Formulation and in-vitro Evaluation of Vildagliptin Microspheres Using Pectin and Xanthan Gum as Polymers

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Abstract

Aim

The aim of the work is a formulation and in vitro evaluation of the vildagliptin microsphere using pectin and xanthan gum.

Objective

Vildagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor extensively associated with the therapy of type-2 diabetes mellitus. A controlled release of the drug in the gastrointestinal tract may aid in maintaining the therapeutic range for an extended period. Sodium alginate, pectin, and xanthan gum are commonly used as biopolymers in drug delivery with favourable biocompatibility and biodegradation.

Materials and method

Sodium alginate-pectin and sodium alginate-xanthan gum beads were developed to deliver vildagliptin. A calcium chloride ion (cacl2) induced ionic gelation technique was employed for synthesizing pH-sensitive beads by varying the ratio of sodium alginate, pectin, and xanthan gum.

Results

The FTIR investigation verified that drugs and polymers are compatible. The developed beads were evaluated for scanning electron microscopic and DSC study, drug content, swelling ratio, and in-vitro dissolution study. The microscopic images exhibited some are in spherical and semi-spherical shaped beads with cracked and rough surfaces. A pH-dependent swelling was seen, indicating that water intake was restricted in an acidic medium and elevated in an alkaline pH environment. The in-vitro dissolution study demonstrated a controlled release of the drug. optimized formulation Exhibiting diffusion release.

Conclusion

The study reported the successful development of vildagliptin microspheres by using sodium alginate, pectin, and xanthan beads for controlled delivery of Vildagliptin.

INTRODUCTION

Patients previously used conventional dosage forms such as tablets and capsules to treat acute and chronic disorders, however, these conventional dosage forms required many doses each day to maintain peak plasma level concentration. To solve these issues, contemporary and sophisticated techniques

known as controlled drug delivery systems (CDDS) are being developed. The main purpose of a Controlled drug delivery system is to ensure optimum plasma drug concentration, thus enhancing efficacy, safety, and bioavailability of drug with improved patient compliance.

Microspheres are carrier drug delivery systems, Microspheres are spherical, free-flowing,[1] and which are biodegradable or non-biodegradable in nature and have a particle size of 1 μm to 1000 μm. This is an important approach in delivering therapeutic substance to the target site with specificity if modified, and to maintain the desired concentration at the site of interest. Controlled drug delivery can be achieved using microspheres [2].

Sodium alginate is soluble in water and forms a reticulated structure which can be cross-linked with divalent or polyvalent cations to form insoluble meshwork. Calcium and zinc cations have been reported for cross-linking of acid groups of alginates. Pectin is a natural polymer with many applications due to its physicochemical parameters and biodegradability [3]. It is registered in the US Food and Drug Administration (FDA) as an additive with no daily consumption limits and thus can be widely used as an excipient in the formulation of matrix tablets, gels, pharmaceutical coatings, etc. [4]. Xanthan gum (XG) is a high molecular weight, an anionic extracellular polysaccharide that is produced by the gram-negative bacterium *Xanthomonas campestris*. It is widely used in food, cosmetics, and pharmaceuticals because of its encouraging reports on safety [5] It facilitates water retention and produces highly viscous solutions even at low concentrations. Furthermore, it was recently reported that xanthan gum has a potential blood-sugar-lowering and stabilizing effect. This last particular property makes xanthan gum an excellent candidate for the administration of active antidiabetic substances [6, 7]. Given that natural polymers are safe, biocompatible, and biodegradable, their contribution to the formulation of dosage forms is noteworthy.[21, 22]

Vildagliptin is a novel dual-type dipeptidyl peptidase-4 (DPP 4) inhibitor, that efficiently regulates endocrine levels in both hypoglycaemia and hyperglycaemia and can be used both as monotherapy and in grouping treatment for the cure of type 2 diabetes mellitus (T2DM) [8]. VLG is not efficient in providing control over drug delivery with usual oral dosage forms because of the short elimination half-life of 1.6 to 2.5 hand rapid metabolism, causing significant variations of drug levels in plasma. It is suggested that patients having (T2DM) should conform precisely to the dosing time and that vildagliptin should be taken twice daily 50 mg dose [9, 10].

In this present study, a microsphere of vildagliptin was prepared with an optimum concentration of all ingredients and pectin, xanthan gum was used as a matrix-building material in combination with sodium alginate to provide a controlled and prolonged release.

MATERIALS AND METHODS

Materials

Vildagliptin (AET Lab Pvt Ltd), Pectin, Calcium chloride, Potassium dihydrogen phosphate (SDFCL (SD of fine-chem Ltd., Mumbai) Xanthan gum, sodium dihydrogen orthophosphate, Hydrochloric acid (NICE Chemicals (P) Ltd).

Preformulation studies

Determination of λmax

The 10mg drug was weighed and taken in a volumetric flask, dissolved in 10 ml phosphate buffer.1ml sample was withdrawn and made up with 100ml of phosphate buffer to get a 10µg/ml concentration.10µg/ml concentration was scanned by using a double beam U V-visible spectrophotometer in the wavelength range from 200 to 400nm.

Preparation of standard calibration curve [11]

10mg drug was weighed and taken in a volumetric flask, dissolved in 10 m1 of phosphate buffer considered as stock-1, and 1ml of stock-1 solution can be withdrawn and made up with 100ml of phosphate buffer in a volumetric flask and considered as a stock 2 solution. Dilutions were prepared by using phosphate buffer in the range of 1µg-10µg concentration and absorbance was noted in the 210nm wavelength, A calibration curve was prepared by plotting the absorbance against the concentration.

Fourier transform infrared spectroscopy (FTIR) study [11]

The FTIR is a technique used to obtain an infrared spectrum of absorption of target material, that can be used to identify the functional groups and molecular structure. Pure vildagliptin and vildagliptin with a mixture of polymer FTIR spectra were recorded. The required quantity of KBR and vildagliptin and vildagliptin with polymer was mixed. Under hydraulic pressure of 600 kg, the disk was created using approximately 100 mg of the mixture. Then the FTIR spectra were recorded between 4000 and 400 cm^{-1}.

METHODOLOGY

Preparation of vildagliptin microspheres using pectin and xanthan gum, The modified method (composition) is taken from **Sreejan Manna et al**[17], and the $cacl_2$ is used as a cross-linker and the parameters, such as the drying process and stirring speed, differ from it (**Sreejan Manna et al).**

Preparation of sodium alginate-pectin microsphere

Sodium alginate-pectin microspheres were prepared by using the ionotropic gelation method, Sodium alginate, and pectin were weighed the accurate amount according to the composition, and dissolved in 25 ml of distilled water with continued stirring and add the vildagliptin drug into it and stirred for 30 mins with high rotational speed using a mechanical stirrer. Drug-polymer mixture was added dropwise into a $cacl_2$ solution and provided 30 mins for hardening, filtered it and placed for air drying. Different

concentrations (F1, F2, F3, F4, F5, F6, F7) of microspheres are prepared according to the composition table no 1.

Preparation of sodium alginate-xanthan gum microsphere

Sodium alginate-xanthan gum microspheres were prepared by using the ionotropic gelation method, Sodium alginate, and pectin were weighed the accurate amounts according to the composition, and dissolved in 25 ml of distilled water with continuous stirring, and add the vildagliptin drug into it and stirred for 30 mins with high rotational speed using a mechanical stirrer. Drug-polymer mixture was added dropwise into a $cacl_2$ solution and provided 30 mins for hardening, filtered it and placed for air drying. Different concentrations (X1, X2, X3, X4, X5, X6) of microspheres are prepared according to the composition table no 2.

EVALUATION PARAMETERS OF MICROSPHERES

Viscosity

The viscosity of solutions at various concentrations was measured by using a Brookfield viscometer. The tests were performed at room temperature with different spindles s64 and s61 and a 100-rpm shear rate.

Physical appearance

All the prepared microspheres were observed visually for colour and uniformity size.

Percentage yield (%)

The prepared microspheres were weighed by using a weighing balance. weight microspheres were divided by the entire amount of drug and polymer used in the preparation of microspheres [12].

$$\mathbf{percentageyield}\,(\%) = \frac{\mathbf{praticalyield}}{\mathbf{theorticalyield}} \times 100$$

Flow properties

Angle of repose

The flow characteristics are measured by the angle of repose. The angle of repose was determined by using the funnel method. In this method, a funnel was secured with its tip at a 1cm height above the paper that was placed on a flat horizontal surface. Microspheres were carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel. Measure the height of the heap pile and the radius of the base with a ruler. The angle of repose was determined by using the equation [13].

$$\mathbf{tan}\backslash\mathrm{varvec}\theta = \frac{\mathbf{h}}{\mathbf{r}}$$

Where,

h = height of the file

R = radius of the base of the pile

Ø = angle of repose

Bulk density and tapped density:

Accurately weighed 1g of microspheres are poured into a 10ml measuring cylinder and the initial volume was observed and volume is noted and the cylinder was tapped continuously up to 100 times and noted the tapped volume.it is expressed in gm/ml and calculated by using the given formula [14].

$$\mathbf{BD} = \frac{\mathbf{Weightofthepowder}}{\mathbf{Initialvolume}} \mathbf{TD} = \frac{\mathbf{Weightofthepowder}}{\mathbf{Tappedvolume}}$$

Carr's index

The compressibility of the granules is determined by Carr's compressibility index.it is indirectly related to the relative flow rate cohesiveness. it was calculated by using the results of bulk density and tapped density. Calculated by using the given formula [15].

$$\mathbf{Carr'sindex}\,(\%) = \frac{\mathbf{TD} - \mathbf{BD}}{\mathbf{TD}} \times 100$$

Hausner's ratio

Hausner's ratio was related to interparticle friction. It was also calculated by using the results of bulk density and tapped density [16].

$$\mathbf{Hausner'sratio} = \frac{\mathbf{TD}}{\mathbf{BD}}$$

Particle size analysis

Particle sizes of microspheres were determined by optical microscopy. The optical microscope was fitted with an eyepiece micrometer which was then calibrated with a stage microm eter. About 100 microspheres were randomly selected from each formulation and then the average size was calculated.

Swelling index

Weighed the beads 0.1g and placed them into 100ml of PH 1.2 Hcl buffer and followed by a PH 6.8 phosphate buffer solution and allow them to stand for 2 hours and then filtered the solution and weighed the microspheres and calculated by using the equation [17].

$$\mathbf{swellingindex} = \frac{\mathbf{finalweight} - \mathbf{initialweight}}{\mathbf{initialweight}} \times 100$$

Drug content

Weighed the beads equivalent to 50mg 0f the drug, crushed them and mixed them with prepared 100 ml of phosphate buffer with 6.8PH and left them aside for 24 hours. After 24 hours the solution was filtered. The filtrate was subjected to suitable dilution. The filtrate was analyzed by using a UV-visible spectrophotometer at 210 nm [18].

Invitro drug release

To determine the amount of drug released over a period. To identify the drug release in stomach and in the intestine, this is carried out in 0.1N HCL (PH-1.2) and phosphate buffer (PH-6.8), using a USP type II dissolution apparatus (rotating paddle).

Accurately weighed 100mg beads and placed them into a dissolution media containing 900ml of 0.1N HCL (PH-1.2) buffer solution for 2 hours at a controlled temperature of 37 degrees. The speed of rotation was fixed at 100 rpm. At the prefixed time intervals, samples are withdrawn and analysed, and replaced with the same volume in it. After 2 hours the beads in an acidic medium were subjected to filtration and collected then subjected to further dissolution in phosphate buffer solution (PH-6.8). at the prefixed time interval sample was withdrawn and replaced same volume buffer. The solution was subjected to dilution and analysed by using a UV-visible spectrophotometer at 210nm [19].

Mechanism of Release Kinetics

Drug release mechanisms and kinetics are two characteristics of the dosage forms that play an essential role in describing drug dissolution profiles from a dosage form and hence understanding they’re in-vivo performance. The dissolution data obtained are fitted to various kinetic equations such as zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas, and find the R^2 values of the release profile corresponding to each model.

Scanning electron microscope (SEM)

Scanning Electron Microscope was used to determine the Surface nature of microspheres. The microspheres were dried completely before examination and SEM was done at different magnifications [20].

Differential scanning calorimetry (DSC)

The pure drug, and optimized formulation, were subjected to DSC studies by using a differential scanning calorimeter equipped with a computer analyzer. Each sample weighing 1 mg was placed into standard aluminium pans and sealed, and a bare aluminium pan was used as a reference, The Scan was carried

out at a heating rate of 10°C / min under a nitrogen atmosphere over a temperature range of 300C-3000°C [20].

Stability studies

The Stability of a pharmaceutical product may be defined as the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic, and toxicological specifications. The stability of all the formulations was carried out at different temperatures as per ICH guidelines. In the present study, stability studies were carried out for a specific time period of up to 90 days for selected formulations at 40ºC ± 20ºC/75%±5% RH in a humidity control oven. The formulations were packed in butter paper, followed by aluminium foil. After 3 months, the selected formulations were analyzed for their physical parameters like appearances, flow properties, swelling index, and performance parameters like drug content uniformity, and Invitro dissolution study.

Statistical analysis

Experimental data were expressed as the mean value ± S.D. Statistical analysis was performed using the normality test and one-way analysis of variance (ANOVA) test using a graph pad prism (version 5, GraphPad Software, San Diego, California, USA). Differences were considered to be statistically significant when $p < 0.05$ for ANOVA and while normality test is well accepted for the above formulations at a level of significance of 5 percent.

RESULTS AND DISCUSSION

Preformulation studies

Absorbance maxima

The sample was prepared with a phosphate buffer. Using a spectrophotometer, the maximum absorbance of the sample was measured, the vildagliptin spectrum gave the highest peak at 210 nm. indicating the wavelength where it absorbs light most strongly fig no 1.

Standard Calibration Curve

From the Standard Calibration Curve vildagliptin, it was observed that the drug obeys Beer's law in the concentration range of 1–10 µg/ml in Phosphate Buffer (PH6.8) and shows linearity ($R^2 = 0.9902$). The linear Regression equation generated was used for the calculation of the amount of drug. Fig no:2

Fourier-transform infrared spectroscopy (FTIR)

FT-IR spectra of vildagliptin and vildagliptin with Polymers are shown in Figs. 3, 4, and 5. Pure vildagliptin showed principal absorption peaks at 735.67 cm^{-1} (C-H Bending), 1034.69 cm^{-1} (C = O stretching), 1399.5 cm^{-1}cm-1 (-NO_2 bending)1651.73cm^{-1} (> C = C < stretching) and2909.90 cm^{-1} (C-H stretching).

The identical peaks of C-H Bending C = O stretching, -NO_2 bending, >C = C < stretching and C-H stretching vibrations were also noticed in the spectra of the drug with polymers. FT-IR spectra revealed that there was no interaction between the drug and the polymer used for formulations.

Viscosity

The viscosity of sodium alginate-pectin microspheres ranged from 150 to 40.25 and viscosity gradually decreased. the viscosity of sodium alginate-xanthan gum microspheres ranges from 180 to 864, viscosity gradually increased due to xanthan gum. The data shown in table 3

Percentage yield

The percentage yield of sodium alginate-pectin microspheres ranged from 89 to 97%. and the percentage yield of sodium alginate-xanthan gum microspheres ranged from 87 to 95%. Values are shown in table 3

Flow properties

Vildagliptin microspheres show excellent flow properties.

The bulk density of all formulations was found to be in the range of 0.77 to 0.55gm/ml, whereas the tapped density was observed between 0.84 to 0.583 gm/ml. From the values of bulk density and tapped density, the values for the compressibility index and Hausner's ratio were calculated. The values for compressibility index were found between 5.17 to14.2%. The values for Hausner's ratio were found in between 1.04 to 1.16. All these values are within the specified limit which indicates good flow properties. The angle of repose was found to be less than 20 which indicates good flow. Overall, these values indicate good flow properties. The results is given in below table 4.

Particle size analysis

The particle size of vildagliptin-loaded microspheres ranges found to be 1671 to 1530µm. and the highest particle size is F7 and lowest is F5 in pectin microspheres. The highest particle size is X4 and the lowest is X2 in xanthan gum microspheres. Values are shown in table 5

Swelling index

Microspheres in phosphate buffer show a high swelling rate showed in table no 5 Compared to acidic buffer. among all formulations F6, and F7 showing are high swelling rates. The data shown in table. 5

Drug content

F1 and F3 formulations show high drug content when compare with other formulations. Result is shown in table 5

Comparative dissolution profile of pectin- vildagliptin microspheres

Comparison of invitro drug release of all microsphere's formulations prepared using sodium alginate-pectin are shown in table no 6 fig no:6 F3 formulation shows the highest amount of drug release of 98.6% when compared to other formulations.

Comparative dissolution profile of xanthan gum-vildagliptin microspheres

Comparison of invitro drug release of all microsphere's formulations prepared using sodium alginate-xanthan gum are shown in table no 7 Fig no 7.X4 and X6 formulation shows the highest amount of drug release of 97.1% and 98.42% when compared to other formulations.

Statistical analysis

The formulated vildagliptin microspheres by using pectin and xanthan gum were subjected to one-way ANOVA and normality tests using Graph pad prism. The statistical analysis of one-way ANOVA produces a p-value that tends to be < 0.0001. At the same time, the normality test is well accepted for all formulations at a significance level of < 0.05%.

Selection of Optimized Formulation

Based on the above results, the two preparations have the two best formulations (F3 and X6), and when comparing the results obtained from various evaluation parameters of the two formulations i.e., percentage yield (91% and 95%), particle size (1590µm and 1615µm), swelling index (1400 and 1620), drug content (31mg and 4.01mg), and drug release (98.6 and 98.4). from this data of two formulations, F3 has good particle size, high drug content, and in-vitro drug release, so, F3 is considered the optimized formulation. Then SEM and DSC were also performed for the optimized formulation to determine the size, and melting point and confirm its functional structure. stability study and a comparative study was done for the optimized formulation.

Drug Release Kinetic Studies

The release data were fitted to zero order, first order, Korsmeyer-peppas model and Higuchi equation to know the mechanism of drug release from the microspheres and drug release mechanism of optimized formulation (F3) is a tablet no 8, fig no 8

The release profile of the optimized formulation F3, following zero order and Higuchi kinetic model.

Scanning Electron Microscopy (SEM)

Optimized formulation is subjected for Scanning electron microscopy, the microspheres appear spherical and hemispherical/ semi spherical in shape observed in fig no. 9 and with diameters ranging from 1.59mm or 1590µm to 1.66mm or 1660µm fig no 9. The surface of the microsphere was demonstrated

as rough in one and smooth in the other observed in fig no 9(a) and cracks or fractures emerging on the surface might be caused by external temperature and stress conditions affecting the microspheres.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) studies were performed for the optimized formulation and drug sample, fig no: 10 DSC of the drug showed a sharp peak at158.88ºC. fig no:11 DSC of optimized formulation of microspheres showing a board peak from 192.43ºC to 222.09ºC and a peak at 196.49ºC, at 302.85°C an exothermic peak was observed such a phenomenon is called cold crystallization.

Comparative study

A comparison study was conducted between the optimized formulation (F3) and the marketed formulation of Vildagliptin tablets (Galvas® (50mg); Norvartis). Data comparisons for these two formulations the optimized formulation (x6) showing better control than the marketed formulation. Drug release of marketed formulation took 2 hours to reach 99.41% and optimized formulation take 8 hours to reach 98.6%. the optimized formulation showing better controlled release and taking long time to release (8 hrs.) than the marketed formulation. table no 9

Stability studies

Stability studies of the prepared microspheres were carried out by storing optimized formulation (F3) at room temperature humidity and 40ºC ± 2ºC/75% RH ± 5% RH in a humidity control oven for ninety days. Stability studies were carried out to predict the degradation that may occur over a prolonged storage period with different temperatures and humidities. Results were decreased when compared with fresh microspheres and results was tableted 10 below.

Inference:

Other evaluation parameters as also done, such as flow properties (bulk density, tapped density, Carr's index, and Hauser's ratio) particle size, and swelling index there is no significant difference in values.

CONCLUSION

The present study has been a satisfactory attempt to formulate microspheres of Vildagliptin, an oral administration of microspheres used to treat type 2 diabetes mellitus. The therapeutic range may be maintained with a controlled release of the medication in the gastrointestinal tract. An ionotropic gelation technique was employed to synthesize both sodium alginate-pectin and sodium alginate-xanthan gum vildagliptin-loaded microspheres. FTIR study confirmed the no drug-polymer interactions. Particle size ranges from 1530µm to 1679µm. The swelling study performed in acidic and alkaline media revealed a good release in alkaline solution PH-6.8 in beads. The in vitro drug release experimental study had succeeded based on the ratio of beads, although all microspheres exhibited satisfactory results, the best results were obtained with optimized formulation F3 of sodium alginate-pectin. Based on exponent

values it shows diffusion release. Performed the SEM and DSC for optimized formulation. when compared with the marketed formulation of vildagliptin tablets (galvus® 50mg) with vildagliptin microspheres are shows greater controlled release than the marketed formulation. stability studies were conducted for optimized formulation for 90 days, there are no significant changes.

The above study concluded that formulated microspheres of vildagliptin would be more efficacious and acceptable than conventional drug delivery of vildagliptin and have a satisfactorily controlled release profile, which may provide increased therapeutic efficacy, decrease dosing frequency, and improves patient compliance.

Future scope

Further the formulations may be suggested for the in-vivo bioavailability studies and in-vivo in-vitro correlation studies are to be done to establish the efficacy of these formulations.

Declarations

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Conflict of Interest

The authors declare that they have no conflict of interest for this study

References

1. Deshmukh PP, Barhate AN. Formulation and evaluation of microspheres of Glibenclamide by inotropic gelation method. Indo-American Journal of Pharmaceutical Research. 2017;7(9).
2. Mane S. Effect of formulation variables on the characteristics of vildagliptin microspheres. IP International Journal of Comprehensive and Advanced. 2022 Jul; 3(1): 134-140.
3. May CD. Industrial pectins: Sources, production and applications. Carbohydrate polymers. 1990 Jan 1;12(1):79-99.
4. Villicaña-Molina E, Pacheco-Contreras E, Aguilar-Reyes EA, León-Patiño CA. Pectin and chitosan microsphere preparation via a water/oil emulsion and solvent evaporation method for drug delivery. International Journal of Polymeric Materials and Polymeric Biomaterials. 2020 May 2;69(7):467-75.

5. Bhattacharya SS, Mazahir F, Banerjee S, Verma A, Ghosh A. Preparation and in vitro evaluation of xanthan gum facilitated superabsorbent polymeric microspheres. Carbohydrate polymers. 2013 Oct 15;98(1):64-72.
6. Fuwa M, Nakanishi Y, Moritaka H. Effect of xanthan gum on blood sugar level of cooked rice. InAbstracts of Annual Congress of The Japan Society of Home Economics 67th Annual Conglress of The Japan Society of Home Economics 2015 (p. 179). The Japan Society of Home Economics.
7. Yahoum MM, Toumi S, Tahraoui H, Lefnaoui S, Kebir M, Amrane A, Assadi AA, Zhang J, Mouni L. Formulation and Evaluation of Xanthan Gum Microspheres for the Sustained Release of Metformin Hydrochloride. Micromachines. 2023 Mar 6;14(3):609.
8. Kumari B, Khansili A. Analytical method development and validation of UV-visible spectrophotometric method for the estimation of vildagliptin in the gastric medium. Drug Research. 2020 Sep;70(09):417-23.
9. Pratley RE, Jauffret-Kamel S, Galbreath E, Holmes D. Twelve-week monotherapy with the DPP-4 inhibitor vildagliptin improves glycemic control in subjects with type 2 diabetes. Hormone and metabolic research. 2006 Jun;38(06):423-8.
10. Kumari B, Khansili A, Kumar M. Development and Optimization of Vildagliptin Loaded Floating Microspheres using Central Composite Design: In vitro and In vivo Evaluation. Annals of the Romanian Society for Cell Biology. 2021 Jul 20:12742-55.
11. Atara SA, Soniwala MM. Formulation and evaluation of pectin-calcium chloride beads of azathioprine for colon-targeted drug delivery system. Int J Pharm Pharm Sci. 2018;10:172-7.
12. Rudhali lihare kfhl. Preparation and evaluation of topiramate-loaded microspheres by ionotropic gelation method. World journal of pharmacy and pharmaceutical sciences.2020 sept;9(12): 1291-1310.
13. Kumar K, Pant NC, Ahmad S, Fateh MV, Rai AK, Verma B, Chaurasia H. Development and evaluation of floating microspheres of curcumin in alloxan-induced diabetic rats. Tropical Journal of Pharmaceutical Research. 2016 Oct 5;15(9):1819-25.
14. Kawashima Y, Niwa T, Takeuchi H, Hino T, Itoh Y. Hollow microspheres for use as a floating controlled drug delivery system in the stomach. Pharm Sci 1992; 81: 135-140.
15. Aulton ME. Pharmaceutics - The Science of Dosage Form Design, Churchill Livingstone, Elsevier Science Ltd; 2002; (2): pp 315-320.
16. Mulla TS, Salunkhe VR, Bhinge SD, Mohire N. Formulation, design and optimization of antidiabetic drug loaded microspheres. Polymer Bulletin. 2023 Jun;80(6):6171-96.
17. Manna S, Aziz A, Das S, Bhowmik M. Ionically Cross-linked Pectin-alginate Hydrogel Beads for Prolonged Release of Glibenclamide. International Journal of Pharmaceutical Investigation. 2022 Oct 1;12(4).
18. Mohapatra DK, Roy H, Pattanayak D, Nandi S, Senapati P. Design and in vitro evaluation of cefuroxime axetil floating microbeads for the treatment of hospital acquired infection. Int. J. Pharm. Sci. and health care. 2012;2(2):36-51. Drug content

19. Naik JB, Waghulde MR. Development of vildagliptin loaded Eudragit® microspheres by screening design: In vitro evaluation. Journal of Pharmaceutical Investigation. 2018 Nov 15;48:627-37.
20. Dewan I, Islam S, Rana MS. Characterization and compatibility studies of different rate retardant polymer loaded microspheres by solvent evaporation technique: in vitro-in vivo study of vildagliptin as a model drug. Journal of drug delivery. 2015;2015.
21. Manubolu K, Munna S, Bonnoth CK. A Study on Extraction and Characterization of Vigna mungo Polymer. , Int. J. Res. Pharm. Sci., 2021, 12(3), 1870-1878.
22. Manubolu K, Munna S, Bonnoth CK, Mohan GR. Design and Optimization of Floating Microspheres Using Natural Polymer.(2021). Int. J. Life Sci. Pharma Res.;11(1):166-83.

Tables

Table no 1: **Composition of sodium alginate-pectin microspheres**

SL. No	Formulation	Sodium alginate (mg)	Pectin (mg)	Drug(mg)	Crosslinker (calcium chloride)
1	F1	900	100	50mg	2.5%
2	F2	800	200	50 mg	2.5%
3	F3	700	300	50mg	2.5%
4	F4	600	400	50mg	2.5%
5	F5	500	500	50mg	2.5%
6	F6	400	600	50mg	2.5%
7	F7	250	750	50mg	2.5%

Table no 2: **Composition of sodium alginate-xanthan gum microspheres**

SL. No	Formulation	Sodium alginate(mg)	Xanthan gum(mg)	Drug(mg)	Crosslinker (calcium chloride)
1	X1	900	100	50mg	2.5%
2	X2	850	150	50 mg	2.5%
3	X3	800	200	50mg	2.5%
4	X4	750	250	50mg	2.5%
5	X5	700	300	50mg	2.5%
6	X6	650	350	50mg	2.5%

Table no. 3: Viscosity, % yield, and appearances of sodium alginate-pectin microspheres

S. No	Formulation	Viscosity (Cp)	Percentage yield (%)	Appearances of beads
1	F1	150	89	Goldish brown, Spherical
2	F2	134	90	olive green, Spherical
3	F3	109	91	olive green, Spherical
4	F4	80	97	Sand brown, Spherical
5	F5	67	94	Sand brown, Spherical
6	F6	52.3	95	Brown, Spherical
7	F7	40.25	96	Brown, Spherical, oval
8	X1	180	88	Pale white, Spherical
9	X2	276	93	Pale white, Spherical
10	X3	368	87	Pale white, Spherical
11	X4	521	93	Pale white, Spherical
12	X5	690	94	Pale white, Spherical
13	X6	864	95	Pale white, Spherical

***Table no. 4*: Flow properties of vildagliptin microspheres**

Formulation	Angle of repose(º)	Bulk density (g/ml)	Tapped density(g/ml)	Carr's index	Hausner's ratio
F1	10.30 ± 0.1	0.73636 ± 0.04	0.81 ± 0.04	9.8	1.109
F2	5.19 ± 0.54	0.7769 ± 0.03	0.8416 ± 0.02	8.3	1.09
F3	14.91 ± 0.2	0.74 ± 0.07	0.7928 ± 0.01	6.3	1.06
F4	13.49 ± 0.4	0.706 ± 0.02	0.74 ± 0.06	5.8	1.04
F5	17.74 ± 0.4	0.715 ± 0.05	0.775 ± 0.05	7.7	1.08
F6	9.4 ± 0.3	0.64705 ± 0.07	0.7333 ± 0.02	12.3	1.14
F7	17.74 ± 0.1	0.55 ± 0.01	0.5833 ± 0.07	5.17	1.05
X1	14.03 ± 0.09	0.5857 ± 0.04	0.6833 ± 0.02	10	1.16
X2	15.16 ± 0.1	0.5863 ± 0.07	0.645 ± 0.01	9.51	1.10
X3	17.35 ± 0.4	0.5857 ± 0.09	0.6473 ± 0.06	10	1.105
X4	11.30 ± 0.07	0.635 ± 0.01	0.7055 ± 0.02	14.28	1.11
X5	14.83 ± 0.5	0.5952 ± 0.03	0.6944 ± 0.04	14.2	1.16
X6	14.89 ± 0.2	0.6047 ± 0.02	0.7055 ± 0.05	14.28	1.16

Table no. 5: Swelling index of pectin-vildagliptin microspheres

SL.NO	Formulation	Mean particle size (µm)	Swelling index of microspheres in phosphate buffer	Swelling index of microspheres in acidic (Hcl) buffer	Drug content (mgs)
1	F1	1619	1520 ± 1.01	210 ± 0.7	47.7 ± 0.10
2	F2	1665	1310 ± 0.9	160 ± 0.3	16.03 ± 0.04
3	F3	1590	1400 ± 0.4	160 ± 0.5	31 ± 0.06
4	F4	1561	1330 ± 0.3	200 ± 0.8	13 ± 0.02
5	F5	1552	1410 ± 1.0	210 ± 0.3	20.8 ± 0.06
6	F6	1622	1680 ± 0.6	320 ± 0.2	5.7 ± 0.02
7	F7	1641	2120 ± 1.21	700 ± 0.9	13.6 ± 0.4
8	X1	1579	1250 ± 1.07	100 ± 0.97	5.6 ± 0.09
9	X2	1530	1200 ± 1.6	50 ± 0.3	5.3 ± 0.10
10	X3	1583	1330 ± 1.2	170 ± 0.9	3.9 ± 0.08
11	X4	1671	1260 ± 0.98	140 ± 0.81	3.9 ± 0.08
12	X5	1602	1590 ± 1.1	190 ± 0.8	4.0 ± 0.02
13	X6	1615	1620 ± 1.02	220 ± 1.2	4.01 ± 0.02

Table no. 6: **Comparative dissolution profile of pectin vildagliptin microspheres**

Time in hrs.	F1	F2	F3	F4	F5	F6	F7
0	0	0	0	0	0	0	0
0.5	1.4 ± 0.10	12.1 ± 0.02	5.4 ± 0.04	16.7 ± 0.10	18 ± 0.02	3.6 ± 0.02	6.3 ± 0.03
1	15.1 ± 0.04	21.8 ± 0.03	12.5 ± 0.03	20.8 ± 0.12	21.3 ± 0.03	11.4 ± 0.04	18.6 ± 0.04
2	26.7 ± 0.06	24.6 ± 0.02	15.4 ± 0.06	39.2 ± 0.03	47.3 ± 0.03	13.3 ± 0.06	27.8 ± 0.1
3	30.3 ± 0.08	43.6 ± 0.04	23.4 ± 0.04	40.9 ± 0.08	52 ± 0.02	29.4 ± 0.06	33.7 ± 0.08
4	45.8 ± 0.06	52.9 ± 0.02	35.0 ± 0.02	43.8 ± 0.06	60 ± 0.04	30.2 ± 0.02	38.9 ± 0.02
5	57 ± 0.08	59.3 ± 0.05	45.6 ± 0.05	65.1 ± 0.12	68.1 ± 0.08	52.3 ± 0.03	53.6 ± 0.07
6	64.8 ± 0.09	75.3 ± 0.01	73.7 ± 0.10	74.7 ± 0.03	81 ± 0.05	63. ±0.08	67.5 ± 0.02
7	73.9 ± 0.01	82 ± 0.04	83.4 ± 0.09	81.6 ± 0.09	94.7 ± 0.02	76.2 ± 0.01	81.4 ± 0.02
8	90.1 ± 0.08	95.2 ± 0.02	98.6 ± 0.03	93.7 ± 0.08	96.9 ± 0.04	91.5 ± 0.09	90 ± 0.03

Table no. 7: Comparative dissolution profile of vildagliptin xanthan gum microspheres

Time in hrs.	X1	X2	X3	X4	X5	X6
0	0	0	0	0	0	0
0.5	4.7 ± 0.01	8.4 ± 0.04	12.57 ± 0.03	11.68 ± 0.02	18.06 ± 0.04	15.89 ± 0.02
1	9.51 ± 0.02	12.2 ± 0.01	22.97 ± 0.04	12.57 ± 0.01	22.75 ± 0.03	18.32 ± 0.04
2	17.4 ± 0.03	15.1 ± 0.01	34.92 ± 0.05	18.76 ± 0.01	30.51 ± 0.04	27.17 ± 0.03
3	25.8 ± 0.02	30.8 ± 0.05	40.01 ± 0.02	29.39 ± 0.02	36.69 ± 0.02	31.16 ± 0.06
4	36.02 ± 0.02	42.8 ± 0.04	59.05 ± 0.03	42.45 ± 0.03	48.42 ± 0.05	38.02 ± 0.04
5	44.9 ± 0.01	51.9 ± 0.01	67.9 ± 0.01	54.4 ± 0.03	57.9 ± 0.06	52.41 ± 0.02
6	47.6 ± 0.03	67.4 ± 0.03	74.54 ± 0.01	76.53 ± 0.01	78.97 ± 0.02	78.97 ± 0.06
7	59 ± 0.03	74.1 ± 0.01	80.96 ± 0.04	87.6 ± 0.02	81.18 ± 0.03	85.61 ± 0.05
8	65.1 ± 0.02	79.3 ± 0.05	86.1 ± 0.01	97.1 ± 0.02	96.67 ± 0.02	98.42 ± 0.02

Table no. 8: Release Kinetics

Formulation	zero order	First-order kinetics	Higuchi release model	Hixson Crowell	Korsmeyer Peppas
F3	0.9808	0.3695	0.8994	0.8844	0.8318

Table no.9: **Comparison study of optimized formulation with a marketed formulation**

Time (hrs)	Marketed formulation (galvus®)	Optimized formulation (F3)
0	0	0
0.1	28.41	0
0.5	63.2	5.4
1	90.57	12.5
2	99.41	15.4
3		23.4
4		35.04
5		45.6
6		73.7
7		83.4
8		98.6

Table no.10*:* Stability studies of optimized pectin formulation

Storage condition	Days	Drug content (mgs)	%Drug release
Room temperature	30	31	98.4
	60	31	98.4
	90	31	98.3
At40ºC AND 75% RH	30	31	98
	60	30.9	97.1
	90	30	97.1

Figures

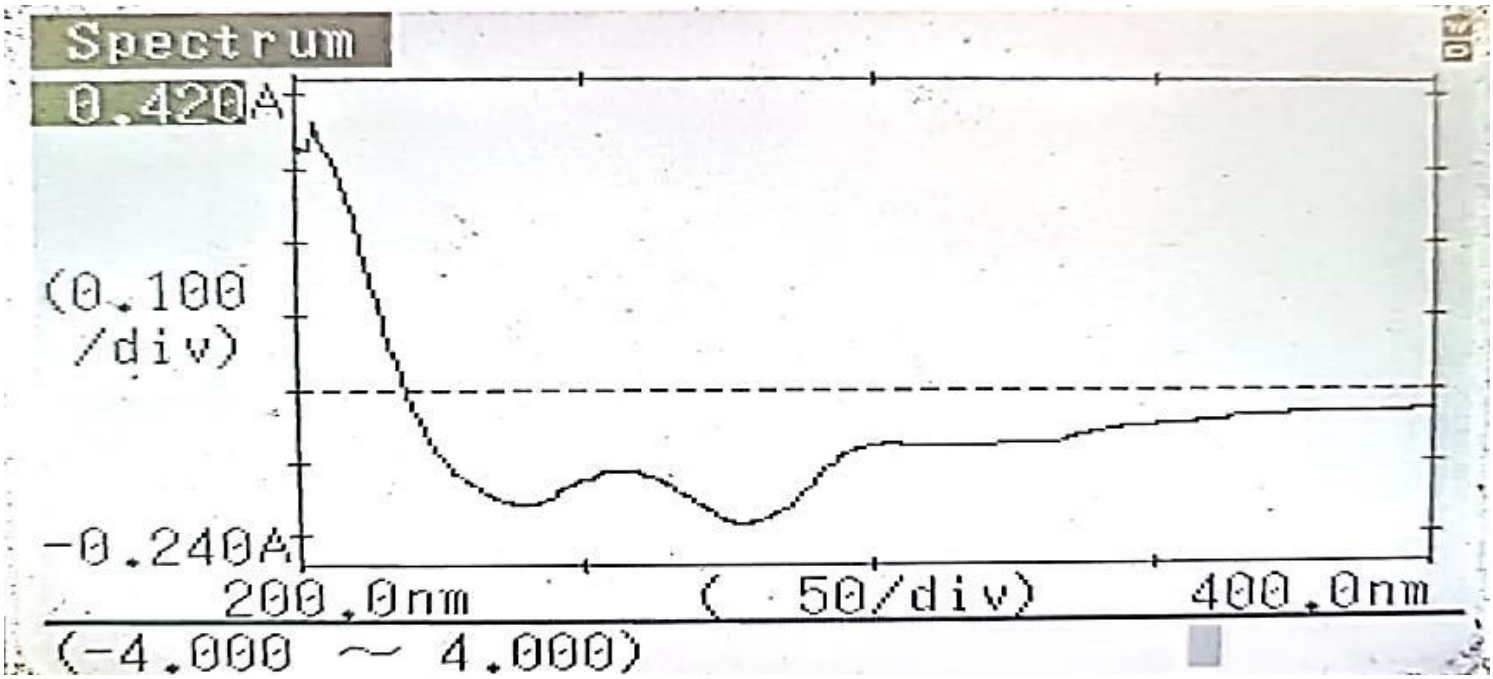


Figure 1

Absorbance maxima of vildagliptin

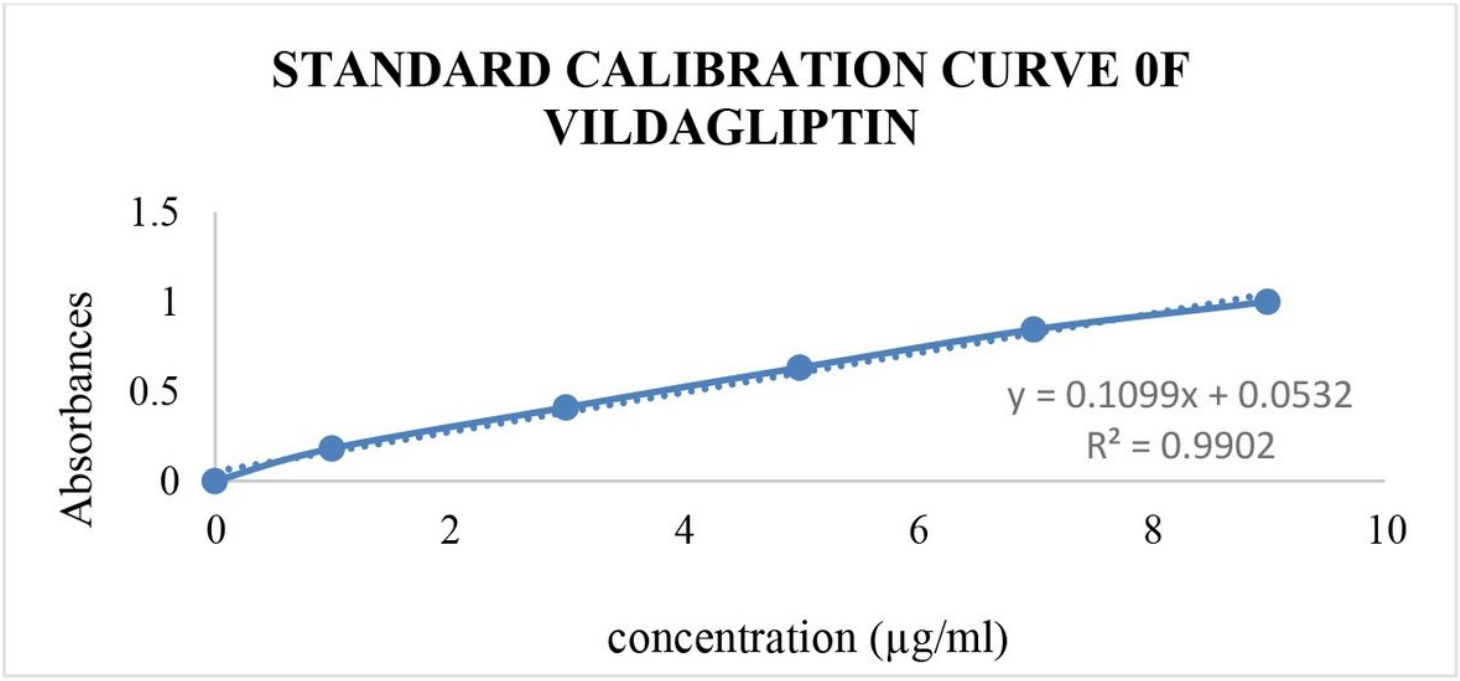


Figure 2

Standard Calibration Curve

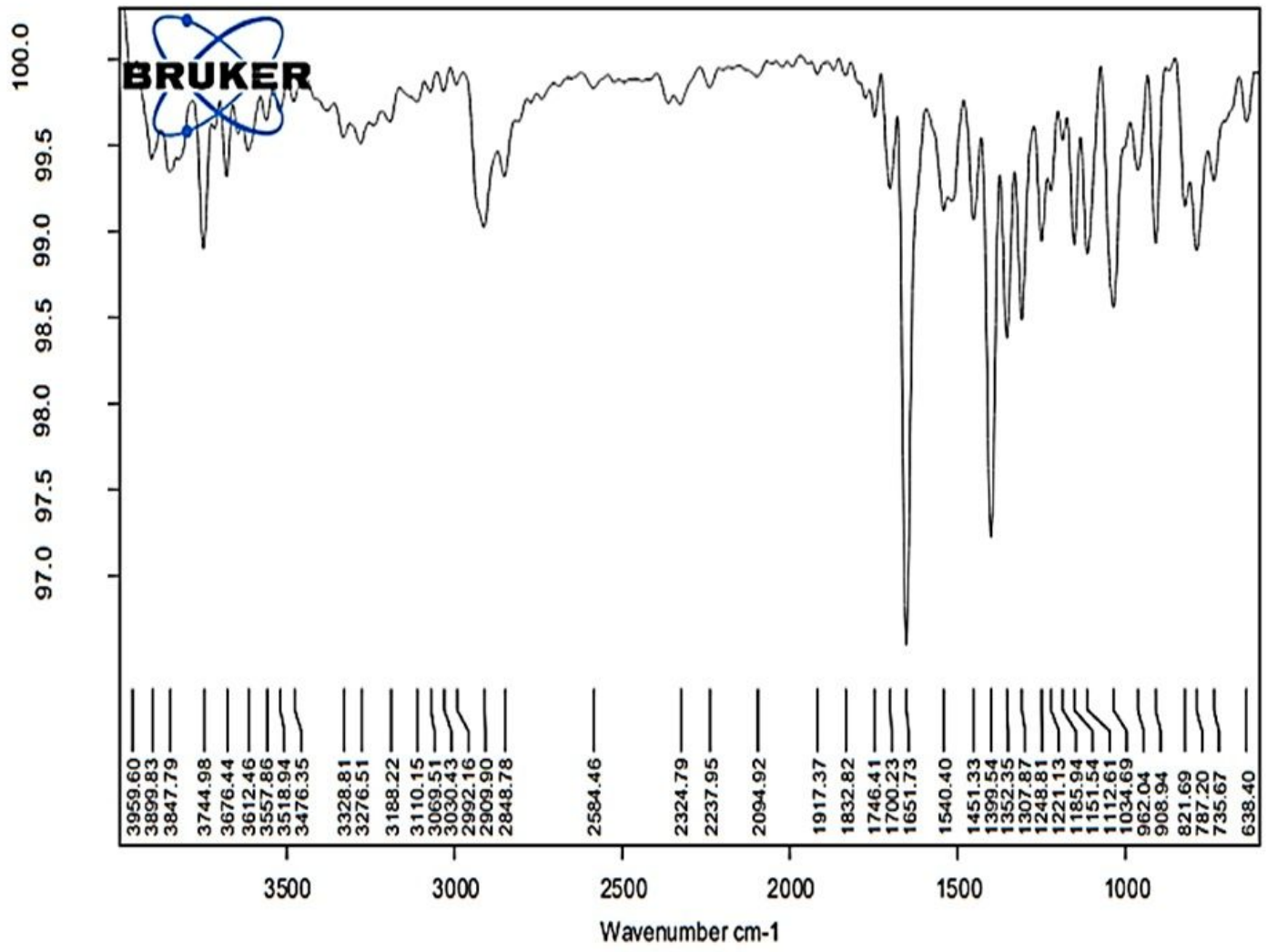


Figure 3

FTIR spectrum of vildagliptin

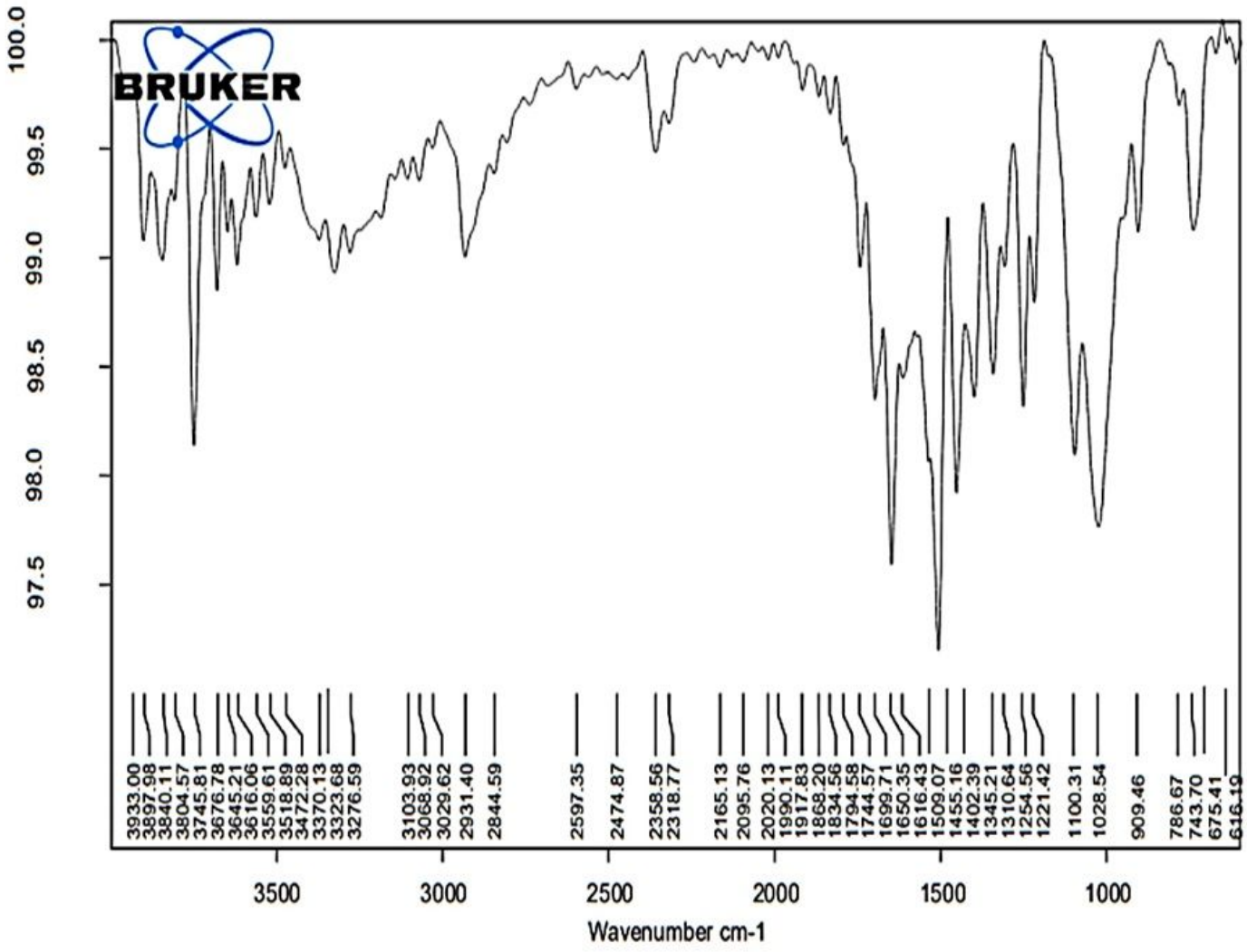


Figure 4

FT-IR spectrum of vildagliptin with pectin

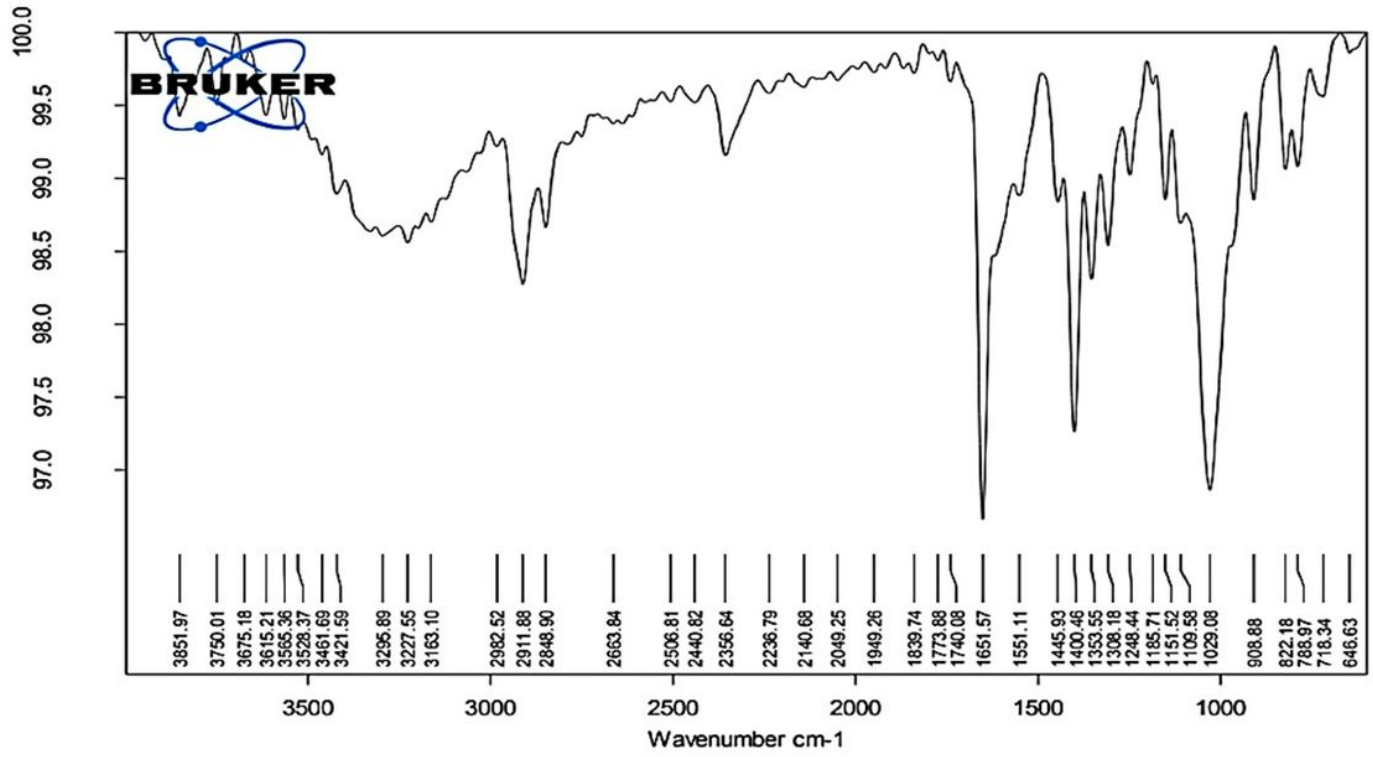
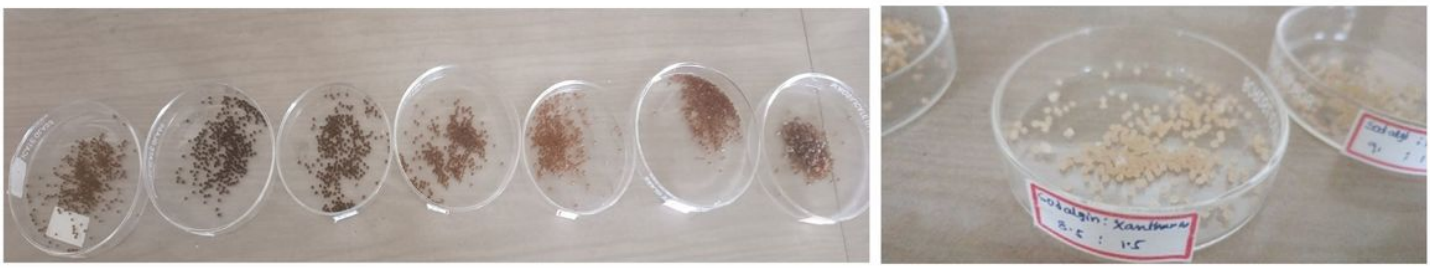


Figure 5

FT-IR spectrum of vildagliptin with xanthan gum



(a) (b)

Figure 6

(a) sodium alginate-pectin beads (b) sodium alginate-xanthan gum beads

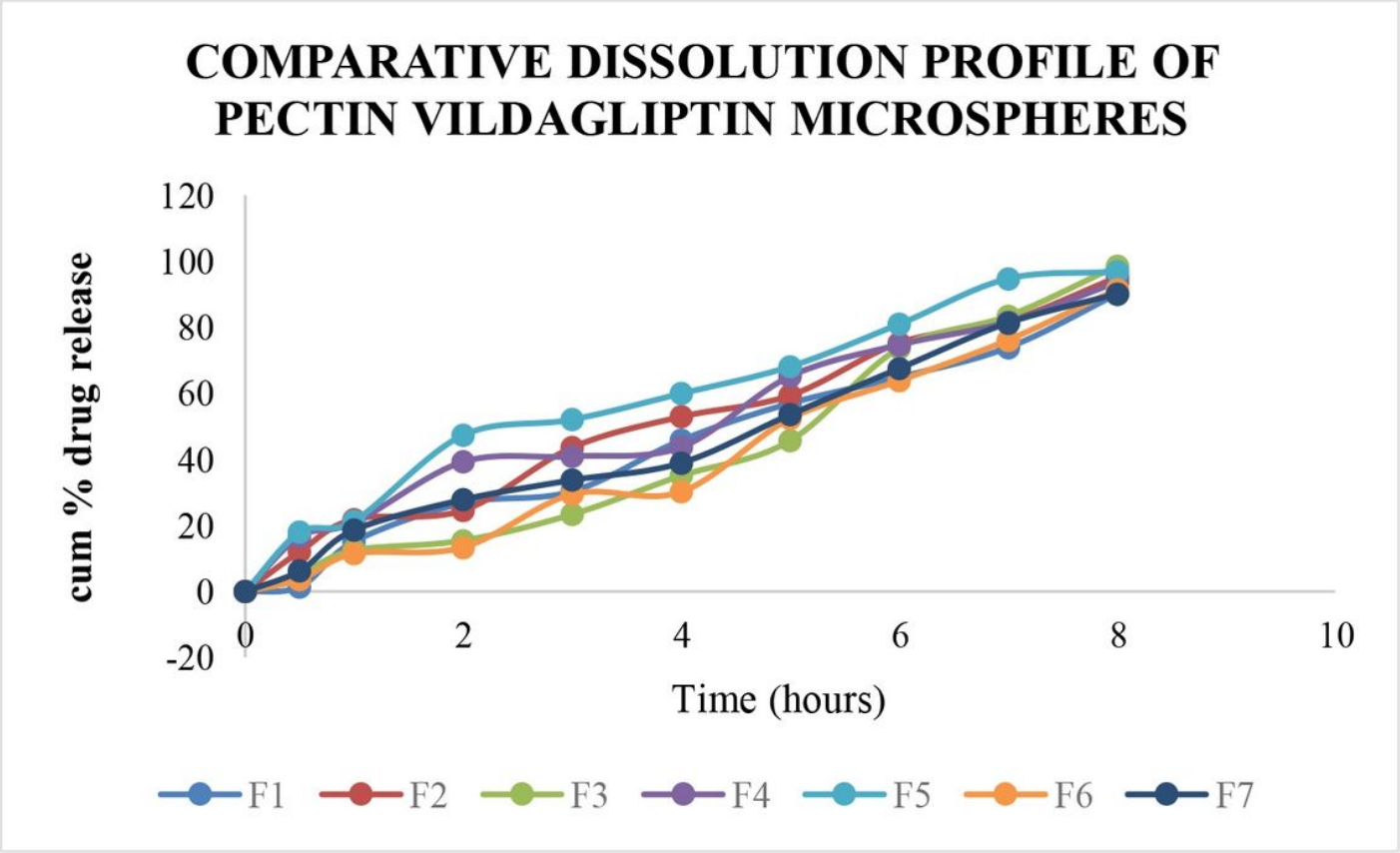


Figure 7

Figure No.6: Comparative dissolution profile of pectin vildagliptin microspheres

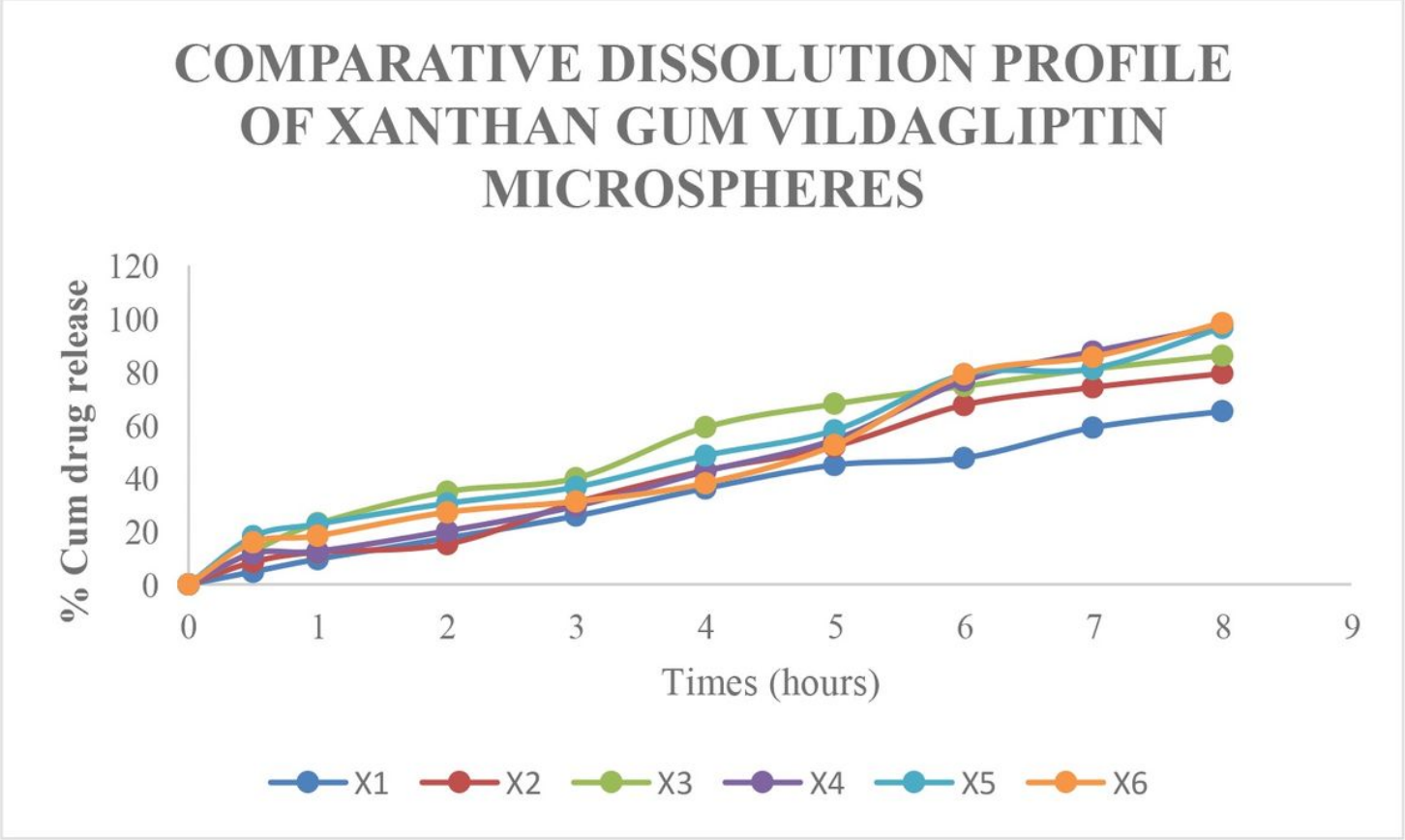


Figure 8

Figure No.7: Comparative dissolution profile of vildagliptin xanthan gum microsphere

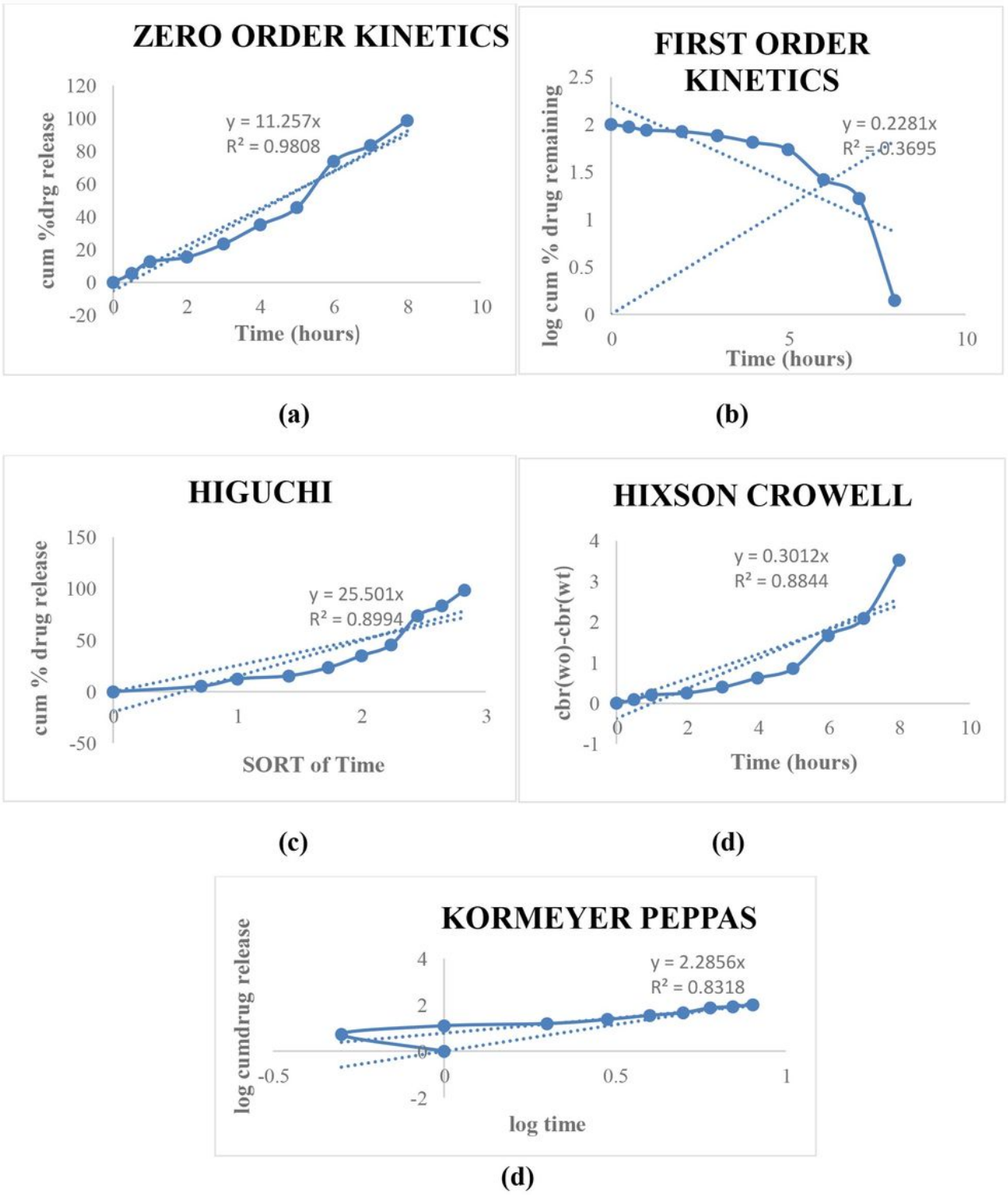


Figure 9

Figure No. 8: In-vitro Drug Release Kinetics for Formulation F3 (a) Zero order Release Kinetics (b) First order (c) Higuchi'smodel (d) Hixson-Crowell's model(e) Korsmeyer-Peppa's model

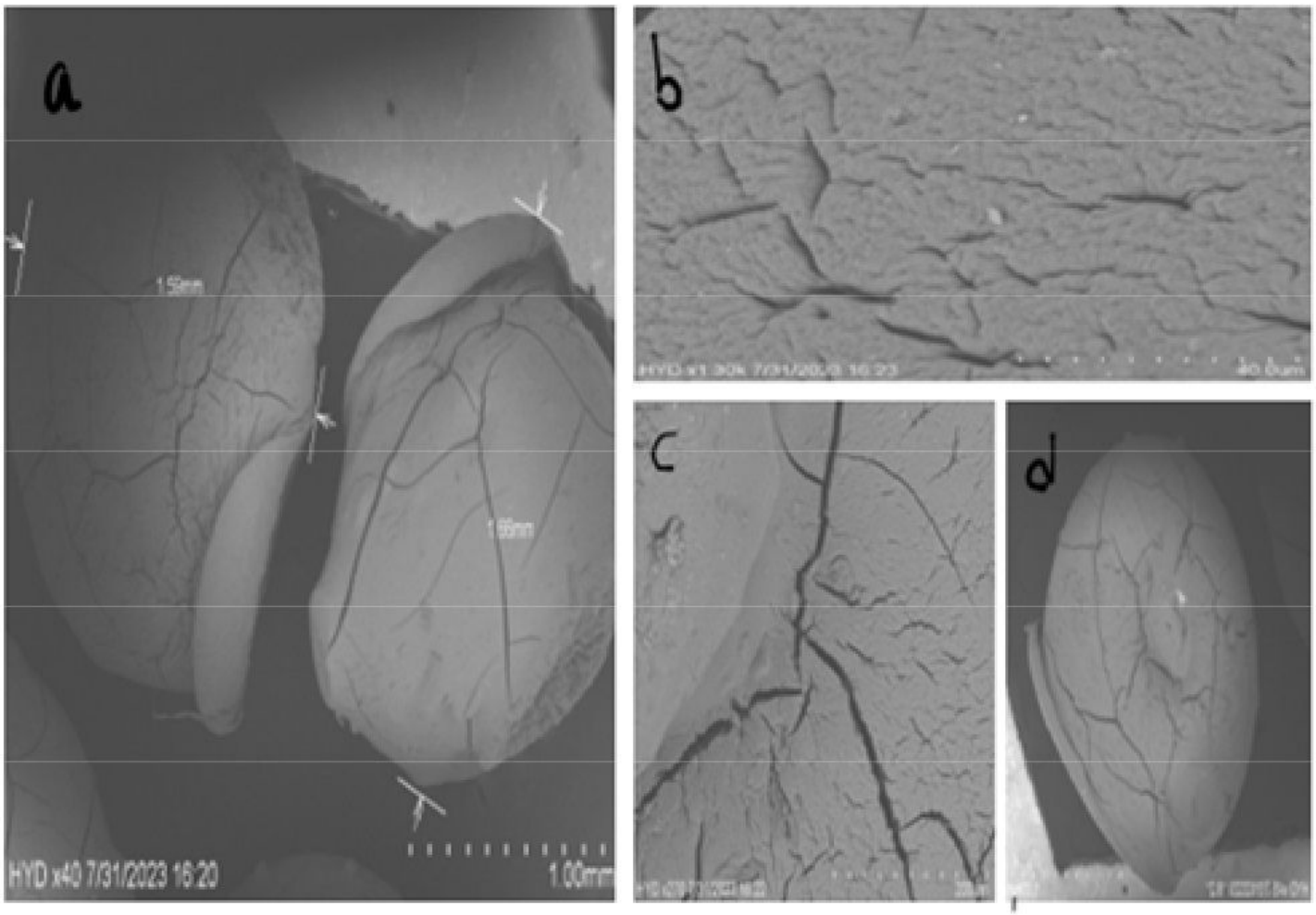


Figure 10

*Figure No.*9: (a) SEM of optimized formulation at 1.00 mm with particle size (b) surface of microspheres at 40µm (c) surface of microspheres at 200µm (d) at1.00mm

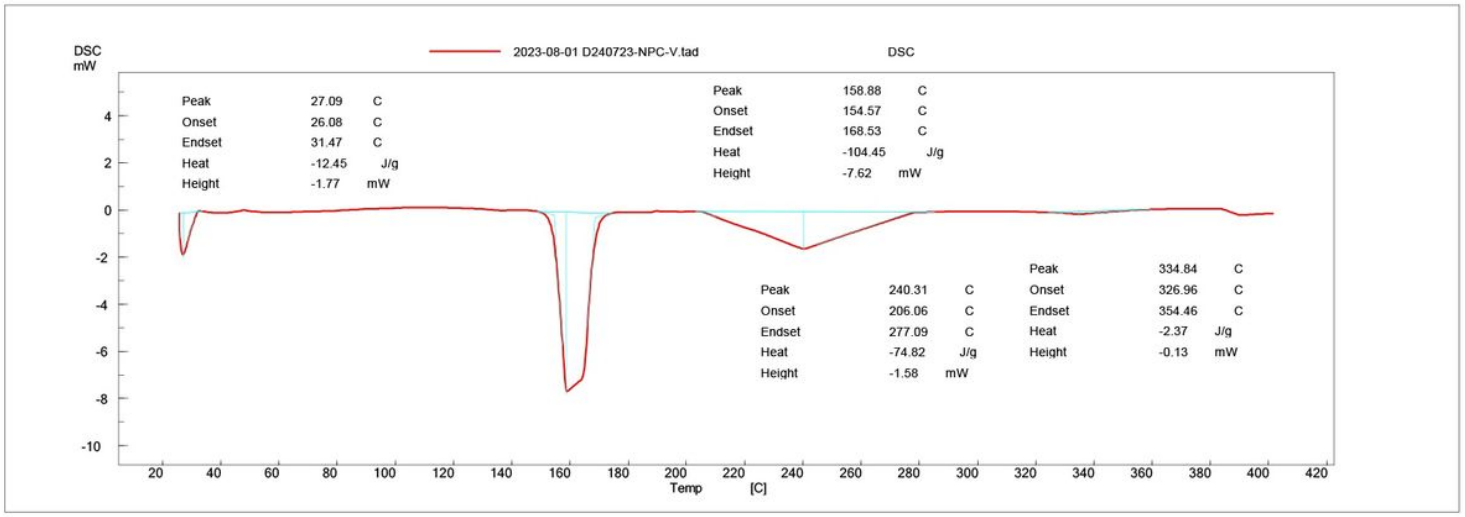


Figure 11

Figure No.10: DSC of vildagliptin

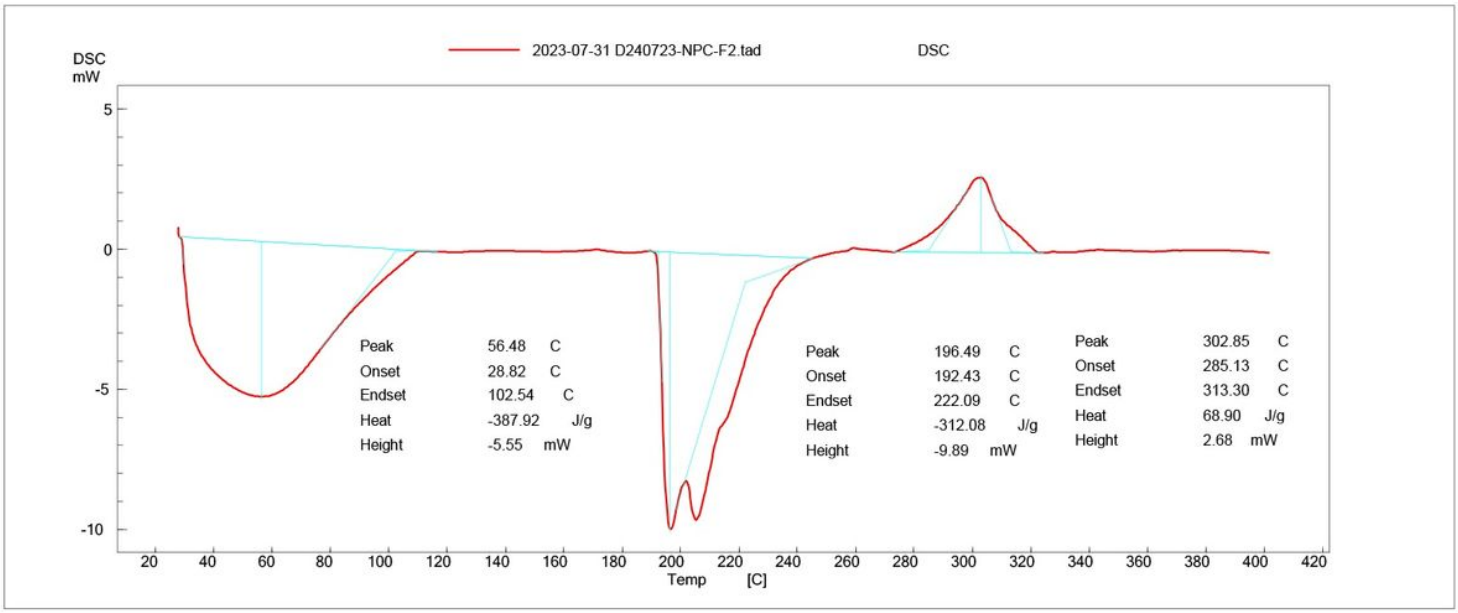


Figure 12

Figure No.11: DSC of vildagliptin microspheres (F3)

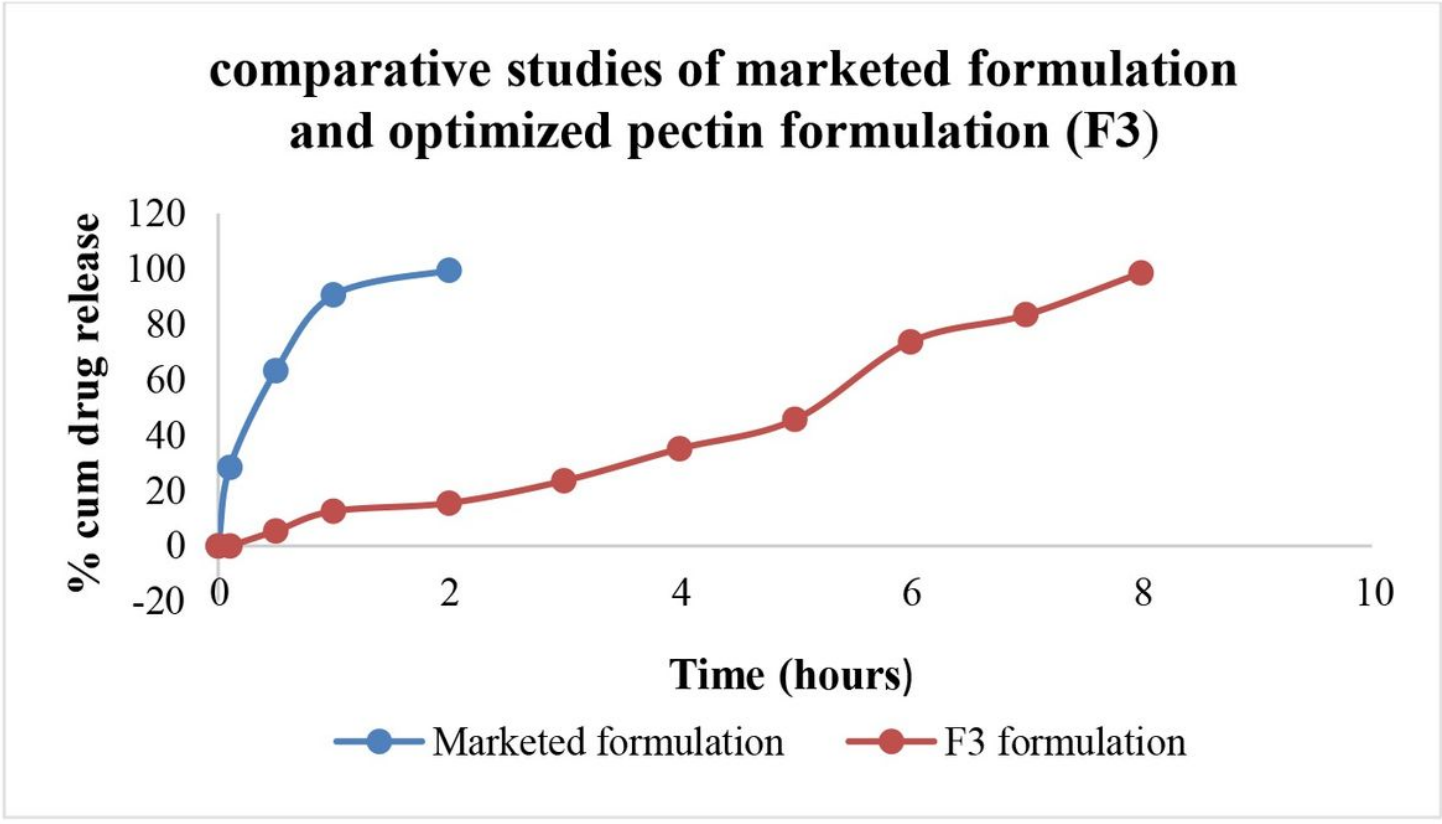


Figure 13

Figure No.12: comparative studies of marketed formulation and optimized pectin formulation (F3)