A REVIEW ON FLOATING MICROSPHERES OF TENELIGLIPTIN

Dr. Roohinaz Ansari (B.Pharm, Pharm D)
Doctor of Philosophy Scholar
Department of Pharmacy
Bhagwant Global University, Kotdwar, Uttarakhand, India

Abstract: Teneligliptin is one of the newly approved gliptins and is effective in treating type-2 diabetes. It is hypothesized that an oral formulation that could substantially retain in the gastrointestinal tract (GIT) and release the drug in a controlled manner could be highly effective than a single dose conventional dosage form. The purpose of the present investigation is the development and characterization of gastro-retentive floating drug delivery system for anti-diabetic drug "Teneligliptin" that would retain the drug in stomach and continuously release the drug in controlled manner up to a predetermined time leading to improved bioavailability. Different formulations of Teneligliptin would be prepared as the floating microspheres using Ethylcellulose polymer by emulsion solvent evaporation technique. The dried floating microspheres would be evaluated for a drug content, particle size analysis, incorporation efficiency, floating behavior and in-vitro drug release studies. The developed gastro retentive floating drug delivery system of Teneligliptin must show excellent physicochemical properties and controlled drug release pattern, thereby improve the bioavailability of the drug and also manage the complicacy of the diabetes in a better manner. In conclusion, the formulation and evaluation of floating microspheres of Teneligliptin represent a promising strategy to enhance drug delivery and therapeutic efficacy in the management of type 2 diabetes mellitus. The integration of innovative formulation techniques and comprehensive evaluation methodologies underscores the potential of floating microspheres as a viable drug delivery system. Future research should focus on addressing challenges such as scalability, stability, and regulatory considerations to facilitate clinical translation and commercialization of these formulations

IndexTerms **- Floating microspheres, Teneligliptin, In-vitro release, Bioavailability, pre-clinical study (in vivo)**

I. INTRODUCTION

Diabetes is a chronic disease that occurs when the pancreas produces insulin inadequately or when the body cannot use the insulin produced by it efficiently. Insulin is a peptide hormone that controls the amount of glucose in the blood, commonly known as blood sugar. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes. Unchecked diabetes, over time, leads to potential complications in various organ systems of the body, which includes heart ailments, kidney damage, and nerve damage. According to The World Health Organization (WHO), about 8.5% of adults aged 18 years and above had diabetes in 2014. During 2016, diabetes was the direct cause of 1.6 million deaths, and in the year 2012, high blood glucose was the leading cause of 2.2 million deaths additionally. Nearly half of all deaths attributable to high blood glucose occur before 70 years of age. WHO estimated the diabetes was the seventh leading cause of death in 2016.

Dipeptidyl peptidase-4 (DPP-4) inhibitors are a comparatively new form of oral diabetes drugs. Also identified as gliptins, they are generally prescribed for people with type- 2 diabetes who do not respond well to medications such as metformin and sulphonylureas. DPP-4 is an enzyme that destroys the hormone incretin. DPP4 inhibitors work by blocking the activity of DPP4. Incretins help the body produce more insulin only when needed and reduce the liver's amount of glucose when it is not required. These hormones are released during the whole day, and levels are increased at mealtimes

Teneligliptin is one of the newly approved gliptins and is effective in treating type-2 diabetes. It is hypothesized that an oral formulation that could substantially retain in the gastrointestinal tract (GIT) and release the drug in a controlled manner could be highly effective than a single dose conventional dosage form. In this context, mucoadhesive drug delivery systems adhere to certain gastrointestinal segments and would offer various advantages. The present study aims to prepare floating microspheres of teneligliptin to prolong the residence time in the gastric and provide control release.

Drugs that are easily absorbed from alimentary canal (GIT) and have short half-lives are eliminated quickly from the circulation. Frequent dosing of those drugs is required to realize suitable therapeutic activity. To avoid this limitation, the event of oral sustained-controlled release formulations is an effort to release the drug slowly into the alimentary canal (GIT) and maintain an efficient drug concentration in the systemic circulation for a long time.

Gastro retentive delivery systems are designed to be retained in the stomach for a prolonged time and release their active ingredients and thereby enable sustained and prolonged input of the drug to the upper part of the gastrointestinal (GI) tract.

Gastro retentive delivery system can be classified as follows.

Bio-adhesive Drug Delivery System
Expandable Drug Delivery System
Floating Drug Delivery System and
High-density systems

1.1.1. Bio-adhesive drug delivery system

The term bio-adhesion is defined as adhesion to biological surface i.e. mucus and/or mucosal surface. In instances when the polymeric system interacts with mucus layer only, it is referred as muco-adhesion. In order to develop an ideal oral bio-adhesive system, it is important to have a thorough understanding of mucosa, bio-adhesive polymers and mucin- polymer interactions in the physiological environment.

1.1.2. Expandable drug delivery systems

A dosage form in the stomach will withstand gastric transit if it is bigger than the pyloric sphincter (Caldwell et al., 1988). However, the dosage form must be small enough to be swallowed, and must not cause gastric obstruction either singly or by accumulation. Thus, three configurations are required, a small configuration for oral intake, an expanded gastro-retentive form and a final small form enabling evacuation following drug release. Unfoldable systems are made of biodegradable polymer; the concept is to make a carrier, such as a capsule, incorporating a compressed system, which extends in the stomach. Caldwell et al., 1988 proposed different geometric forms (tetrahedron, ring or planar membrane (4-lobed, disc or 4- limbed cross form) of biodegradable polymer compressed within a capsule.

1.1.3. Floating drug delivery systems

The concept of FDDS was described in the literature as early as 1962. FDDS have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents the drug is released slowly at the desired rate from the system. This results in an increased GRT and a better control of fluctuations in plasma drug concentration. The device must have sufficient structure to form a cohesive gel barrier; it must maintain an overall specific gravity lower than that of gastric contents (1.004-1.010) and it should dissolve slowly enough to serve as a drug reservoir. Based on the mechanism of buoyancy the two distinctly different technologies have been utilized in the development of FDDS. 1) Non- Effervescent FDDS 2) Effervescent FDDS

1.1.3.1. Non-Effervescent FDDS

The Non-effervescent FDDS is based on mechanism of swelling of polymer or bioadhesion to mucosal layer in GI tract. The most commonly used excipients in non- effervescent FDDS are gel forming or highly swellable cellulose type hydrocolloids, hydrophilic gums, polysaccharides and matrix forming materials such as polycarbonate, polyacrylate, polymethacrylate, polystyrene as well as bioadhesive polymers such as Chitosan and carbopol. The various types of this system are as:

Single Layer Floating Tablets They are formulated by intimate mixing of drug with a gel-forming hydrocolloid, which swells in contact with gastric fluid and maintains bulk density of less than unity. They are formulated by intimate mixing of drug with low-density enteric materials such as HPMC.

Bi-layer Floating Tablets A bi-layer tablet contain two-layer one immediate release layer which releases initial dose from system while another sustained release layer absorbs gastric fluid, forming an impermeable colloidal gel barrier on its surface, and maintain a bulk density of less than unity and thereby it remains buoyant in the stomach.

Alginate Beads Multi-unit floating dosage forms were developed from freeze-dried calcium alginate. Spherical beads of approximately 2.5 mm diameter can be prepared by dropping sodium alginate solution into aqueous solution of calcium chloride, causing precipitation of calcium alginate leading to formation of porous system, which can maintain a floating force for over 12 hours. When compared with solid beads, which gave a short residence time of 1 hour, and these floating beads gave a prolonged residence time of more than 5.5 hours.

Hollow Microspheres Hollow microspheres (microballoons), loaded with drug in their outer polymer shells are prepared by a novel emulsion-solvent diffusion method. The ethanol: dichloromethane solution of the drug and an enteric acrylic polymer is poured into an agitated aqueous solution of PVA that is thermally controlled at 400C. The gas phase generated in dispersed polymer droplet by evaporation of dichloromethane forms an internal cavity in microsphere of polymer with drug. The microballoons float continuously over the surface of acidic dissolution media containing surfactant for more than 12 hours in vitro.

1.1.3.2. Effervescent System

Effervescent systems include use of gas generating agents, carbonates (ex. Sodium bicarbonate) and other organic acid (e.g. citric acid and tartaric acid) present in the formulation to produce carbon dioxide (CO2) gas, thus reducing the density of the system and making it float on the gastric fluid. An alternative is the incorporation of matrix containing portion of liquid, which produce gas that evaporates at body temperature. These effervescent systems further classified into two types. 1. Gas generating systems, 2. Volatile Liquid / Vacuum Containing Systems

1.1.3.3. Gas Generating Systems

Tablets - Floating bilayer tablets with controlled release for furosemide were developed by Ozdemir et al., 2000. The low solubility of the drug could be enhanced by using the kneading method, preparing a solid dispersion with β cyclodextrin mixed in a 1:1 ratio (Singh and Brahma, 2000). One layer contained the polymers HPMC K4M, HPMC K100M and CMC (for the control of the drug delivery) and the drug. The second layer contained the effervescent mixture of sodium bicarbonate and citric acid. The in vitro floating studies revealed that the lesser the compression force the shorter is the time of onset of floating, i.e., when the tablets were compressed at 15 MPa, these could begin to float at 20 minutes whereas at a force of 32 MPa the time was prolonged to 45 minutes.

Floating capsules - Floating capsules are prepared by filling with a mixture of sodium alginate and sodium bicarbonate. The systems were shown to float during in vitro tests as a result of the generation of CO2 that was trapped in the hydrating gel network on exposure to an acidic environment.

Multiple unit type floating pills - The system consists of sustained release pills as 'seeds' surrounded by double layers. The inner layer consists of effervescent agents while the outer layer is of swell able membrane layer. When the system is immersed in dissolution medium at body temp, it sinks at once and then forms swollen pills like balloons, which float as they have lower density. This lower density is due to generation and entrapment of CO2 within the system.

Floating system with Ion-Exchange resin - A floating system using ion exchange resin that was loaded with bicarbonate by mixing the beads with 1M sodium bicarbonate solution (Shweta Arora et al., 2005). The loaded beads were then surrounded by a semipermeable membrane to avoid sudden loss of CO2. Upon coming in contact with gastric contents an exchange of chloride and bicarbonate ions took place that resulted in CO2 generation thereby carrying beads toward the top of gastric contents and producing a floating layer of resin beads.

1.1.3.4. Volatile Liquid / Vacuum Containing Systems

Intra-gastric floating gastrointestinal drug delivery system: These systems can be made to float in the stomach because of floatation chamber, which may be a vacuum or filled with air or a harmless gas, while drug reservoir is encapsulated inside a micro-porous compartment.

Inflatable gastrointestinal delivery systems: In these systems an inflatable chamber is incorporated, which contains liquid ether that gasifies at body temperature to cause the chamber to inflate in the stomach. These systems are fabricated by loading the inflatable chamber with a drug reservoir, which can be a drug impregnated polymeric matrix, encapsulated in a gelatin capsule. After oral administration, the capsule dissolves to release the drug reservoir together with the inflatable chamber. The inflatable chamber automatically inflates and retains the drug reservoir compartment in the stomach. The drug continuously released from the reservoir into the gastric fluid.

Intragastric osmotically controlled drug delivery system: It is comprised of an osmotic pressure-controlled drug delivery device and an inflatable floating support in a biodegradable capsule. In the stomach, the capsule quickly disintegrates to release the intragastric osmotically controlled drug delivery device. The inflatable support inside forms a deformable hollow polymeric bag that contains a liquid that gasifies at body temperature to inflate the bag. The osmotic pressure-controlled drug delivery device consists of two components; drug reservoir compartment and an osmotically active compartment. The drug reservoir compartment is enclosed by a pressure responsive collapsible bag, which is impermeable to vapour and liquid and has a drug

delivery orifice. The osmotically active compartment contains an osmotically active salt and is enclosed within a semipermeable housing. In the stomach, the water in the GI fluid is continuously absorbed through the semipermeable membrane into osmotically active compartment to dissolve the osmotically active salt. The osmotic pressure thus created acts on the collapsible bag and in turn forces the drug reservoir compartment to reduce its volume and activate drug release through the delivery orifice.

The floating support is also made to contain a bio-erodible plug that erodes after a predetermined time to deflate the support. The deflated drug delivery system is then emptied from the stomach.

1.1.4. High density drug delivery systems

Gastric contents have a density close to water (1.004 g /cm3). When the patient is upright small high-density pellets sink to the bottom of the stomach where they become entrapped in the folds of the antrum and withstand the peristaltic waves of the stomach wall. A density close to 2.5 g/cm3 seems necessary for significant prolongation of gastric residence time and barium sulphate, zinc oxide, iron powder, titanium dioxide are used as excipients.

Among these systems, Floating Drug Delivery System has been most commonly used. Floating drug delivery systems is one of the important approaches to achieve gastric retention to obtain sufficient drug bioavailability.

1.2. Recent Advancements in Floating Microsphere Technology:

Novel Polymers: Researchers are exploring new polymers with improved biocompatibility, biodegradability, and mucoadhesive properties to enhance the performance of floating microspheres. This includes the use of natural polymers like chitosan, alginate, and pectin, as well as synthetic polymers like poly (lactic-co-glycolic acid) and Eudragit® polymers. (Kumar et al., 2012) discusses the use of lipoidal soft hybrid biocarriers for drug delivery, which could potentially be adapted for Teneligliptin.

Targeted Delivery: Efforts are underway to develop microspheres that can target specific sites within the gastrointestinal tract, such as the stomach or small intestine. This can be achieved by incorporating ligands or antibodies on the surface of the microspheres that bind to receptors expressed on the target cells. (Noble et al., 2014) discusses ligand-targeted liposome design, a similar concept that could be applied to microspheres.

Stimuli-Responsive Systems: Researchers are developing microspheres that release the drug in response to specific stimuli, such as changes in pH, temperature, or enzymatic activity. This allows for more controlled and targeted drug delivery. (Floating modular drug delivery systems with buoyancy independent of release mechanisms to sustain amoxicillin and clarithromycin intra-gastric concentrations, 2015) describes a floating modular drug delivery system with buoyancy independent of release mechanisms, showcasing advancements in controlled release.

Combination Therapy: Floating microspheres can be used to deliver multiple drugs simultaneously, which is beneficial for treating complex diseases like diabetes. This can improve patient compliance and enhance therapeutic outcomes. (Floating modular drug delivery systems with buoyancy independent of release mechanisms to sustain amoxicillin and clarithromycin intra-gastric concentrations, 2015) also exemplifies this by mentioning a system designed to sustain both amoxicillin and clarithromycin concentrations.

1.3. Challenges in Formulating and Evaluating Floating Microspheres for Teneligliptin:

Drug Stability: Teneligliptin, like many drugs, can be susceptible to degradation during the formulation process or upon storage. Ensuring drug stability within the microspheres is crucial for maintaining therapeutic efficacy.

Controlling Drug Release: Achieving a sustained and controlled release of Teneligliptin from the microspheres can be challenging. The release profile should ideally mimic the pharmacokinetic profile of the drug to maintain therapeutic levels for an extended period.

In Vivo Performance: Predicting the in vivo performance of floating microspheres based solely on in vitro data can be difficult. Factors like gastric emptying time, intestinal motility, and drug absorption can influence the overall effectiveness of the delivery system.

Regulatory Considerations: Meeting regulatory requirements for the approval of new drug delivery systems can be complex and time-consuming.
Despite these challenges, floating microspheres hold significant promise for improving the delivery of Teneligliptin and other drugs for treating type 2 diabetes. Continued research and development in this field are essential to overcome these challenges and translate these advancements into clinically viable therapies.

1.4. Research Gaps:
While floating microspheres offer a promising approach for enhancing the delivery of various drugs, research specifically focusing on Teneligliptin using this technology appears limited based on the provided search results. There's a lack of published studies investigating the feasibility and efficacy of floating microspheres for Teneligliptin delivery. This gap in research presents an opportunity for further investigation.

II. RESEARCH OBJECTIVES AND APPROACH

The main objectives of the proposed research are:

Systematic Literature review
Selection of drug candidates, polymers and other constituents
Formulation development and optimization
Pharmaceutical evaluation of the optimized formulations
Biological evaluation of the optimized formulations (in vitro studies)
Pre-clinical studies of the optimized formulations (in vivo studies on study animal)

The research approach (in floating system) is based on:

Floating systems are low density systems that have maximum buoyancy to float on the gastric material and remain in the stomach for longer period of time. During the system hangover the gastric contents, the drug is released sustain with desired rate, which results in elevated gastric retention time and minimizes fluctuation. A low amount of gastric content is required to permit the right achievement of the buoyancy retention principle; a minimal level of floating force (F) is required to stay the dosage form buoyant on the surface of the gastric content. A floating dosage form is a feasible approach especially for drugs which have limited absorption sites in upper small intestine. The controlled, slow delivery of drug to the stomach provides sufficient local therapeutic levels and limits the systemic exposure to the drug. Drugs that have poor bioavailability due to site specific absorption from the upper part of the GIT are potential candidates to be formulated as floating drug delivery systems thereby increasing their absorption. Floating microspheres are gastro-retentive drug delivery systems supported non-effervescent approach. Hollow microspheres are considered as one of the most promising buoyancy systems, as they possess the unique advantages of multiple unit systems as well as the better floating properties, because of the central hollow space inside the microspheres. These microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, ideally having a size less than 200 micrometer.

III. LITERATURE REVIEW

Hinal Prajapati et al., the present study was aimed to formulate and evaluate floating microspheres of Baclofen. The research work's objective was to retain Baclofen in the stomach for a prolonged period of time, which has absorption window in the upper gastrointestinal tract. The microspheres were prepared by solvent evaporation technique. Results of the multiple regression analysis revealed that in-vitro drug release decreased and particle size, percentage drug entrapment efficiency, percentage buoyancy was increased with increasing the concentration of Eudragit RL100 and Eudragit RS100. In-vitro drug release of Baclofen floating microspheres showed a sustained release up to 24 h. The floating microspheres were free-flowing, porous, and almost spherical in shape. The in-vitro drug release kinetics studies revealed that the Higuchi model was followed by the formulation and drug release by fickian diffusion mechanism.

Beena Kumari et al., the development and optimization of vildagliptin floating microspheres using a central composite design. The selected independent variables were Chitosan, Eudragit RL100, and Sodium lauryl sulphate. The selected dependent variables were mean particle size, entrapment effectiveness, and drug release within 12 h. Further, the microspheres were categorized for particle size, micrometric study, entrapment effectiveness, in vitro drug discharge, kinetic studies for drug release, SEM, DSC, FTIR of the optimized formulation. In-vivo floating capability (X-ray) study and in-vivo antidiabetic action were performed using the Streptozotocin model. The vildagliptin microspheres were found free-flowing with excellent results. SEM has shown a spherical organization of microspheres with soft surface morphology. The results for in vivo antidiabetic activity and in vivo gastric retention for 12 hours were also found very significant. As per results obtained from in vitro and in vivo studies, floating microspheres of vildagliptin may prove to be a potential contender for secure and efficient sustained drug release over an absolute phase of time which can decrease dosing frequency.

Anamika Saxena et al., Oral controlled release systems are designed to release the drug in-vivo with prediction so as to increase efficacy, minimize adverse effects and increase bioavailability of drugs. Floating drug delivery systems (FDDSs) are expected to remain buoyant in a lasting way upon the gastric contents. The various buoyantstudies.1tions include hollow microspheres, granules, powders, tablets, capsules, pills and laminated films. Floating microspheres are especially gaining attention due to their wide applicability in the targeting of drugs to the stomach. Floating microspheres (Hollow Microspheres) are gastro-retentive drug delivery systems based on non-effervescent approach. Hollow microspheres are in strict sense, spherical empty particles without core, free flowing powders consisting of proteins or synthetic polymers, ideally having a size in the range 1-1000 micrometer. Gastro- retentive floating microspheres are low-density systems that have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period. The drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma drug concentration. Floating microspheres to improve patient compliance by decreasing dosing

frequency, better the rapeutic effect of short half-life drugs can be achieved. Enhanced absorption of drugs which solubilize only in stomach, Gastric retention time is increased because of buoyancy. Floating microspheres are prepared by solvent diffusion and evaporation methods to create the hollow inner core.[3]

M. V. Nila et al., designing a sustained release system for Carvedilol to increase its residence time in the stomach. Preparation of floating microsphere by the emulsion solvent diffusion method, studying the effect of various process parameters and optimize the formulation using full factorial design. Different microsphere formulations were prepared by varying the ratio ethanol:dichloromethane (1:0 to 1:1.5), ethyl cellulose: hydroxypropyl methyl cellulose and stirring speed (800–1600 rpm). The effect of these variables on particle size, encapsulation parameters, surface topography, in vitro floatability and drug release were evaluated. Full factorial design was used for the optimization of the formulation. Drug entrapment efficiency, particle size and in vitro drug release were dependent on concentration of ethyl cellulose and stirring speed. Microspheres remained buoyant for more than 10 h and showed sustained release of the drug.

Revathi et al., aim of the present work is to formulate, optimize and evaluate hydrodynamically balanced antidiabetic system incorporated with sitagliptin and phytochemical constituents of Triphala extract for the treatment of constipation associated with diabetes. The Triphala churna of two different ratios, 1:1:1 (TC1) and 1:2:4 (TC2) was subjected to hot percolation using Soxhlet apparatus using methanol as solvent. The floating matrix tablets of Sitagliptin with methanolic Triphala extract was prepared by wet granulation technique using HPMC K4M as polymer, starch/honey as binder and sodium bicarbonate & citric acid as effervescent agents by 24 factorial designs. The compatibility studies showed that there is no chemical interaction between the drug, polymer and the excipients used in the tablets. The prepared floating tablets were subjected to all post compression parameters such as hardness, friability, swelling capacity, buoyancy, total floating time, drug content & in-vitro drug release and were found to be within normal limits. Based on drug content, buoyancy lag time and invitro drug release the formulations F14 and F16 were selected for in-vivo study of the formulation.

Jameel Ahmed et al. The mucoadhesive microspheres areRats9 of the most promising novel techniques for drug delivery. Mucoadhesive systems provide a sustained drug release method, enhancing drug absorption in a site-specific manner. This study aims to prepare teneligliptin mucoadhesive microspheres to increase the residence time in the gastric and offers control release. The teneligliptin mucoadhesive microspheres are prepared by ionotropic external gelation technique using sodium alginate, HPMCK4, HPMC K15, HPMC K100, xanthan gum, guar gum, and carbopol 934. All mucoadhesive microspheres after preparation were characterized for percentage yield, particle Mice8, drug entrapment efficiency, mucoadhesive test, and in vitro release. The results obtained are differed depending on the concentration of polymers and ratios of drug to polymers.

Gadad et al.: Lafutidine Gastroretentive Floating Microspheres Gastroretentive floating microsphere containing Lafutidine, a second-generation histamine H2– receptor antagonist was prepared by ionotropic gelation technique by using sodium alginate, HPMC K4M, ethyl cellulose as polymers, sodium bicarbonate as gas generating agent and calcium chloride as cross linking agent. Objective: To formulate a system to remain in the stomach for prolonged and predictable period in order to enhance the drug bioavailability. Method: They were evaluated for micromeritic study, percentage yield, drug entrapment efficiency, in-vitro buoyancy, surface morphology, in-vitro drug release, in-vivo floating study and stability studies. Results: The micromeritic parameters of floating microspheres were found to be within the acceptable limits. The floating microspheres were spherical in shape with distinct pores, slightly rough surface when observed under scanning electron microscopy. The optimized formulation F4 was floating in rabbit stomach for almost 8 h. All the formulations followed KorsemeyerPeppas kinetics indicating drug release by non-fickian release mechanism. The stability studies showed that floating microspheres were stable at 40 ± 2°C. Conclusion: The optimized formulation showed good floating for 8 h in stomach of rabbit. The formulation was stable at the end of 60 days with stability study.

Vinod et al Much attention has been focused in pharmaceutical research in the area of gastroretensive oral drug delivery systems. Henceforth a wide spectrum of dosage forms has been developed for drugs which are unstable in alkaline pH, soluble in acidic pH, having a narrow absorption window, site of action specific to stomach. This article provides the entire classification of gastroretensive systems, formulation considerations for developing gastroretensive systems, factors affecting gastroretensive systems, merits and demerits, applications in pharmacy and a comparative diagrammatic representation limelight this article. Those gastroretensive systems which depend on liberation of carbondioxide show poor patient compliance because of flatulence and belching.

Kauslya et al. The main intention of this research was to formulate and evaluate floating microspheres of ciprofloxacin using different polymers to prolong gastric residence time. Methods: The microspheres were formulated by the solvent evaporation method using different ratios of polymers like carbopol 940, ethylcellulose, and Hydroxy Propyl Methyl Cellulose K4M. Further, the floating microspheres were evaluated for micromeritic properties like bulk density, tapped density, angle of repose, etc., percentage yield, particle size, entrapment efficiency, floating capacity, in vitro drug release study, release kinetics, drug content, swelling index, and Fourier Transform Infrared Spectroscopy (FTIR) (Compatibility studies). Results: The ciprofloxacin microspheres showed the good flowing property. Conclusion: The floating microspheres were prepared successfully and the results clearly stated that prepared ciprofloxacin microspheres may be safe and effective controlled drug delivery over an extended period which can increase bioavailability, patient compliance, and decrease dosing frequency.

Pandey Manisha et al The purpose of this research was to prepare a floating drug delivery system of famotidine. In the present study, preparation of famotidine floating microspheres, evaluation of Floating Drug Delivery System (FDDS) in vitro, prediction of the release, and optimization of stirring speed and polymers ratio to match target release profile was investigated. Floating

microspheres were prepared by solvent evaporation (Oil-in-water emulsion) technique using hydroxylpropyl methylcellulose (HPMC) and Ethylcellulose (EC) as the rate controlling polymers. Particle size analysis, drug entrapment efficiency, surface topography, buoyancy percentage and release studies were performed. The mean particle size of prepared floating microspheres increased but the drug release rate from the microspheres decreased as the polymer concentration increased. The developed floating microspheres of famotidine may be used in clinic for prolonged drug release in stomach for at least 12 hrs, thereby improving the bioavailability and patient compliance. Keywords: Floating drug delivery system (FDDS), HPMC, Ethyl cellulose, famotidine, in-vitro release.

Miyako Kishimoto el al., Dipeptidyl peptidase-4 (DPP-4) inhibitors have recently emerged as a new class of antidiabetic that show favorable results in improving glycemic control with a minimal risk of hypoglycemia and weight gain. Teneligliptin, a novel DPP-4 inhibitor, exhibits a unique structure characterized by five consecutive rings, which produce a potent and long-lasting effect. Teneligliptin is currently used in cases showing insufficient improvement in glycemic control even after diet control and exercise or a combination of diet control, exercise, and sulfonylurea- or thiazolidine-class drugs. In adults, teneligliptin is orally administered at a dosage of 20 mg once daily, which can be increased up to 40 mg per day. Because the metabolites of this drug are eliminated via renal and hepatic excretion, no dose adjustment is necessary in patients with renal impairment. The safety profile of teneligliptin is similar to those of other available DPP-4 inhibitors. However, caution needs to be exercised when administering teneligliptin to patients who are prone to QT prolongation. One study has reported that the postprandial blood glucose-lowering effects of teneligliptin administered prior to breakfast were sustained throughout the day, and the effects observed after dinner were similar to those observed after breakfast or lunch. Thus, although clinical data for this new drug are limited, this drug shows promise in stabilizing glycemic fluctuations throughout the day and consequently suppressing the progression of diabetic complications. However, continued evaluation in long-term studies and clinical trials is required to evaluate the efficacy and safety of the drug as well as to identify additional indications for its clinical use.

IV. CURRENT WORK AND PRELIMINARY RESULTS

Current work:
Systematic literature review for the selection of drugs, polymers and methodology for the formulation of floating microspheres

4.1. Selection of drug:

Materials and methods

Teneligliptin was obtained as a gift sample from one of known Pharmaceuticals, was procured from one of the known Lab. All the other chemicals, excipients, and solvents used of laboratory grade and analytical grade was procured from reliable sources.

Polymers

Ethyl cellulose (EC) and various types of Eudragit® are the most commonly used polymers for the preparation of the floating microspheres by emulsion solvent diffusion technique. The drug release from the microspheres consists of only EC or Eudragit® is very less.

According to Lee *et al*., many drugs are not released in significant amount from these microparticles at the pH of gastric fluids. So, there is a need of some hydrophilic polymers to be added into the formulation. These polymers cause rapid ingress of the dissolution medium into the microspheres facilitating more drug releases.

4.2. Method of Preparation of Floating Microspheres

Wide ranges of developmental techniques are available for the preparation of Gastroretentive floating microspheres. However, solvent evaporation technique and ionotropic gelation method have been extensively employed by large number of scientific investigators worldwide to explore the different vistas of floating microspheres.

During the preparation of floating controlled release microspheres, the choices of optimal method will utmost relevance for the efficient entrapment of active constituents. Selection of fabrication technique generally depends upon the nature of the polymer, the drug, and their intended use. Characteristic features of materials and the process engineering aspects strongly influence the properties of microspheres and the resultant controlled release rate.

4.2.1. Solvent Evaporation Technique

This technique is widely employed by large number of pharmaceutical industries to obtain the controlled release of drug. This approach involves the emulsification of an organic solvent (usually methylene chloride) containing dissolved polymer and dissolved/dispersed drug in an excess amount of aqueous continuous phase, with the aid of an agitator. The concentration of the emulsifier present in the aqueous phase affects the particle size and shape. When the desired emulsion droplet size will be formed, the stirring rate will be reduced and evaporation of the organic solvent must realize under atmospheric or reduced pressure at an appropriate temperature. The Subsequent evaporation of the dispersed phase solvent would yield to the solid polymeric microparticles entrapping the drug. The solid microparticles would recover from the suspension by filtration, centrifugation, or

lyophilisation. For emulsion solvent evaporation, there would be basically two systems which include oil-in-water (o/w) and water-in-oil (w/o) type.

4.2.2. Oil-In-Water Emulsion Solvent Evaporation

Technique In this process, both the drug and the polymer should be insoluble in water while a water immiscible solvent is required for the polymer. In this method, the polymer will be dissolved in an organic solvent such as dichloromethane, chloroform, or ethyl acetate, either alone or in combination. The drug will either dissolve or dispersed into polymer solution and this solution containing the drug would be emulsified into an aqueous phase to make an oil-in water emulsion by using a surfactant or an emulsifying agent.

After the formation of a stable emulsion, the organic solvent will evaporate either by increasing the temperature under pressure or by continuous stirring. Solvent removal from embryonic microspheres determines the size and morphology of the microspheres. It would be reported that the rapid removal of solvent from the embryonic microspheres leads to polymer precipitation at the o/w interface. This may lead to the formation of cavity in microspheres, thus will make them hollow to impart the floating properties. Oil-in-water emulsion is widely used than water-in-oil due to simplicity of the process and easy cleans up requirement for the final product.

4.2.3. Oil-in-Oil Emulsification Solvent Evaporation Technique

This oil-in-oil (sometimes referred as water-in-oil) emulsification process is also known as non-aqueous emulsification solvent evaporation. In this technique, drug and polymers would be co-dissolved at room temperature into polar solvents such as ethanol, dichloromethane, acetonitrile etc. with vigorous agitation to form uniform drug–polymer dispersion. That solution will slowly be poured into the dispersion medium consisting of light/heavy liquid paraffin in the presence of oil soluble surfactant such as span.

The system would be stirred using an overhead propeller agitator at 500 revolutions per minute (rpm) and room temperature over a period of 2– 3 h to ensure complete evaporation of the solvent. The liquid paraffin will be decanted and the microparticles will be separated by filtration through a Whitman filter paper, washed thrice with nhexane, air dried for 24 h and subsequently will store in desiccators. Span 60 is generally used which is non-ionic surfactant. Span 60 has an HLB value of 4.3 and acts as a droplet stabilizer and prevents coalescence of the droplets by localizing at the interface between the dispersed phase and dispersion medium.

4.2.4. Spray drying technique

Drug loaded microspheres made with a polymeric blend will be prepared by a spray- drying technique. Spray drying techniques involves dispersing the core material in a liquefied coating material and spraying the core-coating mixture in to the environment to effect solidification of coating. Solidification will be accomplished by rapid evaporation of the solvent in which coating material will be solubilized. Organic solutions of two polymers in different weight ratios and of drug will be prepared and sprayed, in different experimental conditions, achieving drug-loaded microspheres.

The process control variables in this technique would be feed material properties, feed rate, method of atomization and drying rate. Spray drying method is rapid, reproducible and easy to scale up. But due to the fast-drying process the polymer may lose its crystallinity.

4.2.5. Ionic gelation technique

The drug will be added to aqueous solution of sodium alginate. The solution will be stirred till a complete solution would be formed. That solution will be added drop wise to a solution containing Ca or Al and chitosan solution in acetic acid. The microspheres formed should be left into the original solution for 24 hours for internal gelification. The product will be then separated by filtration. The particle size must be ranged from 2-4 mm but the encapsulation efficiency would be 98%.

4.2.6. Single emulsion technique

Aqueous solution / suspension of drug and polymer will be stirred with the help of stirrer. That solution will be dispersing in organic phase then the solution will be treated with formaldehyde or butanol to get the microspheres. The preparation of floating microspheres based on lowdensity foam powder, using (a) the solvent evaporation method (b) the soaking method will be done.

4.2.7. Double Emulsion technique

Aqueous solution of drug or polymer will be homogenized with homogenizer. That aqueous solution will be dispersed in organic phase to get single emulsion. Multiple emulsions would be formed by the addition of aqueous solution of PVA in single emulsion. That multiple emulsion should be denatured to get microspheres.

4.2.8. Polymerization technique

Drug polymer and aqueous solution of sodium hydroxide with surfactant should be vigorously stirred by stirrer to get micellar solution of polymer in aqueous media. By the polymerization of the solution microspheres will be formed.

4.2.9. Phase Separation Coacervation technique

This process consists of 3 steps under continuous stirring. The formation of three phases: Dispersing a core material in a solution of coating polymer Immiscible polymer in liquid state (Coating material phase) Coating is accomplished by controlled physical mixing of coating solution and core material in liquid manufacturing vehicle phase. Rigidisation could be achieved by thermal, chemical crosslinking or desolvation techniques

4.2.10. Non aqueous solvent evaporation

Drug and polymer should be mixed and stirred to get slurry. That slurry will then mix with petroleum ether. After the solvent will be evaporated and treated with glutraldehyde to get the microspheres.

4.2.11. Cold homogenization technique

The drug will be dissolved into the melted lipid. After solidification, the mixture will be milled in liquid nitrogen or dry ice with the help of mortar mill. Grinded particles will then disperse into an aqueous surfactant solution heated at 5-10˚C below the lipid melting point. Particles could be disrupted by putting them through the homogenizer once or several times.

4.3. Characterization of Floating Microspheres

Characterization of floating microspheres is an important phenomenon which helps in the evaluation of suitable drug delivery systems. Floating microspheres are characterized by following parameters:

4.3.1. Scanning electron microscopy

A microsphere will be observed under an electron microscope. They would be mounted directly onto the SEM sample stub using sided tape and will be coated with gold film under reduced pressure.

4.3.2. Particle size

Particle size of microspheres will be measured by optical light microscopy.

4.3.3. Drug loading, Drug Entrapment Efficiency

A quantity of microspheres containing equivalent to 50mg of the drug would be taken for evaluation. The amount of drug entrapped will be estimated by crushing the microspheres and extracting with aliquots 100ml of 0.1 N HCl repeatedly. The extract will then transfer to a 100 ml volumetric flask and the volume should be made up using 0.1N HCl. The solution will be filtered and the absorbance will be measured after suitable dilution spectrophotometrically at 232nm against appropriate blank. The amount of drug entrapped in the microspheres will be calculated by the following formula:

Drug Entrapment Efficiency = (Amount of drug actually present / Theoretical drug load expected) X **100**

Drug loading = (Amount of drug actually present/ total weight of microspheres) X **100**

4.3.4. Percent yield

The total amount of microspheres obtained will be weighed and the percentage yield must be calculated taking into consideration the weight of the drug and polymer.

$$\textbf{Percent yield} = \frac{\textbf{practical yield}}{\textbf{Theoretical yield}} \times 100$$

4.3.5. In-Vitro evaluation of floating ability

An in vitro floating study will be carried out using simulated gastric fluid USP containing 1% Tween 80 as a dispersing medium. Microspheres will be spread of over the surface of 900 ml of dispersion medium at 37□0.5□C and agitated by a paddle rotating at 100rpm. Each fraction of microspheres must be floating on the surface and those settled down will be collected at a predetermined time point. The collected samples will be weighted after drying.

% floating microspheres = (Weight of floating microspheres / initial weight of floating microspheres) □ 100

4.4. Compilation of technical database on the drug candidates

4.4.1. Dosage and administration of the drug candidates

In adults, teneligliptin is orally administered at a dosage of 20 mg once daily, which can be increased up to 40 mg per day.

4.4.2. Pharmacodynamics and pharmacokinetic profile of the drug candidates

The plasma concentrations of teneligliptin after the administration of teneligliptin at dosages of 10 or 20 mg once daily for 4 weeks revealed a median time to maximum concentration (Cmax) of 1.0 hour in both groups and a mean $t_{1/2}$ of 20.8 and 18.9 hours, respectively.

The maximum percentage of the inhibition in plasma DPP-4 activity must be achieved within 2 hours after administration and will find to be 81.3% and 89.7% in the 10 and 20 mg teneligliptin groups, respectively.

4.4.3. Clinical records

Since September 2012, teneligliptin has been commercially sold in Japan and has been used for the treatment of type 2 diabetes mellitus when patients do not show sufficient improvement after diet control and exercise or a combination of diet control, exercise, and sulfonylurea- or thiazolidine-class drugs. In adults, 20 mg of teneligliptin may be orally administered once daily. If this dosage is insufficient, the dosage can be increased to 40 mg once daily.

4.4.4. Clinical efficacy and tolerability of the drug candidates

The incidence of adverse events (AEs) was not significantly different between the teneligliptin and placebo groups in the study conducted by ETO Et Al· When AEs were rated by the investigators for intensity and potential relationship to the study drug, two drug-related as per the previous study / literature, AEs, increased levels of alanine aminotransferase and γ-glutamyltransferase, were observed in one patient (2.9%) treated with 10 mg of teneligliptin. No drug-related AEs occurred in the placebo or 20 mg teneligliptin groups. Furthermore, none of the patients in any of the groups experienced hypoglycemic symptoms or serious AEs. In addition, a pharmaceutical company provided information regarding domestic clinical studies that included 1183 patients, of which 118 patients (10.0%) experienced AEs, including abnormalities in clinical examination values such as levels of liver and kidney function, blood cell count, creatinine phosphokinase, and electrolytes.

As per the literature review the main AEs included hypoglycemia (35 patients: 3.0%) and constipation (eleven patients: 0.9%). The pharmaceutical company also warned of serious AEs such as hypoglycemia, which could occur when other antidiabetic drugs were co-administered. In addition, they cautioned that intestinal obstruction could occur with an unknown frequency. GLP-1 is involved in gastrointestinal motility, and the patients with intestinal obstruction had a past medical history of intestinal obstruction or abdominal surgery. Therefore, we should be cautious when administering incretin-related agents to patients with a history of these conditions. Continued assessment of AEs previously reported in clinical trials and post-market monitoring is required to determine the benefit/risk ratio for the drug.

4.5. Interaction Profile of drug candidates

4.5.1. Fourier transform Infrared spectroscopic studies

The advantage of this method brings about significant merits in terms of ease, speed and cost by using FTIR spectroscopy for calculating the amount of desired active ingredient during quality control testing of finished pharmaceuticals. In this method absorbance mode of FTIR will be preferred because of two main difficulties related with transmittance mode. That should be sensitivity in comparison to absorbance mode and development of accurate calibration curve. Therefore, absorbance FTIR is excellent choice for accurate determination of active ingredient without using any solvent.

4.5.2. Identification by HPLC and UV spectroscopy

A new, accurate and selective gradient RP-HPLC and UV-Spectrophotometric method was proposed for the determination Teneligliptin validated as per the ICH guidelines. The method has higher sensitivity towards the determination of related substances. The method must find to be simple, selective, precise, accurate, isolated and characterized using spectral data. The method must be low time consuming due to simply mobile phase composition and relatively short analysis time by considering different system suitability parameters like retention time, etc.

In a study the responses of tablet dosage form were measured at 242 nm for quantification of TEN by using RP-HPLC. The amounts of TEN present in sample solution were determined by the responses into the regression equation for TEN in the method.

According to a study, in this method solutions of Teneligliptin to be prepared in Dimethyl Sulphoxide (DMSO). Teneligliptin standard solution will be scanned in the UV range (400-200nm) in a 1cm quartz cell in a double beam UV spectrophotometer. The standard solution of Teneligliptin must be showed maximum absorption at wavelength 267.2 nm.

Table 1: Optical and Regression characteristics and validation parameters of HPLC method for analysis of TEN

Parameter	Teneligliptin
Calibration range	500- 3000 ug/ml
Regression equation	Y= 1.899 x + 119.9
Slope (m)	1.899
Intercept ()	119.9
Correlation coefficient (R sqare)	0.9983
Inter Day (%RSD)	0.063 - 0.116
Intra Day (%RSD)	0.052 - 0.114
Repeatability (%RSD)	0.327
Detection limit (ug/ml)	9.539
Quantitation limit (ug/ml)	59.210

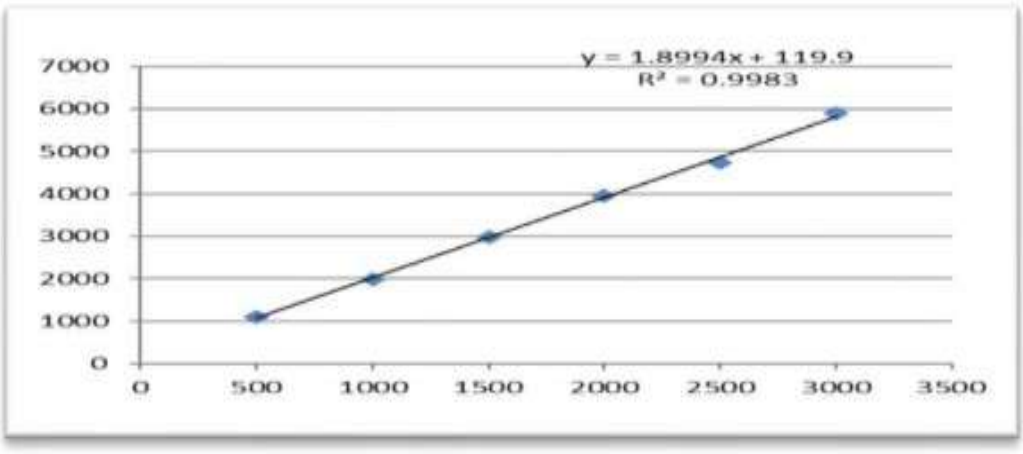
4.6. Preparation of Calibration Curves

In a study a series of standard solutions 500-3000 µg/ml of TEN were prepared. An aliquot of 20µl of each solution was injected 3 times for each standard solutions and peak area was observed.

Plot of average peak area versus the concentration should be plotted and from that the correlation coefficient and regression equation will be generated. The standard calibration data of TEN is given in the table, while Figures represents linearity graphs of drugs.

Table 2: Linearity study data for TEN (according to literature review study)

Sr. No.	Conc. (ug/ml)	Avg. area*+-SD	%RSD
1	500	1100.379 (+- 11.115)	0.998
2	1000	1990.491 (+- 0.246)	0.010
3	1500	2970.993 (+- 12.567)	0.422
4	2000	3960.910 (+- 26.371)	0.664
5	2500	4740.167 (+- 10.046)	0.211
6	3000	5900.652 (+- 7.124)	0.120



Calibration curve of teneligliptin

4.7. Formulation and Evaluation of Floating Microspheres

4.7.1. Selection of methodology

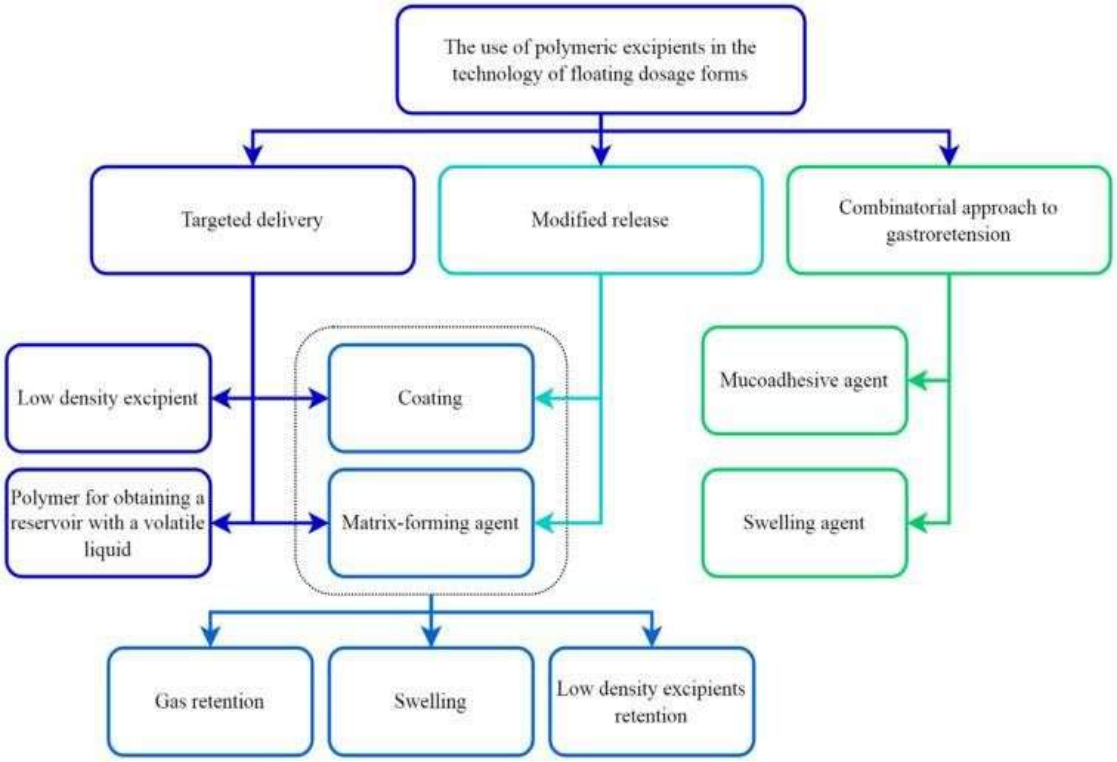
The combination of targeted transport and improvement of the release profile of the active pharmaceutical ingredient (API) is a current trend in the development of oral medicinal products (MP). A well-known way to implement this concept is to obtain floating gastroretentive delivery systems that provide a long stay of the dosage form (DF) on the surface of the stomach contents. Among various methods chooses one suitable, time saving and appropriate method for formulation and evaluation of floating microspheres after trying and testing methods in laboratory.

4.7.2. Selection of sustained release

Natural polymers may be used in floating system are Guar gum, Chitosan, xanthan gum, Gellan gum, Sodium alginate, etc. Synthetic polymers to be used for the floating drug delivery are HPMC, Eudragit, ethyl cellulose, etc.

4.7.3. Selection of various polymers and other constituents

The most important role in the technology of floating dosage forms would be played by polymeric Es, traditionally subdivided on the basis of their origin, the functional properties of which ensure the achievement of the distinctive qualities of these systems: gastroretention, due to flotation, and modified release. Such characteristics are the ability to significant swelling, the formation of stable gels, low density, etc.



4.7.4. Formulation of the drug loaded floating microspheres

Different methods for the formulation of the drug loaded floating microspheres are there. Among them choosing one suitable method and formulate drug accordingly.

4.7.4.1. Optimization of the formulation

Optimization techniques are used in the different formulations of drugs which help to make good products. It involves in the various form of drug product and their process. Optimization techniques are used in the finding solution of a slew of issues relating to the pharmaceutical process and product such as new drug development selection of excipients, formulation, manufacturing and other pharmacy-related problems. Due to the optimization techniques, we can examine the various problems that occur during research.

Optimization technique is helpful to make easy the process and formulation of pharmaceutical products and processes. It provides diverse design quality of formulation and experiment design, as well as systemic and mannered strategies and performance, which are investigated by changing the experimental variable to assess the effect on the specific response. The optimization strategies aid in the development of safe, effective, low-toxicity, and low-adverse-effect drug products, as well as cast effective products that are accessible to everyone.

4.7.4.2. Characterization and evaluation of optimized formulations

Evaluation studies such as Particle Size Analysis, Percentage yield, Percentage Drug Entrapment Efficiency, Percentage Buoyancy Study, Effects of formulation variables (polymer composition, dissolution and profile of optimized formulation etc), in-vitro drug release studies, and in vivo pharmacokinetic studies will be performed for the optimized drug formulation.

4.7.4.3. Stability profile of optimized formulation

For assessing the stability of the prepared optimized batch and to know the shelf life and decomposition rate of the same, stability studies will be conducted for the optimized drug at elevated temperature and relative humidity (RH) storage conditions. As per the ICH guidelines, the optimized drugs must be placed in the stability chambers at specified temperature and relative humidity conditions.

V. PRE-CLINICAL STUDIES

Preclinical studies for teneligliptin microspheres focus on developing sustained-release formulations to enhance the efficacy of this DPP-4 inhibitor, which is typically used for Type 2 Diabetes Mellitus (T2DM). Research often involves creating mucoadhesive or sustained-release microspheres to increase gastric residence time and control drug release.

5.1. Formulation Technique: Mucoadhesive microspheres are frequently prepared using ionotropic external gelation, utilizing polymers like sodium alginate in combination with HPMC (K4, K15, K100), xanthan gum, guar gum, and carbopol 934.

5.2. Characterization: The microspheres typically exhibit a high percentage yield (up to 93.58%) and excellent entrapment efficiency (ranging from 75.25% to 86.52%).

5.3. Particle Size: Particle sizes for teneligliptin-loaded mucoadhesive microspheres are reported to range between 320.22 µ and 448.25 μm, suitable for oral administration.

5.4. Drug Release Mechanism: Studies demonstrate a sustained release, with lower release rates observed when mucoadhesive polymer content (like carbopol) increases, due to enhanced swelling and increased diffusional path length.

5.5. Mucoadhesion Efficiency: Formulations containing carbopol 934P show superior mucoadhesive properties, with some retaining over 35% of microspheres in the gastric region after 8 hours. These studies aim to provide a superior alternative to conventional 20–40 mg daily oral dosing by reducing frequency and improving gastric absorption.

5.6. Animal Models for Efficacy Testing

The selection of an appropriate animal model is critical for the preclinical evaluation of anti-diabetic agents like Teneligliptin. The following models are commonly used to mimic the pathophysiology of type 2 diabetes in humans.

5.6.1. High-Fat Diet (HFD)-Induced Obesity and Insulin Resistance Model

This model is widely used to study impaired glucose tolerance and early-stage type 2 diabetes, as it closely mimics the etiological and pathological progression in most human cases.

Model: mice are to be typically fed a high-fat diet (around 60% of calories from fat) for several weeks to induce obesity, insulin resistance, and hyperglycemia.

Key Features: This model exhibits increased body weight, adiposity, elevated circulating glucose and insulin levels, and impaired glucose tolerance.

5.6.2. Streptozotocin (STZ)-Induced Diabetic Model

This model is characterized by the chemical ablation of pancreatic β-cells, leading to insulin deficiency and hyperglycemia. It is often used to study both type 1 and, with modifications, type 2 diabetes.

Model: A single high dose or multiple low doses of streptozotocin are administered to rodents (rats or mice) to induce diabetes. For a model that more closely resembles type 2 diabetes, nicotinamide is often co-administered to partially protect β-cells.

Key Features: This model displays significant hyperglycemia, hypoinsulinemia, and can be used to study diabetic complications.

5.6.3. Genetic Models of Obesity and Diabetes

These models have genetic mutations that lead to a diabetic phenotype.

db/db Mice: These mice have a mutation in the leptin receptor gene, leading to hyperphagia, obesity, insulin resistance, and subsequent β-cell failure.

Zucker Diabetic Fatty (ZDF) Rats: These rats also possess a leptin receptor mutation, resulting in obesity, hyperlipidemia, and the development of type 2 diabetes.

5.7. Efficacy Data of Teneligliptin in Animal Models The following tables summarize the quantitative data from various studies evaluating the efficacy of Teneligliptin in different animal models.

Table 3: Efficacy of Teneligliptin in High-Fat Diet-Fed Mice

Parameter	Control (HFD)	Teneligliptin (30 mg / kg / day)	Teneligliptin (60 mg / kg / day)
Body weight	Increased	Reduced to 87 % of control	Reduced to 70 % of control
Oxygen consumption	--	-	Increased by 21 %
Mean Adipocyte size	Hypertrophic	-	43 % of control
Hepatic triglycerides	Elevated	-	33 % of control
Plasma insulin	Elevated	-	Reduced to 39 % of control
Glucose infusion rate (Euglycemic clamp)	--	-	Increased by 38 %

Table 4: Efficacy of Teneligliptin in Streptozotocin-induced Diabetic Rats

Parameter	Diabetic control	Teneligliptin treated
Blood glucose (mg/ dl)	- 411 +- 24	Maintained at a low level
Body weight (g)	- 347 +- 18	Showed less impairment in weight gain compared to control
Paw withdrawal threshold (g)	- 29 +- 5	Significantly attenuated the reduction in threshold

Table 5: Efficacy of Teneligliptin in db/db Mice

Parameter	Diabetic control	Teneligliptin (60 mg / kg / day)
Total cholesterol (mM)	6.18	4.92
Triglycerides (mM)	34.5	2.51
Low density lipoprotein cholesterol (mM)	Elevated	Dramatically declined
Escape latency (Morris water maize) (s)	37.9	28.3
Time in platform Quadrant (Morris water maize) (s)	10.9	16.3
SOD activity (U/ mg protein)	21.4	33.4
GSH - PX Activity (U / mg protein)	17.9	28.5
IL - 1 beta (pg/ mg protein)	77.3	57.2
IL - 6 (pg/ mg protein)	114.6	71.8

5.8. Experimental Protocols

Protocol 1: Induction of Diabetes with Streptozotocin (STZ) in Rats

Materials: Streptozotocin (STZ), Citrate buffer (0.1 M, pH 4.5), cold Syringes and needles, Animal scale Glucometer and test strips.

Procedure:
Fast the rats overnight before STZ injection.
Prepare a fresh solution of STZ in cold citrate buffer. A common dose for inducing type 1-like diabetes is a single intraperitoneal (i.p.) injection of 50-60 mg/kg body weight.
Weigh each rat and calculate the required volume of STZ solution.
Administer the STZ solution via i.p. injection.
Monitor blood glucose levels 48-72 hours post-injection and then periodically. Rats with blood glucose levels above 250 mg/dL are typically considered diabetic.
Provide the animals with 10% sucrose water for the first 24 hours after injection to prevent hypoglycemia due to the initial massive release of insulin from damaged β-cells.

Protocol 2: Oral Glucose Tolerance Test (OGTT) in Rodents

Materials: Glucose solution (e.g., 20% or 40% in water), Oral gavage needles, Glucometer and test strips, Timer, Restraining device (optional).

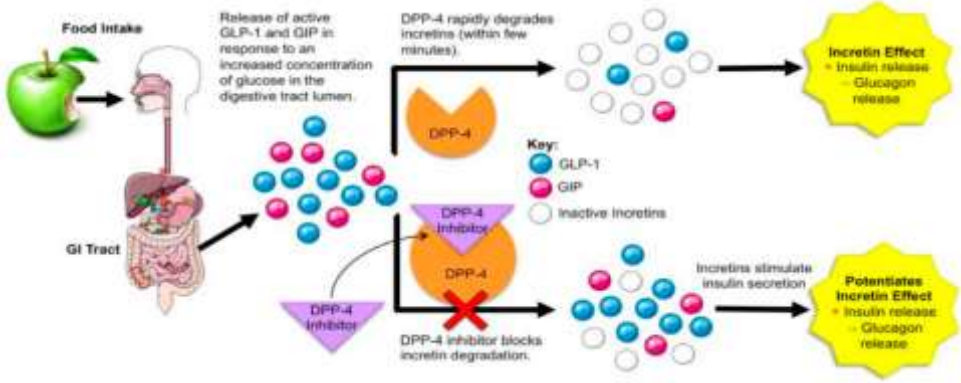
Procedure:
Fast the animals for 4-6 hours (mice) or overnight (rats) with free access to water.
Record the baseline blood glucose level (time 0) from a tail vein blood sample.
Administer a glucose solution orally via gavage. A standard dose is 2 g/kg body weight.
Collect blood samples at specific time points after glucose administration, typically at 15, 30, 60, 90, and 120 minutes.
Measure and record the blood glucose levels at each time point.
The data can be plotted as blood glucose concentration versus time, and the area under the curve (AUC) can be calculated to assess glucose tolerance.

Protocol 3: Assessment of Pancreatic β-Cell Mass

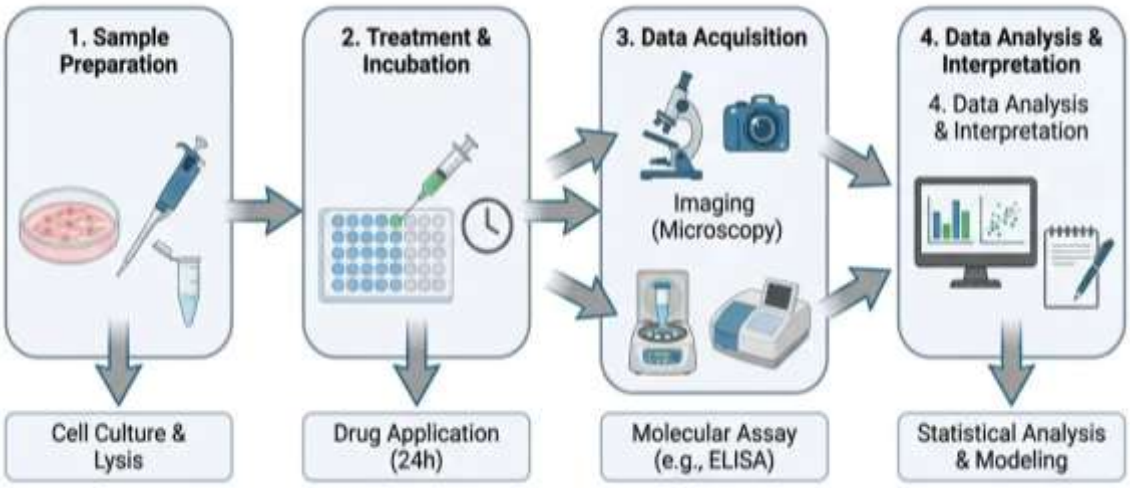
Materials: 4% Paraformaldehyde (PFA), Paraffin, Microtome, Glass slides, Anti-insulin antibody, Secondary antibody (HRP-conjugated), DAB substrate kit, Microscope with a digital camera, Image analysis software.

Procedure:
Euthanize the animal and carefully dissect the entire pancreas.
Weigh the pancreas.
Fix the pancreas in 4% PFA for 4 hours, followed by dehydration and embedding in paraffin.
Cut 5-µm thick sections at regular intervals (e.g., 250 µm apart) throughout the pancreas and mount them on glass slides.
Perform immunohistochemistry for insulin by incubating the sections with a primary anti-insulin antibody, followed by a secondary HRP-conjugated antibody and DAB substrate.
Capture images of the entire pancreatic sections using a slide scanner or a microscope with a tiling function.
Use image analysis software to quantify the insulin-positive area (representing β-cells) and the total pancreatic tissue area in each section.
Calculate the β-cell mass by multiplying the ratio of the insulin-positive area to the total pancreatic area by the total weight of the pancreas.

Signaling Pathways and Workflows



Teneligliptin Mechanism of Action



Experimental Workflow for Teneligliptin Efficacy Testing

5.9. Validated Analytical Methods for the Estimation of Teneligliptin:

Ensuring the quality, safety, and efficacy of pharmaceutical formulations containing Teneligliptin requires robust analytical methods for its quantification. The validation of these analytical procedures is a critical regulatory requirement, demonstrating that a method is suitable for its intended purpose.

Two common analytical techniques for the estimation of Teneligliptin in bulk and pharmaceutical dosage forms: UV-Vis Spectrophotometry and Reverse Phase High-Performance Liquid Chromatography (RP-HPLC), in accordance with the International Council for Harmonisation (ICH) guidelines**.**

Method 1:

UV-Vis Spectrophotometric Estimation of Teneligliptin: This method provides a simple, rapid, and cost-effective approach for the quantification of Teneligliptin in bulk drug and dosage forms.

Solvent Selection: HPLC grade Methanol or distilled water can be used as a solvent.

Preparation of Standard Stock Solution: Accurately weigh 100 mg of Teneligliptin working standard and transfer it to a 100 mL volumetric flask. Dissolve in 50 mL of methanol, sonicate for 10 minutes, and make up the volume to the mark with methanol to obtain a concentration of 1000 µg/mL.

Preparation of Working Standard Solutions (Calibration Curve): From the stock solution, prepare a series of dilutions to get concentrations ranging from 10-50 µg/mL.

Preparation of Sample Solution: Weigh and powder 20 tablets. Take an amount of powder equivalent to 10 mg of Teneligliptin and transfer it to a 100 mL volumetric flask. Add 70 mL of methanol, sonicate for 15 minutes, and dilute up to the mark. Filter the solution through a Whatman filter paper. Further dilute to obtain a final concentration within the linearity range.

Spectrophotometric Analysis: Scan the standard solution from 200-400 nm to determine the wavelength of maximum absorbance (λ max), which is typically observed around 246 nm. Measure the absorbance of all working standard solutions and the sample solution at the determined λ max.

Plot a calibration curve of absorbance versus concentration and determine the regression equation. Calculate the concentration of Teneligliptin in the sample solution using the regression equation.

Summary of Validation Parameters (UV-Vis Spectroscopy)

Table 6: The method is validated according to ICH guidelines to ensure it is fit for its intended purpose.

Validation Parameter	Typical Acceptance criteria	Reported values for Teneligliptin
Wavelength (lamda max)	N/A	245 - 247 min
Linearity range	5 - 90 ug/ ml	10 - 50 ug/ ml
Correlation coefficient (r square)	>_ 0.999	0.995 - 0.998
Accuracy (% recovery)	98 % - 102 %	98.4 % - 101.4 %
Precision (% RSD)	<_ 2.0 %	<_ 2.4 %
Limit of detection (LOD)	Signal- to - noise ratio >_ 3:1	0.284 - 2.158 ug/ ml
Limit of quantification (LOQ)	Signal- to - noise ratio >_ 10:1	1.33 - 6.47 ug / ml

Method 2: RP-HPLC Estimation of Teneligliptin

RP-HPLC methods offer high specificity and sensitivity for the estimation of Teneligliptin, especially in the presence of other components or degradation products.

Chromatographic Conditions: A variety of conditions have been reported. A representative method is described below.

Column: Kromasil C18 (250 x 4.6 mm, 5 µm).

Mobile Phase: Buffer : Acetonitrile : Methanol (65:25:10, v/v/v).

Flow Rate: 1.0 mL/min.

Injection Volume: 10 µL.

Column Temperature: 30°C.

Detection Wavelength: 254 nm.

Preparation of Standard and Sample olutions: Prepare stock and working solutions as described in the UV-Vis method, using the mobile phase as the diluent. The linearity range is typically between 5-30 µg/mL.

System Suitability: Before sample analysis, inject a standard solution multiple times to ensure the chromatographic system is performing adequately. Acceptance criteria typically include a %RSD of 2000, and a tailing factor of <2.

Analysis: Inject the standard solutions to establish a calibration curve, followed by the sample solutions. The quantification of Teneligliptin is based on the peak area compared to the calibration curve. The retention time for Teneligliptin under these conditions is approximately 2.84 minutes.

Summary of Validation Parameters (RP-HPLC)

Table 7: The HPLC method is validated to demonstrate its suitability for routine analysis.

Validation Parameter	Typical Acceptance criteria	Reported values for Teneligliptin
Retention time (t R)	N/A	- 2.37 - 4.2 min
Linearity range	5 - 60 ug/ ml	10 - 50 ug/ ml
Correlation coefficient (r square)	>_ 0.999	0.996 - 0.998
Accuracy (% recovery)	98 % - 102 %	99.4 % - 100.4 %

Precision (% RSD)	<_ 2.0 %	<_ 5 %
Specificity	No interference at the tR of analysis	Peak is pure, separated from degradants
Robustness	% Rsd <_ 2 % after minor change	Method in robust to small change in flow rate, mobile phase composition

Method 3: Stability-Indicating Forced Degradation Studies

Forced degradation studies are essential to develop a stability-indicating analytical method. They demonstrate the specificity of the method to measure the analyte accurately in the presence of its degradation products.

Acid Hydrolysis: Reflux a sample solution with 2N HCl at 60°C for 30 minutes. Neutralize the solution before analysis.

Alkali (Base) Hydrolysis: Reflux a sample solution with 2N NaOH at 60°C for 30 minutes. Neutralize the solution before analysis.

Oxidative Degradation: Reflux a sample solution with 20% v/v H_2O_2 at 60°C for 30 minutes.

Thermal Degradation: Place the drug sample in an oven at 105°C for 6 hours.

Photolytic Degradation: Expose the drug sample to UV light (e.g., 200 Watt hours/m^2) in a photostability chamber for 7 days.

Analysis: After applying the stress, dilute the samples appropriately with the mobile phase and analyze them using a validated stability-indicating HPLC method. The chromatograms are evaluated to see if the degradation product peaks are well-separated from the main Teneligliptin peak.

Summary of Forced Degradation Results

Table 8: The results indicate the stability of the drug under various conditions and the ability of the method to separate the drug from its degradation products.

Stress condition	Reagent/ condition	Observed degradation (%)
Acidic	0.1 N - 2 N HCl	3.67 % - 10.37 %
Alkaline	0.1 N - 2 N NaOH	2.76 % - 11.59 %
Oxidative	3 % - 20 % H2O2	1.02 % - 16.28 %
Thermal	Heat (40 - 105 degree C)	19.53 %
Photolytic	UV light	18.90 %

5.10. Preclinical Pharmacokinetics

Pharmacokinetic studies in preclinical models are crucial for determining the absorption, distribution, metabolism, and excretion (ADME) profile of a drug candidate. The following table summarizes key pharmacokinetic parameters of teneligliptin in rats following oral administration.

5.10.1. In Vivo Efficacy Studies

Teneligliptin has demonstrated significant efficacy in various preclinical models of type 2 diabetes and related metabolic disorders. The following table summarizes representative in vivo studies.

Experimental Protocols

Preparation of Teneligliptin Formulation for Oral Administration

This protocol describes the preparation of a teneligliptin solution for oral administration to preclinical animal models, such as mice and rats, via drinking water.

Materials: Teneligliptin hydrobromide hydrate, Purified water, Calibrated balance, Volumetric flasks, Stir plate and stir bar, Animal drinking bottles.

Procedure:

Calculate the required amount of teneligliptin: Based on the target dose (e.g., 30 or 60 mg/kg/day) and the average daily water consumption of the animals, calculate the total amount of teneligliptin needed.

Dissolve teneligliptin: Accurately weigh the calculated amount of teneligliptin hydrobromide hydrate. In a volumetric flask, add a portion of purified water and the weighed teneligliptin.

Ensure complete dissolution: Place the flask on a stir plate and stir until the teneligliptin is completely dissolved.

Adjust to final volume: Once dissolved, add purified water to the volumetric flask to reach the final desired volume and mix thoroughly.

Administration: Transfer the prepared teneligliptin solution to the animal drinking bottles.

Vehicle Control: For the control group, provide drinking bottles containing only purified water (vehicle).

Monitor water intake: Measure and record the daily water consumption to ensure accurate dosing.

5.10.2. In Vivo Efficacy Study in a Type 2 Diabetes Mouse Model

This protocol outlines a typical in vivo efficacy study to evaluate the anti-diabetic effects of teneligliptin in a streptozotocin (STZ)-induced diabetic mouse model.

Materials: Male mice (8-10 weeks old), Streptozotocin (STZ), Citrate buffer (pH 4.5), Teneligliptin formulation (prepared as described above), Blood glucose meter and test strips, Animal handling and restraint equipment, Oral gavage needles (if alternative dosing method is used)

Procedure:

Animal Acclimatization: House the mice in a controlled environment (temperature, humidity, and light-dark cycle) for at least one week before the experiment. Provide standard chow and water ad libitum.

Induction of Diabetes:

Fast the mice for 4-6 hours.

Prepare a fresh solution of STZ in cold citrate buffer.

Induce diabetes by a single intraperitoneal injection of STZ (dose to be optimized based on literature, e.g., 150 mg/kg).

Return the mice to their cages with free access to food and water.

Confirmation of Diabetes:

Measure blood glucose levels from the tail vein 72 hours after STZ injection.

Mice with fasting blood glucose levels above a predetermined threshold (e.g., >250 mg/dL) are considered diabetic and included in the study.

Grouping and Treatment:

Randomly assign the diabetic mice into two groups:

Vehicle Control Group: Receives purified water.

Teneligliptin-treated Group: Receives teneligliptin in drinking water at the desired dose.

A non-diabetic control group receiving vehicle should also be included for comparison.

Initiate treatment and continue for the specified duration (e.g., 4-12 weeks).

Monitoring:

Measure and record body weight and fasting blood glucose levels weekly.

Observe the general health and behavior of the animals throughout the study.

5.10.3. Endpoint Analysis (example):

Oral Glucose Tolerance Test (OGTT): At the end of the treatment period, perform an OGTT.

Fast the mice overnight.

Administer a glucose solution (e.g., 2 g/kg) orally.

Measure blood glucose levels at 0, 15, 30, 60, and 120 minutes post-glucose administration.

Tissue Collection: At the end of the study, euthanize the animals and collect blood and tissues (e.g., pancreas, liver, adipose tissue) for further analysis (e.g., histology, gene expression).

5.10.4. Safety and Handling

Teneligliptin is a potent pharmaceutical compound and should be handled with appropriate safety precautions. Researchers should wear personal protective equipment (PPE), including gloves, lab coats, and safety glasses, when handling the pure compound or its formulations. All procedures should be performed in a well-ventilated area or a fume hood. Consult the Safety Data Sheet (SDS) for detailed information on handling, storage, and disposal.

VI. NEED OF THE PROPOSED RESEARCH WORK

With conventional dosage forms, the drug is quickly dissolved in the gastrointestinal fluid and builds up in a high concentration and decrease exponentially until the next dose. Because of the kinetics of absorption into and of elimination out of the plasma, the plasma drug level alternates between high peaks and low troughs and the optimal therapy are scarcely attained.

Controlled release dosage forms where the drug is dispersed through polymer deliver the drug in the gastrointestinal tract with a low rate, leading to a more constant plasma level. To overcome these limitations, there is need of novel delivery system such as floating microspheres which provides an ease of administration and improved patient compliance as sustained release action of microspheres increase bioavailability of drug and dose interval.
Floating microspheres are reducing the dose of drug, improve the bioavailability.

VII. CONCLUSION

While floating microspheres offer a promising approach for enhancing the delivery of various drugs, research specifically focusing on Teneligliptin using this technology appears limited based on the provided search results. There's a lack of published studies investigating the feasibility and efficacy of floating microspheres for Teneligliptin delivery. This gap in research presents an opportunity for further investigation.

In conclusion, the formulation and evaluation of floating microspheres of Teneligliptin represent a promising strategy to enhance drug delivery and therapeutic efficacy in the management of type 2 diabetes mellitus. The integration of innovative formulation techniques and comprehensive evaluation methodologies underscores the potential of floating microspheres as a viable drug delivery system. Future research should focus on addressing challenges such as scalability, stability, and regulatory considerations to facilitate clinical translation and commercialization of these formulations

VIII. ACKNOWLEDGMENT

I would like to express my sincere gratitude to all those who supported me throughout the course of this research. I am deeply indebted to my academic advisor, Dr. K Saravanan, for their invaluable guidance, constructive feedback, and unwavering encouragement. My heartfelt appreciation goes to my peers and colleagues, whose insightful discussions and suggestions have greatly enriched my work. On a personal note, I wish to acknowledge the love and patience of my family and friends. Their belief in me and constant support provided the emotional strength required to persevere. Finally, This research represents the collective efforts and inspiration of many, and I am profoundly grateful for the contributions of each individual and group.

IX. REFERENCES

1) Hinal Prajapati, Keyur Patel, Arun Kumar Gupta, Formulation and Evaluation of Floating Microspheres of Baclofen, International Journal of Pharmaceutical Sciences and Research 2021; 12(3): 1482-1494.
2) Beena Kumari1, Aparna Khansili, Manish Kumar. Development and optimization of vildagliptin loaded Floating microspheres using central composite design: In vitro and in vivo evaluation. Annals of the Romanian Society for Cell Biology 2021; 25(4): 12742-12755.
3) Anamika Saxena, Kalpesh Gaur1, Virendra Singh, Rajneesh Kant Singh. Floating Microspheres as Drug Delivery System. American Journal of Pharmacy and Pharmaceutical Sciences 2014; 1(2): 27-36.
4) M. V. Nila1, M. R. Sudhir1, T. A. Cinu1, N. A. Aley kutty, S. Jose. Floating microspheres of carvedilol as gastro retentive drug delivery System: full factorial design and in vitro evaluation. Taylor & Francis 2014; 21(2): 110-117.
5) Revathi S*1, Gopal V2, Jeyabalan G1 Formulation and Evaluation of Floating Tablets of Sitagliptin with Extract of Triphala 1 Sunrise University, Alwar, Rajasthan, India 2 College of Pharmacy, Mother Theresa Postgraduate and Research Institute of Health Sciences, Puducherry, India
6) Jameel Ahmed S Mulla1*, Vijayanand R Aralelimath2, Omkar Tipugade1, Shreyasi S Shinde1, Nisha G Tetgure1, Ayesha A Mulla1, Dinesh D Gavali1 Formulation and Evaluation of Teneligliptin-Loaded Mucoadhesive Microspheres 1 Department of Pharmaceutics, Shree Santkrupa College of Pharmacy, Ghogaon, Karad, 415111, Maharashtra, India 2 Department of Pharmacology, Shree Santkrupa College of Pharmacy, Ghogaon, Karad, 415111, Maharashtra, India
7) Anand Panchakshari Gadad* , Sneha Shripad Naik, Panchaxari Mallappa Dandagi and Uday Baburao Bolmal, Formulation and Evaluation of Gastroretentive Floating Microspheres of Lafutidine Department of Pharmaceutics, KLE University's College of Pharmacy, JNMC Campus, Belagavi-590010, Karnataka, INDIA
8) *Vinod K.R.1, Santhosh Vasa1, Anbuazaghan S2, David Banji1, Padmasri A1, Sandhya S1 Approaches for Gastrotentive Drug Delivery Systems 1nalanda College of Pharmacy, Nalgonda, A.P. India 2chilkur Balaji College of Pharmacy, Hyderabad, A.P., India.
9) Kauslya Arumugam1*, Payal D. Borawake1, Jitendra V. Shinde1 1Formulation And Evaluation Of Floating Microspheres of Ciprofloxacin by Solvent Evaporation Method Using Different Polymers Original Article Department of Pharmaceutics, Pune District Education Association's, Seth Govind Raghunath Sable College Of Pharmacy, Saswad, Pune, Maharashtra, India *Email: Kauslyaa9764@Gmail.Com Received: 23 Feb 2021, Revised And Accepted: 28 May 2021
10) Singh Bandana, Kanoujia Jovita, Pandey Manisha*, Saraf Shubhini A Formulation and Evaluation of Floating Microspheres of Famotidine. Department of Pharmaceutics Babu Banarasi Das National Institute of Technology & Management Sector I, Dr. Akhilesh Das Nagar, Faizabad Road, Lucknow, U.P. India-227105
11) Miyako Kishimoto, Department of Diabetes and Metabolic Medicine, Center Hospital, Tokyo, Japan; Diabetes and Metabolism Information Center, Diabetes Research Center, National Center for Global Health and Medicine, Tokyo, Japan Correspondence: Department of Diabetes and Metabolic Medicine, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan, Patel B, Kushwaha RS and Jain S. Formulation, development and evaluation of floating microsphere of losartan potassium using natural polymer. Journal of Drug Delivery and Therapeutics 2019; 9(3): 223-28.
12) Saroja SP and Sudheer P. Formulation and evaluation of aceclofenac mucoadhesive microspheres for oral controlled drug delivery. Asian Journal of Pharmaceutical and Clinical Research 2019; 12(9): 184-90.
13) Purohit K and Garud N. Formulation and evaluation of floating microspheres of losartan potassium using sodium alginate and HPMC by solvent evaporation method. J of Drug Delivery and Therapeutics 2019; 9(1): 60-66.
14) Patil P, Singh S and Sarvanan J. Preparation and evaluation of microspheres of flurbiprofen. International Journal of Pharmaceutical Sciences and Research 2018; 9(12): 5388-93.
15) Bairagee D, Kulkani S, Choudhary N and Gupta AK. Formulation and evaluation of floating microspheres of amoxicillin trihydrate. International Journal of Pharmacy and Pharmaceutical Research 2018; 11(4): 39-55.
16) Y. M. Jagtap,* R. K. Bhujbal, A. N. Ranade, and N. S. Ranpise. Jagtap, *et al.*: Effect of Polymers on Floating Microspheres. Indian J Pharm Sci. 2012 Nov-Dec; 74(6): 512–520. Effect of Various Polymers Concentrations on Physicochemical Properties of Floating Microspheres,
17) What is the mechanism of Teneligliptin? [synapse.patsnap.com]
18) Frontiers | Efficacy and safety of teneligliptin in patients with type 2 diabetes mellitus: a Bayesian network meta-analysis [frontiersin.org]
19) glucagon.com [glucagon.com]
20) The Assessment of Beta Cell Mass during Gestational Life in the Mouse [bio-protocol.org]
21) The novel dipeptidyl peptidase-4 inhibitor teneligliptin prevents high-fat diet-induced obesity accompanied with increased energy expenditure in mice - PubMed [pubmed.ncbi.nlm.nih.gov]
22) Teneligliptin Co-Infusion Alleviates Morphine Tolerance by Inhibition of Spinal Microglial Cell Activation in Streptozotocin-Induced Diabetic Rats - PMC [pmc.ncbi.nlm.nih.gov]
23) Automated quantification of pancreatic β-cell mass - PMC [pmc.ncbi.nlm.nih.gov]
24) researchgate.net [researchgate.net]
25) Tissue distribution of teneligliptin in rats and comparisons with data reported for other dipeptidyl peptidase-4 inhibitors - PMC [pmc.ncbi.nlm.nih.gov]
26) noblelifesci.com [noblelifesci.com]
27) mdpi.com [mdpi.com]

28) Comparison of the Effects of Teneligliptin and Sitagliptin, Two Dipeptidyl Peptidase 4 Inhibitors with Different Half-Lives, on Glucose Fluctuation and Glucagon-Like Peptide-1 in Type 2 Diabetes Mellitus. | Semantic Scholar [semanticscholar.org]
29) ijbcp.com [ijbcp.com]
30) researchgate.net [researchgate.net]
31) Staining Protocols for Human Pancreatic Islets - PMC [pmc.ncbi.nlm.nih.gov]
32) Teneligliptin alleviates diabetes-related cognitive impairment by inhibiting the endoplasmic reticulum (ER) stress and NLRP3 inflammasome in mice - PubMed [pubmed.ncbi.nlm.nih.gov]
33) Teneligliptin alleviates diabetes-related cognitive impairment by inhibiting the endoplasmic reticulum (ER) stress and NLRP3 inflammasome in mice - PMC [pmc.ncbi.nlm.nih.gov]
34) Accurate measurement of pancreatic islet β-cell mass using a second-generation fluorescent exendin-4 analog - PMC [pmc.ncbi.nlm.nih.gov]
35) Teneligliptin Exerts Antinociceptive Effects in Rat Model of Partial Sciatic Nerve Transection Induced Neuropathic Pain - PMC [pmc.ncbi.nlm.nih.gov]