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Preparation and Evaluation of Alginate–Chitosan Microspheres loaded Hard Gelatin Capsule for Antidiabetic activity

Sanghavi S J[1]* , Satish Kumar Sarankar [2]

[1] *Research Scholar, Faculty of Pharmacy, Mansarovar Global University, Sehore (Madhya Pradesh)
[2] Professor, Faculty of Pharmacy, Mansarovar Global University, Sehore (Madhya Pradesh)

Abstract:

Objective: This research focuses on the development and evaluation of sustained-release (SR) alginate–chitosan microspheres encapsulating glipizide, intended for oral administration via hard gelatin capsules to enhance diabetes management.
Methodology: Microspheres were synthesized using an ionic gelation approach. Aqueous solutions of sodium alginate and chitosan were gently stirred with glipizide, and the resulting mixture was extruded into a 5% calcium chloride solution to form microspheres. This method aimed to ensure controlled drug release and reduce the gastric irritation typically associated with single-unit glipizide formulations. Transitioning to a multi-unit dosage form allows the drug to be evenly distributed across the gastrointestinal tract, potentially minimizing irritation and enhancing absorption.
Results: The prepared microspheres exhibited particle sizes ranging from 200 to 500 µm. Among the formulations, GCS7 demonstrated the highest mucoadhesion at 89.3 ± 0.51% and a swelling index of 28.0%, while GCS6 achieved the highest drug entrapment efficiency at 90.2 ± 0.29%. In vitro drug release for formulations GCS1 and GCS2 ranged from 81% to 91%, indicating efficient sustained release. In vivo studies showed that formulation GCS3 achieved a 50–60% reduction in plasma glucose levels compared to standard formulations.
Conclusion: This study also explored various factors influencing the performance of microspheres in oral delivery systems. Sustained-release formulations are particularly useful for drugs like glipizide, which have a short half-life and are rapidly cleared from systemic circulation. By providing controlled drug release in the gastrointestinal tract, the developed microspheres help maintain more consistent plasma drug levels over an extended period.

Keywords: Microsphere, Capsule, Glipizide, Hard gelatin, Chitosan, Sodium alginate, etc.

Introduction

Diabetes mellitus is a leading cause of death and disability worldwide. In 2000, it was estimated that approximately 171 million people were living with diabetes globally, with projections suggesting this number could rise to 366 million by 2030. According to the American Diabetes Association (ADA), the economic burden of diabetes in the United States was around $132 billion in 2002, with expected costs reaching $192 billion by 2020. Due to its growing prevalence, there is a strong emphasis in the medical field on both preventing and managing diabetes, which is reflected in the increasing volume of research conducted annually.

Diabetes mellitus (DM) refers to a collection of chronic metabolic conditions characterized by elevated blood glucose levels (hyperglycemia) and disruptions in the metabolism of fats, carbohydrates, and proteins. These changes occur because the body either does not produce enough insulin or cannot effectively use the insulin it produces. DM is associated with a shortened lifespan, serious complications affecting small blood vessels (microvascular complications), and a decline in overall quality of life. A fasting blood glucose measurement of 126 mg/dL or higher, or a blood glucose level of 200 mg/dL or above following an oral glucose intake, are commonly used diagnostic criteria for diabetes.
Medications used to manage diabetes work by lowering blood sugar levels. Apart from insulin, exenatide, and pramlintide, most diabetes medications are taken orally and are therefore often referred to as oral hypoglycemic agents. There are several different classes of these drugs, and the choice of treatment depends on factors such as the type of diabetes, the patient's age, and their overall health status. Effective management requires a personalized approach based on an accurate diagnosis of the diabetes subtype.

Drug delivery systems capable of accurately regulating drug release or directing medications to particular areas of the body play a significant role in healthcare. Over the last twenty years, significant progress has been made through the integration of polymer science and materials engineering, leading to the creation of innovative drug delivery technologies [1]. Among various particulate systems, microspheres stand out due to their small size and effective ability to carry drugs. Nevertheless, their limited effectiveness is often attributed to their brief retention time at the absorption site [2]. Because the properties of the active drugs vary widely, microspheres are typically tailored to meet specific

therapeutic requirements [3]. The emulsion-gelation technique is commonly used for preparing microspheres because it is straightforward and cost-efficient [4].
Diabetes mellitus (DM) is a long-term metabolic condition that impacts individuals globally, leading to serious health issues and deaths primarily due to complications involving small and large blood vessels, which affect many essential organs and tissues [5]. Projections indicate a sharp rise in the number of people with diabetes by 2030. The actual prevalence is likely higher, as numerous cases remain undetected due to a lack of symptoms. Glipizide is a medication used to manage Type II diabetes. Because glipizide has a relatively short half-life of 2 to 3 hours, it has been developed into a sustained-release (SR) form to minimize how often it needs to be taken. An ideal dosage form ensures the drug reaches and maintains effective levels in the bloodstream throughout the treatment period. The absorption of glipizide decreases when it is taken with food, so it is recommended to take it on an empty stomach. The sustained-release versions provide a steady level of the drug in the blood for up to 24 hours [6]. Glipizide undergoes extensive liver metabolism (about 90%), with only a small portion (around 10%) eliminated unchanged through the kidneys [7].

In this study, a glipizide formulation was developed to maintain measurable blood concentrations for over 10 hours by utilizing a polymer with expandable, gelling, and swelling properties. The chosen polymer was sodium alginate, a cost-effective and non-toxic substance derived from the brown algae known as kelp. Also referred to as alginic acid or algin, this anionic polysaccharide is commonly found in the cell walls of brown algae and forms a viscous gel when it interacts with water. Sodium alginate has the ability to absorb between 200 to 300 times its weight in water. The chemical composition of alginates varies depending on the species of brown seaweed from which they are extracted, resulting in differences in their physical characteristics. Some sources produce alginates that form strong gels, while others yield weaker gels; some are easily refined into a creamy white powder, whereas others are more challenging to process and are better suited for applications where color is not critical [8,9].

Sodium alginate is commonly utilized as a thickening and gelling agent and is also employed in emulsion preparation due to its ability to lower the interfacial tension between oil and water phases. This substance is a linear copolymer consisting of two types of monomers: D-mannuronic acid and L-guluronic acid. These monomeric units are arranged within the alginate chain in distinct blocks made up solely of one type of monomer (known as M-blocks or G-blocks), or in alternating sequences of both monomers. The unique calcium-binding properties of alginate arise from the specific molecular structure of these blocks. Sodium alginate forms firm gels when exposed to calcium ions or other multivalent cations. While it is straightforward to categorize alginate chains by their M and G components, analyzing the precise molecular arrangement, including block sizes and their distribution, presents a complex challenge. Additionally, microspheres composed of calcium pectinate and coated with chitosan have been explored for sustained drug release or targeted delivery to the colon [10-12].

Capsules are widely used and commonly seen as a solid form of medication. They can serve as effective containers for multiple types of drug delivery systems, including microspheres and pellets. Gelatin is the primary polymer used to manufacture hard capsules because it dissolves in hot water and in the acidic environment of the stomach. Unlike tablets, capsules generally need fewer excipients during production and do not involve compression, which helps preserve the integrity of sensitive materials like microspheres.

Material and Method

Material
Glipizide was kindly provided as a sample by Kreative Organics, Hyderabad. Chitosan was sourced from the Kerala State Chitosan Plant in Kerala, while sodium alginate was acquired from CDH, New Delhi. All other reagents used in the study were of analytical grade.

Preparation of microspheres
Glipizide Chitosan-Alginate Microspheres
In thirty milliliters of distilled water, the 25 mg of sodium alginate was dissolved. 100 mg drug was added, and it was homogenized. 25 mg of chitosan was dissolved in 2% v/v acetic acid, and the pH (5.5) was corrected with a 10% NaOH solution. To it was added a 5% solution of calcium chloride dehydrate. The chitosan-calcium chloride solution was gradually stirred with a thermally controlled magnetic stirrer as the drug alginate combination was introduced drop by drop at a steady rate of 30 ml/h.

After being filtered, the microspheres were cleaned with acetone and dried at 35°C in an oven. Before being employed, the desiccator held the dried formulations in a bottle with an amber tint (Table 1).

Table 1: Ionic gelation technique formulating process codes and parameters for Glipizide microsphere formulation

Code	Drug	Sod. alginate	Chitosan	Distilled water	CaCl2 (5%)	D:P
GCS1	100 mg	25 mg	25 mg	30 ml	100 ml	1:0.5
GCS2	100 mg	50 mg	50 mg	30 ml	100 ml	1:1
GCS3	100 mg	60 mg	60 mg	30 ml	100 ml	1:1.2
GCS4	100 mg	70 mg	70 mg	30 ml	100 ml	1:1.4
GCS5	100 mg	80 mg	80 mg	30 ml	100 ml	1:1.6
GCS6	100 mg	90 mg	90 mg	30 ml	100 ml	1:1.8
GCS7	100 mg	100 mg	100 mg	30 ml	100 ml	1:2

Percentage yield

For every batch, the weight of the microspheres was divided by the combined weight of the medication and polymer to determine the microspheres' percentage yield.

Percentage yield=Practical yield/theoretical yield × 100

Size distribution and size analysis

Using a mechanical shaker with standard sieves, Glipizide and chitosan microspheres were sieved for 10 minutes to divide them into different size fractions in accordance with Indian Pharmacopeia criteria. The distribution of particle sizes was ascertained, and the average particle size of the gel beads was computed using the subsequent formula.

$$\text{Mean particle size} = \frac{\Sigma\left(\text{Mean particle size of the fraction} \times \text{weight fraction}\right)}{\Sigma\left(\text{Weight fraction}\right)}$$

Differential scanning calorimetry (DSC)

Drug, polymer, and microsphere thermograms were acquired with a Mettler Toledo DSC 822e calorimeter. DSC calculates the thermal energy that a sample absorbs or releases during heating, cooling, or maintaining a steady temperature. The thermograph demonstrates that there is no interaction between the medication and the polymers. In the thermogram, the medications and polymers had different peaks.

Scanning electron microscopy (SEM)

The microspheres were sprinkled on one side of the double adhesive stub to create the samples for the SEM investigation. Fine gold dust was then applied to the stub. After that, the microspheres were examined at 15 kV using a SEM (JEOL Model JSM-6390LV). It displayed discrete, uniformly distributed microspheres that were more or less uniform.

Entrapment efficiency

Following filtering and appropriate dilutions, the produced microspheres were dissolved, and the amount of drug present in weighed samples was ascertained by spectrophotometric analysis at 270. In the concentration range of 0–35 µg/ml, the procedure complied with Beer's law. The amount of medicine in dry microspheres, both theoretical and actual, was used to compute the entrapment efficiency.

Drug entrapment efficiency (%) =Calculated drug content/theoretical drug content × 100

Swelling index

A specific buffer solution's microspheres' degree of swelling was measured to calculate the swelling index. In order to guarantee total equilibrium weight, the quantity of microspheres was permitted to expand within a buffer solution. Blotting was used to remove any extra liquid that had adhered to the droplets, and a microbalance was used to weigh the enlarged microspheres. After that, the hydrogel microspheres were dried for five hours at 60°C in an oven until the sample's dried mass remained unchanged. The following formula was used to get the microsphere's swelling index.

Mass of swollen microspheres - Mass of dry microspheres / Mass of dried microspheres × 100

Mucoadhesive studies

A wet glass slide that was adhered to the beaker walls with adhesive was covered evenly with about fifty microspheres. After that, this assembly was added to the USP disintegration device. At regular intervals, the number of microspheres

that were still attached to the glass slide was counted, and their Mucoadhesive nature was evaluated. Each measurement was made three times, and the outcomes are shown as a standard deviation (SD) ±.

In-vitro release studies

Glipizide (50 mg) were dissolved in 100 milliliters of methanol to prepare the standard plot. After that, it was appropriately diluted in a range of 0-35 µg/ml for graded solutions. An ultraviolet (UV) spectrophotometer (Shimadzu 1800) was used to measure the absorbance at λmax 270.

In-vitro release of microspheres

The in-vitro release experiments were carried out by buffer change method using 200 ml of phosphate buffers with 0.1 N HCl (1 hr.), 4 pH (1 hr.), 6 pH (3 hrs.), 6.8 pH (3 hrs.), and 7.4 pH (2 hrs.) in sink conditions using a diffusion cell, at 37±0.5°C and 100 rpm. A precisely weighed specimen of premade microspheres was introduced into the recipient cell. Five milliliter aliquots were removed at predetermined intervals and replaced with an equivalent volume of brand-new dissolving medium. Following the appropriate dilution, the aliquots were examined UV spectrophotometrically at λmax 276 nm for Glipizide.

In-vivo hypoglycemic activity

In-vivo hypoglycemic activity of Glipizide formulation, in normal and hyperglycemic rats

The study investigated the in-vivo hypoglycemic activity in male albino Wistar rats that were both healthy and had been given a hyperglycemic injection. The rats were given a dose of microspheres equivalent to 5 mg/kg body weight of Glipizide orally, and the hyperglycemic effect was measured in relation to the pure drug at the same dose by using model Streptozotocin (STZ) -induced diabetic rats. Pinnacle Biomedical Research Institute's Institutional Animal Ethics Committee (IAEC) gave its approval to all animal research (PBRI). The animals were kept in individual cages with regulated temperature (22 ± 2°C). Water and a standard meal were frequently provided to all animals.

The animals were housed in 12-hour cycles of light and dark. Eight groups of six animals each were created from the remaining animals.

A hypoglycemic injection typically refers to the administration of medication, often in the form of an injection that is used to lower blood glucose levels in individuals with diabetes. The term "hypoglycemic" relates to substances that reduce blood sugar levels.

In diabetes management, insulin is the most common hypoglycemic injection used. Insulin helps lower blood glucose by facilitating the uptake of glucose by cells and inhibiting the liver's production of glucose. Other injectable medications that can be considered hypoglycemic include GLP-1 receptor agonists (like liraglutide) that enhance insulin secretion, inhibit glucagon release, and slow gastric emptying.

It's important to note that while these injections are vital for managing blood sugar levels, they can also cause hypoglycemia (low blood sugar) if the dosage is too high, if meals are skipped, or with excessive physical activity. Monitoring blood sugar levels regularly is therefore essential for individuals using these medications.

Induction of diabetes

1. Rats were given an intraperitoneal injection of 60 mg/kg body weight of streptozotocin (STZ) to cause diabetes. 0.1 M citrate buffer with ice cold STZ dissolved in it.
2. Overnight, the animals were given 5% glucose solution to alleviate the hyperglycemia caused by STZ.
3. If the animals' blood glucose level was more than 200 mg/dl on the third day after receiving STZ injection.
4. They were classified as diabetics. Taking the fourth day following the STZ injection as the first day of treatment, the course of treatment was initiated.
5. The course of treatment was extended to day five. After therapy, blood glucose levels and body weight were measured on days 0, 3, 5, and 7.

Experimentation

Every animal was split up into six groups at random, each containing six animals. Group I was the vehicle control group; Groups II, III, IV, V, VI, and VII were rendered diabetic using STZ. Group I was given normal saline orally only. Groups II, VI, and VII received the optimized formulation (5 mg/kg) orally every day, Groups III and IV received Glipizide, and Group V received saline. The test samples were all dosed orally over the whole trial.

Stability studies of formulations

A crucial step in developing a formulation is stability testing. It produces data regarding the formulations and shelf life of medicinal compounds and suggests suitable storage guidelines. The stability of formulations 1 through 7 remained mostly unchanged. Low polymer concentration formulations showed a loss of medication content.

Organoleptic characteristics and release profile
During a 90-day study period, the prepared microspheres were stable at ambient temperature (32°C), 45°C in the oven, and 5°C in the freezer [40, 41]. Neither the morphology nor the release properties changed much. Due to polymer breakdown, slight color changes were seen at higher temperatures.

Microspheres loaded Capsule preparation and characterization
Preparation of capsules containing drug-loaded microspheres
Microspheres containing the drug were filled into hard gelatin capsules of size #1. Each capsule was filled to capacity, and its weight was subsequently measured.

Evaluation of Hard Gelatin Capsule
Disintegration Time
An accurately weighed quantity of granules, corresponding to 50 mg of drug-loaded microspheres, was dissolved in 100 mL of phosphate buffer (pH 6.8). The solution was then filtered, appropriately diluted, and analyzed for drug content at 270 nm using a UV-visible spectrophotometer.

Drug Content
An accurately weighed quantity of granules, corresponding to 50 mg of drug-loaded microspheres, was dissolved in 100 mL of phosphate buffer (pH 6.8). The solution was then filtered, appropriately diluted, and analyzed for drug content at 270 nm using a UV-visible spectrophotometer.

In-vitro Drug Release Study
The in vitro release profile of glipizide from the microspheres was evaluated using a USP Dissolution Apparatus Type II (paddle type). To begin the test, the microspheres were filled into size #3 empty hard gelatin capsules, which were then carefully positioned in a dry basket. The basket was immersed into the dissolution vessel containing 900 mL of phosphate buffer solution at pH 6.8, maintained at a temperature of 37 ± 0.5°C. The apparatus was operated at a constant stirring speed of 50 rpm. At five-minute intervals over a total duration of 60 minutes, 5 mL samples were withdrawn and immediately replaced with fresh buffer maintained at the same temperature to preserve sink conditions. The withdrawn samples were filtered using Whatman filter paper No. 41, and the absorbance was recorded at 270 nm using a UV-Visible spectrophotometer. The cumulative percentage of drug released at each time point was determined based on a calibration curve generated from standard solutions (refer to Figure 9).

Stability Study as Per ICH Guideline
A stability study in accordance with ICH guidelines was conducted over a period of one month at 40 ± 2°C and 75 ± 5% relative humidity. During this period, the formulation was assessed for disintegration time, drug content, and in-vitro drug release.

Result and Discussion

Drug and excipient interaction study
A. Fourier Transformation Infrared Spectroscopy (FTIR)
FTIR spectrum of Glipizide is shown in following Fig. 1 revealed that the characteristic peaks representing the presence of functional groups claim by its chemical structure. From this we can consider that the Glipizide was of pure quality.

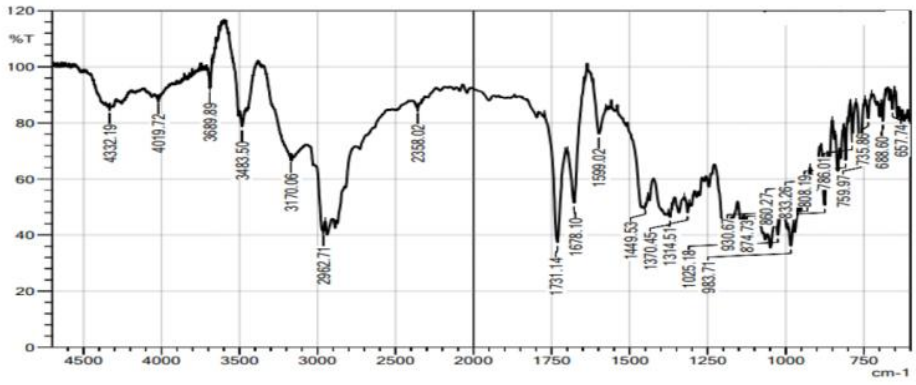

Fig. 1: FTIR spectra of Glipizide

Table 3: Interpretation data of Glipizide

Material	Functional group	Standard IR Ranges (cm^{-1})	Observed IR Ranges (cm^{-1})
Glipizide	C=O Stretching C-N Stretching	1870-1540 1342-1266	1599.02, 1678.10, 1731.14 1314.51

After interpretation of FT-IR Spectrum of Glipizide, it was concluded that all the characteristic peaks corresponding to the functional group present in the molecular structure of Glipizide were found within the reference range and confirming its identity.

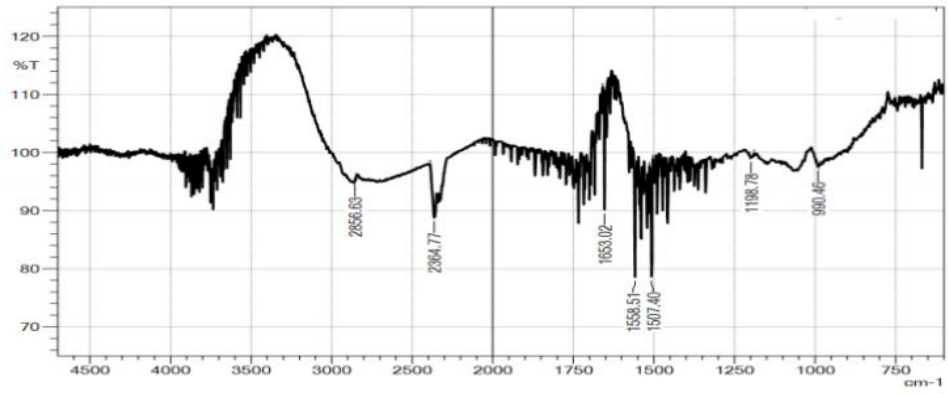

Fig. 2: FTIR Spectra of Chitosan

After interpretation of FT-IR Spectrum of polymer, it was concluded that all the characteristic peaks corresponding to the functional group present in molecular structure of chitosan were found within the reference range, confirming its identity.

Table 4: Polymer Interpretation data of FTIR

Material	Functional group	Standard IR Ranges (cm^{-1})	Observed IR Ranges (cm^{-1})
Chitosan	O-H Stretching C-O Stretching C=O Stretching	3300-2500 1382-1036 1680-1630	2856.63 1198.78 1653.02

B. Differential Scanning Calorimetric analysis (DSC)

The thermal analysis of Glipizide was studied by using DSC as shown in figure respectively. The Glipizide shows an endothermic peak at approximately 205 ℃ and it corresponds to its melting point shown in following figure 3.

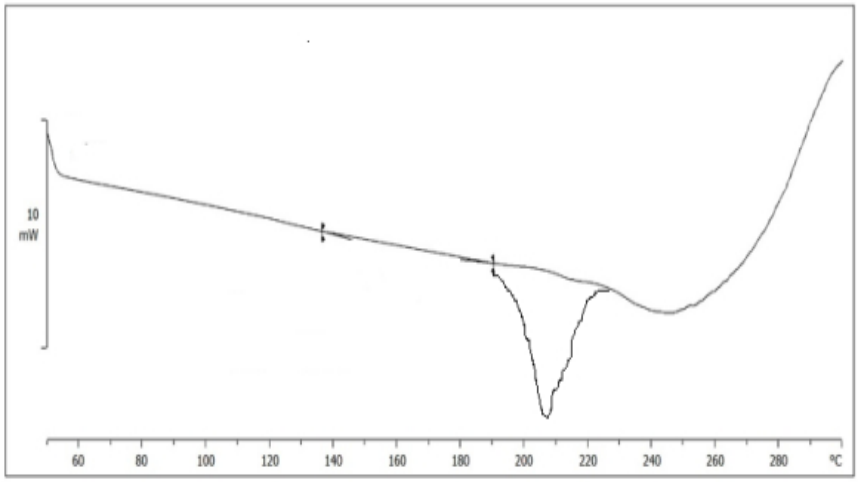

Fig. 3: DSC thermogram of Glipizide

Table 5: Glipizide chitosan alginate microspheres' physical-chemical characteristics (D: P 1: 0.5 1:2), n=3

Code	% yield	% entrapments	Shape	Color	% Swelling index	Mucoadhesive property (%) ± SD
GCS1	91.7	88.2	Spherical	Light brown	12.6	50.2
GCS2	86.3	85.5	Spherical	Light brown	16.8	64.1
GCS3	85.4	90.7	Spherical	Light brown	18.3	66.3
GCS4	93.1	89.5	Spherical	Light brown	20.1	70.6
GCS5	92.2	73.2	Spherical	Light brown	21.6	73.6
GCS6	86.2	90.2	Spherical	Light brown	25.6	82.4
GCS7	84.4	83.1	Spherical	Light brown	28.1	89.3

Size distribution and size analysis

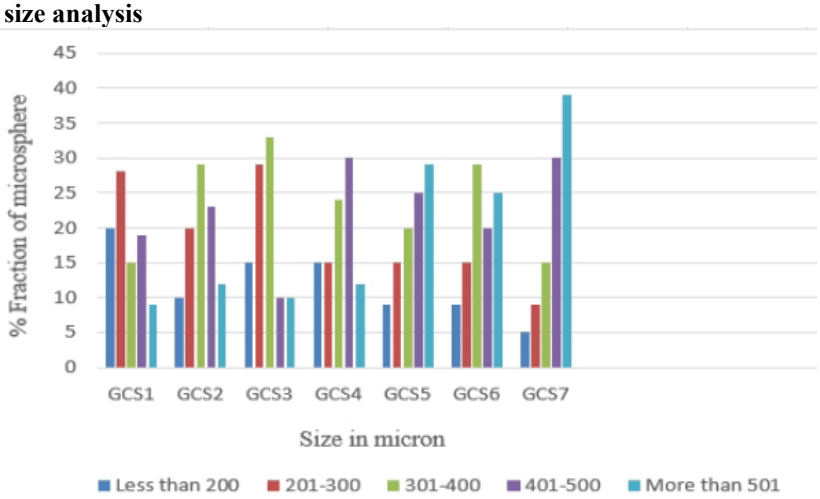

Fig. 4: Glipizide drug's sodium alginate microsphere size distribution (drug to polymer-1: 0.5-1:2)

Differential scanning calorimetry (DSC)

Thermogram demonstrates that no interactions were seen between the medication and polymers. The thermogram (Fig.) displayed distinct maxima for the medicines and polymers.

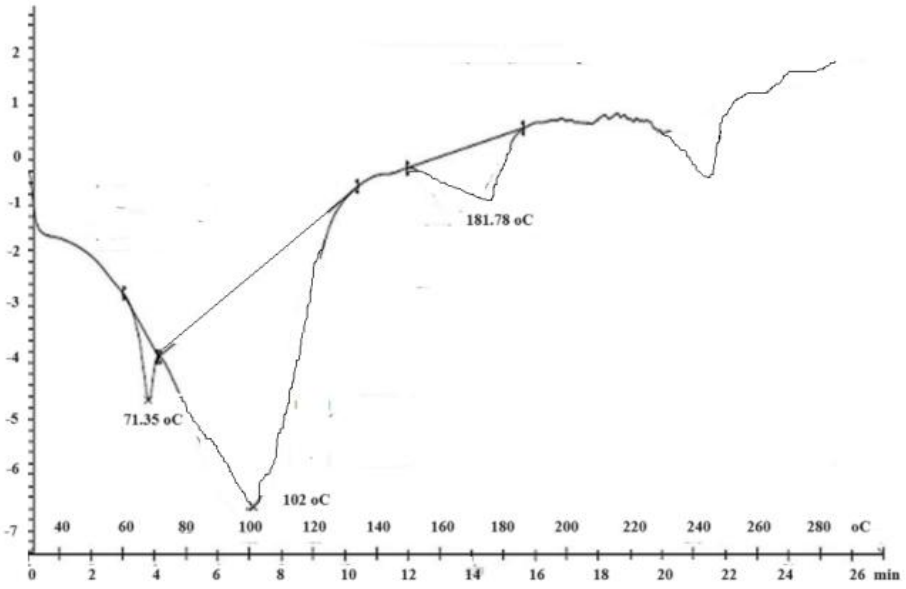

Fig. 5: Differential scanning calorimetry of Glipizide alginate-chitosan microspheres (GCS-4)

Scanning electron microscopy (SEM)

The microspheres were then examined at 15 kV using the JEOL Model JSM-6390LV SEM. It displayed distinct, roughly homogenous, and evenly spaced microspheres (Fig.6).

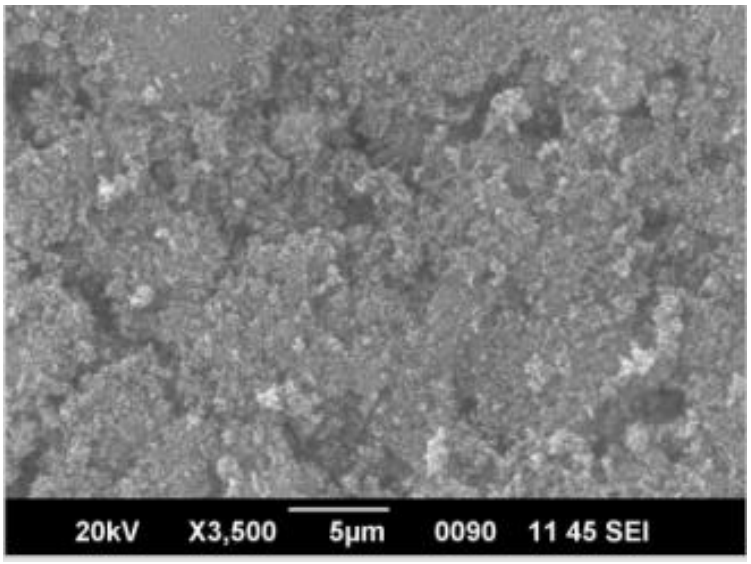

Fig. 6: Scanning electron microscopy of GCS-7

We looked at how several physicochemical, morphological, and in-vitro release properties were affected by process factors. The method's remarkable reproducibility was demonstrated by its low coefficient of variations.
Using the orifice ionic gelation process, Glipizide microspheres were prepared with chitosan and sodium alginate. To entrap the drug, different concentrations of the polymer were employed, and different factors of the process variable were examined.

The matrix type of the microspheres was discrete, free-flowing, and monolithic. The alginate Microparticles' surface additionally developed a layer of chitosan.
As the polymer concentration grew, the microspheres' Mucoadhesive properties increased as well. The distribution of sizes fell between 200 and 500 µm. The size of the microspheres was shown to rise with an increase in the polymer ratio. The dispersion of the insoluble medications in the matrix's interstitial spaces led to a rise in size upon the incorporation of more polymers.

A larger percentage of polymer in the formulation was found to produce smoother microspheres. With an increase in chitosan concentration, the rate of medication release reduced. Microspheres revealed the polymers' natural brown hue. The percentage yield and drug entrapment both improved with an increase in the polymer fraction.

The features of drug release were examined in intestinal and gastric simulated fluid, which was devoid of pepsin to replicate the gastrointestinal milieu. Release happened gradually over a long period of time. The matrix structure of the formulation may be the cause of this. Ineffective water-soluble medications first appear to pour into the polymer's interstitial gaps, where the drug is pushed into the gel-like part of the polymer by the water phase. After then, the expanding hydrophilic membrane functions as a barrier to control flux. So, for sustained drug release, alginate microspheres containing chitosan made using a straightforward ionic gelation technique might be employed. Diffusion and erosion controls were used to regulate the medication release.

In-vitro release of microspheres

Table 6: Data from the Glipizide chitosan alginate microspheres' in vitro release kinetic equation (D: P 1: 0.5 1:2), n=3 for values

Code	Zero order R_0	First order R_1	Higuchi R_H	Korsmeyer–Peppas R_K	Hixson-Crowell R_{HC}
GCS1	0.988	0.956	0.986	0.745	0.983
GCS2	0.992	0.959	0.992	0.755	0.972
GCS3	0.980	0.959	0.977	0.775	0.963
GCS4	0.981	0.969	0.982	0.815	0.979
GCS5	0.977	0.967	0.977	0.843	0.975
GCS6	0.982	0.984	0.981	0.874	0.987
GCS7	0.983	0.975	0.981	0.902	0.956

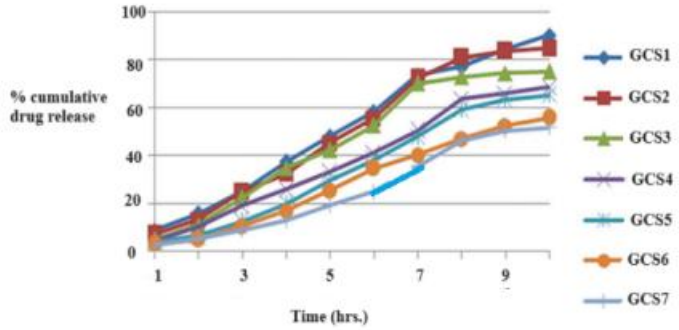

Fig. 7: In-vitro drug release of GCS1-GCS7

In-vivo hypoglycemic activity

Table 7: For in-vivo grouping of animals

Group No.	Treatment	Dose	Number of animals
1	Normal saline	5 ml/kg p.o.	06
2	STZ (STZ control)	60 mg/kg i.p.	06
3	Glipizide	5 mg/kg p.o.	06
4	GCS3	5 mg/kg p.o.	06

Table 8: Blood glucose level (mg/dl)

Sr.no.	Treatment	0 Day	3 Day	5 Day	7 Day
1	Normal saline (5 ml/kg)	88.91	90.61	91.49	90.21
2	STZ control	285.36	290.44	294.93	297.74
3	Glipizide (5 mg/kg)	283.94	223.66	167.91	120.44
4	GCS3 (5 mg/kg)	288.38	241.73	193.24	146.71

STZ: Streptozotocin

Table 9: Reduction of plasma glucose levels in rats following the administration of Glipizide formulation and pure medication

Treatment	1 day	3 day	5 day	7 day
STZ control group Glipizide	19.102	22.014	41.212	51.875
Glipizide (5 mg/kg)	19.214	24.073	44.216	60.752
GCS3 (5 mg/kg)	11.352	17.830	35.598	51.899

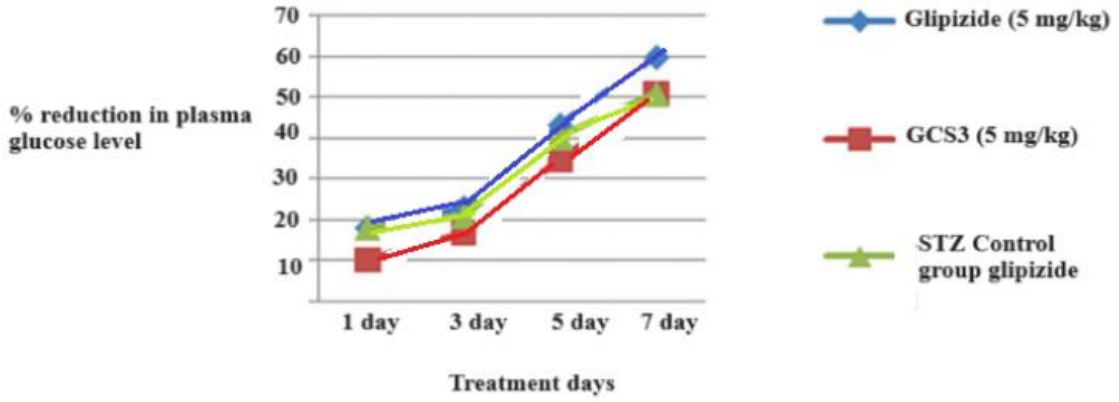

Fig. 8: % reduction in plasma glucose levels in rats with hyperglycemia following the administration of both formulation and pure medication GCS 3

Capsule
In this study, Glipizide-loaded microspheres were formulated into hard gelatin capsules using the wet granulation technique.

Disintegration Time
The time required for the disintegration of hard gelatin capsules ranged from 2.6 ± 0.82 to 3.34 ± 0.47 minutes.

Drug Content
The drug content in all optimized formulations ranged from 97.66 ± 0.632% to 98.15 ± 0.574%, falling within the permissible limits defined by the Indian Pharmacopoeia (IP).

In- vitro Drug Release
Figure 10 illustrates the cumulative percentage of drug release for all formulations. Table 10 presents the findings related to disintegration time, drug content, and in-vitro drug release.

Table 10: Evaluation of Fast Disintegrating Tablets

Batch No.	Disintegration Time (sec)	Drug Content (%)	% Cumulative Drug Release at 60 min
1	2.61 ± 0.107	99.05 ± 0.393	99.77 ± 0.507
2	2.64 ± 0.242	99.17 ± 0.576	99.74 ± 0.622
3	2.55 ± 0.259	98.68 ± 0.634	99.60 ± 0.263

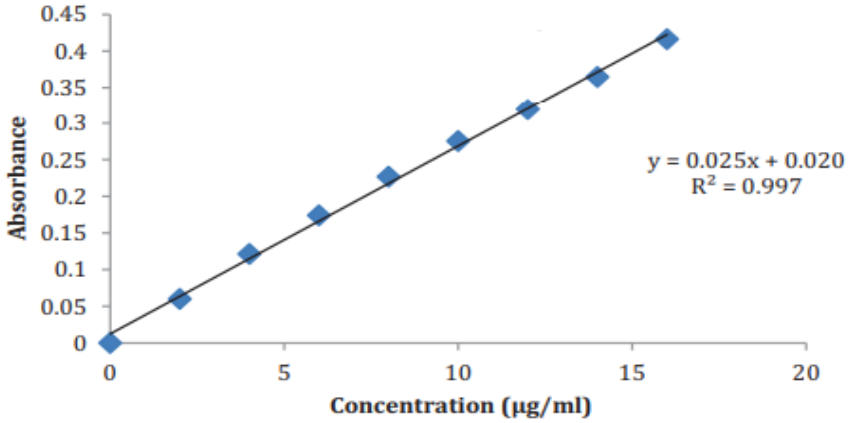

Fig. 9: Calibration curve of Glipizide in phosphate buffer pH 6.8

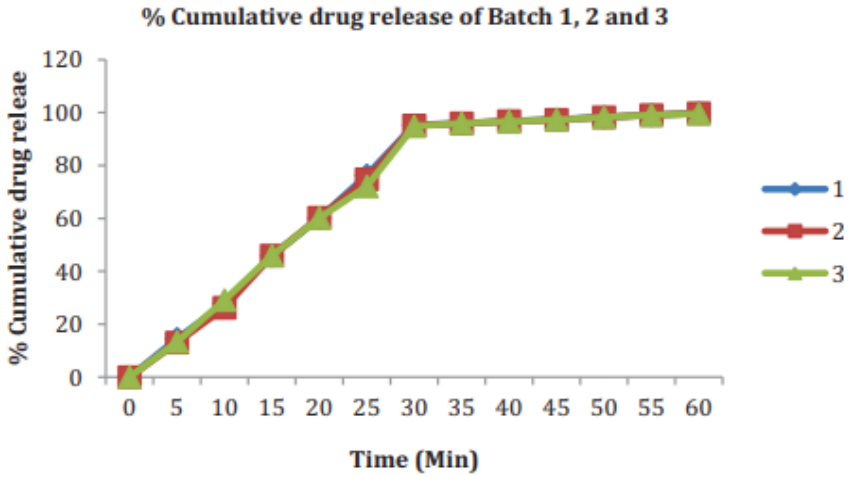

Fig. 10: % Cumulative drug release of batch 1, 2 and 3

Stability Study as per ICH Guideline

A stability study was conducted on hard gelatin capsules containing glipizide-loaded microspheres to assess the impact of temperature and humidity during storage. Evaluations were carried out at the start and after one month, focusing on parameters such as disintegration time, drug content, and in-vitro drug release. The findings, summarized in Table 11, indicated no significant variations in these parameters over the study period.

Table 11: Stability Study Results of Formulation

Test After Time (Mont	Disintegration Time (Seconds)	Drug Content (%)	In-vitro Drug Release (%)
0	2.55	99.17	99.77
1	3.26	98.69	97.58

Conclusion

A new SR system of glipizide microspheres of alginate-chitosan was designed and formulated by an ionic gelation method. It's morphological, and release characteristics were studied. The microspheres were easy to prepare, and the mean diameter of microspheres increased with increase in the amount of the polymers increase. The pore size of microspheres was affected by the concentration of the alginate and chitosan. Stirring at high speed above 200 rpm causes the destruction of microspheres. The microspheres showed excellent in-vivo activity and SR characteristics as compared to the conventional oral dosage forms. Thus, drug entrapment technique is a useful tool for the development of multiparticulate system even for a water-insoluble drug. The hard gelatin capsules filled with Glipizide-loaded microspheres were assessed for their disintegration time, drug content, and in-vitro drug release profile. Stability testing indicated no notable variations in these parameters over time. Based on these findings, the formulation was deemed optimized for the immediate release of Glipizide.

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