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Research Article

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3^2-Factorial Design for Sitagliptin Osmotic Tablets: Characterization, Optimization, and Controlled Release Mechanisms

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Abstract Sitagliptin, a widely used antidiabetic agent, poses challenges in achieving precise and sustained drug release due to its biopharmaceutical properties. In response to these challenges, this study focused on the design and development of an osmotic drug delivery system for Sitagliptin. The research employed a combination of formulation techniques, including drug-polymer compatibility studies and a 3^2-factorial design, to optimize the controlled release profile. The osmotic system was characterized for its *in vitro* drug release. Furthermore, this research demonstrates the potential of osmotic drug delivery systems as a versatile platform for enhancing the performance of challenging drugs like Sitagliptin. The findings underscore the importance of controlled drug release in managing chronic conditions and pave the way for further investigations in this field.

Keywords Sitagliptin, DPP-4 inhibitor, Characterization, Osmotic drug delivery

1. Introduction

Individuals diagnosed with type 2 diabetes may have potential advantages from the use of Sitagliptin, an oral medication designed to reduce glucose levels. Sitagliptin is classified as a member of the DPP-4 inhibitor pharmacological category, exerting its effects through the inhibition of the dipeptidyl peptidase-4 (DPP-4) enzyme [1-3].

The compound known as sitagliptin exists in the form of a white to off-white powder, which exhibits solubility in water that is contingent upon the pH of the solution. While water is capable of dissolving isopropanol, it is unable to dissolve either isopropanol or isopropyl acetate. Alcohols, ketones, ketone esters, acetone, and acetonitrile have limited solubility with it [4-6].

Sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, is hypothesized to mitigate the enzymatic degradation of incretin hormones in individuals diagnosed with type 2 diabetes mellitus. JANUVIA has been found to enhance the concentrations of active intact hormones, hence prolonging and enhancing their physiological effects. During the course of the day, the stomach secretes a continuous flow of incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptide (GIP) [7-8]. The enzyme dipeptidyl peptidase-4 (DPP-4) efficiently inactivates endocrine disruptors. Incretins play a crucial role in the endogenous mechanism that facilitates the regulation of glucose homeostasis within the body [9-10]. The stimulation of insulin synthesis and release from pancreatic beta cells by GLP-1 and GIP occurs through intracellular signaling pathways, notably involving cyclic AMP, in the presence of normal or high blood glucose levels [11-13]. GLP-1 additionally suppresses the secretion of glucagon from pancreatic alpha cells, thereby exerting an inhibitory effect on hepatic glucose synthesis [10-12]. The

glucose-dependent insulin release of JANUVIA exacerbates diabetes, whereas the augmentation and prolongation of plasma levels of active insulin-like growth factor-1 exacerbates insulin insufficiency (IGF-1). In vitro studies have shown that Sitagliptin, at concentrations that are clinically relevant, selectively inhibits the activity of DPP-4, while not affecting the activity of DPP-8 or DPP-9 [14-15].

Sitagliptin is classified as a dipeptidyl peptidase-4 (DPP-4) inhibitor, which is a pharmacological agent known to modulate the activity of incretin hormones by inhibiting their degradation. This mechanism of action is believed to be responsible for the therapeutic effects of sitagliptin in individuals diagnosed with type 2 diabetes. Delaying the release of the drug in the gastrointestinal system would yield benefits, as it would extend the therapeutic period and mitigate potential adverse effects associated with Sitagliptin [1-6].

The objective of this project is to utilize a 3^2-factorial design to develop, analyze, and enhance the formulation of an oral osmotic controlled release tablet containing Sitagliptin. The aim of this study is to optimize the therapeutic efficacy of the pill in managing Diabetes mellitus.

Material and Methods

Analytical method development for Sitagliptin

The analysis of Sitagliptin was conducted using a UV-visible spectroscopy method. A stock solution with a concentration of 1000 micrograms per liter of Sitagliptin was prepared by dissolving 100 mg of the compound in 100 mL of 0.1N hydrochloric acid (HCI). To obtain a standardized solution with a concentration of 100 ng/mL, a 10 mL stock solution was diluted to a final volume of 100 mL. Standard solutions with concentrations of 2, 4, 6, 8, and 10 g/mL were prepared by diluting the standard solution using 0.1N hydrochloric acid (HCl) [16-19]. The absorbance of the solutions was measured at a wavelength of 267.0nm using a twin beam UV visible spectrophotometer. The data was subjected to analysis using the software program Microsoft Excel.

Drug Excipient Compatibility Study

The drug and excipients were combined and subjected to ambient conditions for a duration of 24 hours. The compatibility of drug excipients was assessed through the FT-IR. Because of their near proximity, drug excipients have the potential to interact with one another in any formulation. To investigate potential interactions between drugs and polymers, FT-IR spectroscopy was used. A Fourier Transform Infrared (FTIR) spectrophotometer (Alpha, Bruckerpvt ltd., Japan) loaded with potassium bromide (KBr) pellets was used to analyze drug and polymer combinations. The maximum scanning speed observed was in the range of 400-4000 cm^1 [20-21].

Experimental Design

A full factorial design was used to optimize two factors with three levels each, resulting in a total of 3^2 experimental conditions. The optimization strategy utilized the desirability approach to determine the optimal formulation parameters. The trial batches showed that drug release was significantly influenced by three distinct factors. The concentration of SLS and the concentration of the coating agent (NaCl) were both treated as independent variables. The aim was to optimize the formulation of the drug to ensure that the release occurred at 2 hours, 6 hours, and 12 hours, while also retaining the release exponent. The table displays the two factors that were selected, along with their respective levels and the evaluated response. Additionally, it presents the matrix of the factorial design. In the matrix, every experiment is depicted as a row, and each experiment produces a distinct result or response. This approach resulted in the development of an empirical second-order polynomial model [22-23].

Table 1: Variables in 3^2 full factorial design

Independent Variable	**Low(-1)**	**Middle(0)**	**High(+1)**
X_1 : NaCl (mg)	20	25	30
X_2 : SLS (mg)	10	20	30
Dependent Variable	**Response**		
Y_1 :DR% After 2 hour			
Y_2 : DR% After 6 hour			
Y_3 : DR% After 12 hour			

3^2 Factorial Design for Sitagliptin Osmotic tablets

Table 2: 3^2 Factorial Design for Sitagliptin Osmotic tablets

	Coded Formula	
Experimental Run	**X_1**	**X_2**
1	+1	0
2	0	+1
3	0	+1
4	0	-1
5	-1	-1
6	+1	-1
7	0	0
8	0	0
9	-1	0

Preparation of Tablets

The core pills were produced using a wet granulation technique. In order to efficiently remove all impurities, a #40 sieve was employed. The researchers employed a computerized weighing scale to measure the quantities of ingredients for a total of 25 tablets. The components, excluding PVP K30, magnesium stearate, and talc, were combined in a mortar and pestle utilizing the geometric dilution technique. The PVP K30 compound was first dissolved in isopropyl alcohol and subsequently granulated in the dry blend. After undergoing a drying process at a temperature of 60°C for a duration of six hours, the powder bulk was subsequently subjected to sieving using a #20 mesh sieve. Subsequently, a period of three minutes was allocated for the combination to undergo sedimentation prior to its utilization. The tablets were manufactured using a rotary tablet punching machine in conjunction with a 9mm concave die punch set [24-26].

Method of Preparation of Tablet Coat Solution

A solution was prepared by adding 75% of the total volume of acetone to the mixture, followed by agitation for a duration of 30 minutes at a rotational speed of 35 revolutions per minute using a propeller stirrer. The solution was considered transparent after this process. The process of stirring was thereafter performed following the trituration of magnesium stearate and the coloring component within a mortar. Acetone was employed in order to restore the initial volume [24-26].

Coating of the Core Tablets

The process of coating tablets was achieved by the utilization of tablet coating pans. The inlet air temperature of the coating pan was adjusted to 500°C, while the flow rate was set at 3.2 kg/min, operating at a speed of 30 rpm. The application of the coating solution was conducted at a flow rate ranging from 4 to 5 mL per minute. The maximum allowable batch size was 50 tablets. The test batch tablets and 40 placebo tablets were combined. The empty coating pan was operated at the parameters mentioned above for a duration of five minutes. The container was filled with solid medication forms and allowed to reach a state of rest. The tablets were subjected to a drying period of 5 minutes after the application of the coating solution, following which the solution was reapply at a flow rate of 5 mL/min for a duration of 2-3 seconds. A quantity of approximately 100 mL of coating solution was employed for a batch comprising 50 tablets [24-26].

Characterization of Osmotic Tablet

Hardness. The concept of hardness. The fracture strength of the tablets was determined using a Monsanto tablet hardness tester. Fracture strength refers to the magnitude of force necessary to induce radial compression and subsequently cause the shattering of a tablet. The metric unit employed for calculating the average hardness is kilograms per square centimeter (kg/cm^2).

Friability. The concept of friability refers to the tendency of a substance to crumble or break apart The brittleness of the tablets was assessed using a Roche friabilator. A collection of tablets, each with a predetermined weight (w0), or

a sample consisting of 10 tablets, is introduced into a drum for the purpose of removing dust particles. This dedusting process is carried out for a fixed duration, involving 100 revolutions of the drum. Subsequently, the tablets undergo a second weighing process, denoted as w. The calculation of the percentage of friability involved utilizing the weight reduction, as indicated by the subsequent equation.

Weight Variation Test. The pills were individually weighed using an electronic balance, and subsequently, the average weight for each formulation was determined based on the weights of the 20 tablets. The tablets will successfully pass the IP test if the quantity of tablets surpassing the predetermined percentage threshold does not exceed two, and if there is no tablet exhibiting a percentage difference over this threshold.

Thickness. The measurement of tablet thickness was performed using a Vernier caliper. A total of twenty pills were employed to calculate the mean. The permissible range for tablet thickness should fall within a variation of plus or minus 5%.

Determination of Drug Content. A total of ten tablets were accurately measured in terms of weight prior to their pulverization. A meticulously measured quantity of the powder, corresponding to 100mg of Sitagliptin sodium, was carefully weighed and subsequently extracted in 100mL of water using agitation for a duration of 20 minutes. The samples underwent spectrophotometric analysis at a wavelength of 267 nm following filtration using Whatman filter paper number 1 and subsequent dilution with water. The determination of the drug's concentration was performed utilizing the Sitagliptin calibration curve.

In Vitro Drug Release Study*:* The equipment employed for evaluating the solubility of pharmaceutical substances conforming to the standards set by the United States Pharmacopeia (USP). In a prior experiment, the rate of Sitagliptin release from tablets fabricated in a basket-type configuration was assessed. The disintegration test was conducted under controlled conditions, with a temperature of 37 degrees Celsius and a rotational speed of 100 revolutions per minute. During the experimental procedure, a 900mL solution of hydrochloric acid (HCl) buffer with a concentration of 0.1M and a pH value of 1.2 was employed for a duration of two hours. Subsequently, the experiment was replicated over a duration of ten hours within a phosphate buffer solution, maintaining a pH level of 6.8. A volumetric sample of 1 mL was extracted from the dissolution apparatus and replaced with a fresh solution at regular intervals of 12 hours. Prior to measurement of absorbance at a wavelength of 267 nm, the samples underwent dilution and filtration utilizing Whatman filter paper. The determination of the cumulative percentage release of the drug.

Curve Fitting Analysis. The release data obtained from in vitro experiments were analyzed using several mathematical models, such as the zero-order, first-order, Higuchi, and Korsmeyer and Peppas equations. The investigation focused on the kinetics of drug release from the porous osmotic pump tablet, utilizing the aforementioned models.

Zero-Order Release Kinetics: The equation utilized in this study was employed to simulate the release data in order to investigate the kinetics of zero-order release.

The equation dQ/dt = K0 (2) is derived using a zero-order release rate constant (K0) and a drug release time (t).

The graph depicts the time course of cumulative drug release expressed as a percentage (CDR%).

First-Order Release Kinetics. The subsequent equation is employed to establish a suitable fit for release rate data with the purpose of examining the kinetics of first-order release.

If we divide the fraction of medication released by the first-order release rate constant, we get dQ/dt = K1Q (3).

Higuchi Release Model. The following equation is utilized to optimize the release rate data in order to analyze the Higuchi release model.

The fractional release (Q) of a medication can be mathematically represented as the division of the release rate constant (KH) multiplied by the square root of time (t) by time (t).

The graph depicts the correlation between the percentage of carbon dioxide removal (CDR) and the square of time [27-30].

The standard curve of Sitagliptin in 0.1N hydrochloric acid (HCI) at a wavelength of 267nm.

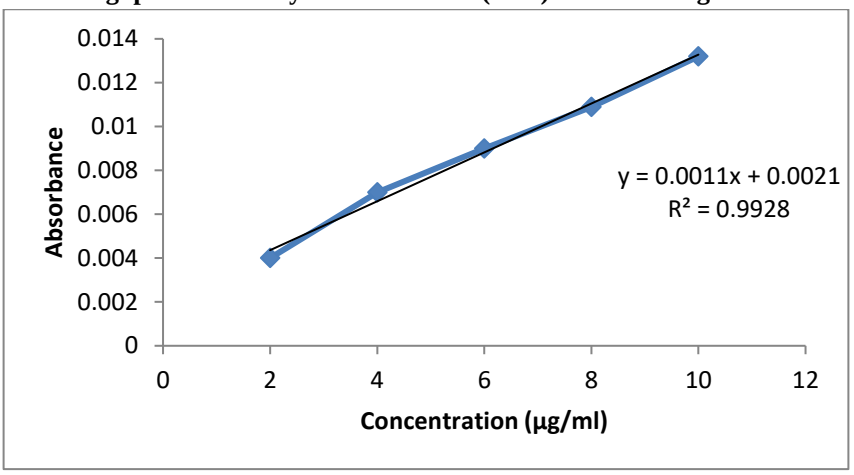
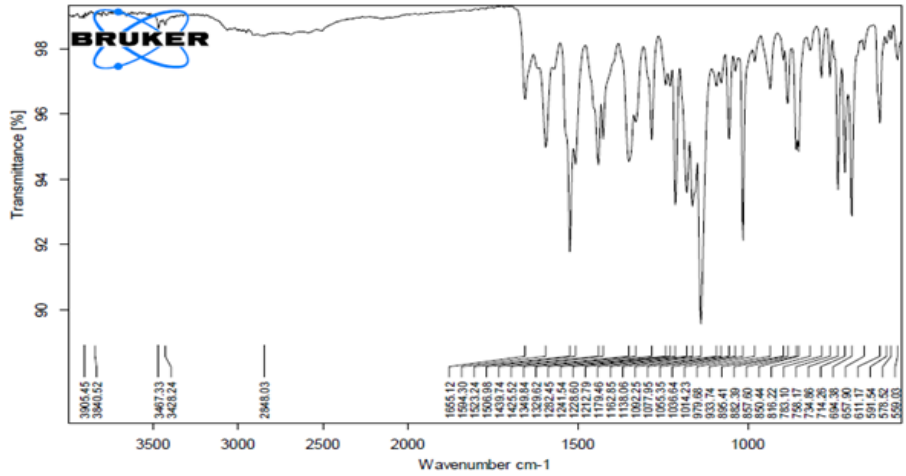
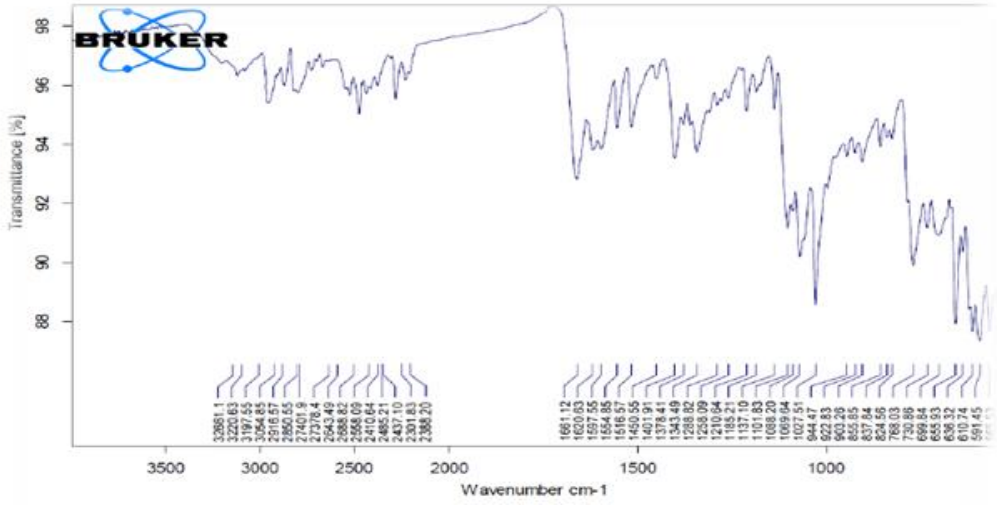


Figure 1: The standard curve of Sitagliptin in 0.1N HCl at a wavelength of 267nm

FT-IR STUDY



(a)



(b)

Figure 2: FTIR Spectra of Sitagliptin (a) and Sitagliptin with excipients (b)

Table 2: Formulation Table for Oral Osmotic Tablet of Sitagliptine

Ingredient (mg)	Formulation								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Sitagliptin	100	100	100	100	100	100	100	100	100
NaCI	20	25	30	20	25	30	20	25	30
SLS	10	10	10	20	20	20	30	30	30
PVP K30	10	10	10	10	10	10	10	10	10
Starch	50	45	40	45	40	35	35	30	25
Mg. stearate	5	5	5	5	5	5	5	5	5
Talc	5	5	5	5	5	5	5	5	5
Total (mg)	200	200	200	200	200	200	200	200	200

Table 3: Composition of Coating Solvent

Ingredients	Composition
Cellulose Acetate	2% w/v
PEG 400	2% w/v
TiO_2	0.2% w/v
Coloring Agent	0.2%w/v
Acetone	Up to 100ml

Table 4: Micrometric Results of different batches of Sitagliptin Osmotic Tablet

Batch	Angle of Repose	Bulk density(gm/cm^3)	Tapped density(gm/cm^3)	Carr'sndex	Porosity (%)
F1	31.03±0.03	0.187±0.13	0.289±0.13	33.63±0.33	11.43
F2	17.43±0.03	0.188±0.03	0.299±0.33	41.26±0.27	13.45
F3	28.13±0.03	0.197±0.06	0.219±0.04	46.39±0.21	12.46
F4	29.13±0.13	0.127±0.11	0.267±0.07	32.58±0.31	10.13
F5	28.17±0.04	0.191±0.06	0.288±0.04	33.63±0.29	12.43
F6	29.67±0.05	0.184±0.03	0.287±0.08	35.89±0.34	10.67
F7	29.13±0.04	0.185±0.04	0.291±0.08	32.63±0.27	10.83
F8	28.66±0.05	0.189±0.02	0.285±0.04	33.45±0.56	11.35
F9	28.33±0.06	0.183±0.06	0.283±0.02	28.56±0.03	10.43

Table 5: Evaluation of Core osmotic tablet of Sitagliptin

Batch	Hardness (kg/cm^2)	Friability (%)	Thickness(mm)	Drug Content (%)	Weight(mg)
F1	8.13	0.31	3.91	91.33	200
F2	8.27	0.85	3.92	93.44	201
F3	9.11	0.71	3.90	92.67	200
F4	8.01	0.43	3.93	94.32	202
F5	8.22	0.75	3.90	97.33	201
F6	7.99	0.76	3.92	98.67	203
F7	8.32	0.86	3.92	96.67	202
F8	9.01	0.87	3.90	97.33	201
F9	9.11	0.33	3.91	98.33	200

Table 6: The in-vitro release profile of various formulations of Sitagliptin Osmotic Tablet

Time (hr.)	% of Drug Release								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
1	20	10	22	12	20	22	20	18	24
2	22	28	31	21	32	32	28	21	36
3	42	32	36	46	56	47	42	38	56
4	45	57	42	52	72	49	57	41	62
6	68	70	51	59	89	69	70	50	78
8	74	86	62	68	98	77	86	68	81
10	86	98	70	74	-	86	99	70	98
12	90	-	82	86	-	90	-	79	100
14	92	-	85	90	-	93	-	86	

Release Profile of Different Formulation Sitagliptin Osmotic Tablet

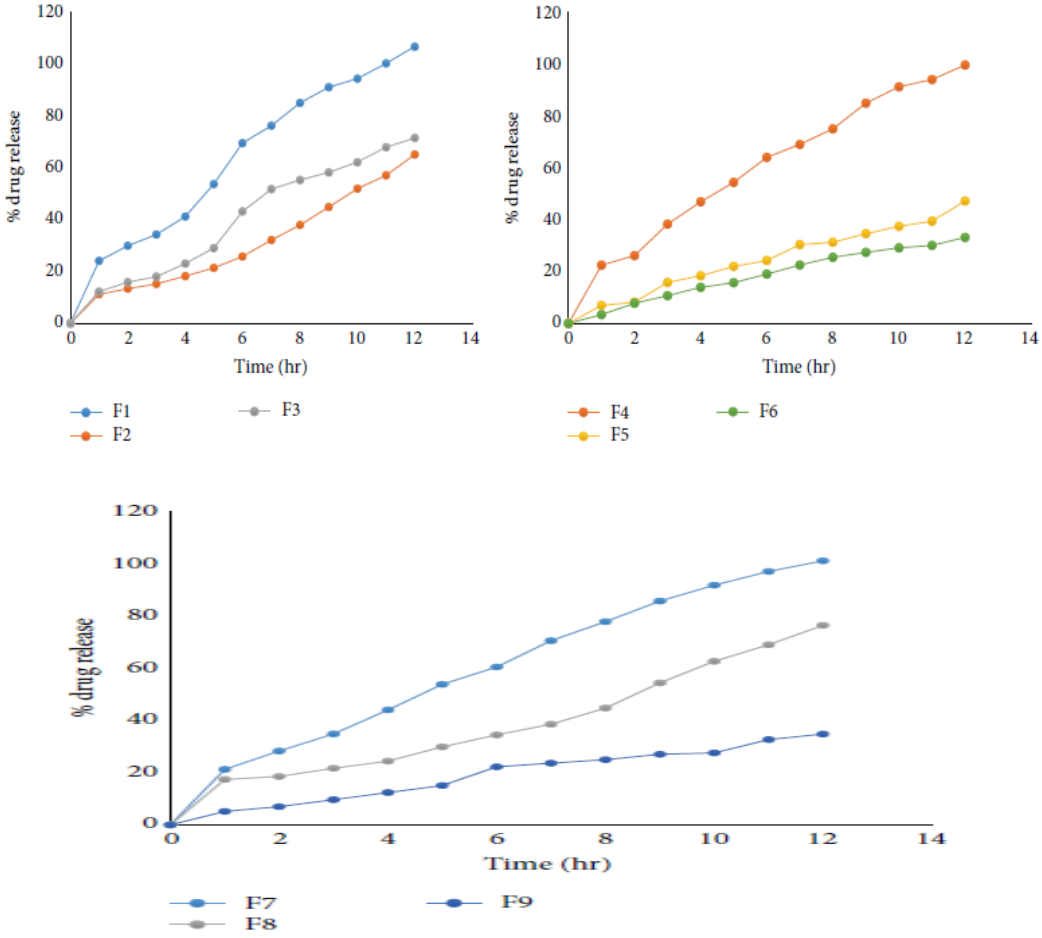


Figure 3: Release Profile of Different Formulation Sitagliptin Osmotic Tablet

Table 7: Kinetic Modelling of Optimized Formulation F9

Parameter	**Zero-order**	**First-order**	**Higuchi**	**Korsmeyer-Peppas**
Sum of residuals	1314.416	346.8983	205.599	75.4920
Correlation coefficient (r)	0.9781	0.9821	0.9905	0.9960
R square ($r2$)	0.940	0.785	0.785	0.838
F	109.534	28.901	17.132	6.89

Release Kinetics Study

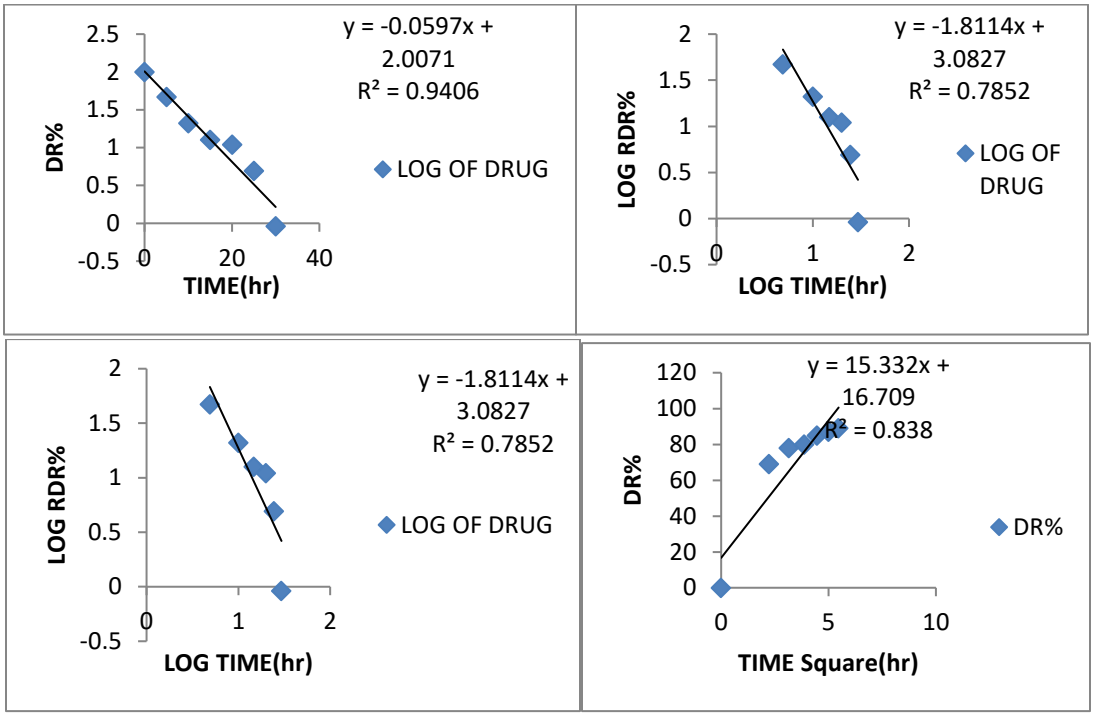


Figure4: Release Kinetic Study of Formulation F9 Sitagliptin Osmotic Tablet

Table 8: Results of Stability Study

Parameter	Initially	After 30 days
Weight variation (n = 10)	200	198
Diameter (mm)	10	10
Thickness (mm)	5	4.9
Hardness (kg/cm^2)	6.8	6.7
$Y3$ (% drug release at 12 hr)	98.72	95.72

3D Plot Drug Release at 12hr

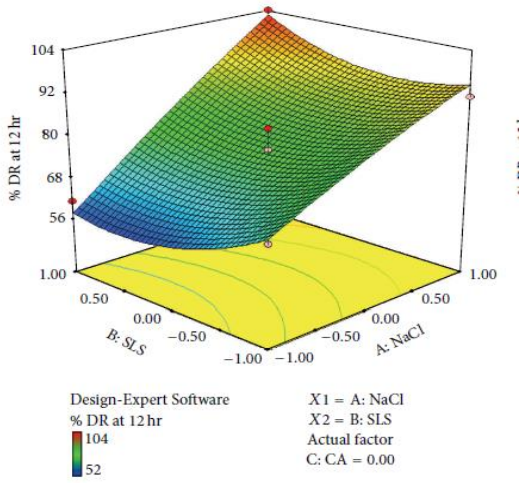


Figure 5: 3D Plot Drug Release at 12hr

3D PLOT Drug Release at 6 hr.

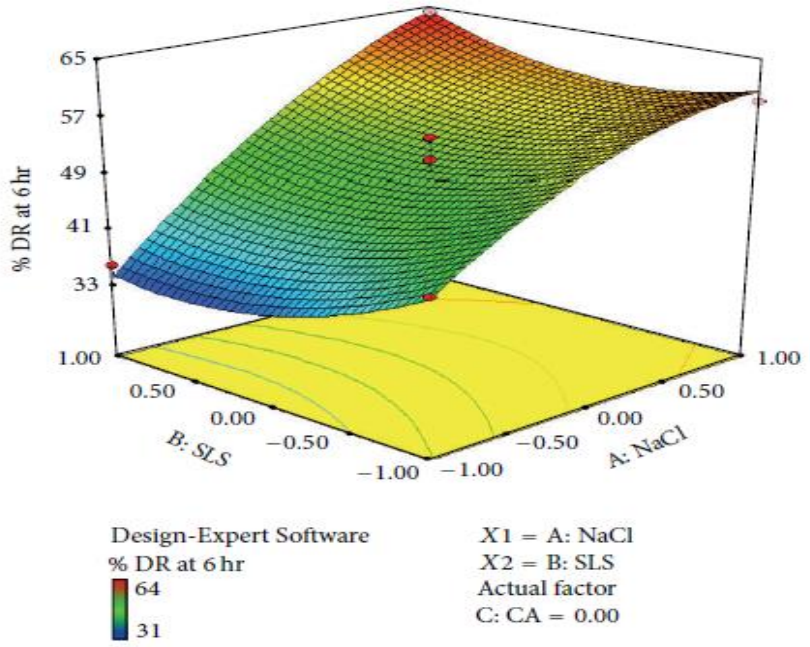


Figure 6: 3D Plot Drug Release at 6hr

3D PLOT Drug Release at 2 hr

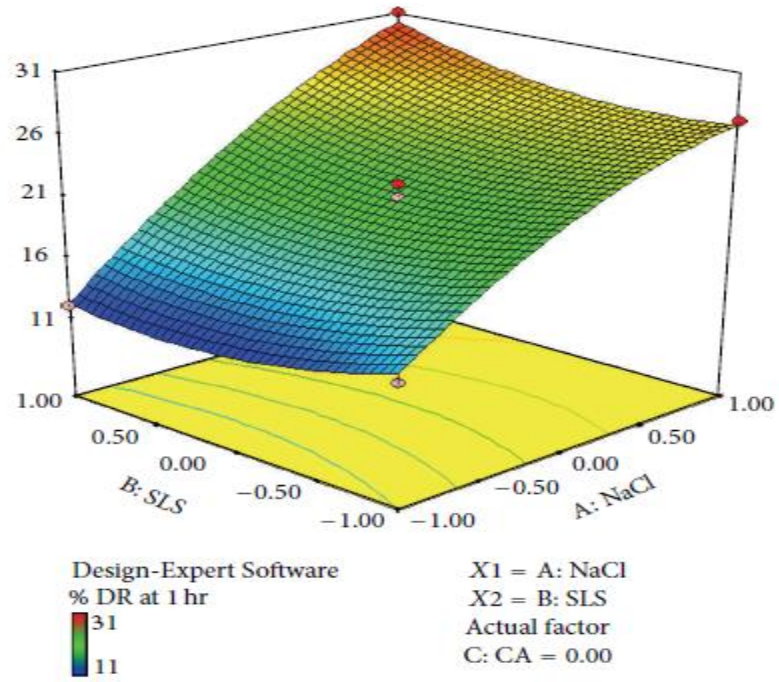


Figure 7: 3D Plot Drug Release at 2hr

Stability Studies

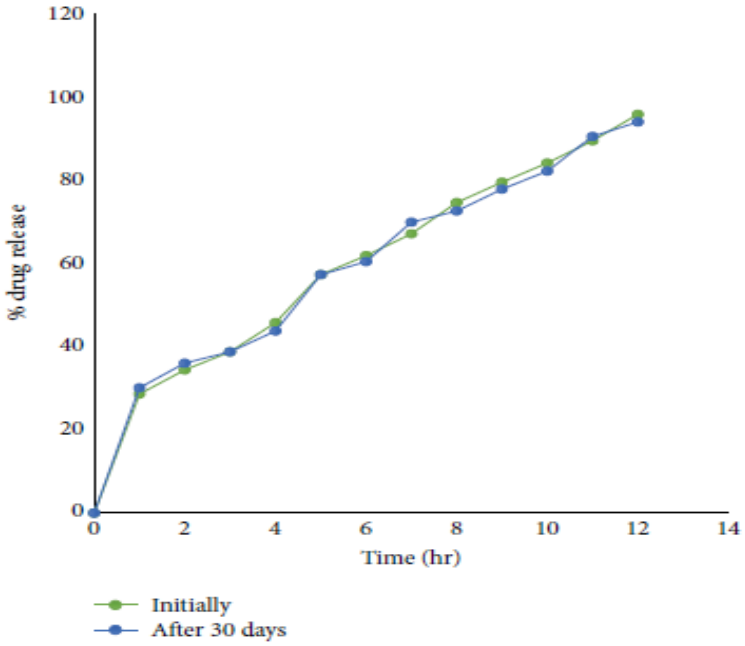


Figure 8: % drug release study of Formulation F9 at initially and after 30 days

Results and Discussion

The standard calibration curve of Sitagliptin in a 0.1N hydrochloric acid (HCI) solution at a wavelength of 267nm was prepared.

A 267 nm wavelength was used to generate the calibration curve for Sitagliptin in 0.1 N hydrochloric acid (HCI). Sitagliptin had a high degree of linearity (r2=0.992) and an intercept of 0.002 in 0.1 N hydrochloric acid (HCI) at concentrations ranging from 2 to 12 g/ml, as measured at a maximum wavelength of 267 nm. Table 6 contains the calibration curve data, and Figure 13 depicts the calibration curve.

Drug -excipient compatibility study

Drug Polymer Compatibility Studies Using FTIR: The physical and chemical interactions that occur between drugs and excipients have been studied using Fourier transform infrared spectroscopy. In this study, different IR grades of KBr were used to combine pure Sitagliptin with Excipients. Following that, the resulting mixtures were scanned using FTIR equipment in the 400-4500 cm-1 spectral range (Alpha, Bruckerpvt ltd, Japan). The amide group, alcohol group, and stretching vibrations of C-H, Ar-O-CH, C=C, and C-O-C bonds are responsible for the peaks observed in the drug's spectrum. The analysis of the infrared spectra of a combination of pharmaceutical substance and pure pharmaceutical substance revealed negligible or minimal changes in the primary peaks associated with the drug. The FTIR investigation results, as shown in Figures 14 and 15, show that there are no physical or chemical interactions between Sitagliptin and the excipient.

Pre formulation study results: In the current research. Pre-compression tests were performed on all nine formulations, including measurements of the angle of repose, bulk and tapped density, compressibility index, and Hauser's ratio. Table 7 shows the results of the pre-compression evaluation. All formulations had an angle of repose ranging between 29.020 and 36.340 degrees, indicating good flow characteristics. The Carr's index of the powders in all formulations ranged from 10.07 to 19.62 percent.

Post compression results: Table 8 shows the results of the physical examination of the tablets. The hardness of the tablets from different batches was consistent, ranging from 4.93 to 5.83 kg/cm2. Friability is an additional parameter used to assess a tablet's durability or strength. The friability test results were found to be within the specified limit.

The weight variation test was used to determine whether or not tablet formulations met the specifications outlined in the Indian Pharmacopoeia (I.P.). The pharmacopoeia limit for percentage deviation for tablets weighing more than 200 mg is 5%. All of the formulations examined in this study met the weight variation test requirements specified in the I.P. The formulations had a high level of drug content uniformity, with a drug content percentage that exceeded 95%. All of the tablet formulations demonstrated acceptable Pharmaco-technical properties.

Effect of Formulation Variable on Drug Release at 2hr (Y1).

A three-dimensional surface graphic was used to help understand the relationship between the formulation variables (X1 and X2) and Y1. Figure 4(b) shows that NaCl (X1) had a greater osmotic impact on drug release, whereas SLS (X2) had a more pronounced effect.

Effect of Formulation Variable on Drug Release at 6 hr. (Y2).

Based on the analysis of the three-dimensional surface plot, it is clear that the use of SLS (X2) had a more pronounced effect on the formation of pores, influencing drug release. The use of NaCI (X1), on the other hand, had no effect on the drug's release.

Effect of Formulation Variable on Drug Release at 12 hr. (Y3).

In comparison to SLS, the factor NaCl (X1) has a more pronounced effect on drug release (X2).

Effect of pH on Drug Release.

In vitro release assays in buffers with varying pH levels and distilled water revealed no significant changes in the release profiles of formulation F9. As a result, variations in fluid levels across different segments of the gastrointestinal tract have no effect on the osmotic system's drug release capacity.

Effect of Agitation Intensity on Drug Release. The study's findings show that changes in agitation intensity had no discernible effect on the release profile of Sitagliptin from the optimized formulation F9.

Stability Study

Following a one-month storage period, the formulation's initial hardness, diameter, thickness, percent drug content, and friability values were found to be nearly indistinguishable. The drug profile matched the initial profile, as expected. There was no discernible change in either the value or the physical appearance. Given the formulation's stability, this statement can be asserted.

Conclusion

The study aimed to identify optimal compositions for the manufacturing of porous osmotic pump tablets containing Sitagliptin, serving as a typical medication model. The osmotic agent utilized in the experiment was sodium chloride, with a concentration of 30mg. The pore-forming agent employed was sodium lauryl-sulfate, also at a concentration of 30mg. Additionally, a coating agent consisting of cellulose acetate was applied, with a concentration of 2 percent. The experimental findings closely aligned with the anticipated values, suggesting that the formulations were effectively adjusted. This observation provides evidence for the effectiveness of the optimization process in the development of these tablets. After careful evaluation, it was concluded that Batch F9 had superior performance compared to other batches. The results of the study indicate that the formulation optimization process yielded steady outcomes, suggesting that the production of porous osmotic pump tablets for diabetic drugs was successful.

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