Formulation & Evaluation of Insulin Microparticles

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Abstract: Objective: To develop and evaluate insulin-loaded microparticles for oral delivery, enhancing bioavailability and patient compliance. This study aimed to formulate and evaluate insulin-loaded microparticles for oral delivery. Microparticles were prepared using sodium alginate, lactate dehydrogenase, and PLGA. Results showed improved insulin loading, controlled release, and stability. In vitro studies demonstrated enhanced bioavailability and safety.

Methods: Insulin-loaded microparticles were formulated using sodium alginate, lactate dehydrogenase, and PLGA via a Polymerisation method. Particle size, zeta potential, insulin loading, and in vitro release were characterized.

Keywords: Diabetes, Insulin, Microparticles.

INTRODUCTION

Diabetes mellitus (DM) It is a metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipidaemia, negative nitrogen balance and sometimes ketonaemia. A widespread pathological change is thickening of capillary basement membrane, increase in vessel wall matrix and cellular proliferation resulting in vascular complications like lumen narrowing, early atherosclerosis, sclerosis of glomerular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency. Enhanced nonenzymatic glycosylation of tissue proteins due to persistent exposure to high glucose concentrations and the accumulation of larger quantities of sorbitol (a reduced product of glucose) in tissues are believed to be causative in the pathological changes of diabetes. The concentration of glycosylated haemoglobin (HbA1c) is taken as an index of protein glycosylation: it reflects the state of glycaemia over the preceding 2–3 months. Two major types of diabetes mellitus are:

Type I : Insulin-dependent diabetes mellitus (IDDM)/juvenile onset diabetes mellitus: There is β cell destruction in pancreatic islets; majority of cases are autoimmune (type 1A) antibodies that destroy β cells are detectable in blood, but some are idiopathic (type 1B)—no β cell antibody is found. In all type 1 cases circulating insulin levels are low or very low, and patients are more prone to ketosis. This type is less common and has a low degree of genetic predisposition.

Type II: Non-insulin dependent diabetes (NIDDM)/new onset diabetes: no loss or moderate reduction in β cell mass; Circulating insulin was low, normal or high. No anti-β-cell antibodies were detected. Genetic predisposition was high. Generally it will be slow. (Past middle age) More than 90% of cases of diabetes are caused by type II DM. The cause may be:

• Abnormality in gluco-receptor of β cells so that they respond at higher glucose concentration or relative β cell deficiency. In either way, insulin secretion is impaired; may progress to β cell failure.

• Reduced sensitivity of peripheral tissues to insulin: The number of insulin receptors decreases, 'down-regulation' of insulin receptors. Many types of high blood pressure are hyperinsulinemia. But it is normal blood sugar. and is related to abnormal fat conditions High levels of uric acid in the blood and abdominal obesity Hyperinsulinemia per se has been implicated in the etiology of angiopathy.

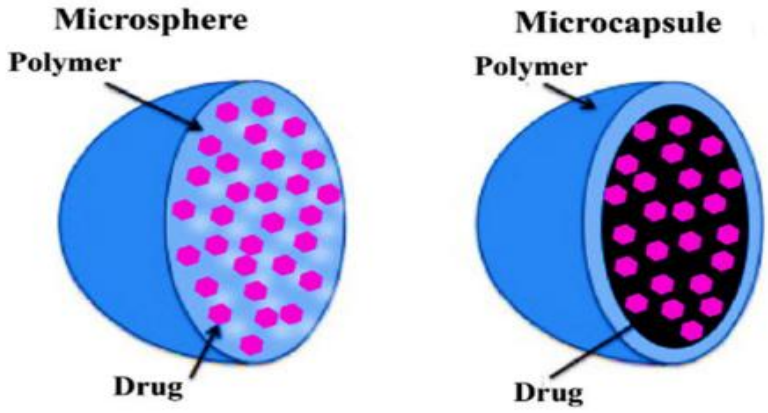
• Excessive hyperglycemia (glucagon, etc.)/obesity causes insulin-β deficiency, cell regression, D.M. [(1)]

• In 2014, 8.5% of adults aged 18 years and older had diabetes. In 2019, diabetes was the direct cause of 1.5 million deaths and 48% of all deaths due to diabetes occurred before the age of 70 years. Another 460 000 kidney disease deaths were caused by diabetes, and raised blood glucose causes around 20% of cardiovascular deaths ,Between 2000 and 2019, there was a 3% increase in age-standardized mortality rates from diabetes. In lower-middle-income countries, the mortality rate due to diabetes increased 13%.By contrast, the probability of dying from any one of the four main noncommunicable diseases (cardiovascular diseases, cancer, chronic respiratory diseases or diabetes) between the ages of 30 and 70 decreased by 22% globally between 2000 and 2019. India is home to the world's second highest number of diabetic patients. Within the age group of 20–79 years, India

has 74.9 million diabetics in 2021 projected to increase to 124.9 million by 2045. [2]

Microparticles:

Microparticles are small spherical particles with diameters ranging from 10 µm to 1000 µm, appearing as free-flowing powders. These particles are developed from different components such as inorganic, polymers and minerals. MP may also exist in different structural structures such as microgranules, micropellets, microcapsules, micros. emulsion magnetic lipid vesicles and: in the form of liposomes and niosomes. The most common type of MP is MP polymer, which is a naturally occurring synthetic or synthetic polymer that Mainly designed in two-structured MP microspheres, the MP matrix is a homogeneous mixture of polymers, copolymers, and active pharmaceutical ingredients (APIs). Meanwhile A microsphere refers to a core composed of a solid and a liquid surrounded by a coating of material that is noticeably different from the polymer core. Most MPs are polymers that determine their structure. and significantly affect their properties. In principle The polymer should be inert, stable, safe, and biodegradable. Biocompatible and has a low cost A variety of polymers are used to create MP from natural and synthetic sources. The table shows examples of different types of polymers. Injecting a high concentration of drug, i.e., with a high network efficiency in the MP, can increase the efficiency. This depends on the type of polymer. Additionally, studies have proven that particle size directly affects the drug loading capacity. Where reducing the particle size reduces the drug loading capacity. and vice versa It can be detected by various methods such as scanning electron microscopy. Surface morphology affects MP properties such as wettability and viscosity. Greater surface diversity and roughness were reported to improve the wettability of MP, while surface roughness was found to have an opposite effect on particle adhesion. As the surface roughness increases The pull-out force is greatly reduced. Therefore, the adhesion properties of MP are reduced. Another important aspect to consider in the preparation and characterization of MPs is the electrical charge of the particles. Zeta potential is a standard analytical method for determining surface charge in colloidal systems. It can be used to determine the long-term and short-term stability of colloidal microparticle dispersions. Colloidal systems with a high zeta potential (negative or positive) are considered electrostable due to the repulsive forces between the particles. A low zeta potential system is at risk for clotting or blood clotting. This can lead to poor physical stability. [3]



Structure of microparticles : A) Matrix Microparticle ; and B) Microcapsule

Need Of Microparticles:

1. Improved Bioavailability - Microparticles enhance insulin absorption, reducing required doses. Increased bioavailability improves glycemic control. [4]
2. Sustained Release -Microparticles provide prolonged insulin action, reducing injection frequency. Sustained release improves patient compliance and reduces hypoglycaemia. [5]
3. Targeted Delivery - Microparticles target insulin delivery to specific tissues or cells. Targeted

delivery improves insulin efficacy and reduces side effects.(6)

4. Reduced Hypoglycemia - Microparticles minimize risk of excessive glucose lowering. Reduced hypoglycemia improves patient safety and quality of life.(7)
5. Alternative Delivery Routes - Microparticles enable oral, pulmonary, or transdermal insulin delivery. Alternative delivery routes improve patient compliance and convenience.(8)
6. Enhanced Stability - Microparticles protect insulin from degradation, improving stability. Enhanced stability extends shelf life and reduces manufacturing costs.(9-10)

Ideal Properties Of Microparticles :

1) Shape and uniformity : Consistent particle size is essential for predictable behavior. This is especially true in drug delivery systems.(11)
2) Surface Area : A high area-to-volume ratio enhances interaction with biological systems and improves responsiveness.(12)
3) Biocompatibility: Microparticles should be non-toxic and compatible with biological tissues for safe medical use.(13)
4) Mechanical Stability: Sufficient strength is required to maintain structural integrity under various conditions. especially during processing.(14)
5) Controlled Release: The ability to release substances at a controlled rate is essential for sustainable drug delivery.(15)
6) Working: Surface modification can increase targeting and effectiveness in medical applications.(16)
7) Electrical Properties: Optimized conductivity or charge can improve performance in sensors and electronic applications.(17)

Advantages Of Microparticles:

1) Sustained Release: Microparticles provide prolonged insulin action.
2) Improved Bioavailability: Enhanced insulin absorption.
3) Reduced Hypoglycemia: Minimized risk of excessive glucose lowering.
4) Targeted Delivery: Specific targeting to pancreatic islets or liver.
5) Improved Glycemic Control: Better blood sugar management.
6) Biocompatibility: Non-toxic and non-inflammatory,
7) Cellular Uptake: Efficient uptake by target cells.
8) Flexibility: Various polymers, stabilizers, and surfactants.
9) Scalability: Easy scale-up for industrial production.
10).Stability: Improved insulin stability.

Disadvantages Of Microparticles:

1) Variable Release Rates: Inconsistent insulin release.
2) Initial Burst Release: Rapid insulin release.
3) Insulin Degradation: Instability during storage
4) Toxicity: Potential toxicity of polymers or excipients.
5) Inflammation: Potential inflammatory response.
6) Complex Manufacturing: Challenging scale-up and production.
7) High Cost: Increased production costs,
8) Hypoglycemia: Risk of excessive glucose lowering.
9) Patient Non-Compliance: Complex treatment regimens.

Application Of Microparicles:

1) Pharmaceuticals

-Controls the release of insulin, vaccines, and other drugs.
-Targeted delivery to specific tissues or cells.
-Improve the absorption and stability of therapeutic substances.

2) Medical Devices

-Implantable devices for sustained drug release
-Wound healing and tissue engineering scaffolds
-Biosensors for glucose monitoring

3) Vaccine distribution

-Encapsulation of vaccine antigens to enhance immunity.
-Targeted delivery to immune cells
-Increase vaccine stability and shelf life.

4) Food and nutrition

-Summary of nutrients and biologically active substances

-and control the release of perfume
-Improve the absorption of dietary supplements

5) Specific uses of insulin delivery
-Oral insulin delivery system
-Insulin delivery system in the lungs
-Transdermal insulin delivery system
-Implantable insulin pump
-Glucose-responsive insulin delivery system

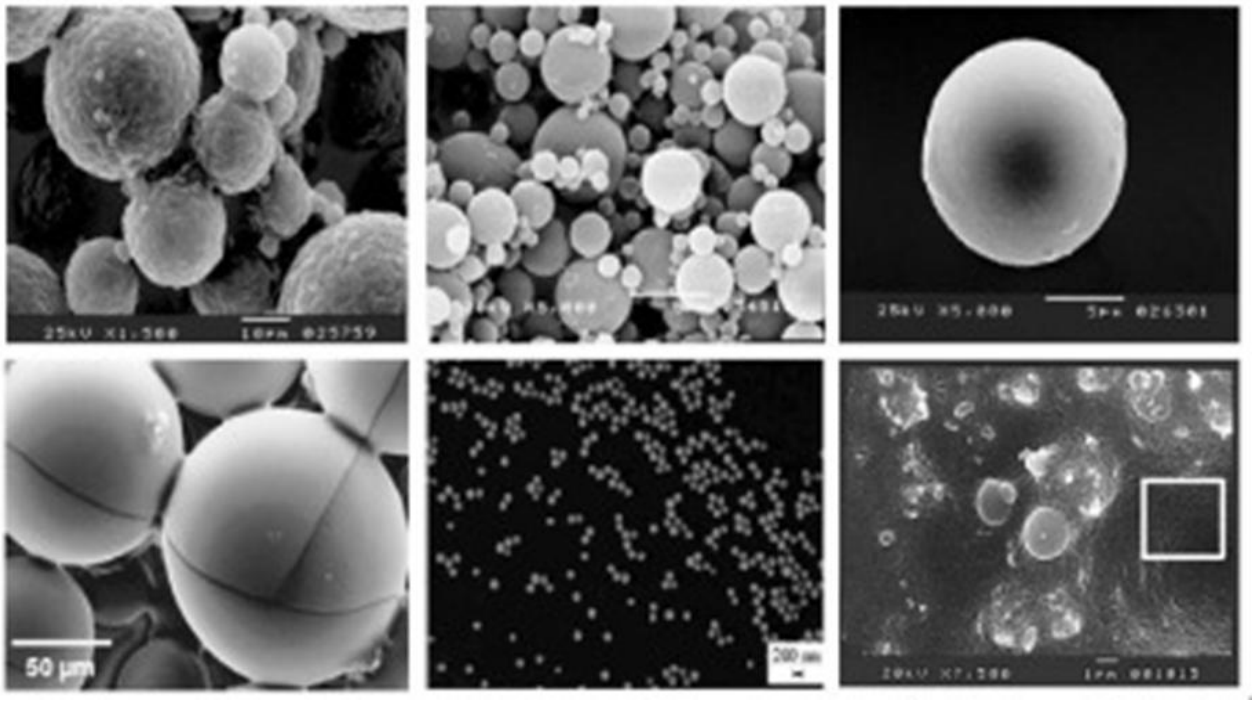
Factor Affecting Microparticles:

1) Size: 1-10 Micrometers
2) Shape: Spherical, Irregular, Porous
3) Surface Charge: Positive, Negative, Neutral
4) Porosity: High, Low, Or Controlled Porosity
5) Density: Affects Sedimentation, Flotation
6) Composition: Homogeneous, Heterogeneous
7) Solubility: Affects Release Kinetics
8) Chemical Stability: Affects Shelf Life
9) Method: Emulsion, Solvent Evaporation, Spray Drying

10.) Concentration: Affects Particle Size, Distribution
11). Temperature: Influences Stability, Release Kinetics
12). Storage Conditions: Affects Shelf Life
13). Scale-Up: Affects Production Costs, Consistency
14). Characterization: Size, Shape, Surface Charge Analysis
15). Regulatory Compliance: Affects Market Approval

Polymeric Microparicles:

Polymeric microparticles are usually formed by a polymer matrix in which a smaller amount of an active compound can be immobilized, With respect to the distribution of the active compound, two different categories of microparticles can be distinguished :"microspheres" and "microcapsules" refers to microparticles composed of a homogeneous mixture of active compound and raw material, while "microcapsules" is the name given to microparticles that present a core (where the active compound is placed) which is delimited by a different material (usually the raw material)The core may be solid, liquid or even gas, Furthermore, one or more discrete domains of active compound may be found in the microcapsule core.[18]



Preparation of insulin microparticles:

Preparation of insulin microparticles prepared by using controlled polymerisation method.

Materials:

SR NO.	Material:
1.	Human Recombinant Insulin
2.	Lactate Dehydrogenase (LDH)

3.	PLGA (Poly(lactic-co-glycolic acid))
4.	Sodium Alginate
5.	Calcium Chloride Dihydrate
6.	Labrasol ALF (a surfactant)
7.	Labrafil M2125 CS (a solubilizer)
8.	L-Glutamine
9.	Fetal Bovine Serum (FBS)

Method :

1.Preparation of Polymer Solution - Dissolve PLGA in an organic solvent (e.g., dichloromethane) to create a polymer solution. If necessary, incorporate Labrasol ALF and Labrafil M2125 CS to enhance the solubilization of insulin.

2. Insulin and Additives Incorporation - Prepare an aqueous phase containing human recombinant insulin, LDH, L-glutamine, and FBS.Mix the aqueous phase into the polymer solution under gentle stirring to form an emulsion.

3. Gelation with Sodium Alginate - Prepare a sodium alginate solution separately.Gradually add the emulsion into the sodium alginate solution while stirring to facilitate gelation.

4. Cross-Linking - Add calcium chloride dihydrate to the alginate mixture to cross-link the sodium alginate, forming microparticles.

5. Hardening and Washing - Allow the mixture to harden for a set time, then wash the microparticles with a buffer solution to remove unreacted materials.

6. Lyophilization (Optional) - If long-term storage is needed, freeze-dry the microparticles to obtain a stable powder form.

Preformulation studies of insulin microparticles:

Preformulation studies are crucial for developing insulin microparticles for diabetes treatment. These preformulation studies ensure the development of a robust and effective insulin microparticle formulation for diabetes treatment.

1. Solubility: These studies can involve adding insulin to deionized water or HBSS with varying concentrations of amino acids. The pH of the solution is measured, and the insulin content is quantified after filtering, Insulin solubility in various solvents (e.g.,water, buffer solutions),Effect of pH, temperature, and ionic strength on solubility.
2. Stability : Thermal stability: Insulin degradation at different temperatures, Chemical stability: Insulin degradation in presence of oxidizing agents, proteases, Photostability Insulin degradation under different light conditions.
3. Partition Coefficient Determination: Insulin distribution between aqueous and organic phases, Effect of pH, temperature, and solvent composition on partition coefficient.
4. Protein Binding : Insulin binding to excipients, containers, and biological matrices, Effect of pH, temperature, and ionic strength on protein binding.
5. Particle Size :The size of a drug substance can affect its dissolution rate and other factors in the drug product, Insulin particle size distribution, Effect of formulation parameters on particle size.
6. Differential scanning calorimetry: This technique can be used to determine the melting point of insulin.
7. Rheological and texture analysis : These studies can be performed on hydrogel formulations to assess their rheological and texture parameters.
8. Interaction : Insulin interactions with excipients, surfactants, and other formulation components, Effect of interactions on insulin stability and activity.

Evaluation of Insulin Microparticles:[19]

1.Particle Size and Shape:

Following techniques are used to determine the particle size and shape of microparticle.

a).Conventional light Microscopy :

This technique is used for evaluating the shape and outer structure of the microparticles. It is used in case of double walled microspheres where the microstructures are visualized before and after coating. The change is evaluated microscopically.

The average particles size of microcapsules is determined by the following formula.

$$P_{average} = \frac{\sum X_i F_i}{F_i}$$

Where,

$P_{average}$ = Average particles size of the microcapsules

X_i = Mean Particle size of the microcapsules

F_i =% of microcapsules Particles retained on the smaller sieve.

b).Scanning Electron Microscopy:

This technique investigates microsphere surfaces. cross-sectioned particles and also double walled

microspheres. Higher resolution is obtained with SEM when compared to LM.

c).Confocal Laser Scanning Microscopy:
It is a nondestructive technique used to visualizz and characterize the surface structure as well as the internal structure. This technique can characterize the internal structure of the microspheres only if the materials are transparent and fluorescently labelled, It is used to depict polymer structures on the surface (using flourescien in water) and release kinetics of the incorporated drug. Laser light scattering and multisize coulter counter are the other techniques which are used to evaluate the particle size and shape of the microspheres.
2.Surface Chemistry:
Attensated Total Reflectance Fourier Transform Infrared Spectroscopy (FTIR) FTIR is used to determine surface composition and degradation of the polymer in matrix. IR beam is introduced onto the attenuated total reflectance (ATR) cell and is reflected several times along the sample, so that the IR spectra of only surface is obtained.
3.Determination of Density:
Microsphere density is determined by multivolume pycnometer. A cup with accurately weighed sample is introduced into a pycnometer. At constant pressure helium is introduced into the chamber and the expansion of helium brings about a pressure change. Difference between the two pressure readings is used to calculate the volume and the density of the microspheres.
4.Isoelectric Point:
Isoelectric point is indirectly measured from the electrophoretic mobility of the microspheres by using an apparatus called microelectrophoresis. This instrument measures the time required for a particle to move a distance of 1 mm under varying pH conditions (3-10) from which the mean particle velocity at different pH values is calculated. This data is used to determine the electrophoretic mobility of the molecules, which is indicative of surface charge, nature of ion absorption and behaviour ionization.
5.Surface Carboxylic Acid Residue:
Radioactive glycine is used to determine the carboxylic acid residue. 14C-glycine ethyl ester HCl is conjugated with microspheres by using water soluble 1-ethyl- 3(3-dimethyl amino) propyl carbidiimide (EDAC) as a linker called radioactive glycine conjugate whose radioactivity is measured using a liquid scintillation counter. This helps in comparison and correlation of carboxylic acid residues on the microspheres. In case of hydrophobic or hydrophilic type of microspheres, free carboxylic acid residue can be measured by indirect estimation.
6. Angle of Contact:
It determines the wetting property of the microspheres which indicates their nature (hydrophilic or hydrophobic). Angle of contact is formed at the solid/air/water interface as the microsphere is subjected to many forces such as Archimedean thrust, gravitational force etc. Angle of contact is measured at 20° within a minute after introducing a droplet in circular cell mounted above the objective of an inverted microscope.
7.Drug Entrapment Efficiency:
Drug entrapment efficiency refers to the amount of drug required to prepare the microsphere and the amount that is found adsorbed on the surface, or as free drug and the amount that is present in the matrix. It is measured by determining the amount of drug entrapped in microsphere, adsorbed on the surface or found in the polymer.
8.In Vitro Release Studies:
In vitro release profiles of microspheres determined from in vitro release studies are indicative of the rate of drug release and efficacy of the microspheres. There are several factors such as method of preparation, formulation conditions and the nature of polymer used which influence the release profile of a drug. In vitro release experiments can be performed by any of the following methods,
(a)Using Rotating Paddle Apparatus:
Rotating paddle apparatus is filled with phosphate saline buffer (pH: 7.4). The sample is added and the paddle is rotated at 100 rpm. Samples are then regularly withdrawn at periodic intervals and the amount of drug released is quantified using a suitable technique mentioned in the monograph. The quantity of sample withdrawn is replaced with fresh saline solution. A graph of the amount of drug released and time taken for drug release is plotted.

(b) Dialysis Method:
This method requires a special dialysis assembly. Initially, accurately weighed quantity of sample is added to a dialysis or a tube with a membrane at its lower end. This is immersed in a larger compartment

containing the dialysing media, which is continuously stirred using a magnetic stirrer. Samples are collected at regular intervals and assayed by suitable methods. The amount of dialysate removed is replaced with fresh dialysing media.

CONCLUSION

This study successfully developed a stable oral delivery system for insulin using sodium alginate, lactate dehydrogenase, and PLGA-based microparticles. The formulated insulin-loaded microparticles demonstrated enhanced in vitro drug release and intestinal absorption, ensuring effective oral insulin delivery. The microparticles provided a high degree of protection against insulin degradation, maintaining its stability and bioactivity.

The formulation exhibited excellent biocompatibility and tolerability, paving the way for a promising oral insulin therapy. This innovative delivery system has the potential to improve patient compliance, convenience, and quality of life for individuals with diabetes, offering a viable alternative to traditional injectable insulin treatments.

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