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Formulation And Evalution Of Bilayer Tablet Of Saxagliptin Solid Dispersion Immediate Release Layer And Dapagliflozin Sustained Release For The Effective Management Of Diabetes Mellitus

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***ABSTRACT**:*
In order to effectively manage the diabetic mellitus type-II hyperglycemic surge, bilayered tablets comprising of Saxagliptin in immediate release layer (IRL) and Dapagliflozin in sustained release layer (SRL) have been designed and fabricated for years. Saxagliptin suffers from reduced aqueous solubility of 0.19 mg/mL which remained a crucial problem for the biological effect as a result of reduced dissolution and bioavailability. Therefore, in this research an effort was done to improve the aqueous solubility of Saxagliptin in a bilayer tablet by forming solid dispersions which will provide prompt release to completely manage the postprandial effectually. In this study, a bilayer tablet of Saxagliptin solid dispersion in IRL and Dapagliflozin in SRL were fabricated by direct compression method, with an intention that the IRL of the formulation will release the Saxagliptin at its earliest to combat the postprandial hyperglycemic level followed by a control of steady state plasma glucose by sustained release Dapagliflozin. The compatibility studies, pre-compression studies, post-compression studies, disintegration studies, dissolution studies, and kinetic release studies were performed. The formulation B3 was found to be highly optimized and demonstrated the highest cumulative drug release where the Dapagliflozin followed the either zero-order or first-order and the Saxagliptin followed anomalous diffusion. Therefore, the designed formulation will offer a better therapeutic regimen and provide patient friendly postprandial hypoglycemic management, where an immediate control and maintenance dose are required.

***Keywords:** Saxagliptin, Dapagliflozin, Bilayer, Solid dispersion, Diabetes mellitus,*

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I. INTRODUCTION:

Insulin is the chief hormone responsible for the maintenance of glucose homeostasis in the human body. It performs by hastening the uptake of glucose molecules into the tissues and concurrently suppresses the glucose production 1 . However, a lack of insulin level leads to several severe chronic metabolic disturbances of carbohydrate, fat and protein, that is often characterized termed as diabetes mellitus (DM) 2 . DM is a classified into two types; Type-I and TypeII, where the former is characterized by high blood sugar as the body does not generate enough insulin that is necessary to convert sugar into energy. In the later phase, cells do not properly use the produced insulin by the body 3 . DM Type-II is the fastest progressing metabolic disorder of this 21st century where 400 million individuals are currently affected 4 . In the due course of time, several anti-diabetic drugs were developed which inhibit diverse enzymatic targets like protein tyrosine phosphatase-1B, dipeptidyl peptidase-4, α-glucosidase, aldose reductase, etc. However, the majority of the drugs on their individual use for the long term management of hyperglycemia seemed inactive 5 . Due to the chronic disease and long term treatment; they usually suffer from low duration of activity, compromised pharmacokinetic profile, and often reduce the quality of life on long usage 6 . The effectiveness of the pharmacotherapy depends on designing drug formulations which provide better postprandial hyperglycemic control as well as day-long duration. The in-vivo secretion of insulin gets stimulated by the glucose, which classically follows a biphasic pattern in the time course 7 . In the first phase secretion, with the increase in the glucose concentration, a brief stimulation occurred which promote insulin secretion. While in the second phase secretion, a steadily rising secondary stimulation is observed 8 . In the case of DM type-II, a disturbance or loss of secretion in the insulin release pattern of the first phase is evidenced. In the second phase secretion, due to the inherent resistance in the cellular level and because of energy-dependent process, a hyperglycemic phase is habitually observed which results in elevated plasma glucose level 9 . In both the cases seen cumulatively, after a heavy meal, hyperglycemia conditions are detected.

along with enhancement of peripheral insulin sensitivity, ultimately leading to decline in fasting, postprandial glucose and glycosolated hemoglobin (HbA1c) levels 13. The drug is having a half-life of 10.4 h and is primarily metabolized extensively by liver to form oxidized and hydroxylated derivates, as well as glucuronic acid conjugates 14. The drug suffers from reduced aqueous solubility of 0.19 mg/mL which remained a crucial problem for the biological effect as a result of reduced dissolution and bioavailability. Therefore, in this research an effort was made to improve the aqueous solubility of Saxagliptin in a bilayer tablet where solid dispersion (SD) of Saxagliptin was skillfully prepared in PEG 6000 containing three different weight ratios; 1:1, 1:2, and 1:5 and suitably fabricated in IRL. The solid dispersion was preferred as the smartest strategy for improving the dissolution properties and subsequently the bioavailability of Saxagliptin by dispersing in PEG 6000 carrier where reduced particle size, surface area enhancement, and transformation in amorphous form improves the solubility 15.

Sodium-glucose transporter (SGLT) 2 inhibitors work by preventing the kidneys from holding on to glucose. Instead, your body gets rid of the glucose through your urine.

Saxagliptin and dapagliflozin combination is used together with proper diet and exercise to treat type 2 diabetes. Saxagliptin helps to control blood sugar levels by making the pancreas gland release more insulin. It also signals the liver to stop producing sugar when there is too much sugar in the blood. Dapagliflozin works in the kidneys to prevent absorption of glucose (blood sugar). This helps lower the blood sugar level. This medicine does not help patients who have insulin-dependent or type 1 diabetes. Type 1 diabetic patients must use insulin injections.

II. MATERIALS AND METHODS:

Chemicals: Dapagliflozin was obtained as a generous gift from Cipla Pharmaceuticals Ltd., Mumbai. Saxagliptin was provided to us as gift sample from Wockhard Pharmaceuticals Ltd., Aurangabad. Leben Laboratories Pvt. Ltd., remained the main supplier for the HPMC polymers of grade K4M, K15M, and K100M. Microcrystalline cellulose, polyethylene glycol 6000, and starch were purchased from Molychem Pharmaceuticals Ltd., Mumbai. Miscellaneous analytical grade chemicals were procured from Qualigens Fine Chemicals Ltd., Mumbai

Preparation of Saxagliptin Solid Dispersions (SDs):

The SD of Saxagliptin in PEG 6000 (Saxagliptin /PEG 6000) containing three different weight ratios; 1:1, 1:2, and 1:5 and here denoted as SD1/1, SD1/2 and SD 1/5, respectively, were prepared by the solvent evaporation method. In this method, to a solution of Saxagliptin in chloroform, an appropriate amount of PEG 6000 was added. The solvent was evaporated under reduced at room temperature by using petridish and the resulting residue dried under vacuum for 3 h. The mixture was stored overnight in a desiccator. The hardened mixture was powdered in a mortar, sieved through a 100-mesh screen, and stored in a screw-cap vial at room temperature until further use 16 .

Characterization of Saxagliptin Solid Dispersions:

Infrared Spectral Analysis:

IR absorption spectrum of pure drug, PEG 6000, physical mixture, and SD formulations were recorded by potassium bromide dispersion technique in the range of 4000 - 400 cm−1. The compounds were scanned at a resolution of 0.15 cm−1 and scan speed was 20 scan/s 17 . Differential Scanning Calorimetric Analysis: The physical state of pure drug, PEG 6000, physical mixture, and SD formulations was characterized by differential scanning calorimeter (DSC) thermogram analysis. The DSC patterns were recorded on a Pyris Diamond TG/DTA Perkin Elmer. Each sample was heated in a platinum crucible along with alpha alumina powder as a reference at a scanning rate of 10 °C/min in an atmosphere of nitrogen (150 mL/min) using the range of 30 - 300 °C. The temperature calibrations were performed periodically using indium as a standard 18 .

Formulation of Bilayer Tablet:

Formulation of Saxagliptin Solid Dispersion Immediate Release Layer (Layer - 1): The immediate release layer was fabricated by direct compression method. Saxagliptin, MCC, croscarmellose sodium, and magnesium stearate were weighed and sifted through a mesh sieve #44. The above weighed ingredients were mixed thoroughly in a mortar except magnesium stearate for 10 min. The magnesium stearate was properly added as a lubricant. The content was compressed by Chamunda® minipress rotary tablet machine using 17 mm concave faced punches. The composition of the immediate release layer is described in Table 1. TABLE 1:

COMPOSITION OF IMMEDIATE RELEASE LAYER

Ingredients G1 G2 G3 Saxagliptin 240 240 240 Cross carmellose sodium 8 12 15 Microcrystalline cellulose 52 48 45 Magnesium stearate 5 5 5 Formulation of Dapagliflozin Sustained Release Layer (Layer - 2):

Dapagliflozin is a very moisture sensitive drug and hence it does not have good flow properties on exposure. To improve the flow properties, the granules were prepared by the wet granulation method. Simultaneously, various batches were prepared in duplicate using the same wet granulation technique. The formulation involved weighing Dapagliflozin HPMC of grades K4M, K15M, and K100M, MCC, and magnesium stearate and sifted through mesh sieve #44. The components were mixed thoroughly in a mortar for 10 min except magnesium stearate. The binder solution was prepared by dissolving starch in purified water (10% starch solution) by gentle warming. The binder solution was added slowly in the mixture and wetted. Subsequently, the wetted mass was forced through mesh sieve #22 to obtain granules. The wet granules were dried in hot air oven for 30 min and sifted through mesh sieve #22 to obtain proper granule size. The magnesium stearate was properly added as a lubricant. Final Compression: Firstly, Dapagliflozin granules were added to die and gently compressed followed by pouring of gliclazide blend. The batches were prepared by following: M7 + G3 = B1; M8 + G3 = B2; and M9 + G3 = B3. Lastly, the final compression was applied to prepare the bilayered tablet using Chamunda® mini press 17 mm concave faced punches.

Evaluation of Prepared Bilayer Tablets:

Compatibility Studies:

The polymers which are to be incorporated into formulation should be compatible with the drug. The compatibility study or interaction study was done using Fourier transformed infrared spectroscopy. In an effort to determine any kind of interaction between drug candidate and the polymer, IR spectra were taken for pure Dapagliflozin and in the combination with polymer. Furthermore, DSC was performed to determine whether any degradation or any incompatibility is identified. Pre-compression Studies: The granules were evaluated suitably for their characteristic parameters such as angle of repose, Hausner's ratio, bulk density, tapped density and Carr's index. The angle of repose was determined by funnel method. Bulk density and tapped density were determined by cylinder method. Carr's index (CI) and Hausner's ratio (HR) was calculated.

Post-compression Studies:

Based on reported standards/procedures, the compressed matrix tablets were characterized for their properties such The prepared granules were evaluated for various pharmacopeia parameters. Later, the granules were compressed using Chamunda® minipress machine of 17 mm size punch to fabricate the tablet layer. The composition of the sustained release layer is mentioned in Table 2. as hardness, friability, thickness, content uniformity and weight variation. The hardness of tablet formulations was determined by using a Monsanto hardness tester. The friability was determined using Roche friability testing apparatus. Likewise, the thickness was determined using Vernier Calipers. The weight variation testing was carried out according to the guidelines mentioned in USP Pharmacopoeia.

Uniformity of Content:

Five tablets Dapagliflozin and gliclazide was powdered in a mortar separately and a quantity equivalent to 500 mg of Dapagliflozin and 80 mg of Saxagliptin was accurately weighed and dissolved in a suitable volume of distilled water / 7.4 pH phosphate buffer and 0.1 N HCl (pH 1.2) respectively. After making suitable dilutions the final solution was analyzed spectrophotometrically at 233 nm and 230 nm, respectively.

Disintegration Test of Immediate Release Layer:

Tablet disintegration was carried by placing one tablet in each tube of the basket and top portion of the each tube was closed with disc and run the apparatus containing (pH 1.2) 0.1 N HCl (gastric fluid) maintained at 37 ± 0.1 °C as the immersion liquid. The assembly was raised and lowered between 30 cycles per minute 19. The time taken for the complete disintegration of the tablet with no palpable mass remaining in the apparatus was measured and recorded. The experiment was carried out in triplicate

TABLE 2: COMPOSITION OF SUSTAINED RELEASE LAYER

Ingredients	M1	M2	M3	M4	M5	M6	M7	M8	M9
Dapagliflozin	500	500	500	500	500	500	500	500	500
HPMC K4M	110	--	--	130	--	--	150	--	--
HPMC K15M	--	110	--	---	130	--	--	150	--
HPMC K100M	--	--	110	--	--	130	--	--	150
MCC	113	113	113	93	93	93	73	73	73
Starch	20	20	20	20	20	20	20	20	20
Mg stearate	7	7	7	7	7	7	7	7	7
Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Dissolution Studies:

The two intact tablets from each batch were taken for dissolution study. The dissolution study was performed on IP-II (Basket) / USP Type-II dissolution test apparatus (Electrolab, TDT 08L, Mumbai, India). The dissolution medium used was 900 mL of 0.1 N HCl for first 2 hr and phosphate buffer (pH 7.4) for the next 10 h at 37 ± 0.1 °C. The paddle speed was kept constant at 50 rpm. Each time, 5 mL of sample was withdrawn at the interval of 5 min for Saxagliptin and 2 mL sample were withdrawn for Dapagliflozin, for first 1 h and thereafter at intervals of 1 h. The withdrawn samples were analyzed spectrophotometrically at 230 nm for Saxagliptin and 233 nm for Dapagliflozin. The same amount of fresh 0.1 N HCl and phosphate buffer pH 7.4 was used to replace the amount withdrawn for respective dissolution media. Percent cumulative release of both drugs from the tablet was calculated 20 .

Kinetic Analysis of Dissolution Data:

In order to determine the mechanism of drug release from the bilayer tablet batches, data obtained from in vitro drug release studies were plotted in various kinetic models like zero-order, first order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas models. The criterion for selecting the most appropriate model was chosen on the basis of the goodness-or fit test. The zero-order kinetic describes the systems in which the drug release rate is independent of its concentration. The first order kinetic describes the systems in which the drug release rate is concentration dependent. Higuchi described the release of drug from an insoluble matrix as the square root of time dependent process. The HixsonCrowell cube root law describes the drug release from systems in which there is a change in the surface area and the diameter of particles present in the tablet. In case of Korsmeyer-Peppas model the drug release from such devices having a constant geometry will be observed till the polymer chains rearrange to an equilibrium state 21 .

III. RESULTS AND DISCUSSION:

Characterization of Saxagliptin Solid Dispersions (SDs): Infrared Spectral Analysis: The FTIR spectrum of Saxagliptin, PEG 6000, physical mixture, and SDs are shown in Fig. 1. The spectrum of Saxagliptin Fig. 1a showed characteristic peaks at 3502, 3274, 3112, 2942, 1708, 1430, 1160 cm-1 , respectively. The spectra of PEG 6000 Fig. 1b displayed no such prominent peaks, however few prominent peaks at 3350, 2773, 1403 cm-1 , etc. were seen. The peaks of the physical mixture Fig. 1c were predominantly seen at 3502, 3278, 3189, and 2942 cm-1 which confirms the presence of drug in unchanged form. The spectrum of SD of Saxagliptin Fig. 1d exhibited analogous results. The observed prominent peaks are 3278, 3112, 1708, 1430, 1160 cm-1 , respectively, which are quite similar to the spectrum of Saxagliptin. This concluded that there were no such drug-polymer interactions in the SDs.

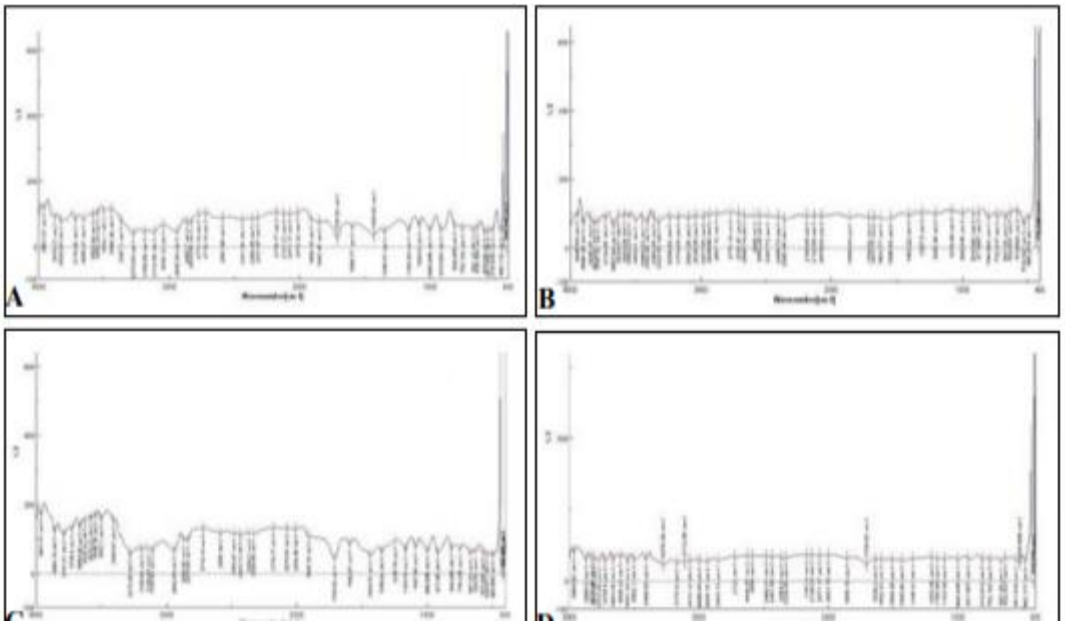

FIG. 1: FT-IR SPECTRA: (A) Saxagliptin (B) PEG 6000; (C) PHYSICAL MIXTURE; AND (D) SOLID DISPERSION OF Saxagliptin

Differential Scanning Calorimeter Analysis:

The DSC thermograms of Saxagliptin, PEG 6000, physical mixture, and SD are shown in Fig. 2. The pure drug showed a very pointed endothermic peak at 174.17 °C with peak onset at 169.26 °C, which corresponds to its melting point Fig. 2a. The sharp endothermic peak of Saxagliptin confirms crystallinity in the structure. In contrast, PEG 6000 exhibits no such endothermic peak over the entire scanning range of 30 - 300 °C, suggesting its polymeric nature Fig. 2b. The physical mixture highlighted the appearance of same drug peak with no difference, suggesting that there is no crossreactivity between the drug and the polymer Fig. 2c. However, no characteristic peak of Saxagliptin was found in SDs over the tested temperature Fig. 2d, which clearly indicated that the drug is in amorphous state or high-energy state after the fabrication. Since amorphous state is considered as a state of high disorder, the solid particles present remain in highly dissolved state in SDs, which further confirms the ability of SDs in maintaining the drug in the dissolved state and thus improves the solubility of the drug

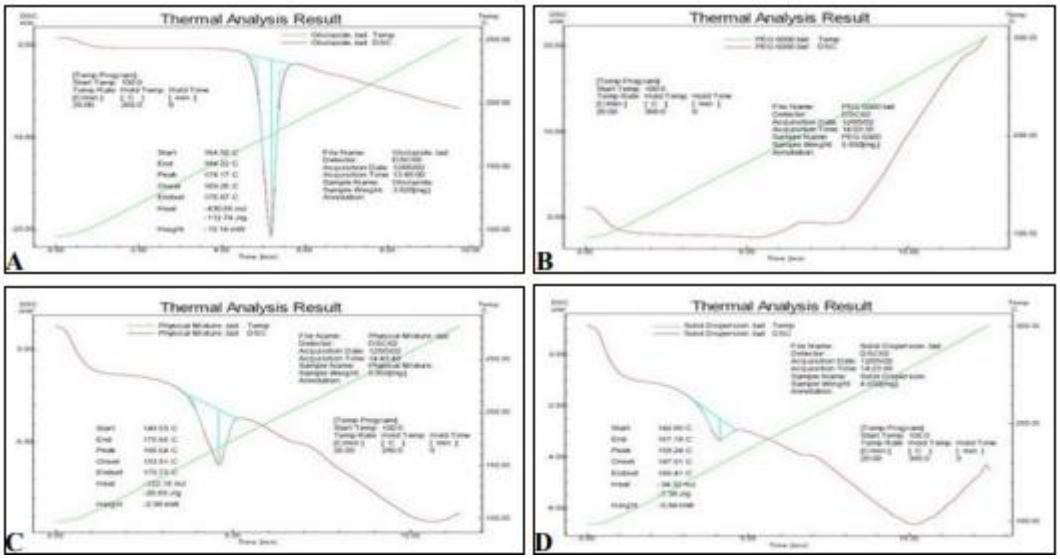

FIG. 2: DSC THERMOGRAM: (A) Saxagliptin (B) PEG 6000; (C) PHYSICAL MIXTURE; AND (D) SOLID DISPERSION OF Saxagliptin

Evaluation of Pre-compression and Post Compression Factors:

Compatibility studies:

Interpretation of drug-polymer interaction was done by viewing the FTIR peaks in the Dapagliflozin, polymer and Dapagliflozin -polymer blend.

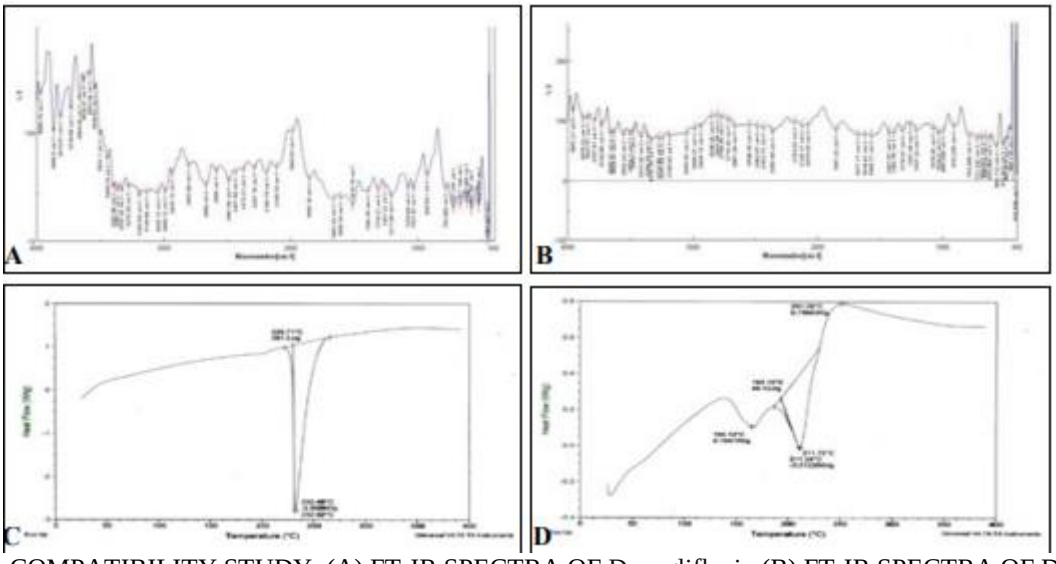

FIG. 3: COMPATIBILITY STUDY: (A) FT-IR SPECTRA OF Dapagliflozin (B) FT-IR SPECTRA OF DRUG-POLYMER BLEND; (C) DSC THERMOGRAM OF Dapagliflozin AND (D) DSC THERMOGRAM OF POLYMER BLEND

Principal peaks of the drug, due to the amines, amide, and aliphatic C-N were observed in wave numbers 3583, 3502, 3332, 2803, 1855, 1392, 1211 cm-1 Fig. 3a. In the drug-polymer blend, prominent peaks were seen in wave numbers 3502, 3332, 2803, 1851, 1392, 1207 cm-1 Fig. 3b. Due to similar observations of the peaks and closeness of peaks in the physical mixture, it may be concluded that no significant drug-polymer interaction occurred. Besides that, the DSC thermograms in heat flow method depicted that the pure drug showed a very pointed endothermic peak at 232.52 °C with an energy of -2.80 w/g Fig. 3c, whereas the polymerdrug blend exhibited a nearly-broad endothermic peak at 211.24 °C with energy of -0.012 w/g Fig. 3d, representing no such interaction of polymer with the drug. It may be believed that a homogenous physical mixture is formed and may be assumed that no such interaction occurred.

Pre-compression Studies:

The pre-compression parameters performed for the immediate release layer of bilayer tablet by direct compression technique are mentioned. For the evaluation of immediate release layer, the bulk and tapped density values of all the three formulations laid between 0.414 - 0.421 g/cm3 and 0.454 - 0.488 g/cm3 . As per pharmacopoeial limit, the bulk and tapped density must be less than 1.2 g/cm3 which indicates a good packing. Therefore, the prepared batches passed the limit prescribed by pharmacopoeia and have the good packing ability. The value of the angle of repose was found to be 25°-21'-28°-45', which indicated satisfactorily acceptable flow property. Hausner's ratio and Carr's index were found to be in range, 1.08 - 1.16 and 8.33 - 13.55%, respectively, which describes acceptable flow property as well as good packing ability. The results for granule characteristics are represented in Table 3.

TABLE 3: PRE-COMPRESSION STUDIES OF POWDER BLEND OF IMMEDIATE RELEASE LAYER

Batches	Bulk density (gm/mL)	Tapped density (gm/mL)	Carr's index (%)	Hausners ratio	Angle of repose (ϕ)
G1	0.414 ± 0.02	0.454 ± 0.02	8.33 ± 0.5	1.08 ± 0.05	28.45 ± 0.5
G2	0.418 ± 0.05	0.478 ± 0.03	12.55 ± 0.1	1.14 ± 0.04	27.56 ± 0.4
G3	0.421 ± 0.01	0.488 ± 0.02	13.55 ± 0.4	1.16 ± 0.05	25.21 ± 0.8

The powder blend used for manufacturing sustained release layer of the bilayer tablet by wet granulation according to the procedure is highlighted. For the evaluation of sustained release layer. The evaluated granules were observed to be acceptable as per the limits. The densities (both bulk and tapped) lie in the range of 0.457 - 0.513 g/cm3 and 0.467 - 0.521 g/cm3 , respectively, which represented an excellent packing

TABLE 4: PRE-COMPRESSION STUDIES OF POWDER BLEND OF SUSTAINED RELEASE LAYER

Batches	Bulk density (g/mL)	Tapped density (g/mL)	Carr's index (%)	Hausners ratio	Angle of repose (ϕ)
M1	0.482 ± 0.04	0.505 ± 0.05	3.7 ± 0.28	1.03 ± 0.02	28.33 ± 1.02
M2	0.513 ± 0.05	0.533 ± 0.04	3.8 ± 0.3	1.04 ± 0.05	26.12 ± 1.08
M3	0.470 ± 0.03	0.487 ± 0.02	3.45 ± 0.08	1.03 ± 0.02	22.34 ± 0.2
M4	0.457 ± 0.06	0.467 ± 0.03	2.20 ± 0.1	1.02 ± 0.01	24.37 ± 0.2
M5	0.503 ± 0.05	0.517 ± 0.06	2.75 ± 0.2	0.97 ± 0.05	25.24 ± 0.7
M6	0.498 ± 0.05	0.511 ± 0.02	2.6 ± 0.1	0.97 ± 0.05	23.01 ± 0.9
M7	0.496 ± 0.03	0.520 ± 0.02	3.14 ± 0.06	0.96 ± 0.05	26.90 ± 0.4
M8	0.505 ± 0.05	0.521 ± 0.02	3.0 ± 0.1	0.96 ± 1.05	24.53 ± 0.4
M9	0.496 ± 0.04	0.508 ± 0.01	2.4 ± 0.1	0.97 ± 0.05	20.90 ± 0.5

of the granules. The angle of repose was observed to be in range of 20°-90'-28°-33' for the batches which indicated reasonably satisfactory flow property. The calculated Hausner's ratio and Carr's index were in the order of 0.96 - 1.04 and 2.4 - 3.7%, respectively which may be interpreted as relatively good packing ability. In short, the tablet blend demonstrated fairly good micromeritic attributes essential for exhibiting sustained release characteristics. The results for physical properties of granules are portrayed in Table 4

Post-compression Studies: The tablets were subjected to evaluation tests according to IP standards. The hardness of tablets from each batch of formulation were found to be in the range 3.96 - 4.26 kg/cm2 . The friability was less than 1% for all the batches of tablets. The above parameters stated that the formulations are having required strength and resistibility. The weight variation was less than 4% and all tablets fall under the pharmacopoeial range of 304.1 - 305.8 mg. Practically, uniform drug content in the range 95.19 - 97.16% were detected among all the batches of the Saxagliptin immediate release layer of tablet. The evaluation values are depicted in Table 5.

TABLE 5: POST-COMPRESSION STUDIES OF IMMEDIATE RELEASE LAYER OF TABLET

Batches	Thickness (mm)	Hardness (kg/cm^2)	Weight variation (mg)	Friability (%)	Drug Content (%)
G1	2.45 ± 0.01	4.12 ± 0.2	304.5 ± 0.5	0.13	96.05 ± 0.72
G2	2.51 ± 0.02	4.26 ± 0.2	304.1 ± 0.8	0.28	95.19 ± 0.65
G3	2.57 ± 0.03	3.96 ± 0.2	305.8 ± 0.3	0.28	97.16 ± 0.72

The evaluation parameters revealed that the prepared sustained tablet batches presented the essential attributes. All the fabricated batches displayed the necessary hardness of more than 5.63 - 6.98 kg/cm2 along with friability value of less than 1%, ultimately representing the required strength and resistibility of the sustained layer. The starch, microcrystalline cellulose, and polymeric combination were found to exhibit a profound role in imparting hardness to the layer. The drug content was identified to be in the range of 94.01 - 97.19%. A weight variation of < 4% was observed for the prepared batches, indicating almost uniform drug content in all the fabricated batches. The postcompression parameters are described in Table 6.

TABLE 6: POST-COMPRESSION STUDIES OF POWDER BLEND OF SUSTAINED RELEASE LAYER

Batches	Diameter (mm)	Thickness (mm)	Hardness (kg/cm^2)	Weight variation (mg)	Friability (%)	Drug Content (%)
M1	17	5.72 ± 0.31	5.83 ± 0.1	752.24 ± 1.53	0.55	94.35 ± 0.9
M2	17	5.46 ± 0.17	5.63 ± 0.3	751.85 ± 0.33	0.79	94.01 ± 0.005
M3	17	5.17 ± 0.55	6.96 ± 0.18	749.37 ± 1.12	0.12	96.27 ± 0.5
M4	17	5.11 ± 0.64	6.77 ± 0.15	751.64 ± 2.89	0.20	94.92 ± 0.13
M5	17	5.31 ± 0.43	6.67 ± 0.15	749.82 ± 0.71	0.20	96.61 ± 0.5
M6	17	5.42 ± 0.32	5.86 ± 0.11	750.95 ± 0.92	0.11	95.37 ± 0.61
M7	17	5.22 ± 0.16	6.06 ± 0.05	753.76 ± 1.73	0.23	97.19 ± 1.05
M8	17	5.19 ± 0.22	6.53 ± 0.17	754.16 ± 1.28	0.31	95.82 ± 0.6
M9	17	5.54 ± 0.29	6.98 ± 0.15	754.94 ± 0.53	0.15	97.17 ± 0.3

Disintegration Test of Immediate Release Layer:

The disintegration time of batches G1 - G3 was found to be in the range of 1 - 3 min for Saxagliptin tablet. The disintegration time for Saxagliptin was found to be maximum for batch G1 and minimum for batch G3 containing the maximum amount of croscarmellose sodium. Table 7 highlighted the disintegration time of the immediate release layer batches.

TABLE 7: DISINTEGRATION DATA OF THE IMMEDIATE RELEASE LAYER

Batch code	Disintegrating time (min)
G1	2.46 ± 0.65
G2	1.66 ± 0.25
G3	1.45 ± 0.35

IV. Dissolution Studies:

Sustained Release Layer Containing Dapagliflozin: All the formulations subjected to in-vitro dissolution studies, revealed that tablets containing release modifiers exhibited controlled release of Dapagliflozin as compared with conventional tablets, which release the whole drug content in 4 - 5 h. Generally, fast dissolution takes place in case of highly water-soluble molecules, where drugs diffuse out of the matrix forming pores for entrance to solvent molecules. Formulation M1 and M2 displayed a cumulative release of nearly 91.8% in 8 h where 110 mg concentrations of polymers HPMC K4M and K15M were employed. In contrast, the formulation M3 exhibited better controlled release profile of 90.6% at 10 h Fig. 4a. Here, the polymer HPMC K100M showed a better rate retardant ability than the above two variants at the same concentration. The hydration rate of HPMC increases with an increase in the hydroxylpropyl content and the solubility of HPMC is pH independent 22. The water-repelling property of higher variants of HPMC retarded the drug release from the matrix by preventing penetration of solvent molecules. The high level of microcrystalline cellulose, the binder cum disintegrant in the formulations also played imperative role in the drug release. In the development of successive batches M4 - M6, the polymer concentration was elevated to 130 mg. The results revealed a diminutive retarded release of drug in the media owing to the concentration of release modifiers in the formulations. Batch M4 and M5 presented the cumulative drug release of 92.53% and 91.21% in 9 h Fig. 4b, respectively.

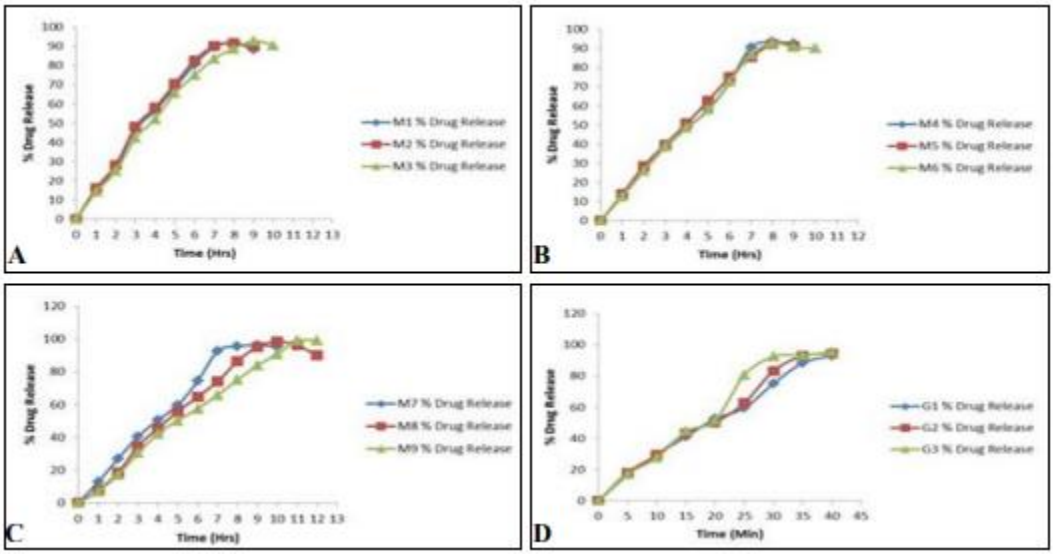

FIG. 4: IN-VITRO DRUG RELEASE: (A) SUSTAINED RELEASE LAYERS OF M1-M3 BATCH; (B) SUSTAINED RELEASE LAYERS OF M4-M6 BATCH; (C) SUSTAINED RELEASE LAYERS OF M7-M9 BATCH; AND (D) IMMEDIATE RELEASE LAYERS OF G1-G3 BATCH

This indicates that as the polymer concentration increased, the drug release rate was found to be retarded 23 . The batch M6 offered a better sustained release profile with 130 mg HPMC K100M polymer mix, leading to release of 90.1% in 10 h Table 8. HPMC K100M was judiciously selected in preparation due to its profound ability to form a strong viscous gel on contact with aqueous media, which helps in controlling the delivery of highly water-soluble drugs 24. The formulation batches M7-M9 having a polymer concentration of 150 mg represented 95.28%, 96.19%, and 99.04% at 11 h Fig. 4c, respectively. The reason of the release profile is due to the optimization of release modifier concentration along with the amount of microcrystalline cellulose.

Time (h)	M1	M2	M3	M4	M5	M6	M7	M8	M9
1	15.54	16.15	14.22	12.59	13.81	12.79	12.79	7.72	7.00
2	27.12	28.13	25.09	26.00	28.44	26.20	27.02	18.48	17.26
3	46.72	48.14	42.35	38.39	39.81	38.80	40.42	34.63	30.37
4	56.58	58.20	52.21	48.45	50.79	48.75	50.48	45.50	42.25
5	68.66	70.49	65.62	57.69	62.57	58.00	59.72	55.76	50.28
6	80.65	82.68	74.96	72.32	75.06	72.62	74.35	64.80	57.49
7	89.59	90.30	83.70	90.60	85.22	86.85	92.64	74.05	65.72
8	91.72	91.82	88.78	93.55	92.33	92.94	95.58	86.44	75.06
9	88.37	89.79	92.84	92.53	91.21	90.71	96.29	95.28	83.90
10	-	-	90.60	-	-	90.10	95.28	98.43	90.91
11	-	-	-	-	-	-	-	96.19	99.04
12	-	-	-	-	-	-	-	90.10	99.04

TABLE 8: IN VITRO RELEASE OF METFORMIN FROM THE SUSTAINED RELEASE LAYER

Immediate Release Layer Containing Saxagliptin:

The in-vitro drug release profile of SDs were studied and compared with the pure drug of Saxagliptin. In all the SDs prepared by PEG 6000 (Saxagliptin /PEG 6000) containing three different weight ratios; 1:1, 1:2, and 1:5, the last batch (G3) having 1:5 drug: polymer ratio demonstrated highest drug release of 95.59% as compared to the 93.13% drug release from batch G1 Fig. 4d. The fact is due to that the SDs prepared with higherratios of polymer could offer more available space for surrounding of hydrophobic drug particle resulted in rapid hydration of drug molecules and consequently better wettability and enhancement in the dissolution 25. Moreover, the transformation of the crystalline nature of pure Saxagliptin into the amorphous form as affirmed by the DSC and FT-IR results facilitates higher drug release rate over the pure drug. In addition to the fact that SD promoted significant improvement in dissolution, the formulations contained superdisintegrant croscarmallose sodium, which provided a burst release leading to exposed surface area endorsing further release of drug in the media 26. From above comparative in vitro drug release, it is concluded that from the above mentioned formulations G1- G3, the last batch, G3 formulation performed the best release of Saxagliptin Table 9.

Time (min)	G1	G2	G3
5	16.62	18.01	17.08
10	29.55	29.32	27.59
15	41.10	43.41	44.56
20	52.64	50.33	51.49
25	59.45	62.69	80.58
30	75.16	83.24	92.47
35	88.32	92.82	93.40
40	93.13	94.55	95.59

TABLE 9: IN-VITRO RELEASE OF Saxagliptin FROM THE IMMEDIATE RELEASE LAYER

Evaluation of Prepared Bilayer Tablets:

Appearance: The dimension of the tablets fabricated was 17 × 7.5 mm. The physical characteristics of the tablet were found to be acceptable. The tablets were free from any defects like capping, picking and chipping. Physical Characteristics: All the formulations were assessed for their desired characteristic attributes as per standard procedures for evaluation. The average weight of tablets from all the formulation were found to be in the range of 1050.84 - 1055.15 mg, which indicates that the all batches have the average standard weight as per the official standards. The drug content in all the batches of the formulation was in the range of 96.35 - 98.21% of Dapagliflozin and 95.69 - 96.57% of Saxagliptin, respectively, reflecting uniform drug content practically. The thickness of the tablet was in the range of 7.37 - 7.63 mm. All the batches have good hardness and friability resistance as per standards. The hardness of the fabricated tablets was found to be in the range 5.84 - 6.3 kg/cm2 and the friability of the batches was less than 1%. The parameters have stated that the prepared batches had the required strength to withstand wear and tear. The evaluation parameters of the prepared batches are illustrated in Table 10.

TABLE 10: EVALUATION PARAMETER OF BILAYER FORMULATIONS

	B1	B2	B3
Thickness (mm)	7.37 ±0.05	7.52 ±0.11	7.63 ±0.2
Hardness (kg/cm^2)	5.84 ±0.5	6.01 ±0.01	6.3 ±0.1
Weight variation (mg)	1054.35 ±0.5	1055.15 ±0.8	1050.84 ±1.41
Friability (%)	0.592	0.471	0.503
Drug Content (%)	97.33 (M) 96.57 (G)	98.21 (M) 95.69 (G)	96.35 (M) 96.12 (G)

Bilayered Tablet Containing Saxagliptin and Dapagliflozin:

The bilayered tablet comprising of Saxagliptin (in the immediate release layer) and Dapagliflozin (in the sustained release layer) was evaluated for the drug release characteristics. The majority of the release controlling agent containing formulations displayed a steadily sustained release attribute as compared to conventional dosage forms. The release of drug from the matrix involves a complex interaction between drug dissolution, solubilization, diffusion, and erosion mechanism(s). The examined formulation B3 represented a sustain release pattern of drug release up to 12 h, in contrast to other two formulations; B1 and B2. Both the formulations (B1 and B2) demonstrated cumulative drug release of 92% Fig. 5a.

Time (in h)	B1	B2	B3
1	15.22	12.75	8.89
2	27.09	25.22	19.36
3	44.30	39.89	31.27
4	54.21	49.75	42.25
5	64.62	60.26	53.28
6	75.86	71.68	60.59
7	86.67	84.75	70.69
8	89.71	92.94	81.06
9	93.92	94.91	86.93
10	91.60	92.10	92.91
11	-	-	98.04
12	-	-	98.09

TABLE 11: IN-VITRO RELEASE OF Dapagliflozin FROM THE BILAYERED TABLET

The reason may be due to large concentrations of binder as compared to batch B3. The formulation B3 consisting of highest concentration of the polymer effectively retarded the release of drug (98.1%) up to 12 h Table 11. The immediate release layer of B2 released the highest amount of Saxagliptin (94.58%) in the dissolution media after 40 minutes Table 12. The formulations B1 exhibited 93.89% release and B3 expressed 93.59% of drug release after 40 min Fig. 5b. Due to the presence of the highest amount of super-disintegrant, a burst release was observed in all the cases and the observations were so much closer in precision that all the tablet batches have equal attribute in the uniform release of the drug 27 . When we compared the release of drug from bilayered tablet and release of drug from individual layers, we observed that the cumulative drug release of Dapagliflozin was retarded from 99.04% to98.09%. Quite similarly, the release of Saxagliptin from individual layer was 95.59%, while from bilayered tablet it was < 94.5% of all the cases. Therefore, it may be concluded that the punching and manufacturing processes might have a crucial role in the modification of drug release.

Time (in min)	B1	B2	B3
5	16.58	19.65	17.08
10	26.48	26.98	27.59
15	44.10	43.54	44.56
20	51.0	50.25	51.49
25	78.48	79.25	80.58
30	92.11	91.89	92.47
35	93.02	92.89	93.04
40	93.89	94.58	93.59

TABLE 12: IN-VITRO RELEASE OF Saxagliptin FROM THE BILAYERED TABLET

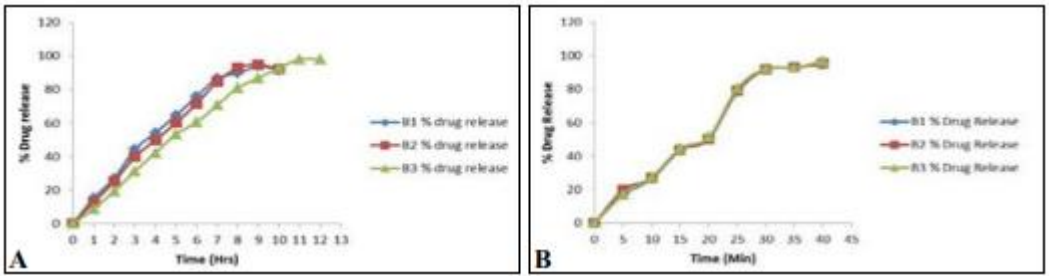

FIG. 5: IN-VITRO DRUG RELEASE FROM BILAYERED TABLET BATCHES (B1-B3): (A) Dapagliflozin AND (B) Saxagliptin

Kinetics Release Profile:

The release mechanism of Dapagliflozin and Saxagliptin from bilayered optimized formulation B3 was studied by fitting the data obtained from in-vitro release studies into zero-order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell models.

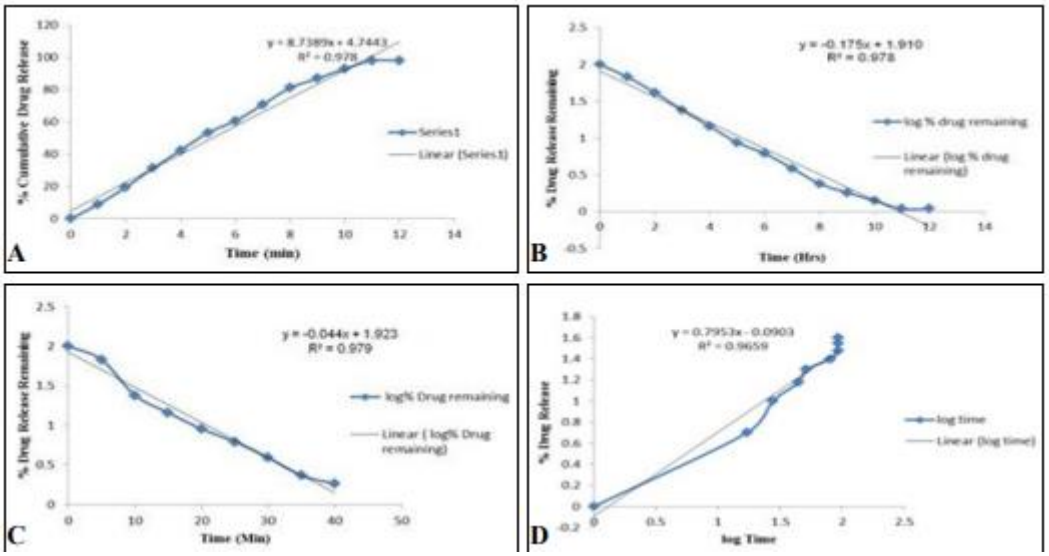

FIG. 6: KINETICS RELEASE PROFILE: (A) RELEASE OF DRUG FROM OPTIMIZED FORMULATION B3 BY ZERO-ORDER; (B) RELEASE OF DRUG FROM OPTIMIZED FORMULATION B3 FROM FIRST-ORDER; (C) Saxagliptin FROM IMMEDIATE RELEASE LAYER BY FIRST-ORDER; AND (D) Saxagliptin FROM IMMEDIATE RELEASE LAYER BY KORSMEYER-PEPPAS

On the application, it was found that the optimized formulation B3 followed the either zero-order Fig. 6a or first-order Fig. 6b based sustained drug release as indicated by the similar regression coefficient (r2) values of 0.978 in the case of Dapagliflozin. The pharmacokinetic data for Dapagliflozin was established according to the Acceptance Table of Test 4 given in USP-33 for the 12 h with dosing correlation coefficient (r2) value 0.998, this batch was selected for the further studies. Thus, the sustained - release tablet formulation displayed release of required quantity of drug with predetermined kinetics in order to maintain an effective drug plasma concentration. The pharmacokinetic data of the Dapagliflozin release from the bilayer tablet are described in Table 13.

Time (h)	Sq. rt. time	Log time	Conc. (µg/mL)	C. C (mg/mL)	% release	Log % release	% drug remaining	Log % drug remaining
0	0.000	0.000	0.000	0.000	0.00	0.00	100	2
1	1.000	0.000	1.980	5.948	8.89	0.948	91.11	1.83
2	1.414	0.301	3.456	10.36	19.36	1.286	80.64	1.62
3	1.732	0.477	5.034	15.10	31.27	1.495	75.75	1.38
4	2.000	0.602	7.176	21.54	42.25	1.625	68.73	1.16
5	2.236	0.698	9.912	29.70	53.28	1.726	46.72	0.94
6	2.449	0.778	12.70	38.11	60.59	1.782	39.41	0.79
7	2.646	0.845	15.25	45.75	70.59	1.849	29.31	0.59
8	2.828	0.903	16.86	50.60	81.06	1.908	18.94	0.38
9	3.000	0.954	19.43	58.29	86.93	1.939	13.07	0.26
10	3.162	1.000	20.32	60.96	92.91	1.968	7.09	0.15
11	3.317	1.041	21.98	65.93	98.04	1.991	1.96	0.04
12	3.464	1.079	21.98	65.93	98.04	1.991	1.89	0.01

Sq. rt., square root; Conc., concentration; C. C., cumulative concentration

TABLE 13: PHARMACOKINETIC PARAMETERS FOR Dapagliflozin RELEASE

In the case of Saxagliptin from immediate release layer, there were two probabilities of drug release based on the regression coefficient; either firstorder Fig. 6c or Korsmeyer-Peppas Fig. 6d order release which show constant drug release rate with time. The former expressed r2 value of 0.979 and the latter exhibited 0.9659. In more detail, it may be believed that drug release was controlled by more than one process; somewhat an intermediate of both diffusion and erosion mechanism (called anomalous diffusion) after the burst due to the presence of super disintegrants 28. The pharmacokinetic drug release data of Saxagliptin from the tablet are elaborated in Table 14

Time (min)	Sq. rt. time	Log time	Conc. (µg/mL)	C. C. (mg/mL)	% drug release	Log % release	% drug remaining	Log % drug remaining
0	0.000	0.000	0.000	0.000	0.00	0.00	100	2
5	2.236	0.688	11.708	35.125	17.08	1.232	82.92	1.995
10	3.162	1.000	12.759	38.277	27.59	1.440	72.41	1.979
15	3.872	1.176	14.456	43.368	44.56	1.648	55.44	1.976
20	4.472	1.301	15.149	45.447	51.59	1.711	48.51	1.956
25	5.000	1.397	18.058	54.174	80.58	1.906	19.42	1.732
30	5.477	1.477	19.247	57.741	92.47	1.966	7.53	1.422
35	5.916	1.544	19.304	57.942	93.04	1.968	6.60	1.236
40	6.324	1.602	19.559	58.677	93.59	1.971	4.41	0.977

Sq. rt., square root; Conc., concentration; C. C., cumulative concentration

TABLE 14: PHARMACOKINETIC PARAMETERS FOR Saxagliptin RELEASE

V. CONCLUSION:

The present study involved in designing and fabricating an oral bilayer hypoglycemic tablet formulation which has attributed to deliver a first impulse of the dose in the shortest time possible (a few min) and a second fraction of the dose in a prolonged time at a constant rate for the duration of more than 10 h. The bilayer tablet comprised of the first layer formulated with Saxagliptin solid dispersion to obtain a prompt release of the drug, often regarded as the loading dose) whereas the second layer was a prolonged released hydrophilic matrix which designed to maintain an effective plasma level for a prolonged period of time, regularly called as maintenance dose. The formulation B3 was found to be highly optimized and demonstrated the highest cumulative drug release where the Dapagliflozin followed the either zero-order or firstorder and the Saxagliptin followed anomalous diffusion. Therefore, the designed formulation will offer a better therapeutic regimen and provide patient friendly postprandial hypoglycemic management, where an immediate control and a maintenance dose is required.

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