IJBPAS, August, 2021, 10(8): 2766-2782

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

FORMULATION DEVELOPMENT AND EVALUATION OF GASTRO RETENTIVE FLOATING BIOADHESIVE BEADS FOR ANTI-DIABETIC AGENT

PATHAN VT[*1], GULECHA VS[1], ZALTE AG[1] AND JADHAV AG[2]

1: School of Pharmaceutical Sciences, Sandip University, Nashik, India

2: Sandip Foundations, Sandip Institute of Pharmaceutical Sciences, Nashik, India

***Corresponding Author: Pathan VT: E Mail: vasimpathan.256@gmail.com**

Received 7th Sept. 2020; Revised 10th Oct. 2020; Accepted 19th Nov. 2020; Available online 1st Aug. 2021

https://doi.org/10.31032/IJBPAS/2021/10.8.5590

ABSTRACT

Background/Aim: The purpose of the study was to prolong the gastric residence time of vildagliptin by designing its gastro-retentive floating Bioadhesive beads.

Materials and Methods: Vildagliptin bio-adhesive and/or floating beads were prepared by using magnetic stirrer successfully. Preliminary trial batches were formulated successfully with varying quantity of sodium alginate, 3 preliminary batches were formulated and further evaluated. All the three preliminary batches characterized for important parameters including external Appearance and Size Uniformity Studies, Swelling Index Studies, Entrapment Efficiency Studies, In Vitro Vildagliptin Release Studies, Scanning Electron Microscopy Studies: etc.

Results: On the basis of preliminary batches, the F1 batch giving more drug release. So that the F1 batch is used for DoE process. Formulation F5 was selected as an optimum formulation. Results showed that F5 batch of vildagliptin beads containing maximum amount (gave 97.89 ± 1.35) of both variables released for the period of 10 hrs and was falling in the yellow region of overlay plot. Percentage Entrapment Efficiency was found to be in the range of 71.71± 1.20, Estimation of Vildagliptin was found to be in range of 93.03% to 99.12% , buoyancy time was 22 ± 1.45 hours etc. Hence, vildagliptin was considered as optimized gastro-retentive bio-adhesive Beads.

Conclusion: It can be concluded from this study that the prepared Gastro-retentive beads could reduce the dosage frequency, dose related side effects, and improve the bioavailability.

Key words: Vildagliptin, Floating Beads, Design Expert, Gastro-Retentive Drug Delivery System, Sodium Alginate

INTRODUCTION

The GIT absorption is a critical procedure and is reliant on various factors. Usually, it is recognized that the level of GIT drug absorption is identified when comes closer to the small intestinal mucosa. Therefore, small intestine transport duration from the intestine is a significant variable for those drugs which get absorbed moderately. Gastro-retentive deliveries can stay inside the area of GI for less duration (minutes) and thus fundamentally extend the drug residence in GIT. Extended gastric residence progresses bioavailability, diminishes misuses of a drug, and enhances drug solubility with poor solubility in a basic (more than 7) pH condition. Local medication at the abdominal and small intestine is a significant application of it. Gastro retention assists with better accessibility of novel materials with their therapeutic outcomes and significant advantages. The retention of controlled gastric solid dosages might be accomplished due to muco-adhesion, buoyancy, sedimentation, increase adjusted shape arrangements, or since the synchronous organization of pharmacologists who postpone gastric emptying by removing the food present in the stomach which gain significant importance **[1, 2]**.

Oral delivery is a popular and helpful route for different medications. Oral delivery is supposed as a perfect route with two principal properties: it is ought to be in a single dosage for longer achievement, it must be conveying API at the objective location. Such contemplations prompted the advancement of a controlled/sustained drug delivery. This system depicts an extended-release drug delivery. The fundamental reason for such arrangements is to improve the security of an item to broaden its action. Some of the disadvantages of these systems, for example, delayed therapeutic blood ranges, differences in bioavailability, improved first-pass effect, and dose clearance. The above systems are typically costly comparing with the traditional systems. As these materials are composed for the greater people, and not for a person, they can have an increased or decreased consistent stage drug level in various people. On the occasion that the therapeutic range of

medication is sufficiently wide, it can't create any issue. Regardless of their drawbacks, an examination is preceded right now; more extension to additionally enhance at presently accessible frameworks. Oral controlled release drug delivery systems (OCRDDS) retaining for a more drawn out duration in the stomach have several benefits in comparison with a formulation that gives sustained release. This system releases the drug in a controlled and extended way giving consistent supply to the absorption site of upper GIT which is one of the very important applications of this system over others **[3-5]**.

Diabetes mellitus is a group of metabolic diseases characterized by hyper-glycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyper-glycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the -cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyper-glycemia.

Symptoms of marked hyper-glycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyper-glycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyper-glycemia with ketoacidosis or the nonketotic hyperosmolar syndrome **[6]**.

Vildagliptin is a novel antidiabetic agent. It belongs to the dipeptidyl peptidase IV (DPP-4) inhibitors. It acts on the incretin system. An incretin hormone Glucagon-like peptide 1 (GLP-1) is released in the gut wall after food ingestion from the L-cells. This hormone inhibits glucagon secretion and stimulates insulin secretion and rapidly eliminated by DPP-4 **(Figure 1)**.

Vildagliptin inhibits DPP-4 therefore results in increased GLP-1 concentrations and decreased glucose concentrations. This drug is a potent and selective inhibitor of dipeptidyl peptidase-IV (DPP-4). It is an orally active drug and improves glycaemic control in patients with type 2 diabetes (T2DM) by increasing pancreatic (α and β) islet function **[7]**.

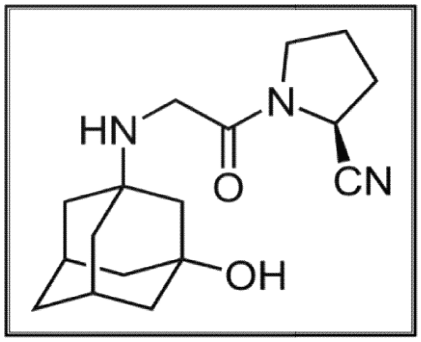

Figure 1: Structure of Vildagliptin

Alginate is an unbranched binary copolymer constituted of (1, 4) linked a-Lguluronic acid and b-D-mannuronic acid. It is a high-molecular mass polysaccharide extracted from kelp. Edward Stanford discovered alginate in 1883. Alginate is abundantly available in nature. It can be found as a structural component (cell wall and intercellular regions) of marine brown algae such as Macrocystispyrifera, Asco phyllumnodosum, and Laminariahyperborea from which it is generally extracted. To extract alginate, usually dried milled seaweed is macerated with a dilute sodium carbonate solution, and the resulting pasty mass is diluted with sufficient soft water to make practicable the separation of insoluble matter. Soft water is essential to avoid the precipitation of insoluble alginates **[8]**.

MATERIALS & METHODS

The drug (Vildagliptin), Sodium bicarbonate, $Cacl_2$, Caco3&other reagents are of analytical or pharmaceutical grade and deionized water was obtained by reverse osmosis. All other chemicals used in the studies were of analytical grade.

Preliminary study:

Fourier Transforms Infrared Spectroscopy (FT-IR)

The drug and polymer interactions were studied by Fourier Transform Infrared Spectroscopy by KBr disc method. FTIR spectra help to confirm the identity of the drug and to detect the interaction of the drug with the excipients **(Figure 2, 3)**.

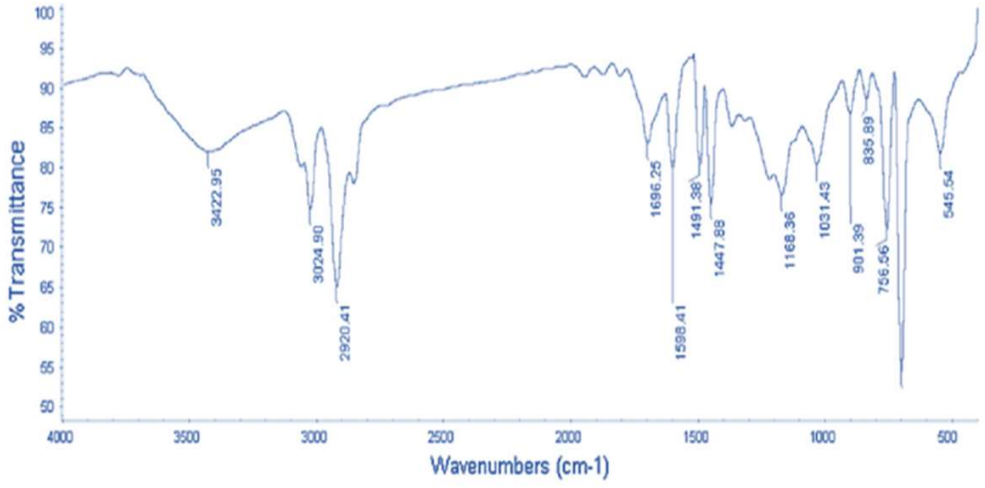

Figure 2: FTIR Spectra of API Vildagliptin

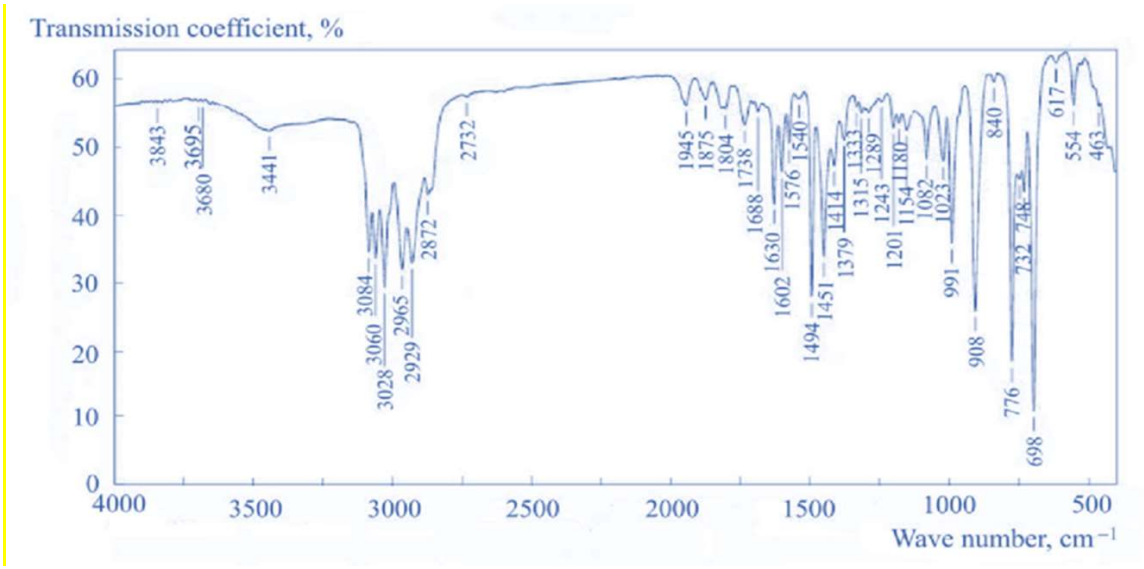

Figure 3: FTIR Spectra of Vildagliptin and Excipients (Sodium alginate, $CaCl_2$ solution, $CaCO_3$)

Table 1: Functional groups and wave numbers observed in FTIR for beads

Sr. No	Bond	Frequency cm^{-1}	Functional group	Pure Drug (Vildagliptin)	Functional group in the formulation
1	=CH(Stretching)	3200-3500	Alcohol	3294.91	3441
2	O-H	3650-3590	Carboxylic Acid	3650-3590	3680
3	C-H	920-675	Aromatics	917.5	776
4	C=O(Stretching)	1680-1620	Alkenyl	1639.53	1602

The FTIR spectra of physical mixture of Vildagliptin and Sodium alginate, $CaCl_2$ solution, $CaCO_3$ showed presence of characteristic peaks at 3441cm^{-1}, 3680cm^{-1}, 776cm^{-1}, 1602cm^{-1}, which indicated no interaction between the drug and excipients when physically mixed in the ratio of 1:1 (drug to polymer).

Drug-Polymer Compatibility Study by Differential Scanning Calorimetry (DSC):

The DSC measurements were performed on DSC-60 (SHIMADZU) differential scanning calorimeter with thermal analyzer (TA-60WS). Pure Vildagliptin sample of 7.8 mg was placed in aluminium pan and sealed before heating under nitrogen flow (300 ml/min) at a scanning rate of 10°C min^{-1} from 30°C to 550°C. An empty aluminium pan was used as reference.

Formulation of Preliminary Batches of vildagliptin beads:

Sodium alginate solutions of different concentrations were prepared by dissolving required amount of alginate in 100 ml of deionized water under gentle agitation. Vildagliptin and calcium carbonate (as gas forming agent) were dispersed in alginate solution under constant stirring for uniform

mixing. The dispersion was sonicated for 30 minutes to remove any air bubbles. The resultant dispersion was dropped through a 22 gauge syringe needle into 100 ml of 1% (w/v) calcium chloride solution containing 10% (v/v) acetic acid at room temperature. Then the beads formed were allowed to remain in the stirred solution for 10 min. The beads were filtered and subsequently oven-dried at 50ºC for 4 hour **[18] (Table 2)**.

Evaluation of preliminary batches of Floating-Bioadhesive Beads:

All the three preliminary batches characterized for important parameters includingexternal appearance and Size Uniformity Studies, Swelling Index Studies, Entrapment Efficiency Studies, In Vitro Vildagliptin Release Studies, Scanning Electron Microscopy Studies: etc. and results are as discussed in **Table 3, 4 and Figure 4**.

Factorial Batches:

Selection of Suitable Design of Experiment (Optimization by 2^3 full factorial design):

A 2^3 randomized full factorial design was used in the present study. In this design, three independent factors were evaluated, each at two levels, and experimental trials were performed for all eight possible combinations. The concentrations of Sodium alginate (X_1), $CaCO_3$ (X_2), and $CaCl_2$(X_3) were chosen as independent variables in 2^3 full factorial design **(Table 5)**.

Evaluation of Factorial Batches of Floating Bio-adhesive Beads:

1. **Entrapment Efficiency Studies of Factorial Batches of Floating Bio-adhesive Beads:**

Percentage drug content in formulation F1-F8 was found to be in range between 54.46±1.23, 72.98±0.98which showed in below **Table 6**. It showed uniform dispersion of drug in polymer system.

2. In Vitro Vildagliptin Release Studies Factorial Batches of Floating Bio-adhesive Beads: [21]

The dissolution conditions used for studying the drug release from bio-adhesive vildagliptin tablet are: Apparatus: USP Type 2 (paddle) Agitation speed (rpm): 50 Medium: Buffer pH 1.2 for 0 to 2 hours and Buffer pH 7.4 for 2 to 8 hours for 900 ml. Temperature: 37.0 ± 0.5 ^{0}C Time: 1, 2, 3, 4, 5, 6, 7, and 8hr Wavelength: 244 nm **(Table 7)**.

Optimization of Final Batch:

From the above results, it is clear that batch number F5 showed the best results, thus optimized for the further formulation. The optimization was performed based on reaction modelling on surface by utilizing the graphical and numerical advancement

technique. Attractive quality is a target work that ranges from zero outside of the cut-off focuses to one at objective.

Evaluation of Optimized Batch:

The optimized batch was evaluated for all important parameters, effect of important DOE variables: % drug entrapment and % Drug release by TWO WAY ANOVA, and the effect of other parameters, and results obtained are shown in **Table 8**.

Effect of DOE variables on the formulation [9] (Table 9).

A)) % Drug Release model

2FI Kinetic Model is Follow % Drug Release

ANOVA analysis of the coefficients of the polynomial equations and the F- value 30.05 implied that the quadratic model is significant.

Y1=+55.09+3.24A+1.54B+11.87C+1.28AB-2.70AC+4.33BC+0.6546 A2-1.46 B2+7.02 C2 **(Figure 5, 6)**.

B) % Entrapment Efficiency:

Model of linear kinetic followed by % Entrapment Efficiency

Coefficients of polynomial equations (eq2) and the model F- value of 45.99 implies model is significant.

Y2=+304.96+0.6863 A+ 4.35 B+2.10 C-2.38 AB-1.63AC-1.13BC-3.86A2 -0.1983 B2+3.20C2 **(Figure 7, 8)**.

External Appearance and Size Uniformity Studies of sodium alginate bead of Vildagliptin:

Size uniformity of vildagliptin beads and external appearance was done by optical microscope.

The mean diameter of 50 dried beads was determined by optical microscopy. The microscope eyepiece was fitted with a micrometer by which the size of the beads could be determined **[21]**

Swelling Index Studies of sodium alginate bead of Vildagliptin:

Swelling index of optimized formulation F5 was carried out in 2 different medias. 0.1N HCl pH 1.2 and phosphate buffer pH7.4.When compared with phosphate buffer swelling index of sodium alginate beads containing Vildagliptin was lower in acidic medium. This is because of the shrinkage of alginate at acidic medium **[22] (Table 10)**.

Scanning Electron Microscopy Studies of sodium alginate bead of Vildagliptin [23]

The SEM photograph of the surface of the sodium alginate bead of Vildagliptin exhibited very hard surface with characteristic massive wrinkles and cracks. These cracks and wrinkles may be caused via partly collapsing the polymeric gel network during drying. Due to the migration of beads

along with water to the surface during drying will result in the formation of drug crystals **(Photo 1)**.

Estimation of Vildagliptin of sodium alginate bead of Vildagliptin [24]

The Estimation of Vildagliptin was found to be in range of 93.03% to 99.12% indicated that the drug was uniformly dispersed in the sodium alginate bead of Vildagliptin

Entrapment Efficiency Studies of sodium alginate bead of Vildagliptin:

Percentage Entrapment Efficiency in optimized batch F5 was found to be in the range of 71.71±

1.20. It showed good entrapment efficiency in the sodium alginate bead of Vildagliptin.

In Vitro Vildagliptin Release Studies of sodium alginate bead of Vildagliptin [19] (Table 11, Figure 9).

X-ray Photographic Studies in Rabbits of sodium alginate bead of Vildagliptin: [10-14] (Figure 10).

The in vivo (X-Ray) evaluation showed X-ray before administration (0 hour) of sodium alginate bead of Vildagliptin. Beads can be seen in the stomach. Next image, taken at 1 hour shows change in position of beads; this shows that floating tablet did not adhere to gastric mucous. Next image, **Figure 10, 11**, taken at 2 h and 4 h after administration of beads showed that beads were still found to be buoyant on gastric content, respectively.

Accelerated Stability Studies of sodium alginate bead of Vildagliptin:

Stability protocol

Packaging material – Tablets wrapped inside foil of aluminium.

Storage condition – Exposed the tablets for stability according to conditions of ICH given in the following table. Kept the samples in the chamber of stability (Thermo lab, TH 200S).

From the results of dissolution studies after 0 and 4^{th} week of the stability studies in **Table 13, Figure 12**. It is clearly seen that no difference was seen in drug release profile of the formulation. Thus, it can be stated as a stable batch after 4 weeks.

Table 2: Composition of preliminary batches of floating Vildagliptin alginate beads

Formulation Ingredients	F1	F2	F3
Sodium alginate (gm)	2.0	2.5	3.0
Vildagliptin (gm)	1.0	1.0	1.0
$CaCO_3$(gm)	1.5	1.5	1.5
$CaCl_2$ solution (%)	1.0	1.0	1.0

Table 3: Entrapment Efficiency Studies of Floating Bio-adhesive Beads [19]

Sr. No.	Batch code	% Entrapment Efficiency
1	F1	73.55±0.67
2	F2	70.88±0.78
3	F3	71.11±0.54

Table 4: In Vitro Vildagliptin Release Studies of Floating-Bioadhesive Beads [20]

Time (hr.)	Percentage cumulative drug release		
	F1	F2	F3
0	0.0±0.0	0.0±0.0	0.0±0.0
1	20.43±0.18	20.45±0.15	22.91±6.83
2	31.34±0.16	32.90±0.56	45.34±0.33
3	43.43±0.15	49.04±0.56	50.34±0.45
4	58.98±0.21	55.23±1.23	55.23±1.50
5	68.49±0.43	65.35±0.10	68.23±2.75
6	78.33±0.45	73.45±0.23	77.12±2.61
8	88.23±0.76	84.04±0.21	88.54±0.34
10	96.25±2.35	92.42±3.23	90.56±1.41

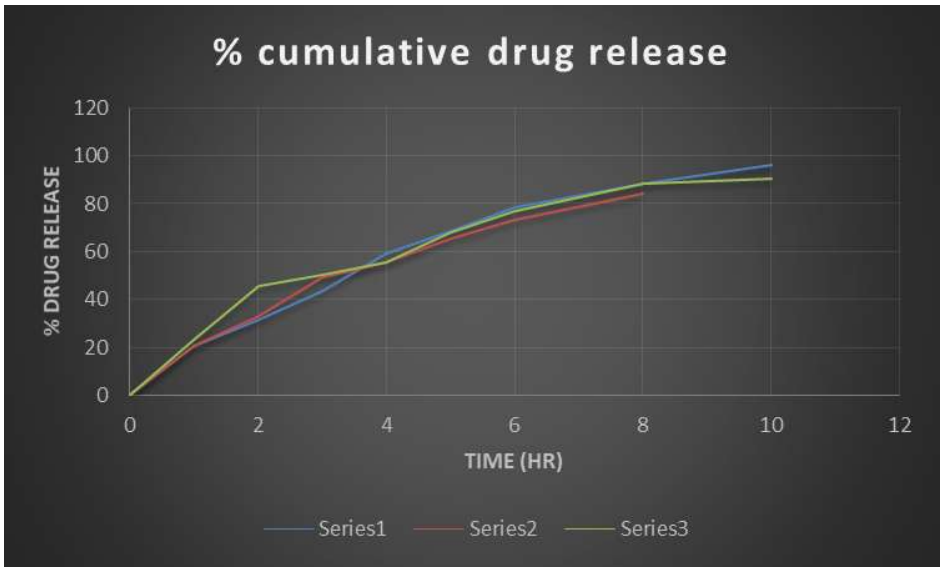

Figure 4: Determination of Percentage cumulative drug release of the preliminary batches of Floating Bio-adhesive Beads

Table 5: Design expert software actual design of Floating Bio-adhesive Beads

Std Run	A: Sodium alginate	B:$CaCO_3$ (mg)	C:$CaCl_{2\,3}$	Response 1: % Drug Release	Response 2: % Entrapment Efficiency
1	2	1	0.5	86.32±2.34	65.23±0.34
2	2	1.5	0.5	90.45±0.24	70.34±1.23
3	2.5	1	0.5	84.37±1.33	66.23±0.43
4	2.5	1.5	0.5	88.6±2.45	69.45±0.83
5	2	1.5	1	99.45±1.98	72.98±0.98
6	2.5	1	1	80.174±2.34	69.78±2.8
7	3	1	1	85.56±1.22	54.46±1.23
8	3	1.5	1	81.45±0.34	59.34±3.25

Table 6: Percentage Entrapment Efficiency of Factorial Batches of Floating Bio-adhesive Beads

Sr. No.	Batch code	% Entrapment Efficiency
1	F1	65.23±0.34
2	F2	70.34±1.23
3	F3	66.23±0.43
4	F4	69.45±0.83
5	F5	72.98±0.98
6	F6	69.78±2.8
7	F7	54.46±1.23
8	F8	59.34±3.25

Table 7: Percentage cumulative drug release from factorial batches of Floating-Bio-adhesive Beads

Time(hr.)	Formulations (mean ± SD)							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0.0 ±00	0.0 ±00	0.0 ±00	0.0 ±00	0.0 ±00	0.0 ±00	0.0 ±00	0.0 ±00
1	18.17 ±1.03	23.65 ±0.87	24.74 ±1.24	25.51 ±1.57	22.58 ±0.18	18.72 ±0.84	22.00 ±0.86	19.56 ±0.84
2	26.04 ±1.89	33.45 ±1.08	39.29 ±1.12	34.51 ±1.09	37.05 ±0.58	26.96 ±0.25	36.91 ±0.58	29.17 ±1.55
3	37.34 ±0.95	34.48 ±0.93	43.42 ±0.96	42.56 ±1.099	48.05 ±0.587	36.94 ±0.25	46.917 ±0.589	34.72 ±0.666
4	55.24 ±0.789	52.48 ±0.933	43.424 ±0.965	45.43 ±1.359	58.08 ±0.344	46.18 ±1.02	52.06 ±1.024	42.63 ±0.479
5	68.31 ±0.867	63.94 ±0.795	58.99 ±0.687	59.09 ±2.011	65.29 ±0.988	54.26 ±0.521	61.98 ±0.279	55.93 ±0.589
6	72.00 ±0.85	73.85 ±0.52	69.85 ±1.65	63.8 ±0.98	72.75 ±0.78	60.51 ±0.15	72.77 ±0.45	64.80 ±0.61
7	7988 ±0.76	84.38 ±1.07	76.076 ±0.42	78.915 ±1.842	88.81 ±0.555	69.602 ±1.089	79.120 ±1.087	73.907 ±0.357
8	86.32 ±2.34	90.45 ±0.24	84.37 ±1.33	88.6 ±2.45	99.45 ±1.98	80.174 ±2.34	85.56 ±1.22	81.45 ±0.34

Table 8: Two way ANOVA for % Drug Release

Source of Variation	Sum of squares	Degree of Freedom	Mean square	F ratio
Model	1063.3	2	489.56	155.57
Sodium alginate	445.45	1	456.78	145.12
$CaCO_3$	530.45	1	4650.67	134.02
Residual	20.49	6	3.48	---
Source of Variation	P value summary		Significant.	
Sodium alginate	*** (P<0.0001)		Yes	
$CaCO_3$	*(P<0.0001)		Yes	

Table 9: Two ways ANOVA for % Drug Entrapment

Source of variation	Sum of squares	Degree of freedom	Mean square	F ratio
Model	28182	3	8584.25	68.01
Sodium alginate	22555	1	33545	167.5
$CaCO_3$	3367	1	3187	29.88
AB	1240.5	1	1260.5	9.69
Residual	602.25	5	120.45	-
Source of Variation	P value summary		Significant?	
Model	*** (P=0.0002)		Yes	
Sodium alginate	*** (P<0.0001)		Yes	
$CaCO_3$	*** (P=0.0028)		Yes	
AB	*** (P=0.0265)		Yes	

Sodium alginate and $CaCO_3$ impact on SI was observed highly significant.

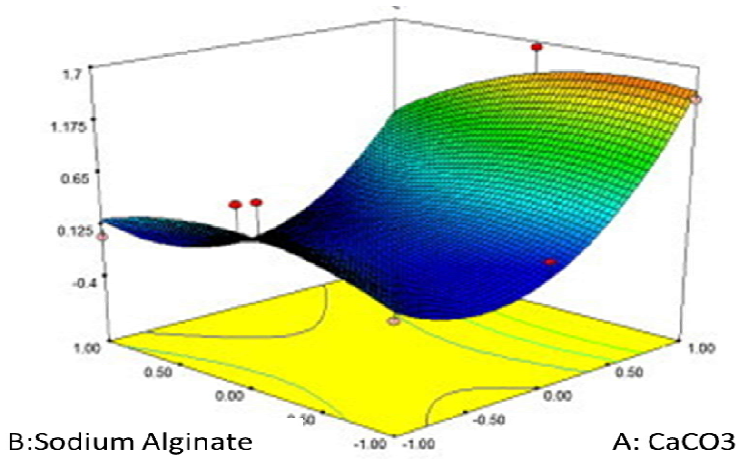

Figure 5: 3-D Response annova plot of % Drug Release of Vildagliptin beads of Optimized batch

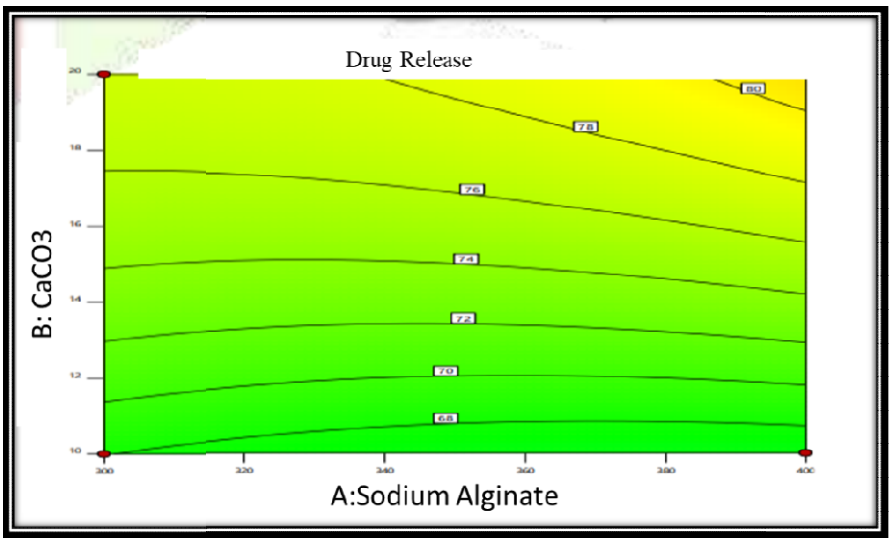

Figure 6: Counter Plot of % Drug Release of Vildagliptin beads of Optimized batch

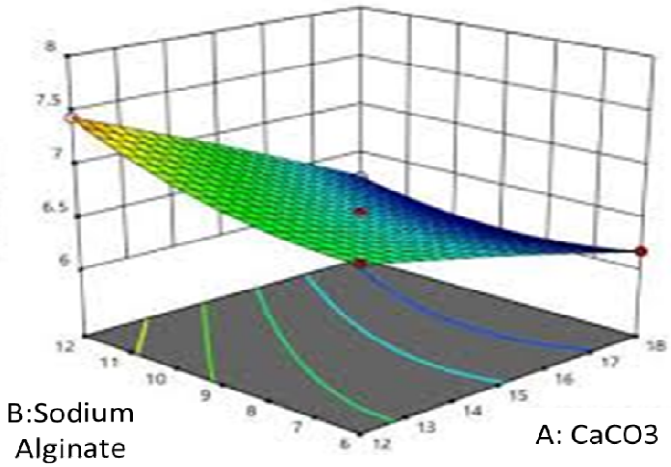

Figure 7: 3-D Response plot of % Entrapment Efficiency of Vildagliptin beads of Optimized batch

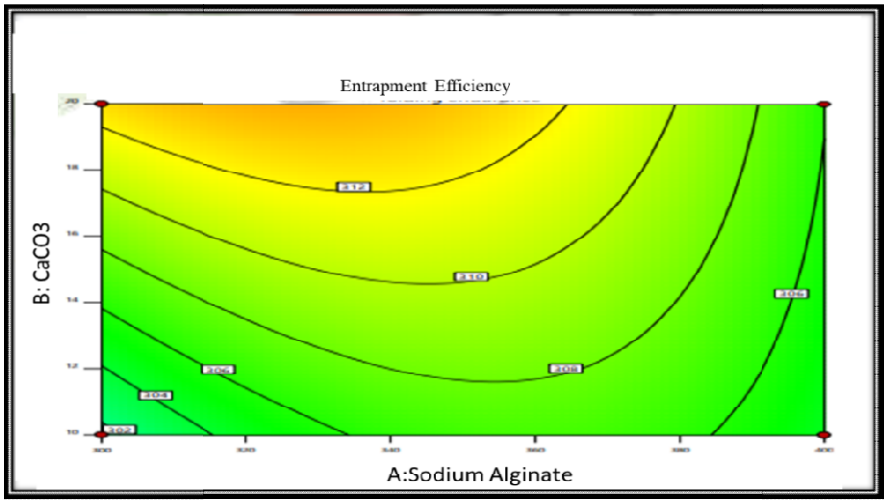

Figure 8: Counter Plot of % Entrapment Efficiency of Vildagliptin beads of Optimized batch

Table 10: Determination of % swelling index of optimized batch of sodium alginate bead of Vildagliptin

Sr. No.	Percentage swelling index			
	4 hr.	8 hr.	16 hr.	24 hr.
1.	224.17±1.1	345.45±0.29	446.31±1.57	536.24±2.21

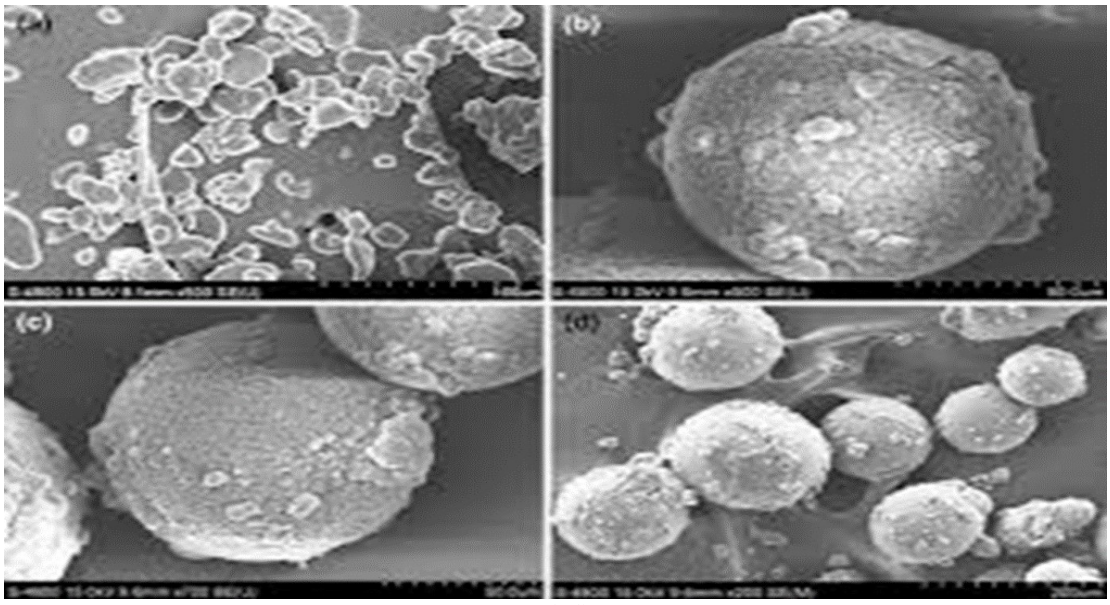

Photo 1: SEM

Table 11: Cumulative drug release of optimized batch of sodium alginate bead of Vildagliptin

Sr. No.	Time in hours	% rug release
1	0	0.00± 0.00
2	1	10.45±1.87
3	2	20.45± 1.22
4	3	32.51± 0.34
5	4	45.34± 1.34
6	5	56.87± 0.97
7	6	69.32± 1.56
8	8	82.47± 3.96
10	10	97.89 ±1.35

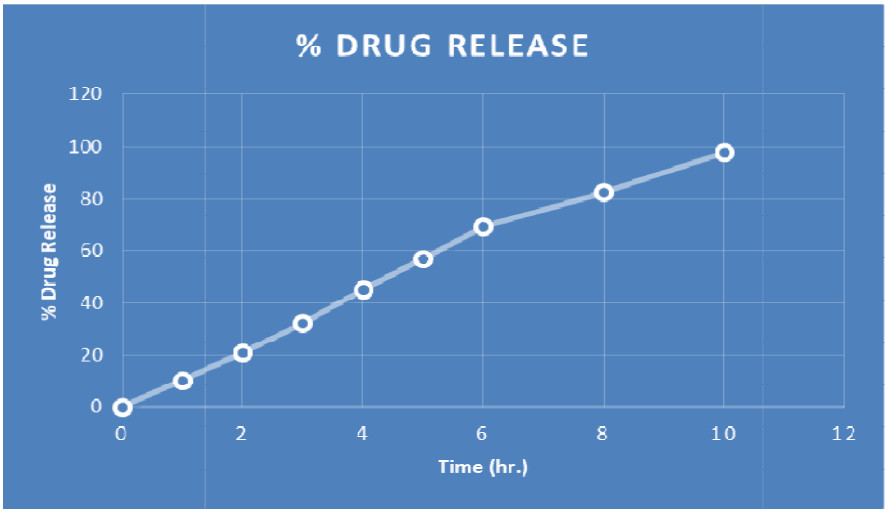

Figure 9: Dissolution profile of optimized batch of sodium alginate bead of Vildagliptin

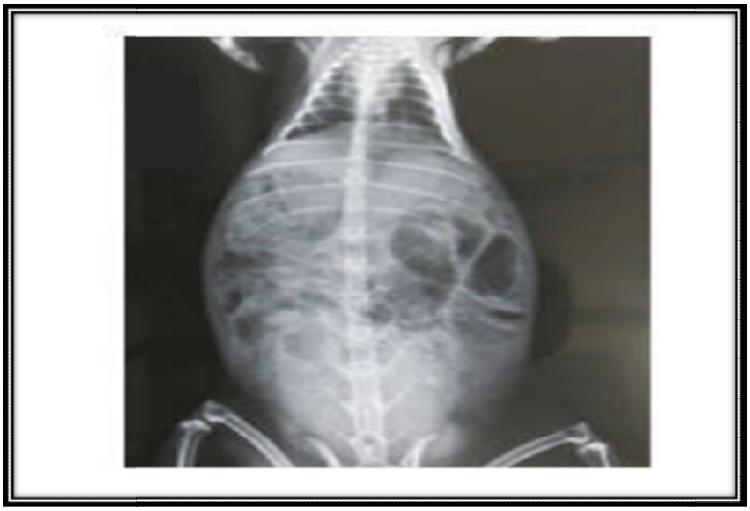

Figure 10: X-ray photograph of rabbit before treatment (0 hour, before administration of beads) from abdomen portion (Control)

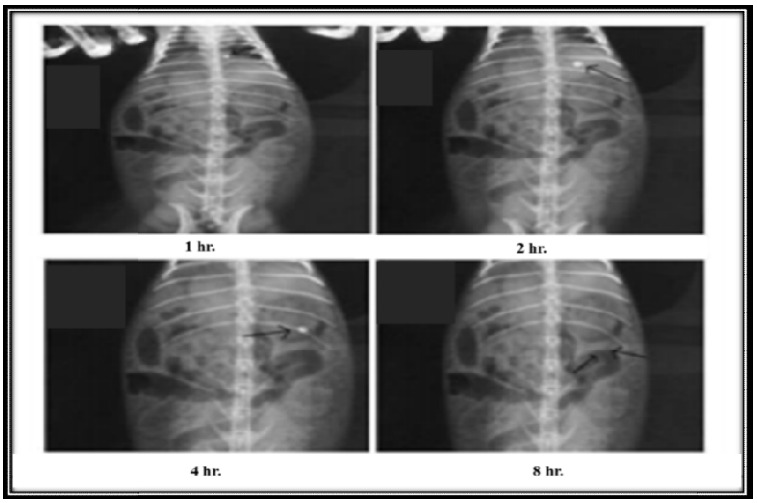

Figure 11: X-ray photograph of rabbit of sodium alginate bead of Vildagliptin After 1 hr., 2 hr., 4 hr., 8 hr

Table No.12: Storage conditions for the Stability study [15-17]

Description	Storage conditions
Accelerated testing	40°C / 75 % RH

Table 13: *In vitro* drug release study of sodium alginate bead of Vildagliptin kept for stability at 40^0C /75%RH

Time(h)	0 Week	4 Week
0	0.00± 0.00	0.0±000
1	10.45±1.87	12.98± 0.19
2	20.45± 1.22	19.40± 0.56
4	32.51± 0.34	30.99± 0.20
5	45.34± 1.34	44.86± 0.56
6	56.87± 0.97	58.29± 0.67
7	69.32± 1.56	68.5± 0.76
8	82.47± 3.96	81.52± 0.52
10	97.89 ±1.35	96.45±1.54

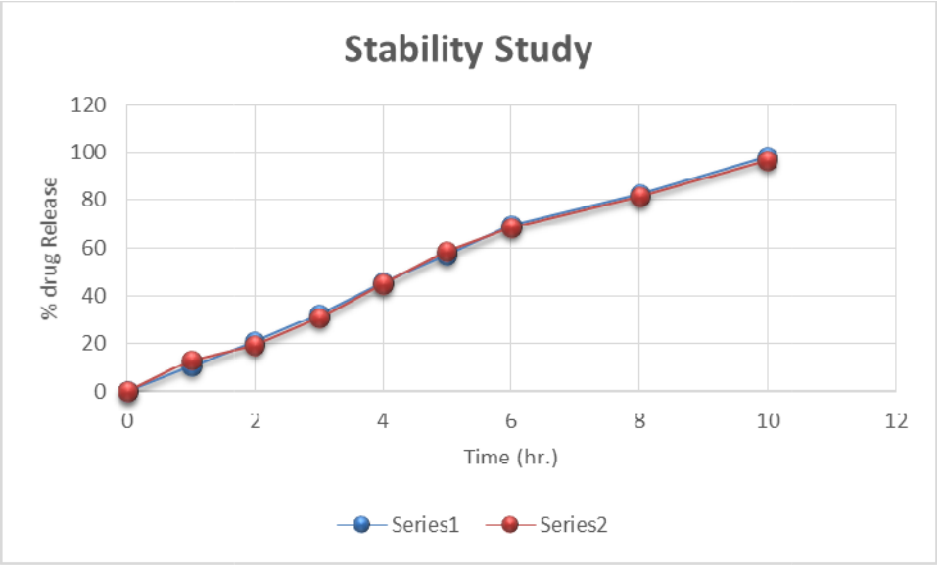

Figure 12: Dissolution study of optimized batch of sodium alginate bead of Vildagliptin before after 0 and 4 week time interval

REFERENCES

[1] Mayavanshi AV, Gajjar SS. Floating drug delivery systems to increase gastric retention of drugs: A Review. Research Journal of Pharmacy and Technology. 2008 Oct; 1(4): 345-8.

[2] Hirtz J. The gastrointestinal absorption of drugs in man: a review of current concepts and methods of investigation. British journal of clinical pharmacology. 1985 Apr; 19(S2): 77S-83S.

[3] Sugito K, Ogata H, Goto H, Noguchi M, Kogure T, Takano M, Maruyama Y, Sasaki Y. Gastrointestinal transit of non-disintegrating solid formulations in humans. International

journal of pharmaceutics. 1990 Apr 30; 60(2): 89-97.

[4] Gad SC, Gad-McDonald S. Biomaterials, medical devices, and combination products: Biocom-patibility testing and safety assessment. CRC Press; 2015 Dec 1.

[5] Simler R, Walsh G, Mattaliano R, Guziewicz N, Perez-Ramirez B. Maximizing data collection and analysis during preformulation of biotherapeutic proteins. BioProcess Int. 2008 Nov; 6(10).

[6] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care. 2009 Jan 1; 32(Supplement 1):S62-7.

[7] Halimi S, Schweizer A, Minic B, Foley J, Dejager S. Combination treatment in the management of type 2 diabetes: focus on vildagliptin and metformin as a single tablet. Vascular health and risk management. 2008 Jun; 4(3): 481.

[8] Shilpa A, Agrawal SS, Ray AR. Controlled delivery of drugs from alginate matrix. Journal of Macromolecular Science, Part C: Polymer Reviews. 2003 Jan 6; 43(2): 187-221.

[9] Jain A, Jain SK. Formulation and optimization of temozolomide nanoparticles by 3 factor 2 level factorial design. Biomatter. 2013 Apr 1; 3(2): e25102.

[10] Zhang X, Zhang Y, Han H, Yang J, Xu B, Wang B, Zhang T. Formulation optimization of gastro-retention tablets of paeonol and efficacy in treatment of experimental gastric ulcer. Chemical and Pharmaceutical Bulletin. 2017: c16-00993.

[11] Ahmed SM, Ali AA, Ali AM, Hassan OA. Design and in vitro/in vivo evaluation of sustained-release floating tablets of itopride hydrochloride. Drug design, development and therapy. 2016; 10: 4061.

[12] Huang H, Wu Z, Qi X, Zhang H, Chen Q, Xing J, Chen H, Rui Y. Compression-coated tablets of glipizide using hydroxypropylcellulose for zero-order release: in vitro and in vivo evaluation. International journal of pharmaceutics. 2013 Mar 25; 446 (1-2): 211-8.

[13] Dey SK, De PK, De A, Ojha S, De R, Mukhopadhyay AK, Samanta A.

Floating mucoadhesive alginate beads of amoxicillin trihydrate: A facile approach for H. pylori eradication. International journal of biological macromolecules. 2016 Aug 1; 89: 622-31.

[14] Ananthakumar R, Chitra K, Kumar SS. Formulation, in vitro and in vivo X-ray study of gastroretentive drug delivery system of risedronate sodium tablet using natural polymer guar gum. Drug Invention Today. 2019 Nov 1; 11(11).

[15] Mazzo DJ, editor. International stability testing. CRC Press; 2020 Aug 26.

[16] Purnima DA, Namita SR. Formulation and Evaluation of Extended Release Nimesulide Tablets. INDIAN DRUGS-BOMBAY-. 2008; 45(2): 105.

[17] Carstensen JT, Rhodes CT. Drug stability, revised, and expanded: principles and practices. CRC press; 2000 Jul 28.

[18] Rasel MA, Hasan M. Formulation and evaluation of floating alginate beads of diclofenac sodium. Dhaka University Journal of Pharmaceutical Sciences. 2012 Nov 4; 11(1): 29-35.

[19] Nagpal M, Maheshwari DK, Rakha P, Dureja H, Goyal S, Dhingra G. Formulation development and evaluation of alginate microspheres of ibuprofen. Journal of Young Pharmacists. 2012 Jan 1; 4(1): 13-6.

[20] Rasel MA, Hasan M. Formulation and evaluation of floating alginate beads of diclofenac sodium. Dhaka University Journal of Pharmaceutical Sciences. 2012 Nov 4; 11(1): 29-35.

[21] Sanap Gajanan S. Formulation and evaluation of mucoadhesive beads of glipizide using 23 factorial design. Journal of Pharmacy Research. 2009 May; 2(5).

[22] Chowdary KP, Sundari GB. Design and Evaluation of mucoadhesive controlled release oral tablets of Glipizide. Indian journal of pharmaceutical sciences. 2003; 65(6): 591.

[23] Ranvirsingh TA, Basavaraj BV, Bharath S, Deveswaran R, Madhavan V. Formulation and Evaluation of Floating Alginate Beads of an Anti Ulcer Drug. International Journal of Pharmaceutical Sciences Review and Research. 2013; 21(2): 120-4.

[24] Mandal S, Kumar SS, Krishnamoorthy B, Basu SK. Development and evaluation of calcium alginate beads prepared by sequential and simultaneous methods. Brazilian Journal of pharmaceutical sciences. 2010 Dec; 46(4): 785-93.