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"Development and Characterization of Repaglinide Microballoons: In-vitro and In-vivo Assessment of Gastric Retention and Drug Release"

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ABSTRACT

This study focuses on evaluations of floating gastroretentive microballoons as a drug delivery system for Repaglinide.It is an oral hypoglycemic agent and the first member of the meglitinide class to be used for the treatment of diabetes mellitus 2. The microballoons were formulated by a solvent evaporation method using ethylcellulose (EC) and hydroxypropylmethylcellulose (HPMC) as polymers, along with alcohol and dichloromethane as solvents. Microballoon optimization was performed using Design of Experiments (DoE) with a Box-Behnken design.The important parameters, including Percentage Buoyancy (PB) and Entrapment Efficiency (EE), were evaluated to assess the performance of the microballoons.*In vitro*and *in vivo*evaluations involved analyzing flow ability parameters, release characteristics of repaglinide using a dissolution apparatus and antidiabetic activity of the microballoons formulation using Streptozotocin-induced diabetic rats as a model. The resulted formulation exhibited high entrapment efficiency (81.12%) and buoyancy (65.02%), suggesting favorable sustained drug release and increased floatability characteristics. Additionally, the formulation showed improved flow ability with a lower Carr's index value of 7.89. It also displayed a lower angle of repose of 25.2 ± 1.7º, indicating enhanced powder cohesiveness and stability. *Invitro*evaluation of repaglinide microballoons demonstrated sustained release action with B5 optimized batch showing 95.48 % drug in 12 hrs. *In vivo* studies revealed reduced blood glucose levels up to 10 hours (103.67 mg/dl) for B5 optimized batch, highlighting the potential therapeutic efficacy of the repaglinide microballoons. In conclusion, in vitro and in vivo evaluation of repaglinide floating gastroretention microballoons offers an effective solution for the treatment of diabetes mellitus.

KEYWORDS: Repaglinide, Microballoons, Box-Behnken design,Buoyancy,Entrapment Efficiency

INTRODUCTION

Oral administration is the most convenient and advantageous route of drug delivery into the systemic circulation. Diabetes mellitus (DM) is a group of metabolic diseases in which there are long-term high blood sugar levels. It includes symptoms such as frequent urination, increased thirst and increased hunger. Serious long-term complications include heart disease, stroke, kidney failure; leg ulcers and eye damage. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Diabetes is one of the leading causes of death and disability in the world. The latest WHO estimate for the number of people with diabetes worldwide in 2000 is 171 million, which is likely to be at least 366 million by 2030. [1, 2].

Repaglinide is an oral BCS class II antidiabetic that belongs to the meglitinide group. It is primarily used to treat type 2 diabetes mellitus [6]. It works by stimulating the release of insulin from the pancreas, thereby lowering blood glucose levels. Effective control of diabetes type-II requires the administration of Repaglinide 0.5-4 mg three times a day. Due to its short biological half-life (1 hour) and low bioavailability (63%); it is necessary to develop a controlled-release dosage form of repaglinide that will release the drug in a prolonged manner [3].

Optimizing the rate of drug release and residence time in the gastrointestinal tract is essential for the development of oral drug delivery systems. Many efforts have been made to develop sustained-release formulations that offer extended clinical effects and reduced dosing frequency. Various approaches have been used to increase the gastric residence time (GRT) and retention of dosage forms in the stomach, which include the use of floating systems, high density systems, mucoadhesive systems, magnetic systems, unfolding systems, stretchable or swellable systems, superporous systemsand hydrogel systems. Floating Drug Delivery Systems (FDDS) are among several approaches that have been developed to increase the gastric residence time (GRT) of dosage forms. Both single and multi-unit systems have been developed. Single-unit floating systems are more popular, but have the disadvantage that the all-or-nothing emptying process leads to high variability in gastrointestinal transit time. Still, multiunit dosage forms may be preferable because they are claimed to reduce intersubject variability in absorption and reduce the likelihood of dose release. Such a dosage form can be widely distributed in the gastrointestinal tract (GIT), which provides the possibility of a longer and more reliable release of the drug from the dosage form. Drugs that are easily absorbed from the GIT and have a short half-life are rapidly eliminated from the bloodstream, so they require frequent dosing. To overcome this disadvantage, sustained-release oral formulations have been developed in an attempt to release the drug slowly into the GIT and maintain an effective serum drug concentration for a longer period of time. [2]

Microballoons are gastroretentive floating systems for drug delivery with a non-effervescent approach. Microballoons (Hollow microsphere) are, in the exact sense of the word, empty spherical particles without a core. These microspheres are typically free-flowing powders containing proteins or synthetic polymers, ideally less than 200 micrometers in size. [4]Both natural and synthetic polymers were used to prepare floating microspheres. These systems allow prolonged residence time of drug forms in the stomach and the achievement of constant plasma levels. Microballoons can encapsulate poorly soluble drugs in their polymer matrix; encapsulation protects the drug from degradation and improves its solubility, making it more bioavailable. This allows sustained drug release, ensures optimal therapeutic levels and minimizes side effects. The unique structure of microballoons makes them suitable for various pharmaceutical applications, including the delivery of drugs classified as BCS (Biopharmaceutics Classification System) class II. [5],

like repaglinide which are characterized by low solubility and high permeability, posing challenges for their effective delivery. Hence the aim of the study was to*in vitro* and *in vivo*Characterization ofRepaglinide microballoons for controlled release action.

MATERIALS AND METHODS

Repaglinide was obtained from ChempurPharma, Mumbai, Hydroxy Propyl Methyl Cellulose K4M and Hydroxy Propyl Methyl Cellulose K15M, Ethyl Cellulose; Tween 80 was purchased from Ranbaxy Fine chemicals, Mumbai. All other chemicals used were of analytical grade.

Preformulation Studies (Drug Identification and Characterization)

Preformulation studies involve a comprehensive evaluation of the physicochemical properties of a drug candidate before formulation. These studies aim to understand the drug's characteristics, such as solubility, stability, polymorphism, particle size, and compatibility with excipients, in order to optimize the formulation and ensure the drug's effectiveness, safety, and stability. Preformulation studies provide valuable insights into the drug's behavior under different conditions and guide the selection of suitable formulation strategies, enabling the development of robust and successful products.

1. Organoleptic properties [8]

The obtained drug samples were examined for their condition, appearance, color, smell, taste, etc.

2. Melting Point Determination [9]

Melting points of Repaglinide were determined on a melting point apparatus. The detected value was compared with the reported value.

3. Fourier Transform Infra-Red (FTIR) analysis [10]

About 5 mg of the sample was mixed with 100 mg of potassium bromide (KBr) and pressed into pellets, this mixture was then scanned in the wavenumber range of 4000 to 400 cm-1 and analyzed for possible interaction. FTIR spectra of the drug were compared with reference values.

4. UV Spectrophotometric Analysis [11]

Determination of λ_{max}

Accurately weighed 10 mg of repaglinide was dissolved in 100 mL of 0.1 N HCl and scanned on a UV spectrophotometer between 200 to 400 nm to determine λmax.

Stock solution: Accurately weighed repaglinide (10 mg) was dissolved in 0.1N HCl and then diluted to 100 mL, resulting in a concentration of 100 µg/mL. This was used to prepare labor standards.

Working standards: -Different concentrations (0.4, 0.8, 1.2, 1.6, 1.8, 2.0, 2.4, 3.0, 3.6 µg) were prepared from the stock solution (100 µg/ml /ml) by diluting the appropriate volumes of stock solutions to 100 ml of 0.1 N HCl. Absorbance was measured at 243 nm. A calibration curve was plotted for concentration versus absorbance.

5. Solubility determination [12]

Drug solubility studies were performed by adding an excess of repaglinide to distilled water, phosphate buffer pH 6.8, and 0.1N HCL, and the solutions containing the flasks were kept on a rotary shaker for 24 hours. After 24 hours, the solutions were analyzed using a UV spectrophotometer at 243 nm.

6. Drug: Excipients Compatibility Study [10]

The pure drug and its physical mixtures were subjected to IR spectral studies using an FTIR spectrophotometer in the wavenumber region from 4000 cm-1 to 400 cm-1. Spectra obtained for pure drug and physical mixtures were compared

7. Physical Observation

Using a glass frit, 10 mg of the drug is mixed with various polymers and triturated separately for 15 minutes. The mixture was packed in sealed vials using butter paper and placed in accelerated environmental conditions (40 °C/75% RH). Any physical change was observed visually after each week for 4 weeks.

Formulation of Microballoons of Repaglinide

Microballoons were prepared previously using a solvent evaporation technique based on the Box-Behnken design. Repaglinide (50 mg) was combined with different ratios of HPMCK15M and EC, keeping the total weight of HPMC and EC per 100 mg Repaglinide constant at 200 mg. As a solvent, a mixture of alcohol and dichloromethane was used in different proportions and kept at a total volume of 100 ml. The process was carried out at room temperature. The resulting polymer solution was poured into 250 ml of distilled water containing 0.01% v/v. Tween 80 and kept at different temperatures. The solution was then stirred at a stirring speed ranging from 200 to 1600 rpm for 20 minutes to evaporate the volatile solvent. Then the formed microballoons were filtered, washed with distilled water and dried.

13 batches (B1 – B13) were prepared by the above method and their flow properties (angle of Repose, Carr index and Hausner ratio) were evaluated. Based on these flow characteristics, an optimized formulation was selected for further evaluation. The optimized batch was then subjected to in vitro and in vivo evaluation. In the in vitro evaluation, the release characteristics of repaglinide were studied using a dissolution device. For in vivo evaluation, streptozotocin-induced diabetic rats were used as a model for measuring antidiabetic activity.

EVALUATION OF MICROBALLOONS

1. Determination of Flow Properties

1.1 Bulk density [11, 13]

It is calculated by dividing the material's particle mass by the total volume that they occupy. Calculations are made using a formula,

$$\text{Bulk Density} = {}^{\text{Mass}}/_{\text{Volume}}$$

1.2 Tapped density [11, 13]

Shaking density is the increased bulk density achieved after mechanically tapping the container containing the powder sample. By mechanically tapping the measuring cylinder containing the powder sample, the shaking density is established. The graduated cylinder is tapped mechanically after the initial powder volume is observed and the volume is read until little further change in volume is seen.

Tapped Density = Mass / change in volume after tapping

1.3 Carr's Index or Compressibility index [11, 13]

The compressibility of the powder can be determined using the Carr index. It is named after Ralph J. Carr Jr., a scientist. The Carr index is widely used as a measure of powder flowability. The bulk density and tapped density in the free-flowing powder would approach the value resulting in a low Carr index.

$$Carr's\ Index = \frac{Tapped\ Density - Bulk\ Density}{Tapped\ Density}\ x\ 100$$

Table 1: Interpretation of Carr's Index Value for powder flow properties

Carr's Index	% Flow
5-12	Excellent
12–16	Good

18–21	Fair to passable
23–25	Poor
33–38	Very poor
>40	Very very poor

1.4 Hausner Ratio [11, 13]

An indicator of a powder or granular material's ability to flow is the Hausner ratio. It bears the engineer Henry H. Hausner's name (1900–1995) the formula, used to determine the Hausner ratio,

$$Hausner\ Ratio = \frac{Tapped\ Density}{Bulk\ Density}$$

Table 2: Interpretation of Hausner ratio value for flow properties

Hausner ratio	Properties
0-1.2	Free flowing
1.2-1.6	Cohesive powder

1.5 Angle of repose [11, 13]

The steepest angle of inclination from the horizontal at which granular materials can be piled without collapsing is known as the angle of repose. Cone piles are formed when large quantities of granular materials are placed on a horizontal surface. The angle of incidence, sometimes referred to as the internal angle between the surface of the pile and the horizontal surface, depends on the density, surface area, shapes and coefficient of friction of the material. Using the formula, it is calculated,

$$Angle\ of\ Repose\ (\Theta) = tan^{-1}\left(\frac{h}{r}\right)$$

Where, h = Height of Pile and r = radius of Pile

Table 3: Powder flow properties based on angle of repose

Angle of repose	Flow ability
< 25	Excellent
25-30	Good
30-40	Passable
> 40	Very poor

2. Determination of Buoyancy, Entrapment Efficiency and Percentage Yield

2.1*In vitro*buoyancy study [14]

In vitro buoyancy was determined by placing 50 mg of the formulation in 100 ml of SGF (pH 1.2) containing Tween 20 (0.02 w/v %) stirred at 100 rpm using a magnetic stirrer. The

layer of floating microballoons was separated from the microballoons which settled after 12 hours by filtration. Both particles obtained were dried and weighed separately. Using the formula below, the buoyancy of the microballoons was determined.

$$Buoyancy\ (\%) = \frac{Wf}{W} x\ 100$$

Where Wf and W are the respective weights of the floated and total weight of Microballoons added.

2.2 Entrapment Efficiency and Percent Yield [14]

50 mg microballoons accurately weighed and crushed in a glass mortar and pestle. The powder microballoons were suspended in 10 mL of ethanol, then the solution was filtered and the volume was made up to 50 mL with 0.1 N HCl. The filtrate was analyzed for drug content at 243 nm. Corresponding drug concentrations in the samples were calculated from a calibration plot created by regression of the data. The percent (%) yield and sample Entrapment efficiency were calculated using Equations 2 and 3. The average of three determinations was considered as the result of the assay performed.

$$\%\ \text{Yield} = \frac{\text{Total Weight of Microballons}}{\text{Total Weight of Drug And Excipients}} \times 100 \qquad (2)$$

$$\text{Entrapment efficiency} = \frac{\text{Weight of Repaglinide in Microballoons}}{\text{Initial weight of Repaglinide}} \times 100 \qquad (3)$$

3. *In vitro*Dissolution Study [15, 16]

The in vitro release study of the prepared microballoons was performed on a USP type II (paddle type) apparatus using 900 mL of dissolution medium at 37 ± 0.5 °C. A 5 ml sample was taken at the specified time interval and replaced with fresh medium. The samples were analyzed by UV-Vis spectrophotometry by measuring the absorbance of the sample at 243 nm to determine the amount of dissolved drug. All determinations were performed in triplicate.

***4. Invivo*Antidiabetic Study [17, 18, 19, 20, 21, 22, 23, 24]**

Objective of this study is to evaluate and compare the antidiabetic activity of B5-Optimized batch test formulation (Repaglinide microballoons) with a marketed tablet in diabetic Wistar rats.

Animals

Experimental study was carried out using adult healthy Wistar Rats. Rats were procured from LacsmiBiofarms Pvt. Ltd., Pune having CPCSEA No. 1277.

Approval of Experimental protocols

Ethical clearance was obtained for procuring of rats and for "Formulation and Evaluation of Novel Gastroretentive Microballoons of Repaglinide"

CPCSEA Registration No. 1942/PO/Re/S/17/CPCSEA/2019/01/08/02,

Dated: 10/08/2019

Animal Maintenance (Housing and feeding condition)

Experimental studies were performed using normal adult healthy Wistar rats (150-250 g). Rats were housed in clean and disinfected polypropylene cages under standard environmental conditions. All rats were acclimatized and habituated to laboratory

conditions for seven days prior to the experiment to minimize non-specific stress conditions. Rats aged 8-10 weeks and body weight 150-250 g were used in this study. Rats were housed in standard polypropylene cages, fed a standard rat pellet diet, water ad libitum, and maintained under standard laboratory conditions; air conditioning with sufficient supply of fresh air.

Environment: Temperature 22 ± 3 °C, Relative humidity 30% to 70%.

Induction and Assessment of Diabetes: Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared streptozotocin (STZ-60 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5) in a volume of 1 ml/kg body weight [7]. At 48 h after administration of streptozotocin, blood glucose levels were estimated in rats after an overnight fast. Rats with blood glucose levels greater than 250 mg/dL were considered diabetic and used for experiments.

Rationale: In this study, the rats are grouped based on their diabetic status and the treatment they will receive. The rationale for grouping is to compare the antidiabetic activity of Repaglinide microballoons with a marketed tablet in diabetic Wistar rats, and to evaluate their effect on blood glucose levels.

Grouping: Twenty adult healthy Wistar Rats divided equally (n=5) in four group; Group I (Normal control), Group II (Positive Control), Group III (B5- Optimized batch test formulation) and, Group IV (marketed tablet formulation) were used in this experiment.

The grouping of rats in this study is as follows:

Group I: Normal Control - This group consists of non-diabetic rats, which will not receive any treatment. This group serves as a Normal control, allowing for the assessment of normal physiological parameters in healthy rats.

Group II: Positive Control - This group consists of diabetic rats, which will receive the vehicle alone (0.5%, w/v solution of Sodium Carboxy Methyl Cellulose, NaCMC). This group serves as a positive control to evaluate the progression of diabetes in the absence of treatment.

Group III: Diabetic rats treated with B5- Optimized batch test formulation- This group consists of diabetic rats which will receive Repaglinide microballoons (B5- Optimized batch test formulation) at a dose of 0.2 mg/kg body weight in a 0.5%, w/v Sodium Carboxy Methyl Cellulose (NaCMC) aqueous suspension form through oral gavage. This group is the experimental group, which will be used to assess the antidiabetic activity of the Repaglinide microballoons.

Group IV: Diabetic rats treated with the marketed tablet- This group consists of diabetic rats which will receive the marketed tablet at a dose of 0.2 mg/kg body weight in a 0.5%, w/v Sodium Carboxy Methyl Cellulose (NaCMC) aqueous suspension form through oral gavage. This group serves as a reference group to compare the antidiabetic activity of Repaglinide microballoons with marketed tablet.

The normal control and positive control groups will help to establish the baseline for comparison, while the treated groups will help compare the therapeutic effects of Repaglinide Microballoons against available Market Standard Tablet.

Table 4: Groupings of rats

Groups (n=5)	Dose
Group I: Normal Control	No Treatment
Group II: Positive Control	1 ml of vehicle (0.5%, w/v solution of NaCMC)
oup III: Diabetic Rats treated with	2mg/kg in a 0.5%, w/v NaCMCaqueous

- Optimized batch test formulation	suspension form through oral gavage.
roup IV: Diabetic rats treated with marketed tablet	2mg/kg in a 0.5%, w/v NaCMCaqueous suspension form through oral gavage.

Calculation of Dose for test and Standards formulation Formula: -

- Human Equivalent Dose $(\text{mg}/kg) = \frac{\text{Human Maximum Daily Dose}}{\text{Human Average Body Weight}} = \frac{\mathbf{2}}{\mathbf{60}} = \mathbf{0.033}\ \text{mg}/kg$
- **Conversion of Human dose to Rat dose**
- Human Equivalent Dose (HED)$(\text{mg}/kg) =$

  Rat equivalent dose $(\text{mg}/kg)\ x\ \frac{\text{Km factor of Rat}}{\text{Km Factor of Human}}$
- Rat equivalent dose $\left(\frac{\text{mg}}{kg}\right) = \frac{\text{Human Equivalent Dose}\frac{\text{mg}}{kg}}{\frac{\text{Km factor of Rat}}{\text{Km Factor of Human}}} = \frac{0.033}{6/37} = \mathbf{0.2}\frac{\text{mg}}{kg}$

	
dministration of B5- Optimized tch test formulation (0.2 mg/kg body weight)	Administration of arketed tablet (0.2 mg/kg body weight)

Figure 1: Administration of Dose to different groups of Rats

Blood sampling and Determination of blood Glucose Levels:

The treatment was administered orally in a 0.5%, w/v NaCMC aqueous Suspension form through oral gavage to all the rats. Small aliquot of blood sample was collected from tail vein at 0, 1, 2, 4, 6, 8, 10 and 12hours. Immediately after blood collection it was used for determination of blood glucose level using glucometer (Accu-Chek, Roche diagnostics India Pvt. Ltd., Mumbai). Blood glucose levels were expressed as mg/dl.

	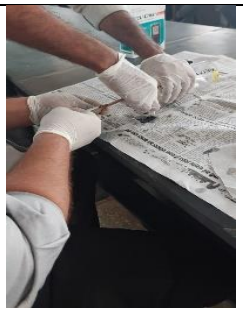
ood sample collection from tail ein for B5- Optimized batch test formulation	ood sample collection from tail vein for marketed tablet

Figure 2: Blood sample collection from different groups of Rats

Statistical analysis: All values of the pharmacodynamics studies were expressed as mean ± SD. The data were statistically evaluated using two-way analysis of variance (ANOVA) followed by Dunnette's Multiple Comparison Test using Graph Pad Prism computer software (Version 8.0.1)

RESULTS AND DISCUSSIONS

Preformulation Studies (Drug Identification and Characterization)

1. Organoleptic properties

The drug sample was found to be white to off white in color, crystalline and odorless powder. This observation matches with the specified description of Repaglinide.

2. Melting Point Determination

Melting point of drug was determined by melting point apparatus and compared with reference value. Melting point was found to be in range as reference value, which confirmed the purity and identity of the drug.

- Reference Melting Point [25]: - 130-131ºC
- Observed melting point: - 130ºC

3. Fourier Transform Infra-Red (FTIR) analysis

The FTIR study of pure drug sample was carried out using FTIR Spectrophotometer. Peaks observed in FTIR spectra of sample drug were recorded and were similar to reported reference values. FTIR spectra confirmed the identity and purity of Repaglinide. The drug shows peaks due to ketonic group, secondary amine, alcohol group, terminal CH_3, C=O stretching in COOH and CONH group.

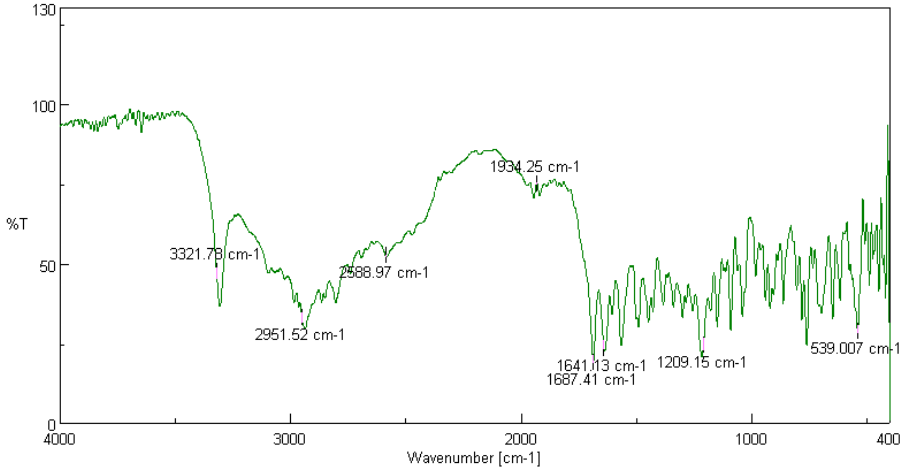

Figure 3: FTIR spectra of Repaglinide sample [28]

Table 5: FTIR spectra of Repaglinide

r. o.	Functional Groups	eference eak [26,27] (cm^{-1})	bserved Peak (cm^{-1})
.	-H stretching vibration	200-3400	3321.78
.	-H stretching vibration	300-3000	2951.52
.	C=O stretching	600-1800	1687.41
.	N-H bending	625-1650	1641.13
.	H3 stretching vibration	200-1250	1209.15

4. UV spectrophotometric analysis

Determination of λ_{max}

The UV spectrophotometric scanning of Repaglinide in 0.1N HCl was done and $\lambda_{max}$was found to be 243 nm.

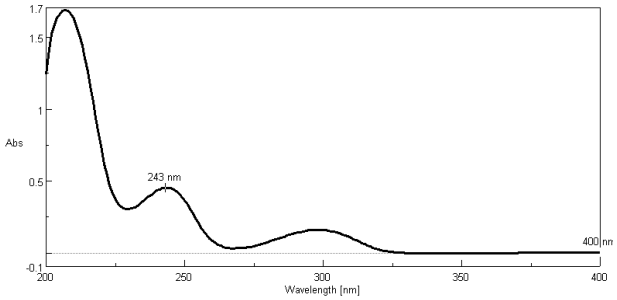

Figure 4: UV Spectrum of Repaglinide

Calibration Curve:

The calibration curve of Repaglinide in 0.1N HCl at 243 nm was developed and absorption values are given in Table 6. It was found to obey Beer's law in prepared concentration range.

Table 6: Absorbance values of various concentrations solution of Repaglinide in 0.1N HCl

centration µg/ml)	orbance 243 nm)
0.4	.2164
0.8	.3914
1.2	.5583

1.6	.7464
2	9335
2.4	.1306
3	4148
3.6	.7016

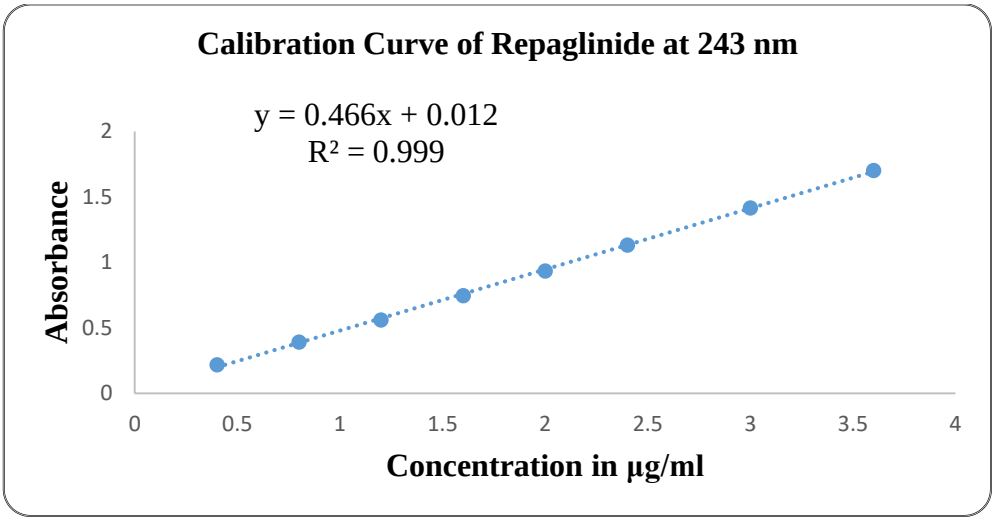

Figure 5: Calibration curve of Repaglinide in 0.1N HCl

5. Solubility determination

The solubility of Repaglinide was evaluated by equilibrium solubility method in rotary shaker. The result of solubility study is given in table 7. Solubility of Repaglinide was found to increase due to ionization at higher pH value.

Table 7: Solubility evaluation of Repaglinide

epaglinide	olubility in stilled water (µg/ml)	olubility in PBS pH 6.8 (µg/ml)	ubility in0.1 N HCl (µg/ml)
	21.4	19.4	23.7

6. Drug: Excipients Compatibility Study

FTIR spectrum of repaglinide, polymers and physical mixture of drug with polymerswere recorded separately using FTIR spectrophotometer to study any possible interaction between drug and polymer. FTIR of pure repaglinide showed characteristic sharp peaks at 3321.78 cm^{-1} due to N-H stretching, 2951.52 cm^{-1} showing C-H stretching, 1687.41 cm^{-1} due to carbonyl group, 1641.13 cm^{-1} due to N-H bending.15 cm^{-1} vibration 120 cm^{-1} These peaks were observed in the FTIR spectrum of the physical mixture of drug and polymer, indicating the compatibility between the drug and the polymers to be used for formulation development

Figure 6: FTIR Spectra of Repaglinide [28]

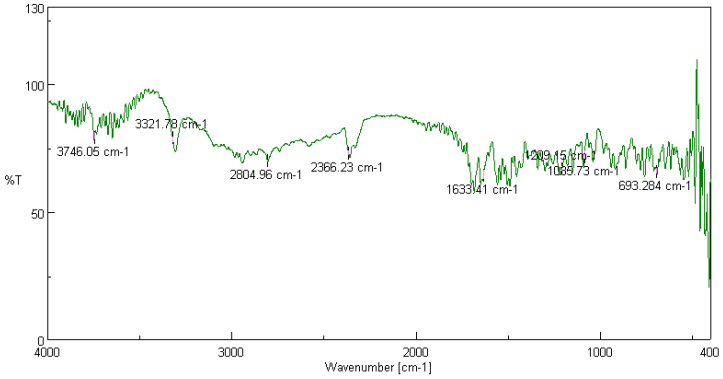

Figure 7: FTIR spectra Repaglinide + Sodium Alginate

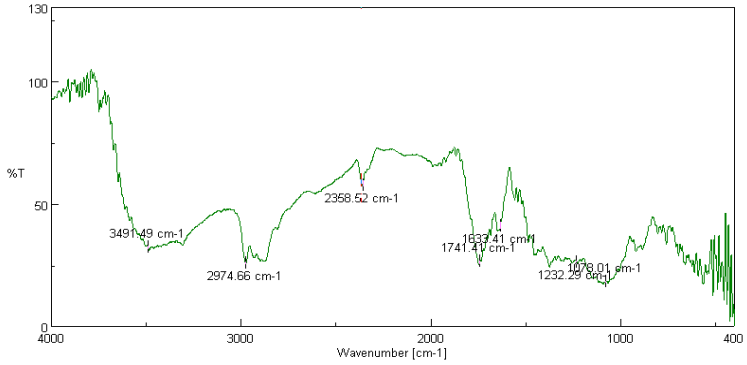

Figure 8: FTIR spectra of physical mixture of Repaglinide + Ethyl Cellulose

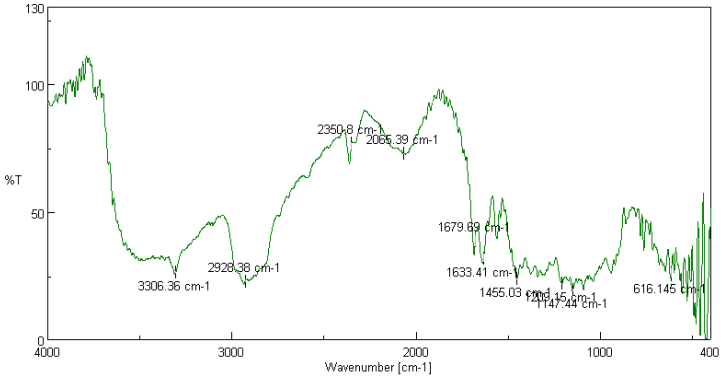

Figure 9: FTIR spectra of physical mixture of Repaglinide+ HPMC K4M

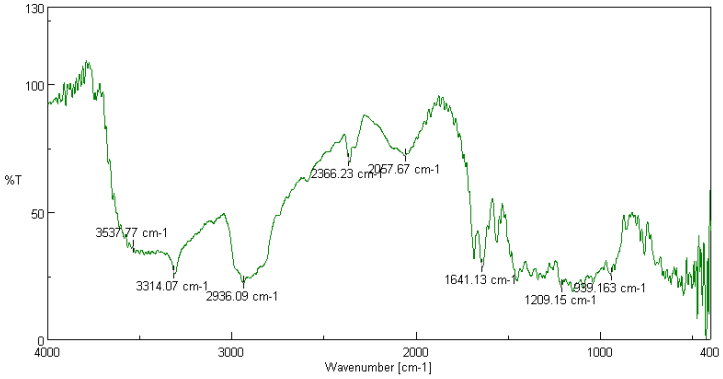

Figure 10: FTIR spectra of physical mixture of Repaglinide + HPMC K15M

Table 8: Characteristic peaks of Repaglinide alone and in combination with polymers

Composition	N-H etching 00-3400 Cm^{-1})	C-H 00-3000 Cm^{-1})	C=O 00-1800 Cm^{-1})	N-H ending 25-1650 Cm^{-1})	CH3 00-1250 Cm^{-1})
Repaglinide	321.78	951.52	687.41	641.13	209.15
Repaglinide + SA	321.78	804.96	702.84	633.41	209.15
Repaglinide +EC	491.49	974.66	741.41	633.41	232.29
glinide + HPMC K15M	314.07	936.09	641.13	633.41	209.15
aglinide +HPMC K4M	306.36	928.38	679.69	633.41	209.15

Table 8 shows that the characteristic peaks of repaglinide observed in its pure form are also present in physical mixtures of the drug with polymers. This indicates that there is no significant interaction or chemical reaction between the drug and the polymers. NH stretching peak (3321.78 cm^{-1}), C-H stretching peak (2951.52 cm^{-1}), carbonyl group peak (1687.41 cm^{-1}), N-H stretching peak (1641.13 cm^{-1}) and stretching peak CH3 (1209.15 cm^{-1}) of repaglinide are preserved in the FTIR spectra of drug-polymer mixts. This further indicates the compatibility between the drug and the polymers used for formulation development. When repaglinide is combined with various polymers such as sodium alginate (SA), ethyl cellulose (EC), hydroxypropyl methyl cellulose (HPMC) K15M and K4M, there are no significant shifts or changes in the characteristic peaks compared to the pure drug spectrum. This strengthens the conclusion that the drug and polymers are compatible with each other. Overall, based on the FTIR analysis results, it can be concluded that Repaglinide is compatible with the tested polymers (SA, EC, HPMC K15M and HPMC K4M), indicating their suitability for formulation development without any observed drug-polymer interactions.

7. Physical Observation

Physical mixtures were observed for any change in color, odor, or formation of lumps or signs of liquefaction. No significant change was observed in the mixture during four weeks of storage. The results are shown in Table 9.

Table 9: Drug Excipient Compatibility evaluation by physical observation when stored at 40ºC and 75% RH

..	Content	mposition	week	d week	l week	n week
	epaglinide	1	No nange	No nange	No nange	No nange
	epaglinide + SA	1:1	No nange	No nange	No nange	No nange

	epaglinide + EC	1:1	No hange	No hange	No hange	No hange
	epaglinide HPMCK4M	1:1	No hange	No hange	No hange	No hange
	epaglinide HPMCK15M	1:1	No hange	No hange	No hange	No hange

Formulation of Microballoons of Repaglinide

Microballoons of repaglinide were previously prepared using the solvent evaporation method, and the Box-Behnken design was employed to optimize the formulation. Thirteen batches (B1 – B13) were prepared using the design, and their flow properties were evaluated. The flow properties, including the angle of repose, Carr's index, and Hausner ratio, were assessed for all batches to identify the optimized batch. Batch B5 was identified as the optimized batch based on the flow properties, and it was selected for further evaluation. *In vitro*and *in vivo*evaluations were conducted using batch B5 to assess its release characteristics and antidiabetic activity. The *in vitro*release of repaglinide from batch B5 was studied using a dissolution apparatus, and the % drug release for 12 Hrs. was analyzed. For *invivo*evaluation, Streptozotocin-induced diabetic rats were used to measure the antidiabetic activity of batch B5 by monitoring blood glucose levels. By following this methodology, the study aimed to optimize the formulation of repaglinide microballoons and evaluate their flow properties, *in vitro*release, and *in vivo*antidiabetic activity.

Evaluation of Microballoons

1. Determination of Flow Properties

The flow behavior of Microballoons was analyzed by determining Carr's Index, Hausner ratio and Angle of repose.

Table 10: Evaluation of microballoons related to Flow Properties

ormulation code	**Bulk density gm/cm³**	**Tapped density gm/cm³**	**Angle of epose (θ)**	**arr's ndex**	**ausner Ratio**
B1	.76± 0.01	83 ± 0.03	3.9 ± 1.2º	3.43	1.09
B2	.76± 0.03	.84± 0.03	0.2± 1.6º	9.52	1.10
B3	.76± 0.03	.85± 0.04	1.8 ± 1.4º	0.58	1.11
B4	.77± 0.03	.86± 0.05	1.7 ± 2.1º	0.46	1.11
B5	.76±0.02	.82± 0.03	5.2 ± 1.7º	7.89	1.12
B6	.77±0.02	.85± 0.02	8.2± 1.9º	9.41	1.07
B7	.77±0.02	.85± 0.05	7.4± 1.1 º	9.41	1.10
B8	.77± 0.02	.85± 0.03	0.5± 1.4º	9.41	1.10
B9	.76± 0.03	.86± 0.04	0.2± 1.7º	1.62	1.13

B10	76 ± 0.01	83 ± 0.03	9.8 ± 1.1º	3.43	1.09
B11	.76± 0.03	.84± 0.03	9.9± 1.1º	3.43	1.09
B12	.76± 0.03	.84± 0.04	0.2 ± 0.8º	9.52	1.10
B13	.77± 0.03	.86± 0.05	0.7 ± 1.0º	0.46	1.11

Values are Mean ±SD, n=3

2. Determination of Buoyancy, Entrapment Efficiency and Percentage Yield

The *in vitro*buoyancy behavior and drug entrapment study was done on formulations of repaglinide microballoons.

Table 11: *In vitro*evaluation of microballoons

ormulation code	**Buoyancy (%)**	**Entrapment fficiency (%)**	**ercentage Yield**
B1	69.83±2.51	80.26 ± 3.51	1.03±1.26
B2	1.41 ±2.86	79.52 ± 2.92	1.63±1.37
B3	64.27±3.21	79.40 ± 2.98	0.01± 1.28
B4	9.97 ±3.46	78.21 ± 2.87	1.01±1.33
B5	5.02 ±3.16	81.12 ± 2.58	2.36±1.92
B6	4.18 ±1.56	78.23 ± 4.01	0.34± 1.22
B7	68.22±1.98	74.25 ± 3.54	9.81± 2.33
B8	4.73± 2.15	74.89 ± 3.62	0.94±2.16
B9	2.34 ±3.66	73.26 ±2.94	8.94± 2.16
B10	67.83±2.51	79.54 ± 2.77	0.13±1.52
B11	62.30±2.86	79.72 ± 1.73	1.23±1.87
B12	63.57±3.21	78.40 ± 1.25	0.66± 1.88
B13	0.72± 2.06	78.38 ± 1.69	1.56± 1.93

Values are Mean ±SD, n=3

Table no. 10 and 11 shows that among the different formulations (B1 to B13), Batch B5 demonstrated exceptional performance in multiple aspects. It exhibited the highest entrapment efficiency of 81.12 ± 2.58%, indicating effective trapping of the desired substance within the microballoons. Additionally, B5 displayed a high buoyancy percentage of 65.02 ± 3.16%, suggesting a greater tendency to float, which is advantageous for sustained-release drug delivery systems. The formulation also yielded a high percentage of the desired substance at 92.36 ± 1.92%, indicating efficient production and reduced wastage. Moreover, B5 showcased better flow ability with a lower Carr's index value of 7.89, making it easier to handle and process during manufacturing. Furthermore, it displayed a lower angle of repose of 25.2 ± 1.7º, indicating enhanced powder cohesiveness

and stability. As a result of these favorable characteristics, Batch B5 was selected for further investigation.

3. *In vitro*Dissolution Study

*In vitro*dissolution studies are conducted to understand the release kinetics and behavior of the microballoons. These studies simulate the conditions of the human body to evaluate the % drug release profile and determine factors such as release rate, duration, and overall release efficiency. The dissolution profile obtained from these studies provides valuable information on the release behavior of the microballoons.

Table 12: Percentage of *in vitrodrug* release of Repaglinide Microballoons

Time	Percentage of *in vitro drug* release in various batches (B1-B13) of Repaglinide microballoons												
Hrs.)	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**B11**	**B12**	**B13**
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	5.18 1.34	5.64 0.74	6.84 0.74	2.54 0.46	5.75 0.25	4.44 0.87	4.04 0.62	3.24 0.63	5.24 0.53	1.64 0.53	9.24 0.97	0.48 0.53	8.04 0.97
2	0.38 1.87	0.75 0.53	1.65 0.53	8.65 0.63	3.35 0.33	9.85 0.72	8.73 0.25	8.95 0.86	0.47 0.63	5.25 0.63	3.45 0.95	4.38 0.63	2.55 0.95
3	7.56 1.98	8.02 0.63	9.22 0.63	4.35 1.87	1.26 0.64	6.82 0.75	6.42 0.63	5.62 1.75	7.62 0.53	4.02 0.25	1.62 0.97	2.86 1.87	0.42 0.53
4	7.39 0.46	0.51 0.82	1.09 0.25	8.24 0.53	8.97 0.93	0.04 0.97	1.34 0.25	9.35 0.79	1.35 0.63	9.04 0.25	6.74 0.95	6.87 0.53	5.58 0.64
5	6.74 0.57	6.09 0.36	7.43 0.64	4.62 0.64	5.31 0.86	4.75 0.95	5.47 0.63	5.42 0.63	6.81 0.64	2.79 0.82	0.11 0.63	1.49 0.64	8.77 0.64
6	0.56 0.67	2.45 0.67	3.47 0.75	0.15 0.82	2.87 0.97	1.43 0.46	9.59 0.25	0.41 0.63	0.61 0.53	7.55 0.36	5.51 0.53	6.56 0.36	4.49 0.75
7	9.48 0.76	9.38 0.76	0.65 0.97	7.73 0.36	0.17 0.95	8.11 0.57	8.27 0.25	6.84 1.87	9.54 0.63	5.73 0.67	3.19 0.63	4.05 0.67	1.92 0.97
8	5.91 0.93	8.24 0.93	9.24 0.95	5.39 0.67	4.35 0.63	7.28 0.67	5.52 0.53	6.32 0.53	5.96 0.82	3.08 0.76	1.16 0.97	2.15 0.76	0.02 0.25
9	2.03 0.82	4.07 0.82	4.93 0.63	2.27 0.25	1.18 0.53	3.21 0.63	0.85 0.63	2.35 0.64	2.09 0.53	8.37 0.93	6.65 0.95	7.54 0.53	5.79 0.63
10	0.36 0.36	2.41 0.36	3.41 0.53	8.12 0.25	9.36 0.36	1.41 0.53	9.41 0.82	0.41 0.82	0.41 0.64	7.41 0.67	5.41 0.63	6.44 0.67	4.41 0.53
11	6.44 0.53	7.54 0.46	9.14 0.63	2.15 0.87	1.97 0.53	5.07 0.82	5.44 0.87	4.65 0.53	6.49 0.87	3.34 0.53	1.24 0.63	2.32 0.76	0.19 0.67
12	1.04	1.07	3.76	5.13	5.48	8.12	0.86	7.53	2.57	9.13	6.23	8.48	4.15

	0.63	0.57	0.64	0.72	0.64	0.53	0.72	0.63	0.72	0.64	0.53	0.72	0.25

Values are Mean ± SD, n=3

The table 12 shows the percentage of *in vitro*drug release from different batches of microballoons (B1-B13) over a period of 12 hours. Overall, the data in the table suggests that the microballoons are a promising delivery system for Repaglinide. The percentage of drug release increases as the time progresses for all samples. This indicates that the release of the drug from the samples occurs gradually over the testing period. Comparing the percentage of drug release among different samples at each time point, variations in drug release can be observed. The samples of batch B5, B7, and B9 consistently show higher percentages of drug release compared to other samples across most time points. Generally, the percentage of drug release tends to increase over time for all samples, indicating sustained drug release.

4. *Invivo*Antidiabetic Study

Blood Glucose Estimation

The blood glucose levels of rates in all the groups were determined by using glucometer and are given in following table.

Table 13: Blood glucose levels in rats of different groups

ne r.)	**roup-I ormal ontrol ng/dl)**	**roup-II ositive ontrol ng/dl)**	**Group-III Optimized batch st formulation mg/dl)**	**Group-IV Marketed Tablet mg/dl)**
	72±5.15	.38±6.44	243.80±12.23*	7.17±6.14ns
	52±7.13	.02±6.63	05.60±5.07***	33±10.75***
	42±5.51	16±11.87	86.25±5.64***	.31±18.9***
	73±7.26	.11±6.32	31.39±10.27***	.53±5.13***
	45±6.62	.05±2.17	23.83±6.82***	.61±6.16***
	2±12.32	.55±2.09	15.52±5.03***	.94±4.52***
0	02±7.7	.83±4.58	03.67±3.07***	.45±4.12***
2	1±10.34	.11±5.11	18.80±4.43***	.06±9.54***

Values are Mean ±SD, n=5,

p<0.12(ns), p<0.033(*), p<0.001(***) when Group-III and Group-IV are compared with Group II by using two-way ANOVA followed by Dunnette's multiple comparison test.

The table 13 shows the blood glucose levels in rats at various time intervals (0, 1, 2, 4, 6, 8, 10 and 12 hours) for four different groups, including a Normal Control (Group I), a Positive Control (Group II), a B5- Optimized batch test formulation (Group III), and marketed tablet (Group IV). The blood glucose levels of group III and IV were compared with Group II using two-way ANOVA followed by Dunnette's multiple comparison tests. Overall, the results presented in above table suggest that the B5- Optimized batch test formulation have potential hypoglycemic effect in rats, which makes them promising candidates for further development as treatments for diabetes. However, it is important to note that these findings are limited to rat models and may not necessarily translate to humans. Further studies are needed to determine the safety, efficacy, and optimal dosing of these treatments in humans.

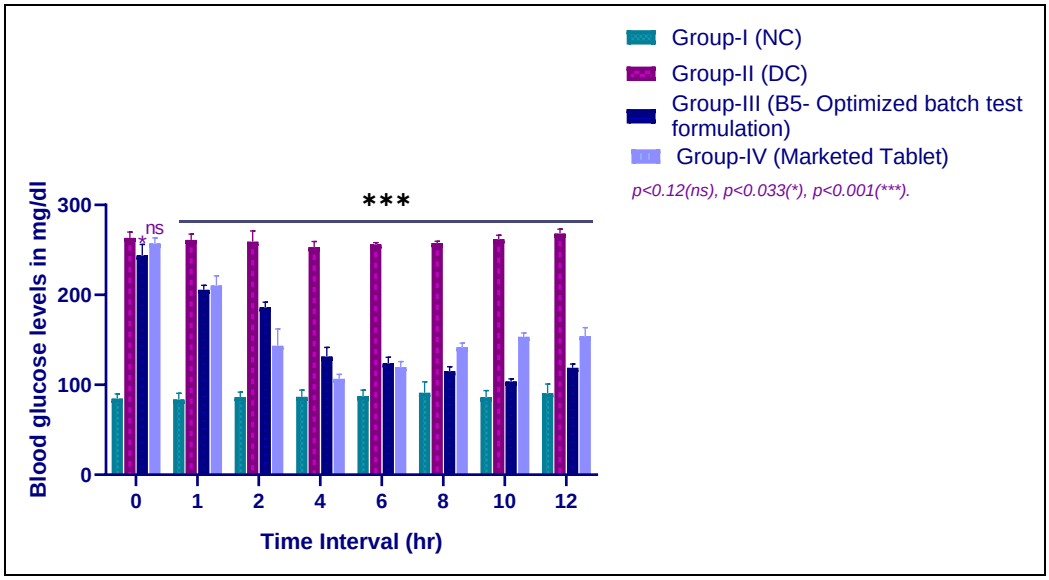

Figure 11: Blood Glucose Levels in Rats of Different Groups.

SUMMARY AND CONCLUSION

The prepared microballoons were subjected to flow properties,*in vitro*and *in vivo*evaluations to assess their performance. The results indicated that the microballoons exhibited uniform particle size, smooth surface morphology, high drug encapsulation efficiency. *In vitro* and *in vivo*evaluation demonstrated that the microballoons provided a prolonged and controlled release of repaglinide as compared to conventional dosage forms. Moreover, the microballoons were found to be well-tolerated with minimal adverse effects, emphasizing their safety profile.

In conclusion, the previously formulated repaglinide microballoons using the solvent evaporation method and Box-Behnken design was successful. The optimized formulation

exhibited favorable characteristics, including uniform particle size, high drug encapsulation efficiency, and sustained drug release. *In vitro*and *in vivo*evaluations confirmed the enhanced therapeutic efficacy of repaglinide when formulated as microballoons. These findings highlight the potential of repaglinide microballoons as a promising strategy for the treatment of diabetes, offering controlled drug release and improved patient compliance. These findings contribute to the development of an effective drug delivery system for repaglinide, addressing the challenges associated with its poor solubility and low bioavailability. Overall, microballoons hold great potential in improving the management of type 2 diabetes mellitus through enhanced drug delivery.

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