10 µg/ml). The working solutions were scanned within the 200–400 nm range to determine the λ_{max}.

2.2.3. Overlay spectra of sitagliptin phosphate and metformin HCl in 0.1 M HCl and pH 6.8 buffer

This study was performed to check whether the absorption peaks of sitagliptin and metformin were distinct or not.

A 10 µg/mL solution of sitagliptin (IR) and metformin (SR) each in 0.1 M HCl was made. Then 5 ml of each solution was taken and mixed, then scanned in a UV spectrometer at wavelengths ranging from 200 to 400 nm.

A 10 µg/mL solution of sitagliptin and metformin each was made in pH 6.8 buffer. Then 5 ml of each solution was taken and mixed, and the mixture was then scanned at wavelengths ranging from 200 to 400 nm using UV-spectrophotometer [9,10].

2.2.4. Construction of standard graphs of sitagliptin and metformin HCl in 0.1M HCl

Sitagliptin and metformin HCl stock solutions were made by dissolving 10 mg of each drug in 10 ml of methanol. Dilutions were done with 0.1 M HCl (2-10 µg/ml concentrations) and absorbance was recorded at 267 nm and 232 nm, respectively, using UV -Visible spectrophotometer.

2.2.5. Construction of calibration curve of sitagliptin and metformin HCl in 6.8 pH phosphate buffer

Sitagliptin and metformin HCl stock solutions were made by dissolving 10 mg of each drug in 10 ml of methanol. Dilutions were done with 6.8 pH phosphate buffer (2-10 µg/ml concentrations) and absorbance was recorded at 266 nm and 233 nm, respectively, using UV-Visible spectrophotometer.

2.2.6. Compatibility studies

The FT-IR analysis were carried out to study physico-chemical interactions between the drugs and excipients. Thus, the FT-IR spectra of pure drugs of sitagliptin phosphate and metformin HCl with physical mixture were recorded using FT-IR Spectrophotometer [11].

2.2.7. Formulation and evaluation of bi-layer tablets

To develop bi-layer tablets, fast-release and sustained-release layers were first prepared individually to evaluate their dissolution profiles and compared with marketed formulations accordingly. This helped in selecting the optimal combination of excipients for each layer. The optimized formulations were then compressed into bi-layer tablets, and the in vitro drug dissolution profiles were compared to the marketed product.

2.2.8. Formulation of sitagliptin phosphate IR layer

Sitagliptin IR tablets were prepared using the direct compression method. Sitagliptin (50 mg) was blended with *Aegle marmelos* gum (disintegrant) and diluent and lubricated with antiadherents as shown in Table 1. The blend was compressed into tablets using a 6 mm round punch, with a hardness of 4-5 kg/cm^2 to ensure mechanical strength and accurate dosing [12-14].

Table 1. Formulation of sitagliptin IR layer

Ingredients (mg)	SP1	SP2	SP3	SP4	SP5	SP6	SP7
Sitagliptin phosphate	50	50	50	50	50	50	50
Aegle marmelos gum	1	1.5	2.5	5	7.5	10	15
Micro crystalline cellulose	64	63.5	62.5	60	57.5	55	50
Magnesium stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Talc	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Total weight ofs tablet (mg)	120	120	120	120	120	120	120

Table 2. Coded Value layout of metformin SR using 32 factorial design

Independent Variables	Codes	Values		
		Lower value (-1)	Middle value (0)	Higher value (+1)
Calcium starch Polymer	X1	40	50	60
HPMC K100	X2	225	250	275

Table 3. Formulation of metformin (SR) layer by 32 factorial design

Ingredients(mg)	MF1	MF2	MF3	MF4	MF5	MF6	MF7	MF8	MF9
Metformin HCl	500	500	500	500	500	500	500	500	500
Calcium starch	40	40	40	50	50	50	60	60	60
HPMC K 100	225	250	275	225	250	275	225	250	275
Micro crystalline cellulose	70	45	20	60	35	10	50	25	-
PVP K30	40	40	40	40	40	40	40	40	40
Magnesium stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Talc	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Total weight of tablet(mg)	880	880	880	880	880	880	880	880	880

2.2.9. Formulation and optimization of SR layer of metformin HCl

For optimization of SR layer 32 full factorial design were taken using Stat-ease software. Independent variables studied included X1 – concentration of calcium starch polymer and X2 – concentration of HPMC K 100 and their impact on the dependent variables which were the %drug release at 1 hr (Y1), 6 hr (Y2) and 12 hr (Y3) [15-17].

The independent variables with their levels given by 32 full factorial design are mentioned in Table 2. The resultant responses were assessed by using statistical model by incorporating in polynomial equation generated from experimental design using Design Expert 7.1.6 software, State-Ease Inc., given below:

$$Y = b0 + b1X1 + b2X2 + b11X12 + b22X22 + b12X1X2$$

Where, Y is a response (dependent variable), b0 is an intercept, b1 to b2 are regression coefficients and X1 and X2 are independent formulation variables. Total 9 experiments were carried out as given in Table 3.

The Metformin SR layer was prepared using the wet granulation method, by mixing metformin with calcium starch, HPMC K 100 polymers, MCC and PVPK30 in different ratios. To maintain constant weight in tablet MCC quantities was adjusted in the formulation. A damp mass was prepared by adding water which was passed through a sieve to create uniform granules. Dry granulation was done at 40-45°C for about 40 minutes to remove moisture and the granules were compressed into tablets using 12 mm round punches, achieving a hardness of 7-9 kg/cm^2.

2.2.10. Characterization of IR and SR layers

Prior to compression, powder blends of metformin HCl and sitagliptin were characterized for angle of repose, bulk density, tapped density, compressibility index, and Hausner's ratio.

2.2.11. *In-vitro* disintegration test for Sitagliptin IR

Disintegration test was conducted for Sitagliptin IR tablets to determine time (in seconds) needed for a tablet to disintegrate [18].

2.2.12. *In-vitro* dissolution studies

Dissolution study was done using a USP dissolution apparatus with 900 mL of 0.1 M HCl for 1 hour for sitagliptin and for metformin for first hour in 0.1 M HCl, followed by pH 6.8 buffer for the remaining hours, at 37±0.5°C and 50 rpm. Samples were taken and analyzed using a UV-spectrophotometer at 267 nm for sitagliptin and 232 nm for metformin.

2.2.13. Preparation of bi-layer tablets

The optimized formulations obtained basing on the dissolution profile were prepared by pouring SR granules into the die cavity and compressed to form a SR layer and above it the IR powder mixture of sitagliptin was placed and subjected to final compression, to get a bi-layer tablet. No layer separation was noticed. Weight of the tablet was 1000 mg for all formulations [19].

2.2.14. Evaluation of bi-layer tablets

The powder blends of metformin HCl and sitagliptin were characterized through post compression parameters, including average weight of tablet, hardness, friability and thickness. These parameters were measured to assess to ensure tablet uniformity and strength of the tablet [20,21].

2.2.15. Dissolution study of bi-layered tablets

The dissolution test was performed using a USP type-II apparatus with 900 mL of 0.1 M HCl for the first hour and pH 6.8 buffer for 12 hrs with temperature at 37 ± 0.5°C at 50 rpm. 10 mL samples of sitagliptin were withdrawn at time intervals of 5, 10, 15, 20, 30, 40, 50, 60 minutes during the first hour and for the subsequent hours at intervals of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hrs and were analyzed using a UV spectrophotometer at wavelengths of 267 nm and 232 nm [22].

2.2.16. Kinetic studies of sustained release layer

In vitro data was fitted into various kinetic models like zero-order kinetics, first-order kinetics, Higuchi model and Korsmeyer-Peppas model which collectively help to predict the drug's behavior in the body [23].

3. Results and Discussion

3.1. Wavelength λ_{max} of sitagliptin phosphate and metformin HCl

The λ_{max} was observed at a wavelength of 267.02 nm in 0.1 M HCl and 266.99 nm in pH 6.8 buffer for sitagliptin and 232 nm at 0.1 M HCl and 233 nm at pH 6.8 phosphate buffer for metformin, as given in Fig. 1-4, by using UV-visible spectrophotometer.

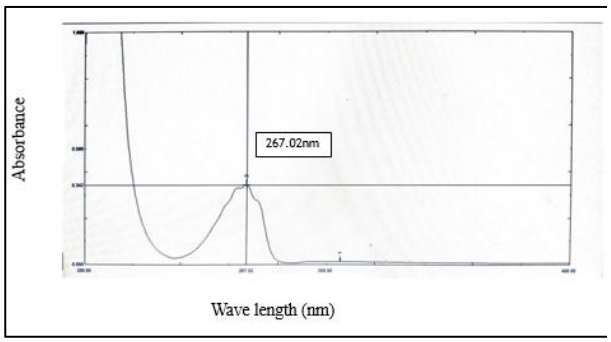

Fig. 1. Sitagliptin phosphate λ_{max} at 267 nm in 0.1 M HCl

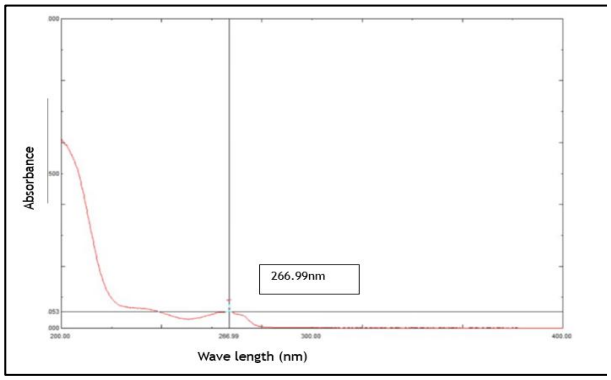

Fig. 2. Sitagliptin Phosphate λ_{max} at 266 nm in pH 6.8 buffer

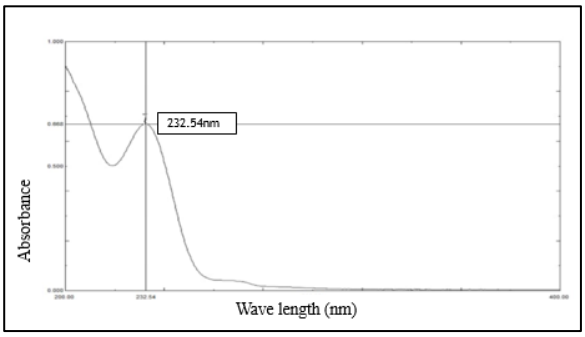

Fig. 3. Metformin HCl λ_{max} at 232 nm in 0.1 M HCl

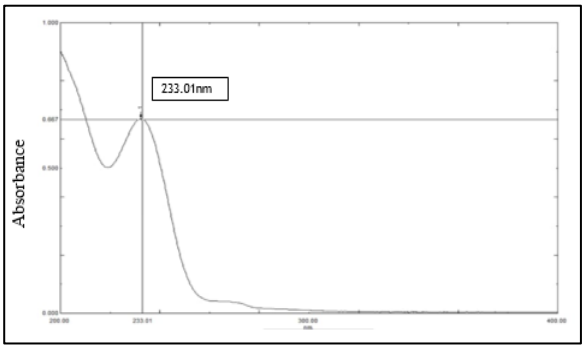

Fig. 4. Metformin HCl λ_{max} at 233 nm in pH 6.8 buffer

3.2. Overlay spectra

This study showed that the absorption peaks of sitagliptin and metformin were distinct, indicating that their release profiles did not overlap or interfere with each other. This confirmed that both drugs can be effectively released from the bi-layer tablet without any negative interaction, ensuring their individual efficacy shown in Fig. 5-6.

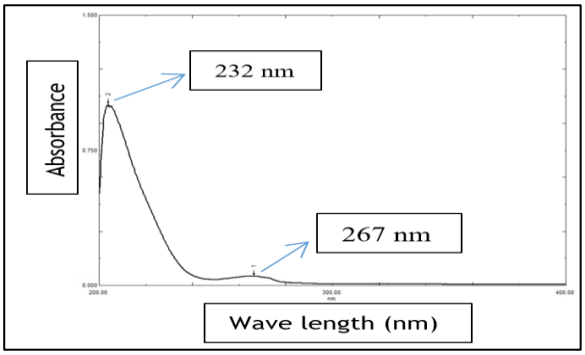

Fig. 5. Overlay spectra of sitagliptin (267 nm) and metformin (232 nm) in 0.1 M HCl

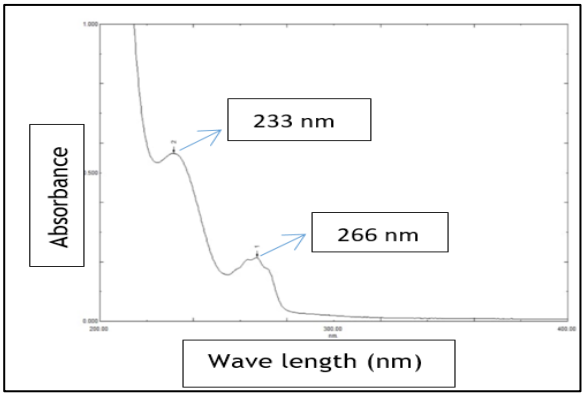

Fig. 6. Overlay spectra of sitagliptin (266 nm) and metformin (233 nm) in pH 6.8 buffer

3.5. Calibration curve

The calibration curve in 0.1 M HCl and pH 6.8 phosphate buffer demonstrated linearity within the 2-10 µg/ml range according to Beer-Lambert's law for both sitagliptin and metformin HCl.

3.4. Interaction study

To determine compatibility, FT-IR experiments were conducted on both the pure medication (sitagliptin phosphate) and its physical mixture. In the sitaglipitin API, there was a slight change in the absorbance from 3059.20 to 2956.97, which was caused by C=C stretching. The amino group was mostly retained, as indicated by the absorbance with a minor shift from 3416.05 to 3421.83. The carbonyl group, C=O-N, showed an absorbance shift from 1670.41 to 1610, which was caused by interactions with hydrogen bonding or other molecules. Lastly, the amine group band showed a minor shift from 1276 to 1273.06 which was caused by a slight change in bonding. Hence, interactions were not observed between sitagliptin pure drug and its physical mixture shown in Fig. 7 and 8.

The FT-IR of metformin HCl and its physical mixture was performed to determine the compatibility. The FT-IR spectrum of pure drug showed an absorbance shift from 1626 to 1629 in physical mixture due to C=N stretching where minor interactions were noticed, -NH_2 group showed a minor shift from 3371 to 3371.68 and C-N group showed the absorbance at 1273 to 1273.06 in physical mixture. Thus, FT-IR spectrum has shown no interactions between the pure drug of metformin HCl and physical mixture given in Fig. 9 and 10.

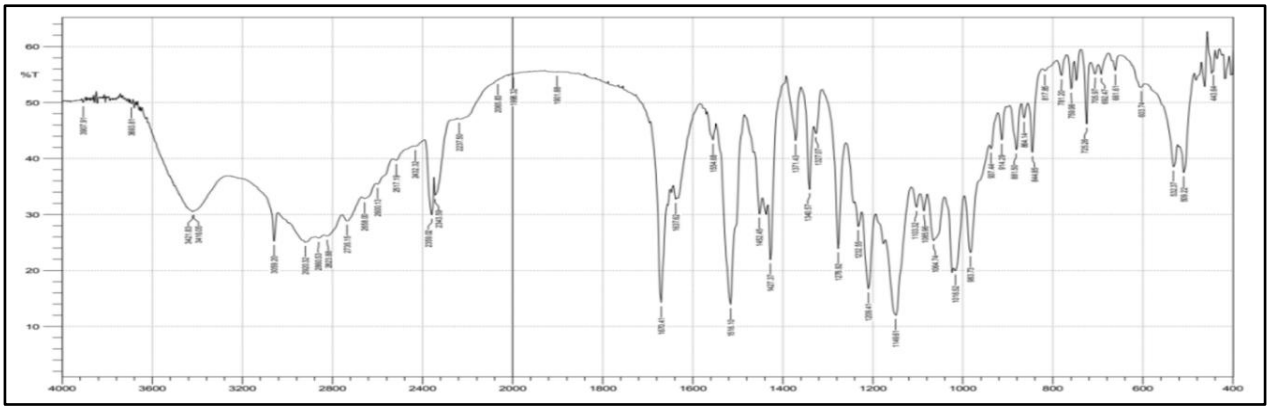

Fig. 7. FT-IR of sitagliptin (API)

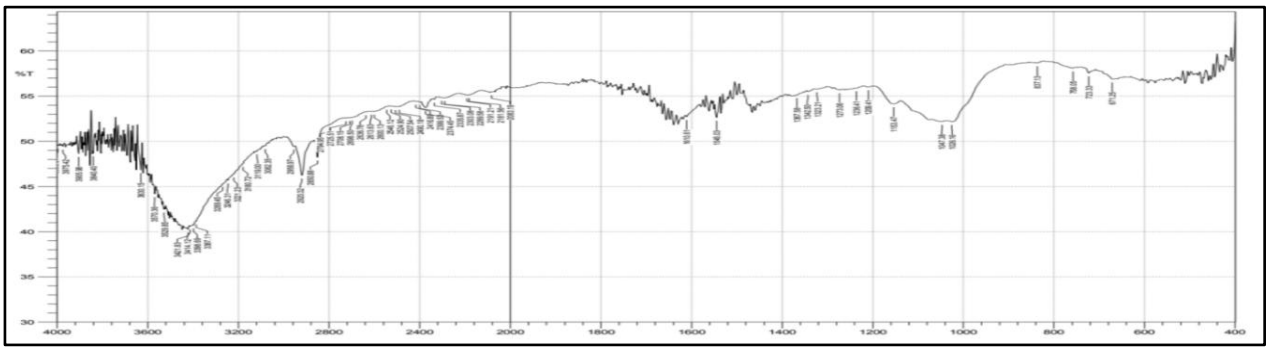

Fig. 8. FT-IR of sitagliptin physical mixture

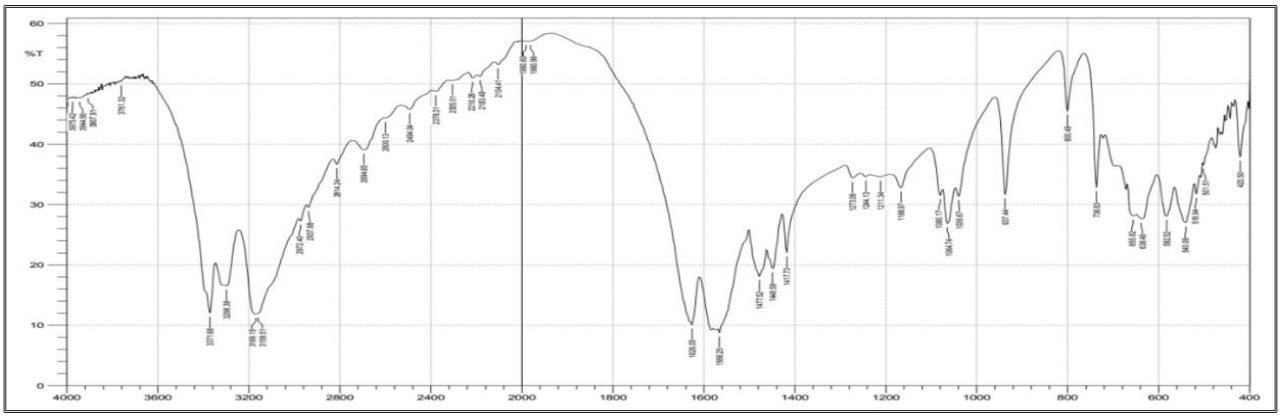

Fig. 9. FT-IR of metformin HCl (API)

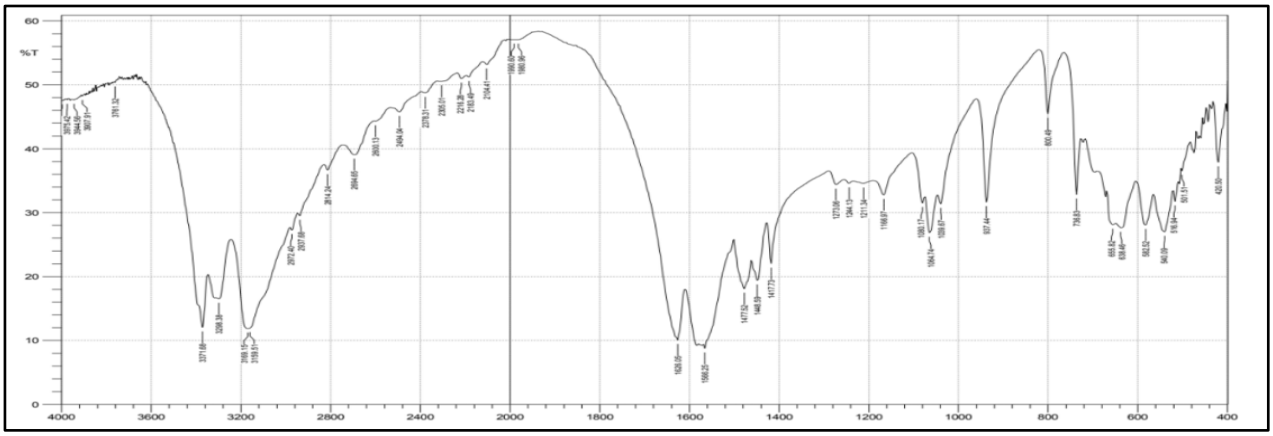

Fig. 10. FT-IR of metformin HCl and physical mixture

3.5. Pre & post compression parameters

Sitagliptin IR layer: the angle of repose for all seven sitagliptin IR formulations (SP_1-SP_7) ranged from 22.69 ± 0.09 to 25.06 ± 0.08, indicating good flow properties. Bulk density values are between 0.73 ± 0.07 g/cm^3 and 0.88 ± 0.08 g/cm^3, while tapped density ranged from 0.96 ± 0.05 g/cm^3 to 0.99 ± 0.11 g/cm^3. The Carr's index varied from 9.27 ± 0.12 to 23.95 ± 0.06, reflecting poor to excellent flow properties across the formulations. Hausner's ratio was observed between 1.10 and 1.25, further confirming fair to excellent flow characteristics as given in Table 4.

Metformin SR layer: The angle of repose for all nine formulations (MF1–MF9) ranged from 20.06 ± 0.08 to 25.83 ± 0.12, indicating good flow properties for the sustained-release layer. Bulk density values were between 0.735 ± 0.12 g/cm^3 and 0.843 ± 0.09 g/cm^3, while tapped density ranged from 0.836 ± 0.08 g/cm^3 to 0.965 ± 0.11 g/cm^3. The Carr's index ranged from 9.35 ± 0.07 to 20.82 ± 0.06, indicating fair to excellent flow properties, and Hausner's ratio was between 1.10 ± 0.05 and 1.26 ± 0.09, suggesting fair to good flow properties given in Table 5.

Post–compression hardness, friability, and percent drug content results of Sitagliptin IR and Metformin SR results were within the acceptable range as given in Table 6 and 7. Among all IR formulations of sitagliptin SP3 formulation was selected given the fastest disintegration time (DT) of 128 seconds in a bi-layer formulation given in Table 6.

Table 4. Precompression parameters of sitagliptin phosphate IR

Formulation	Angle of Repose (θ)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index	Hausner's ratio
SP1	24.45 ± 0.11	0.78 ± 0.09	0.98 ± 0.09	20.04 ± 0.08	1.25 ± 0.04
SP2	25.06 ± 0.08	0.83 ± 0.07	0.96 ± 0.05	13.54 ± 0.01	1.15 ± 0.07
SP3	23.37 ± 0.16	0.86 ± 0.12	0.96 ± 0.08	10.41 ± 0.02	1.11 ± 0.06
SP4	24.47 ± 0.12	0.81 ± 0.09	0.99 ± 0.11	18.18 ± 0.07	1.22 ± 0.05
SP5	23.68 ± 0.09	0.88 ± 0.08	0.97 ± 0.09	9.27 ± 0.12	1.10 ± 0.07
SP6	22.69 ± 0.09	0.73 ± 0.14	0.96 ± 0.11	23.95 ± 0.06	1.31 ± 0.02
SP7	21.73 ± 0.12	0.78 ± 0.09	0.96 ± 0.13	18.75 ± 0.13	1.23 ± 0.07

Table 5. Pre-compression parameters of metformin HCl SR

Formulation	Angle of Repose (θ)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index	Hausner's ratio
MF1	21.80 ± 0.26	0.781 ± 0.09	0.899 ± 0.09	13.12 ± 0.08	1.15 ± 0.04
MF2	20.06 ± 0.12	0.754 ± 0.07	0.878 ± 0.05	14.12 ± 0.01	1.16 ± 0.07
MF3	20.33 ± 0.52	0.735 ± 0.12	0.836 ± 0.08	12.08 ± 0.02	1.13 ± 0.09
MF4	22.97 ± 0.37	0.843 ± 0.09	0.9307 ± 0.11	9.35 ± 0.07	1.10 ± 0.05
MF5	22.68 ± 0.01	0.782 ± 0.08	0.891 ± 0.09	12.23 ± 0.12	1.13 ± 0.07
MF6	21.62 ± 0.09	0.764 ± 0.14	0.965 ± 0.11	20.82 ± 0.06	1.26 ± 0.09
MF7	25.83 ± 0.12	0.767 ± 0.09	0.883 ± 0.13	13.13 ± 0.13	1.15 ± 0.07
MF8	23.16 ± 0.11	0.792 ± 0.15	0.902 ± 0.08	12.19 ± 0.04	1.13 ± 0.05
MF9	21.75 ± 0.13	0.781 ± 0.12	0.894 ± 0.91	12.63 ± 0.17	1.14 ± 0.06

Table 6. Post compression parameters of sitagliptin IR

IR Formulation	Average Weight (mg) (n=20)	Hardness (kg/cm^2) (n=3)	Thickness (mm) (n=3)	Friability % (n=20)	Drug Content %	DT (sec)
SP1	120.01 ± 0.64	3.3 ± 0.41	2.0 ± 0.06	0.48	98.23 ± 0.001	170
SP2	119.02 ± 0.92	4.0 ± 0.23	2.0 ± 0.04	0.38	97.65 ± 0.012	190
SP3	120.02 ± 0.02	4.0 ± 0.03	2.0 ± 0.11	0.56	99.12 ± 0.001	128
SP4	121.07 ± 0.83	3.8 ± 0.51	2.0 ± 0.08	0.66	95.43 ± 0.023	158
SP5	119.12 ± 0.92	4.2 ± 0.36	2.0 ± 0.08	0.43	92.53 ± 0.002	226
SP6	118.05 ± 0.61	3.7 ± 0.01	2.0 ± 0.12	0.59	94.95 ± 0.052	339
SP7	120.06 ± 0.53	4.0 ± 0.02	2.0 ± 0.13	0.51	93.16 ± 0.023	362

Table 7. Post compression parameters of Metformin SR

SR Formulation	Average Weight (mg) (n=20)	Hardness (kg/cm^2) (n=3)	Thickness (mm) (n=3)	Friability % (n=20)	Drug Content %
MF1	880.04 ± 0.12	8.2 ± 0.1	6.0 ± 0.02	0.52	95.23 ± 0.04
MF2	879.09 ± 0.54	8.2 ± 0.6	6.0 ± 0.01	0.47	96.23 ± 0.06
MF3	881.05 ± 0.61	8.5 ± 0.2	6.0 ± 0.02	0.36	99.46 ± 0.02
MF4	874.02 ± 0.34	8.0 ± 0.9	6.0 ± 0.04	0.54	99.62 ± 0.04
MF5	869.05 ± 0.48	8.4 ± 0.3	6.0 ± 0.08	0.52	99.54 ± 0.01
MF6	876.08 ± 0.01	8.3 ± 0.2	6.0 ± 0.03	0.38	93.26 ± 0.03
MF7	880.01 ± 0.43	8.5 ± 0.4	6.0 ± 0.02	0.46	99.92 ± 0.09
MF8	872.03 ± 0.17	8.2 ± 0.1	6.0 ± 0.01	0.54	98.84 ± 0.02
MF9	875.04 ± 0.24	8.0 ± 0.4	6.0 ± 0.09	0.42	97.32 ± 0.05

3.6. *In vitro* dissolution study of IR and SR

The in vitro dissolution studies of all seven batches of IR formulations showed different drug release pattern. The Fig. 11 shows drug release pattern for batches SP1 to SP7 and compared with marketed formulation (Januvia 50 mg).

SP3 formulation has shown 99.31% release within 10 min, drug release is similar to the marketed formulation (99.38%). Among the drug release profiles for all optimization batches, MF7 formulation has shown 98.52% release within 12 hrs, drug release is similar to the marketed formulation Glycomet (95.88%) given in Fig. 12.

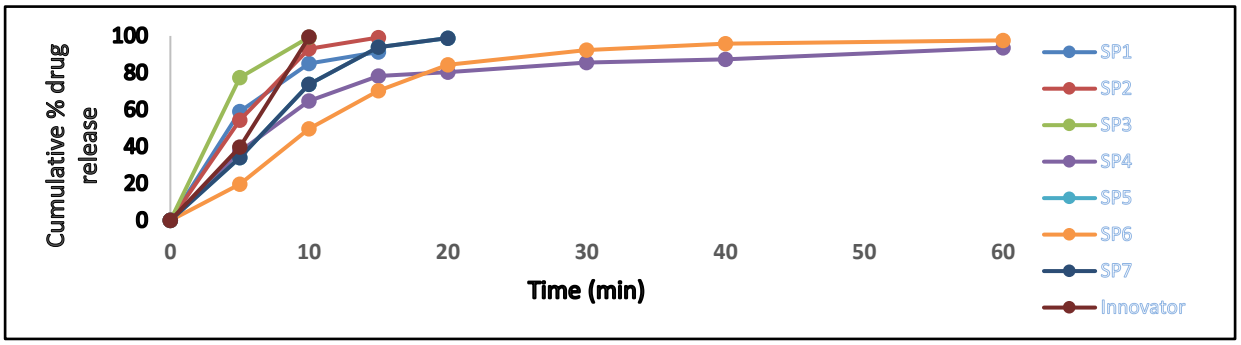

Fig. 11. Drug Release of IR (SP1-SP7) and Innovator

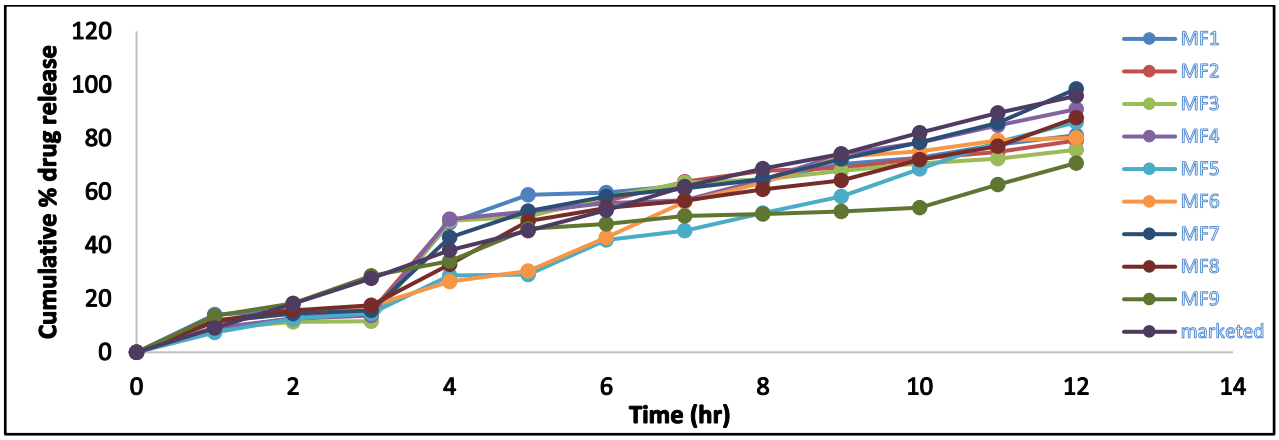

Fig. 12. Drug Release of SR (MF1-MF9) and Innovator

3.7. 32 Full Factorial Design for metformin HCl

For sustained release formulations 32 factorial design was selected for further study of optimization. All polynomial equations were found to be statistically significant ($P < 0.005$), as determined by ANOVA. The equations include coefficients for the intercept, first-order main effects, interaction terms, and higher-order effects. The sign and magnitude of the main effects indicate the relative influence of each factor on the response. Calculating the F value for % drug release at 1, 6, and 12 hours was recommended by the findings of the ANOVA. Plan of experiments and their coded levels are given in Table 8.

Table 8. Plan of experiments by coded level of variables given by 32 factorial design along with responses of SR

Formulation	Variables and Codes		Factor:1	Factor:2	Response 1	Response 2	Response3
			A. Calcium starch (mg)	B. HPMC K100 (mg)	% Dissolution at 1 h (Y1)	% Dissolution at 6 h (Y2)	% Dissolution at 12 h (Y3)
	X1	X2					
MF1	-1	-1	40	225	14.13	59.74	81.19
MF2	-1	0	40	250	8.63	56.68	79.24
MF3	-1	1	40	275	8.83	58.11	75.73
MF4	0	-1	50	225	9.27	55.81	90.93
MF5	0	0	50	250	7.43	42.03	86.03
MF6	0	1	50	275	11.27	43.01	80.24
MF7	1	-1	60	225	11.53	58.27	98.51
MF8	1	0	60	250	11.9	53.96	87.75
MF9	1	1	60	275	13.64	48.01	70.79

3.8. Results of the ANOVA

3.8.1. Dependent variable: dissolution at 1 hr

A mathematical model was evaluated based on statistical terminologies and a polynomial equation was generated given below in Equation 1.

Eq. 1. $Y = 387.7 - 4.07841X1 - 2.23623X2 + 0.023172X12 + 0.003716X22 + 0.007410X1X2$

The findings (Equation 1) of the analysis of variance (ANOVA) indicate that the linear model is significant having p value less than 0.005 and F value 14.07. A high regression R2 value of 0.9095 indicates good fit of data which were given in Table 9 and Fig. 13. From the equation it was observed that there was a decreasing trend of dissolution profile at 1st hour with increase in the concentration of calcium starch and concentration of HPMCK100M as shown in 3D surface plot of drug release which was given in Fig. 14.

Table 9. Results of the ANOVA for dependent variables dissolution at 1 hr

Source	Sum of squares	df	Mean square	F-Value	p-Value	
Model	66.98	5	13.40	14.07	0.0016	Significant
A-Conc of calcium starch polymer	5.01	1	5.01	5.26	0.0556	
B- Conc. of HPMCK100M	0.2360	1	0.2360	0.2479	0.6338	
AB	13.73	1	13.73	14.42	0.0067	
A2	14.83	1	14.83	15.58	0.0056	
B2	14.89	1	14.89	15.65	0.0055	
Residual	6.66	7	0.952			
Lackof Fit	6.66	3	2.22			
Pure error	0.000	4	0.000			
Cor Total	73.65	12				

Fit Statistics			
Std. Dev.	0.9757	**R^2**	0.9095
Mean	9.72	**Adjusted R^2**	0.8449
C.V.%	10.04	**Predicted R^2**	0.1150
		Adeq. Precision	10.8728

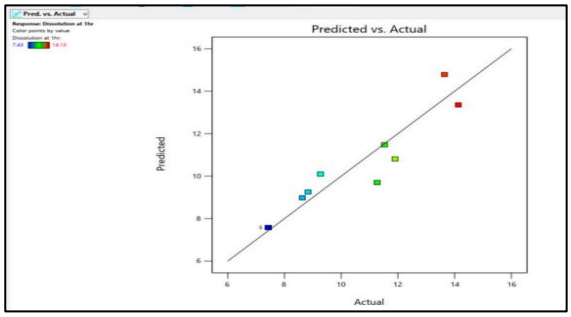

Fig. 13. Predicted vs actual value of dissolution at 1 hr

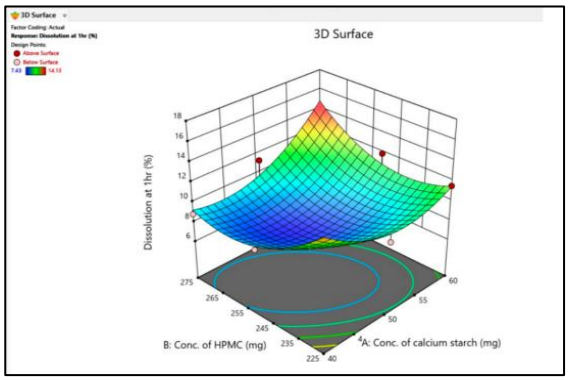

Fig. 14. 3D surface showing the effect of
A. Concentration of calcium starch (mg)
B. Concentration of HPMC K100M (mg)

Eq. 2. % Cumulative Drug Release at 6 hr

$$Y = 656.03 - 8.15187X1 - 3.06207X2 + 0.100712X12 + 0.006658X22 - 0.008630X1X2$$

The findings (Equation 2) of the analysis of variance (ANOVA) indicate that model is significant having p value less than 0.005 and F value 18.15 as given in Table 10. A high regression R2 value of 0.9284 indicates good fit of data that were given in Table 10 and Fig. 15. From the equation it was observed that as the concentration of calcium starch and concentration of HPMC increased, dissolution profile at 6th hour is decreased as shown in 3D surface plot of drug release at 6 hr which is given in Fig. 16.

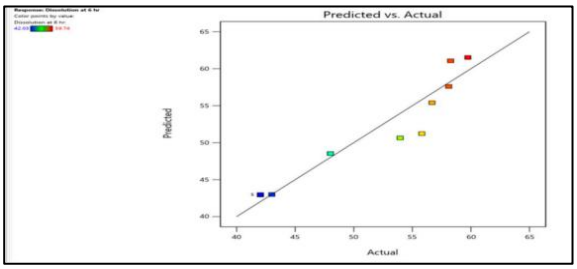

Fig. 15. Predicted vs actual value of dissolution at 6 hr

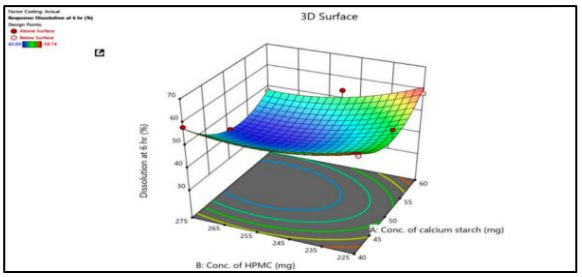

Fig.16. 3D surface showing the effect of
A. Concentration of calcium starch (mg)
B. Concentration of HPMC K100 (mg)

Table 10. Results of the ANOVA for dependent variables dissolution at 6 hr

Source	Sum of squares	df	Mean square	F-Value	p-Value	
Model	641.05	5	128.21	18.15	0.0007	Significant
A-Conc of calcium starch polymer	34.03	1	34.03	4.82	0.0642	
B- Conc. of HPMCK100M	101.60	1	101.60	14.38	0.0068	
AB	18.62	1	18.62	2.64	0.1485	
A2	280.14	1	280.14	39.65	0.0004	
B2	47.82	1	47.82	6.77	0.0353	
Residual	49.45	7	7.06			
Lack of Fit	49.45	3	16.48			
Pure error	0.0000	4	0.0000			
Cor Total	690.50	12				

Fit Statistics			
Std. Dev.	2.66	**R2**	0.9284
Mean	49.52	**Adjusted R^2**	0.8772
C.V.%	5.37	**Predicted R^2**	0.4202
		Adeq.Precision	10.2847

Eq. 3. % Cumulative Drug Release at 12 hr:

Y = -334.395 + 9.169X1 + 1.7539X2-0.0325X12 - 0.001867X22 - 0.022260X1X2

The findings (Equation 3) of the analysis of variance (ANOVA) indicate that model is significant having p value less than 0.005 and F value 0.07 as given in Table 13. A high regression R2 value of 0.9743 indicates good fit of data as is given in Table 11 and Fig. 17, respectively. From the equation it was observed that as the concentration of calcium starch and concentration of HPMC increased, dissolution profile at 12th hour was also increased as shown in 3D surface plot of drug release at 12 hr in Fig. 18.

Table 11. Results of the ANOVA for Dependent variables dissolution at 12 hr

Source	Sum of squares	df	Mean square	F-Value	p-Value	
Model	565.39	5	113.08	52.99	<0.0001	Significant
A-Conc of calcium starch polymer	72.73	1	72.73	34.08	0.0006	
B- Conc. of HPMCK100M	320.76	1	320.76	150.30	<0.0001	
AB	123.88	1	123.88	58.05	0.0001	
A2	29.29	1	29.29	13.73	0.0076	
B2	3.76	1	3.76	1.76	0.2261	
Residual	14.94	7	2.13			
Lack of Fit	14.94	3	4.98			
Pure error	0.0000	4	0.0000			
Cor Total	580.33	12				

Fit Statistics			
Std. Dev.	1.46	**R2**	0.9743
Mean	84.19	**Adjusted R^2**	0.9559
C.V.%	1.74	**Predicted R^2**	0.7468
		Adeq.Precision	25.9489

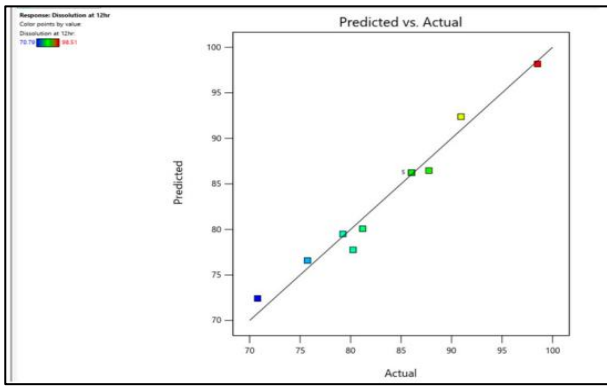

Fig. 17. Predicted vs Actual value of Dissolution at 12 hr

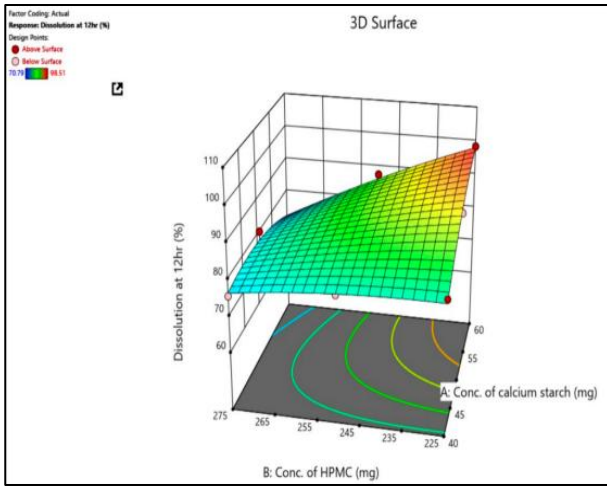

Fig. 18. 3D surface showing the effect of
A. Concentration of calcium starch (mg)
B. Concentration of HPMC (mg)

In all the equations the magnitude of X1 is higher indicating its major impact on the response. That is, concentration of calcium starch plays a crucial role in sustained release of drug at 1, 6 and 12th hr.

3.9. Bi-layer tablet evaluation

The prepared bi-layer tablet has an average weight of 1000.02 mg indicating consistency in tablet weight and the tablet demonstrates good physical characteristics for further studies and use, as given in Table 12.

The final bi-layer tablet released 96.15% of the drug from the immediate release layer in 10 minutes and 97.16% over 12 hours from the sustained release layer and the marketed tablet, on the other hand, released 99.38% from the IR layer and 95.88% over 12 hours from SR layer shown in Fig. 19 and 20.

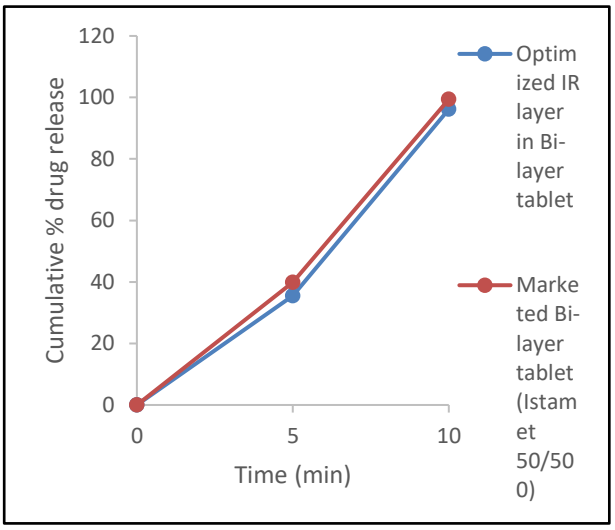

Fig. 19. Drug release graph of optimized IR layer SP3 in a bi-layer tablet and innovator

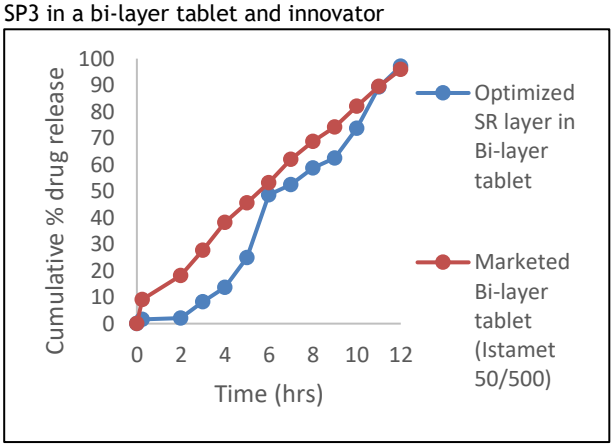

Fig. 20. Drug release graph of optimized SR MF7 layer in a bi-layer tablet and innovator

3.10. Drug Release Kinetics

Results have shown that bi-layered formulation followed first order kinetics (R^2 0.9832). The high R^2 value confirms a good fit, implying release is concentration dependent. The n-value which lied between 0.5 and 1, i.e. 0.765, indicates non-Fickian or anomalous diffusion, involving a combination of diffusion and erosion mechanisms, as given in Table 13.

Table 12. Post compression results of bi-layer tablets

Bi-layer tablet Formulation	Avg weight (n=20) (in mg)	Hardness kg/cm^2 (n=3)	Thickness mm (n=3)	Friability% (n=20)
SP3(IR) & MF7 (SR)	1000.02 ± 0.18	8.6 ± 0.02	7.5 ± 0.047	0.84

Table 13. Release kinetics of SR in a bilayer tablet

Formulation code	Zero Order Kinetics	First Order Kinetics	Higuchi Model	Peppas Model	Hixson Crowell
R^2	0.9684	0.9832	0.9384	0.9363 (n value=0.765)	0.9799

4. Conclusion

Bilayer tablets of metformin as SR layer and sitagliptin as IR layer were successfully formulated. Overlay plot has shown no interference. No interactions between drugs and excipients were detected by the FTIR. All the formulation batches tested for physical parameters and all were found to be within the limits. Bi-layer tablet formulation was prepared by selecting the optimized formulations of SP3 (IR layer) and MF7 (SR layer optimized by 32 factorial design). The final bi-layer tablet was within limits in terms of its post compression properties and exhibited a drug release of 96.15% for the IR layer and 97.16% over 12 hours for the SR layer, similar profile with marketed formulation Istamet. Optimized bi-layer formulation followed first-order kinetics (R^2=0.9832) with non-fickian mechanism (n value = 0.765). Bi-layered tablets of sitagliptin phosphate and metformin HCl were successfully developed which can reduce side-effects, dosage frequency and have good patient compliance over individual marketed products.

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