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A REVIEW ON GASTRO RETENTIVE DRUG DELIVERY SYSTEM FORMULATION AND ITS EVALUATION PARAMETERS ROLE IN DRUG DELIVERY SYSTEM

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ABSTRACT

Gastro retentive drug delivery is an approach to prolong the gastric residence time which helps to target the site-specific drug release in the upper gastrointestinal tract for local or systemic effects. Gastro retentive dosage forms can remain in the gastric region for long periods and hence significantly prolongs the gastric retention time of various medications. Several gastro retentive drug delivery approaches are being designed and developed which helps to retain the drug in the bottom of the stomach, low density (floating) systems that causes buoyancy in gastric fluid. Oral route of drug administration is the most preferable route due to its flexibility in formulation, ease of administration, and more patient compliance. This type of approach has certain limitations like limited gastric residence time for sustained drug delivery system and for the drugs which are absorb from specific region of gastrointestinal tract. To overcome gastro retentive drug problems various approaches have been proposed to increase the gastric retention time of the delivery system in the upper part of gastrointestinal tract. The gastro retentive drug delivery technologies includes high density (sinking), floating, bio or muco adhesive, expandable, unfoldable, super porous hydrogel,

magnetic systems are is use to design the drug formulation to enhance its drug action in gastrointestinal tract.

KEYWORDS: Gastro retentive drug delivery, gastric fluid, stomach, gastrointestinal tract.

INTRODUCTION

Anatomy and physiology of gastrointestinal tract

The anatomy and physiology of GIT should be understood, while developing GRDDS. Factors affecting GI motility like pH, nature and volume of gastric secretion and gastric mucus plays important role while developing GRDDS. The stomach is J shaped enlargement of the GIT directly inferior to the diaphragm in the epigastric umbilical and left hypochondriac regions of the abdomen. It is situated in the left upper part of the abdominal cavity immediately under the diaphragm.[1-4] The stomach is a temporary storage area that mixes food with water and gastric juices to produce chyme, breaks food down physically and chemically, and controls release of the chyme into the small intestine via regulation of the pyloric sphincter.

Specialized cells located throughout the gastric mucosa produce varioussubstances

- Goblet cell secretes mucus that protects the gastric mucosa.
- Cardia is the oesophagus-gastric junction and lacks the sphincter.
- Fundus is the portion above the horizontal line drawn across the oesophagus-gastric junction.
- Body is the middle portion of the stomach between the fundus and the pyloricantrum.
- Pyloric antrum is the distal third of the stomach. The pyloric sphincter has adiameter of 12.8±7 mm in humans and acts as a sieve as well as a mechanical stricture to the passage of large particles.
- Pylorus is the junction of distal end of the stomach with the duodenum. The Fundus and body region store the undigested food and act as reservoir then starts the digestion and release the resulting chyme slowly through the pylorus into theduodenum. Antrum is major site for mixing, motion and acting as pump for gastric emptying.[5-8]
- Its size varies according to the amount of distention: up to 1500 ml following a meal; after food has emptied, a ‘collapsed’ state is obtained with a resting volumeof only 25-50 ml.
- Fasting gastric pH is usually steady and approximates 2, but there are short periods of 7±

6 min characterized by higher values. Food buffers and neutralizes gastric acid, thus increasing the pH up to about 6.5. After meal-ingestion is completed, the pH rapidly falls back below 5 and then gradually declines to fasting state values over a period of a few hours. In the elderly population approximately 20% are hypochlorhydric i.e. with reduced but not absent gastric secretion, whereas the remainder has acid production similar to young people.[9]

Potential candidates for gastroretentive drug delivery system

- Drugs that are primarily absorbed in thestomach e.g. Amoxicillin.
- Drugs that are poorly soluble in alkaline pH e.g. Furosemide, Diazepam.
- Drugs that have narrow absorption window e.g. Levodopa, Methotrexate.
- Drugs that degrade in the colon e.g. Ranitidine, Metformin HCL.
- Drugs that disturb normal colonic microbes e.g. Antibiotics against Helicobacter pylori.
- Drugs rapidly absorbed from the GI tract e.g. Tetracycline.
- Drugs acting locally in the stomach

Gastric motility and emptying

The motility of the stomach is mostly contractile, which causes food grinding into smaller particles, mixing with gastric juices, forward and backward movements of gastric contents and emptying, with all of the actions occurring together. There is a marked difference between motility in the fasting state and the fed state: the motoric activity in the fasting state, termed Inter digestive Myoelectric Motor Complex (IMMC) is a 2 hrs cycle of peristaltic activity that is generated in the stomach and progresses to the ileocecal junction. Its aim is to clear the stomach and the small intestine of indigested debris, swallowed saliva and sloughed epithelial cells.[10-13]

IMMC is composed of four phases

Post phase 1 lasts 45-60 min, is quiescent, with rare low amplitude contractions;

Phase 2 with a length of 30-45 min, has intermediate amplitude contractions, and involves bile secretion.

Phase 3 is also termed ‘housekeeper wave’ and extends for 5-15 min. It is initiated in the stomach in most cases (71%), or in the duodenum. Very high amplitude contractions, with a frequency of 4-5 per min, and maximal pyloric opening, characterize this phase. This enables efficient evacuation of the stomach contents.[14]

Phase 4 has a length of less than 5 min and connects between the maximal amplitude contractions to the basal phase**.** The motor activity in the fed state is induced 5-10 min after ingestion of a meal and persists as long as food remains in the stomach.[15]

Factors affecting gastric emptying

The gastric retention time (GRT) of dosage forms is controlled by several factors such as density and size of the dosage form, food intake, nature of the food, posture, age, sex, sleep and disease state of the individual (e.g. gastrointestinal diseases and diabetes) and administration of drugs such as prokinetic agents.

1. Density-gastric retention is a function of dosage form buoyancy which in turn depends on density.
2. Size-dosage form units with diameter more than 7.5mm are reported to have increased gastric retention as compared to those having 9.9mm diameter.
3. Single or Multiple unit formulation: Multiple unit formulation show a more predictable release profile and insignificant impairing of performance due to failure of units, allow co-administration of units with different release profiles or containing incompatible substances.
4. Fed or unfed state-as these states determine gastric motility it is important from gastric retention point of view.
5. Nature of meal -feeding of indigestible polymers or fatty acids can change the motility pattern of stomach to a fed state, thus decreasing the gastric emptying rate and prolonging drug release
6. If caloric content of the food is high gastric retention is increased.
7. Gender-Mean ambulatory GRT in males is less compared to their age and racematched female counter parts regardless of weight, height and body surface.
8. Posture-GRT can vary between supine and upright ambulatory states of patient.
9. Concomitant administration of drugs that affects GI motility e.g. anti cholinergic affects efficiency of GRDDS.

Classification of gastro retentive drug delivery system:

A. High-density systems.
B. Floating systems

1. Hydrodynamically balanced systems: HBS
2. Gas-generating systems

3. Raft-forming systems
4. Low-density systems

C. Expandable systems.
D. Superporous hydrogel.
E. Mucoadhesive or Bioadhesive system
F. Magnetic systems.
G. Module assemblage technology.

High-density systems

Gastric contents have a density close to water (~1.004 gcm^{-3}). 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.[16-19] A density close to 2.5g cm^{-3} seems necessary for significant prolongation of gastric residence time and barium sulphate, zinc oxide, iron powder, titanium dioxide are used as excipients.

Floating systems

These have a bulk density lower than the gastric content. They remain buoyant in the stomach for a prolonged period of time, with the potential for continuous release of drug. Eventually, the residual system is emptied from the stomach. Gastric emptyingis much more rapid in the fasting state and floating systems rely heavily on the presence of food to retard emptying and provide sufficient liquid for effective buoyancy.[20-22]

Hydrodynamically balanced systems: (HBS)

These are single-unit dosage forms, containing one or more gel-forming hydrophilic polymers. HPMC is the most common used excipient, although hydroxyl ethyl cellulose (HEC), hydroxyl propyl cellulose (HPC), sodium carboxy methyl cellulose (NaCMC), agar, carrageenans or alginic acid are also used. The polymer is mixed with drug and usually administered in a gelatin capsule. The capsule rapidly dissolves in the gastric fluid, and hydration and swelling of the surface polymers produces a floating mass. Drug release is controlled by the formation of a hydrated boundary at the surface. Continuous erosion of the surface allows water penetration to the inner layers, maintaining surface hydration and buoyancy. Incorporation of fatty excipients gives low-density formulations and reduced penetration of water, reducing the erosion.

Gas-generating systems

Floatability can also be achieved by generation of gas bubbles. CO_2 can be generated *in-situ* by incorporation of carbonates or bicarbonates, which react with acid either the natural gastric acid or co-formulated as citric or tartaric acid. An alternative is to incorporate a matrix with entrapped of liquid, which forms a gas at body temperature, the approach has been used for single and multiple unit systems.[23-29] In single unit systems, such as capsules.[21] or tablets,[22] effervescent substances are incorporated in the hydrophilic polymer, and CO_2 bubbles are trapped in the swollen matrix. bilayer or multilayer systems have also been designed.[23] Drug and excipients can be formulated independently and the gas generating unit can be incorporated into any of the layers.[30-34] The main difficulty of such formulation is to find a good compromise between elasticity, plasticity and permeability of the polymer. Multiple unit system comprised an inner effervescent layer (bicarbonate and tartaric acid) and an outer swellable membrane (polyvinyl acetate and shellac).

Raft-forming systems

Here, a gel-forming solution (e.g. sodium alginate solution containing carbonates or bicarbonates) swells and forms a viscous cohesive gel containing entrapped CO_2 bubbles, on contact with gastric fluid.[35-37] Formulations also typically contain antacids such as aluminium hydroxide or calcium carbonate to reduce gastric acidity. Because raft-forming systems produce a layer on the top of gastric fluids, they are often used for gastroesophageal reflux treatment.

Low-density systems

It involves use of low density materials, entrapping oil or air. Involves multiple unit system such as microspheres, microballons and micro sponges etc.

Expandable systems

A dosage form in the stomach will withstand gastric transit if it is bigger than the pyloric sphincter. 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 polymers. The concept is to make a carrier, such as a capsule, incorporating a compressed system made of bioerodible polymer which extends in the

stomach. Another approach involves use of swellable systems which are retained because of their mechanical properties. The swelling is usually results from osmotic absorption of water.[38-40] The dosage form is small enough to be swallowed, and swells in gastric liquids. The bulk enables gastric retention and maintains the stomach in a ‘‘fed’’ state, suppressing housekeeper waves.

Super porous hydro gels

Though these are swellable systems, they differ sufficiently from the conventional types to warrant separate classification. With pore size ranging between 10 nm and 10 Am, absorption of water by conventional hydrogel is a very slow process and several hours may be needed to reach an equilibrium state during which premature evacuation of the dosage form may occur. Superporous hydrogel, average pore size >100 Am, swell to equilibrium size within a minute, due to rapid water uptake by capillary wetting through numerous interconnected open pores. Moreover, they swell to a large size (swelling ratio 100 or more) and are intended to have sufficient mechanical strength to withstand pressure by gastric contraction.This is achieved by co- formulation of a hydrophilic particulate material, Ac-Di-Sol (croscarmellose sod).[28,29]

Mucoadhesive or bio adhesive systems

The basis of mucoadhesion is that a dosage form can stick to the mucosal surface by different mechanisms. Different theories are involved to explain these mechanisms. Firstly, the electronic theory proposes attractive electrostatic forces between the glycoprotein mucin network and the bioadhesive material. Secondly, the adsorption theory suggests that bioadhesion is due to secondary forces such as Van der Waals forces and hydrogen bonding. The wetting theory is based on the ability of bioadhesive polymers to spread and develop intimate contact with the mucus layers, and finally, the diffusion theory proposes physical entanglement of mucin strands and the flexible polymer chains, or an interpenetration of mucin strands into the porous structure of the polymer substrate. Materials commonly used for bioadhesion are poly (acrylic acid) (carbopol, polycarbophil), chitosan, gantrez\ (Polymethyl vinyl ether/maleic anhydride copolymers), cholestyramine, tragacanth, sodium alginate, HPMC, sephadex, sucralfate, polyethylene glycol, dextran, poly (alkyl cyanoacrylate) and polylactic acid.[41-42]

Magnetic systems

This system is based on a simple idea: the dosage form contains a small internal magnet, and

a magnet placed on the abdomen over the position of the stomach assists in gastric retention.

Module assemblage technology

This technology is based on modules or release units, such as swellable matrices possessing their own delivery program. Such matrices, manufactured by compression, were shaped as a disc with curved bases, i.e., one concave and the other convex. Dueto the cupola-like shape of the convex base, the technology was named dome Matrix®. The dome shape made straightforward the assembling of two or more modules by stacking, so obtaining multimodal's' systems. In the basic assembly, the convex base of one module was stuck into the concave base of a second one in such a way that a firm pile of modules was obtained.[43-44] Later, it was discovered that sticking two modules concave base against concave base produced a system with an internal void space. This assembly, named void configuration, was characterized by an immediate floatation when plunged in water.[32]

Pharmacokinetic and pharmacodynamic aspects of gastro retentive dosageforms

Pharmacokinetic aspects

Absorption window—validation that the drug is within the category of Narrow Absorption Window (NAW) agents

Appropriate candidates for CR-GRDF are molecules that have poor colonic absorption but are characterized by better absorption properties at the upper parts of the GI tract. In the case of absorption by active transporters that are capacity limited, the efficacy of the transport activity may increase following sustained presentation of the drug to the transporting enzymes in comparison to non-CR mode of administration.

Enhanced bioavailability

Once it has been ascertained that the compound in question is defined as NAW, the possibility of improving bioavailability by continuous administration of the compound to the specific site should be tested.[45-46] Several different processes, related to absorption and transit of the drug in the gastrointestinal tract, act concomitantly and influence the magnitude of drug absorption.

Enhanced first pass biotransformation

In a similar fashion to increased efficacy of active transporters exhibiting capacity limited activity, the pre-systemic metabolism of the tested compound may be considerably increased

when the drug is presented to the metabolic enzymes (cytochrome P450, in particular CYP3A4) in a sustained manner, rather than by a bolus input.

Improved bioavailability due to reduced P-glycoprotein (P-gp) activity in the duodenum

In apparent contrast to the higher density of CYP3A4 at the upper part of the intestine, P-gp mRNA levels increase longitudinally along the intestine such that the highest levels are located in the colon. Therefore, for drugs that are P-gp substrate and do not undergo oxidative metabolism, such as digoxin, CR-GRDF may elevate absorption compared to the immediate and CR dosage forms.

Reduced frequency of dosing

For drugs with relatively short biological half-life, sustained and slow input from CR- GRDF may result in a flip-flop pharmacokinetics and enable reduced dosing frequency. This feature is associated with improved patient compliance, and thereby improves therapy.

Targeted therapy for local ailments in the upper GI tract

The prolonged and sustained administration of the drug from the GRDF to the stomach may be advantageous for local therapy in the stomach and the small intestine. By this mode of administration, therapeutic drug concentrations may be attained locally while the systemic concentrations, following drug absorption and distribution, are minimal.

Pharmacodynamic aspects[34]

Reduced fluctuations of drug concentration

Continuous input of the drug following CR-GRDF administration produces blood drug concentrations within a narrower range compared to the immediate release dosage forms. Thus, fluctuations in drug effects are minimized and concentration dependent adverse effects that are associated with peak concentrations can be prevented. This feature is of special importance for drugs with a narrow therapeutic index.

Improved selectivity in receptor activation

Minimization of fluctuations in drug concentration also makes it possible to obtain certain selectivity in the elicited pharmacological effect of drugs that activate different types of receptors at different concentrations.

Reduced counter-activity of the body

In many cases, the pharmacological response which intervenes with the natural

physiologic processes provokes a rebound activity of the body that minimizes drug activity. Slow input of the drug into the body was shown to minimize the counter activity leading to higher drug efficiency.

Minimized adverse activity at the colon

Retention of the drug in the GRDF at the stomach minimizes the amount of drug that reaches the colon. Thus, undesirable activities of the drug in colon may be prevented. This pharmacodynamic aspect provides the rationale for GRDF formulation for beta- lactam antibiotics that are absorbed only from the small intestine, and whose presencein the colon leads to development of microorganism's resistance. In most cases, due to complexity of pharmacokinetic and pharmacodynamic parameters, *in-vivo* studies are required to establish the optimal dosage form for a specific drug. For a certain drug, interplay of its pharmacokinetic and pharmacodynamic parameters will determine the effectiveness and benefits of the CR-GRDF compared to the other dosage forms.

Applications of GRDDS

Floating drug delivery offers several applications for drugs having poor bioavailability because of the narrow absorption window in the upper part of the gastrointestinal tract. It retains the dosage form at the site of absorption and thus enhances the bioavailability. These are summarized as follows.

Sustained drug delivery

HBS systems can remain in the stomach for long periods and hence can release the drug over a prolonged period of time. The problem of short gastric residence time encountered with an oral CR formulation hence can be overcome with these systems. These systems have a bulk density of G1 as a result of which they can float on the gastric contents. These systems are relatively large in size and passing from the pyloric opening is prohibited.[47]

Site-specific drug delivery

These systems are particularly advantageous for drugs that are specifically absorbed from stomach or the proximal part of the small intestine, e.g., riboflavin andfurosemide.

Absorption enhancement

Drugs that have poor bioavailability because of site specific absorption from the upper part of the gastrointestinal tract are potential candidates to be formulated as floating drug delivery

systems, thereby maximizing their absorption.

Localized action

FDDS also serves as an excellent drug delivery system for the eradication of *H.pylori*, which causes chronic gastritis and peptic ulcers. The treatment requires high drug concentrations to be maintained at the site of infection that is within the gastric mucosa. By virtue of its floating ability these dosage forms can be retained in the gastric region for a prolonged period so that the drug can be targeted.

Advantages of GRDDS

- Gastro retentive drug delivery formulations provide various advantages such as
- The GRDF's are advantageous for drugs absorbed through the stomach e.g. ferrous salts and for drugs meant for local action in the stomach and treatmentof peptic ulcer disease e.g. antacids.
- The efficacy of the medicaments administered utilizing the sustained release
- principle of HBS has been found to be independent of the site of absorption of the particular medicaments.
- Administration of a prolonged release floating dosage form tablet or capsule will result in dissolution of the drug in gastric fluid. After emptying of the stomach contents, the dissolved drug available for absorption in the smallintestine. It is therefore expected that a drug will be fully absorbed from the floating dosage form if it remains in solution form even at alkaline pH of theintestine.
- When there is vigorous intestinal movement and a short transit time as might occur in certain type of diarrhea, poor absorption is expected under suchcircumstances it may be advantageous to keep the drug in floating condition instomach to get a relatively better response.
- Gastric retention will provide advantages such as the delivery of drugs with
- Narrow absorption windows in the small intestinal region. Many drugs categorized as once-a-day delivery have been demonstrated to have suboptimal absorption due to dependence on the transit time of thedosage form, making traditional extended release development challenging. Therefore, a system designed for longer gastric retention will extend the time within which drug absorption can occur in the small intestine.

Types of drugs can benefit from using gastro retentive devices

These include

- Drugs acting locally in the stomach;
- Drugs those are primarily absorbed in the stomach;
- Drugs those are poorly soluble at an alkaline pH
- Drugs with a narrow window of absorption;
- Drugs absorbed rapidly from the GI tract; and
- Drugs those degrade in the colon.

Limitations

Gastro retentive drug delivery systems have following disadvantages

- One of the major limitations of GRDDS is that they require a sufficiently high level of fluids in the stomach for the drug delivery buoy to float therein and to work efficiently.
- Floating systems are not feasible for those drugs that have solubility or stability problems in gastric fluids.
- Not applicable to drugs which are irritant to gastric mucosa.
- For systems based on swelling patient factors like achlorhydria, improper swelling may occur resulting in inadequate absorption.
- Furthermore drugs that are absorbed equally well throughout the GI track willnot benefit from GRDDS.
- Drugs which undergo first pass metabolism may not be desirable candidate for GRDDS since slow gastric emptying may lead to reduced system bioavailability.

In-Situ gel forming polymeric drug delivery systems

In the past few years, increasing number of In-situ gel forming systems have been investigated and many patents for their use in various biomedical applications including drug delivery have been reported. Smart polymeric systems represent promising means of delivering the drugs; these polymers undergo sol-gel transition, once administered. In-situ gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. Various natural and synthetic polymers are used for formulation development of In-situ forming drug delivery systems[48]. Depending on the route of administration, these In-situ polymeric systems may be classified as illustrated in following sections.

Advantages of *in-situ* gel forming polymeric systems[38]

1. Reduced frequency of administration.
2. Improved patient compliance & comfort.
3. Ease of administration.
4. Easy drug loading.
5. Dose adjustment.
6. Simple manufacturing processes.

***In-situ* gel forming polymeric drug delivery system can be classified are asfallows[39]**

- In-situ gel forming polymeric system for oral administration.
- In-situ gel forming polymeric system for ocular delivery
- In situ gel forming polymeric system for rectal and vaginal delivery
- In-situ gel forming injectable drug delivery system
- In-situ gel forming nasal drug delivery system.

In-situ gel forming polymeric systems for oral administration

For pediatric patients and the elderly who have difficulty swallowing solid dosage forms, suppositories, syrups or chewable tablets may be more suitable dosage forms. Several oral formulations have been designed to achieve a more sustained delivery of this drug. Pectin, xyloglucan and gellan gum, sodium alginate, are the polymers used for *In-situ* forming oral drug delivery systems. The formulation adopted was a gellan solution containing calcium chloride (as a source of Ca2+ ions), and sodium citrate, which complexes the free Ca^{2+} ions and releases them only in the highly acidic environment of the stomach. In this way, the formulation remains in liquid form until it reaches the stomach, when gelation is instantaneous.

In-situ gel forming polymeric systems for ocular delivery

In-situ gels based ocular delivery; most commonly uses natural polymers such as gellan gum, alginic acid and xyloglucan. Local ophthalmic drug delivery has been used for various compounds such as antimicrobial agents, anti-inflammatory agentsand autonomic drugs used to relieve intraocular tension in glaucoma. Conventional delivery systems often result in poor bioavailability and therapeutic responses because high tear fluids turn over and dynamics cause rapid elimination of the drug from the eyes. So, to overcome bioavailability problems, ophthalmic *In-situ* gels were developed. Aqueous solution of gellan dropped into

the eye undergoes transition into the gel state due to the temperature and ionic condition (Ca^{2+}) in the tear fluid. Drug release from these *In-situ* gels is prolonged due to longer precorneal contact times of the viscous gels compared with conventional eye drops.In addition to oral, alginic acid, also used in ocular delivery, alginic acid canbe chosen as a vehicle for ophthalmic formulations, since it exhibits favorable biological properties such as biodegradability and nontoxicity. A prolonged precorneal residence of formulations containing alginic acid was looked for its abilityto gel in the eye as well as because of its mucoadhesive properties.

Various water soluble polymer such as carbopol system- hydroxyl propyl methyl cellulose (HPMC) system, poly (methacrylic acid) and poly (ethylene glycol) come under the category of pH-induced *In-situ* precipitating polymeric systems. Carbopol is a well known pH dependent polymer, which stays in solution form at acidic pH but forms a low viscosity gel at alkaline pH. HPMC is used in combination with carbopol to impart the viscosity to carbopol solution, while reducing the acidityof the solution.

In-situ gel forming polymeric systems for rectal and vaginal delivery

In-situ gels also possess a potential application for drug delivery by rectal and vaginal route. The vagina has a rich system of defences and dynamic microbiology. It has a rich vascular plexus that makes it ideal for absorbing drugs. The opening and lower one third of the vagina are narrow and open into a larger space after passing throughthe urogenital and pelvic diaphragms, which act like sphincters. This explains why the vagina can securely retain drug delivery system investigated the use of xyloglucan based thermoreversible gels for rectal drug delivery of indomethacin[43]. Administration of indomethacin loaded xyloglucan based systems to rabbits indicated broad drug absorption peak and a longer drug residence time as compared to that resulting after the administration of commercial suppository. In addition, a significant reduction of drug C_{max} was observed after administration of *In-situ* polymeric system thus indicating the avoidance of adverse effects of indomethacin on nervous system.

In-situ gel forming injectable drug delivery systems

The development of injectable *In-situ* forming drug delivery systems has received a considerable interest over the last decade. Chitosan is a biodegradable, thermosensitive, polycationic polymer obtained by alkaline deacetylation of chitin, a natural component of

shrimp and crab shell. Chitosan is a biocompatible pH dependent cationic polymer, which remains dissolved in aqueous solutions up to a pHof 6.2. Neutralization of chitosan aqueous solution to a pH exceeding 6.2 leads to the formation of a hydrated gel like precipitate. The main problem with chitosan is its non-biodegradability[45].The pH gelling cationic polysaccharides solution are transformed into thermally sensitive pH dependent gel forming aqueous solutions, without any chemical modification or cross linking by addition of polyol salts bearinga single anionic head such as glycerol, sorbitol, fructose or glucose phosphate salts to chitosan aqueous solution.

In-situ gel forming nasal drug delivery systems

In-situ gelling approach can also be utilized for intranasal delivery of actives. Intranasal delivery of mometasone furoate was developed[47] and evaluated for its efficacy for the treatment of allergic rhinitis where gellan gum and xanthan gum were used as *In-situ* gel forming polymers. Animal studies were conducted using an allergic rhinitis model and the effect of *In-situ* gel on antigen induced nasal symptoms in sensitized rats was observed where *In-situ* gel was found to inhibit the increase in nasal symptoms as compared to marketed formulation nasonex (mometasone furoate suspension 0.05%). Intact ciliated respiratory epithelium and normal goblet cell appearance indicated from histopathology of rat nasal cavity proved that these formulations were safe for nasal administration.[49]

Approaches of *in-situ* gel drug delivery

***In-situ* gel formation based on physiological stimuli**

Thermally trigged system

A temperature-responsive polymer is a polymer which undergoes a physical change when external thermal stimuli are presented. The ability to undergo such changes under easily controlled conditions makes this class of polymers fall into the categoryof smart materials. Poloxamer is most cited example. An 18% poloxamer solution remains liquid at room temperature while it gels near ambient temperatures. Similarly, chitosan, a natural bio-degradable polymer shows thermo reversible gelation near physiological temperature, after neutralization with glycerophosphate. After numerous investigations of poly (N-isopropylacrylamide) (poly-NIPAAM), there was a sparked interest in the applications of this and many other stimuli-responsive polymers.[49] Temperature-sensitive hydrogels are probably the most commonly studied class of environment-sensitive polymer systems in drug delivery research. The use of biomaterial whose transitions from sol-gel is triggered

by increase in temperature is an attractive way to approach *in-situ* formation. The ideal critical temperature rangefor such system is ambient and physiologic temperature, such that clinical manipulation is facilated and no external source of heat other than that of body is required for trigger gelation. A useful system should be tailorable to account for small differences in local temperature, such as might be encountered in appendages at the surface of skin or in the oral cavity.

pH triggered systems

pH sensitive or pH responsive polymers are materials which will respond to the changes in the pH of the surrounding medium by varying their dimensions. Such materials swell or collapse depending on the pH of their environment. This behavioris exhibited due to the presence of certain functional groups in the polymer chain. There are two kinds of pH sensitive materials: one which have acidic group (-COOH,- SO_3H) and swell in basic pH and others which have basic groups (-NH_2) and swell in acidic pH. Polyacrylic acid is an example of the former and chitosan is an example of the latter. The response is triggered due to the presence of ionizable functional groups (like -COOH, -NH_2) which get ionized and acquire a charge (+/-) in a certain pH. The polymer chains now have many similarly charged groups which cause repulsion and hence the material expands in dimensions. The opposite happens when pH changes and the functional groups lose their charge hence the repulsion is gone and the material collapses back. These materials are being extensively used in controlled drugdelivery systems and biomimetics.

***In-situ* gel formation based on chemical reactions**

Chemical reactions that results *In-situ* gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes and photo-initiatedprocesses.

Ionic cross-linking

Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones. While k-carrageenan forms rigid, brittle gels in reply of small amount of K^+, i-carrageenan forms elastic gels mainly in the presence of Ca^{2+}. Gellan gum commercially available as Gelrite® is an anionic polysaccharide that undergoes *In-situ* gelling in the presence of mono- and divalent cations, including Ca^{2+}, Mg^{2+}, K^+ and Na^+. Gelation of the low-methoxy Pectin can be caused by divalent cations, especially Ca^{2+}. Likewise, alginic acid undergoes gelation in

presence of divalent/polyvalent cations e.g. Ca^{2+} due to the interaction with glucoronic acid block in alginate chains.

Enzymatic cross-linking

In-situ formation catalyzed by natural enzymes has not been investigated widely but seems to have some advantages over chemical and photochemical approaches. For example, an enzymatic process operates efficiently under physiologic conditions without need for potentially harmful chemicals such as monomers and initiators. Cationic pH-sensitive polymers containing immobilized insulin and glucose oxidase can swell in response to blood glucose level releasing the entrapped insulin in a pulsatile fashion.

Photo-polymerization

Photo-polymerization is commonly used for *in-situ* formation of biomaterials. A solution of monomers or reactive macromer and initiator can be injected into a tissues site and the application of electromagnetic radiation used to form gel. Acrylate or similar polymerizable functional groups are typically used as the polymerizable groups on the individual monomers and micromeres because they rapidly undergo photo-polymerization in the presence of suitable photo initiator. Typically long wavelength ultraviolet and visible wavelengths are used. Short wavelength ultravioletis not used often because it has limited penetration of tissue and biologically harmful. A ketone, such as 2, 2 dimethoxy-2-phenyl acetophenone, is often used as the initiator for ultraviolet photo-polymerization, where as camphorquinone and ethyl eosin initiators are often used in visible light systems. These systems can be designed readily to be degraded by chemical or enzymatic processes or can be designed for long term persistence *in-vivo* Photopolymerizable systems when introduced to thedesired site via injection get photo cured. *In-situ* with the help of fiber optic cables and then release the drug for prolonged period of time.

Temperature triggered system

Temperature-sensitive hydrogels are probably the most commonly studied class of environment-sensitive polymer systems in drug delivery research. The use of biomaterial whose transitions from sol-gel is triggered by increase in temperature is anattractive way to approach in-situ formation. The ideal critical temperature range for such system is ambient and physiologic temperature, such that clinical manipulation is faccilated and no external source of heat other than that of body is required for trigger gelation. A useful system should be tailorable to account for small differences in local temperature, such as might be

encountered in appendages at the surface of skin or in the oral cavity. Three main strategies are exists in engineering of thermoresponsive sol-gel polymeric system. For convenience, temperature-sensitive hydrogels are classified into negatively thermosensitive, positively thermosensitive, and thermally reversible gels. Negative temperature-sensitive hydrogels have a lower critical solutiontemperature (LCST) and contract upon heating above the LCST.

Drugs for gastroretentive *in situ* gel drug delivery for eradication of *H.pylori*

Helicobacter pylori *(H.pylori)* are reported to be an important etiologic factor in the development of the gastritis, gastric ulcer and carcinoma in human stomach. *H.pylori* resides mainly in the gastric mucosa layer and epithelial cells of the antral region of the stomach. There are two major reasons for the failure of *H.pylori* eradication with conventional dosage forms of antimicrobials. One reason may be the degradation of antimicrobial agents by gastric acid, therefore, the administration of high doses of antimicrobial agents on a daily basis is necessary for *H.pylori* eradication, but they are usually accompanied by adverse effects and poor patient compliance. Another reason for incomplete eradication is the probably that residence time of antimicrobial agents in the stomach is so short, that effective antimicrobial concentrations cannot be achieved in the gastric mucosa layer or epithelial cell surfaces where *H.pylori.*

Drug and Excipient Profile

Metronidazole

Metronidazole is an oral synthetic antiprotozoal and antibacterial agent, 1- (ß-hydroxyethyl) - 2 -methyl - 5 - nitroimidazole, which has the following structural formula:

Molecular formula: $C_6H_9N_3O_3$

Structural formula

Chemical name: 2-(2-Methyl-5-Nitroimidazole-1-yl)Ethanol

Melting point: 159-161^{0}c

Molecular weight: 171.15 gm/mol

Odour: Odorless powder

Solubility: a). Acetic acid 0.1M clear faintly yellowb). Water solubility <0.1g/ml at 20^{0}c

Storage: Protected from sunlight.

Storage temp: 2-8^{0}c

Pharmacodynamic properties

Pharmacotherapeutic group: Nitroimidazole derivatives

Mechanism of action

Metronidazole has antiprotozoan and antibacterial effects. It is effects against Trichomonas vaginalis, Gardnerella vaginalis and other protazoa including Entamoeba histolytica, Gardia lamblia and anaerobic bacteria.

Pharmacokinetic properties

Absorption

Metronidazole is readily absorbed following administration by mouth and bioavailability is 90-100%. Peak plasma concentrations of approximately 5µg/ml and 10µg/ml are achieved an average of 1-2 hours after single doses of 250mg and 500mg respectively. Some accumulation and consequently higher concentrations occur when multiple doses are given. Absorption may be delayed, but is not reduced overall, by administration with food.

Distribution

Metronidazole is widely distributed. It appears in most body tissues and fluids. It also crosses the placenta and rapidly enters foetal circulation. No more than 20% is bound to plasma proteins.

Biotransformation

Metronidazole is metabolised in the liver by side-chain oxidation and glucuronide formation. The plasma elimination half-life of metronidazole is about 6-9 hours; that of the hydroxy metabolite is slightly longer. The half-life of metronidazole is reported to be longer in neonates and in patients with severe liver disease.

Elimination

The majority of a dose of metronidazole is excreted in the urine, mainly as metabolites; a small amount appears in the faeces.

Mechanism of action

The antimicrobial activity of Metronidazole is due to the reduction of the nitro group to a more reactive amine that attacks microbial DNA, brings about loss of helical structure of DNA and subsequent DNA breakage, thus inhibiting further synthesis and causing degradation of existing DNA.

Indications

Metronidazole is used in the treatment of hepatic and intestinal amoebiasis, giardiasis, trichomoniasis of urogenital tract and bacterial vaginosis. Also used in the treatment and prophylaxis of susceptible anaerobic infections in dental and gastrointestinal surgery and in other mixed aerobic-anaerobic infections. Metronidazole is also advocated in the management of *H.pylori* duodenal ulcer in combination with other drugs.

Contraindications

Metronidazole is contraindicated in patients hypersensitive to the drug. There is no evidence of accumulation when used in pregnant women. Therefore dosage regimen requires no adjustment during pregnancy.

Adverse reactions

The most frequently encountered side effect is dizziness, alone or in combination with other adverse reactions. The other side effects occurring to a lesser extent are nausea, pyrosis, intestinal spasms and metallic taste, vertigo, fatigue and other discomforts such as loose stools, insomnia, skin rash and headache have also been reported.

Dose: *H.*pylori infection for single dose 500 mg.

Polymers

Sodium Alginate

Synonym: Manugel, sodium polymannuronate

Chemical name: Sodium alginate Alginic acid

Molecular formula: $NaC_6H_7O_6$

Category: Suspending agent, tablet binder, viscosity enhancing agent

Description: Sodium alginate occurs as an odorless and tasteless, white to pale yellowish brown colored powder.

Solubility

Practically insoluble in ethanol, ether. Also practically insoluble in other organic solvents and acids in which the pH of the resultant solution is less than 3.

Viscosity: A 1 % w/v aqueous solution will have a viscosity of 20-400 cps. Viscosity varies

depending on concentration, pH, temperature or presence of metal ions. Above pH 10, viscosity reduces.

Stability and storage: Sodium alginate is a hygroscopic material. It is stable if stored at relatively low humidity and cool temperature. Aqueous solution of sodium alginate is most stable between pH 4-10. Below pH 3, alginic acid is precipitated. Sodium alginate is susceptible on storage to microbial spoilage, which may affect solution viscosity.

Safety: It is non-toxic and non-irritant material.

Applications

1. It can be used in variety of oral and topical pharmaceutical formulations.
2. In preparation of sustained release oral formulations which delays the drugreleases from tablets, capsules and aqueous suspensions.
3. As a thickening and suspending agent in a variety of pastes, creams andgels.
4. Recently in preparation of aqueous microencapsulation and nanoparticles.
5. Novel delivery system as ophthalmic solutions that form a gel in situ when administered to the eye.

Methylcellulose

It is a chemical compound derived from cellulose. It is a hydrophilic white powder in pure form and dissolves in cold (butnot in hot) water, forming a clear viscous solution or gel. It is sold under a variety of trade names and is used as a thickener and emulsifier in various food and cosmetic products, and also as a treatment of constipation. Like cellulose, it is not digestible, nottoxic, and not an allergen.

Methyl cellulose does not occur naturally and is synthetically produced by heating cellulose with caustic solution (e.g. a solution of sodium hydroxide) and treating it with methyl chloride. In the substitution reaction that follows, the hydroxyl residues (- OH functional groups) are replaced by methoxide ($-OCH_3$ groups). Different kinds of methyl cellulose can be prepared depending on the number of hydroxyl groups substituted. Cellulose is a polymer consisting of numerous linked glucose molecules, each of which exposes three hydroxyl groups. The *Degree of Substitution* (DS) of a given form of methyl cellulose is defined as the average number of substituted hydroxyl groups per glucose. The theoretical

maximum is thus a DS of 3.0, however more typical values are 1.3–2.6. Different methyl cellulose preparations can also differ in the average length of their polymer backbones.

Solubility and temperature

Methyl cellulose has a lower critical solution temperature (LCST) between 40 °C and 50 °C. At temperatures below the LCST, it is readily soluble in water; above the LCST, it is not soluble, which has a paradoxical effect that heating a saturated solution of methyl cellulose will turn it solid, because methyl cellulose will precipitate out. The temperature at which this occurs depends on DS-value, with higher DS-values giving lower solubility and lower precipitation temperatures because the polar hydroxyl groups are masked.

Preparing a solution of methyl cellulose with cold water is difficult however: as the powder comes into contact with water, a gel layer forms around it, dramaticallyslowing the diffusion of water into the powder, hence the inside remains dry. A better way is to first mix the powder with hot water, so that the methyl cellulose particles are well dispersed (and so have a much higher effective surface area) in the water, and cool down this dispersion while stirring, leading to the much more rapid dissolution of those particles.

Hydroxypropylmethylcellulose

Hydroxypropyl methyl cellulose is propylene glycol ether of methyl cellulose, hydroxypropyl and methyl combine with anhydrous glucose ring by ether bond. It is white or pale white cellulose powder or particles. The characteristics of cold water dissolution and hot water insoluble are similar with methyl cellulose. Solubility in organic solvents is superior than water soluble, can be dissolved in anhydrous methanol and ethanol solution, also soluble in chlorinated hydrocarbons and ketones inorganic solvents. Soluble in water, its water solution has a surface activity, the formation of the film after drying, heated and cooled, in turn, from the reversible conversion of sol to gel.

It can be used alone in the cold drink, also can be used with other emulsifier, stabilizer. To cold drink, the maximum amount is 1%. Hydroxypropyl methyl cellulose and other water-soluble high weight compounds use mixture, become transparent, higher viscosity. The gelation temperature of low viscosity products is higher than high viscosity of products. Its solution is stable at room temperature. In recent years, It has been widely used in petroleum chemical industry, papermaking, leather, textile printing and dyeing, pharmaceutical, food, cosmetics and other industries, and as the dispersing agent, thickening agent, adhesive,

excipient, capsule, oil resistant coating and packing etc.

Sodium carboxy methyl cellulose

Carboxymethyl cellulose (**CMC**)

It is a cellulose derivative with carboxymethyl groups (-CH_2-COOH) bound to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone. It is often used as its sodium salt, sodium carboxymethyl cellulose. CMC is also used in pharmaceuticals as a thickening agent, for example as the lubricant in lubricating eye drops, and acts as a viscosity modifier and water retention agent.

Preformulation studies

Identification test

Identification test for Metronidazole was carried out by using IR spectroscopy & UV absorbance spectra. For IR studies KBr powder was dried at 60 ^{o}C for one hour. The dried KBr powder was uniformly mixed with drug and IR spectra was taken for this mixture. For UV identification of Metronidazole, the solution of concentration of 10µg/mlwas prepared in 0.1 N HCl. The solution was scanned from 200 to 400 nm and a spectrum was observed for absorption maxima.

Solubility

Slightly soluble in water, alcohol, acetic acid and in dichloromethane; very slightly soluble in ether.

Melting Point: The melting point of the drug was determined by using capillary method.

U V Method of Analysis

Construction of standard calibration curve

- Solvent employed : 0.1 N HCl
- 100 mg of drug was dissolved in 100 ml of 0.1 N HCl to give 1000 µg/ml stock solution.
- 10ml of above solution was then diluted 100 ml with 0.1N HCl to get 100µg/ml stock solution.
- Further dilutions ranging from 5-30 µg/ml were prepared and analysed by UV Spectrophotometer.
- Spectrum was obtained by scanning the solution [15 µg/ml] over Range from 200-

400nm.

- Absorption Maxima (λ_{max}) = 277 nm was derived.
- The graph of absorbance v\s concentration in µg/ml was plotted. The r^2 value of this graph was calculated to check the linearity of the absorbance against concentration.

Solubility study of the drug

Solubility is an important parameter for Preformulation studies because:

- It affects the dissolution of drug.
- Bioavailability of drug is directly affected by dissolution and absorption of drug by oral administration.
- Particle size, shape, surface area may affects the dissolution characteristics of drug henceit should be determined during Preformulation.

Methods for formulation of in-situ gel

There are various mechanism for the in gel formulation: physiologically changes (temperature and pH), Chemically stimulates (ionic cross linking), physical change in biomaterial (diffusion of solvent and swelling).

In-Situ Gel Formation Based on Chemical Stimulation

Ion sensitive polymer (sodium alginate,calcium alginate,gellan gum,pectin) undergo phase transition in present of various monovalent and divalent cation(ca+2,mg+2,N a+ ,k+) for the formation of gel. For e.g: gelation of low methoxypectin in present of,k+) for the formation of gel. For e.g: gelation of low methoxypectin in present of divalent cation(ca+2). Alginate contain molecule (sodium alginate) under go gelation in presence of di/poly valent cation e.g.ca+2 interact with guluronic acid block in alginate side chain.

Temperature triggered system

Temperature-sensitive hydrogels are probably the most commonly studied class of environment-sensitive polymer systems in drug delivery research. The use of biomaterial whose transitions from sol-gel is triggered by increase in temperature is an attractive way to approach in-situ formation. The ideal critical temperature range for such system is ambient and physiologic temperature, such that clinical manipulation is faccilated and no external source of heat other than that of body is required for trigger gelation. A useful system should be tailorable to account for small differences in local temperature, such as might be encountered in appendages at the surface of skin or in the oral cavity. Three main strategies

are exists in engineering of thermoresponsive sol-gel polymeric system.

For convenience, temperature-sensitive hydrogels are classified into negatively thermosensitive, positively thermosensitive, and thermally reversible gels. Negative temperature-sensitive hydrogels have a lower critical solution temperature (LCST) and contract upon heating above the LCST. Polymers with low critical temperature (LCST) transition between ambient and physiologic temperature is used for this purpose. One of the most extensively investigated polymers that exhibit useful LCST transition is poly (Nisopropylacrylamide) (PNIPAAm). A positive temperature sensitive hydrogel has an upper critical solution temperature (UCST), such hydrogel contracts upon cooling below the UCST. Polymer networks of poly (acrylic acid) (PAA) and polyacrylamide (PAAm) or poly (acrylamide-co-butyl methacrylate) have positive temperature dependence of swelling.

pH triggered systems

Another formation of in situ gel based on physiologic stimuli is formation of gel is induced by pH changes. All the pH-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH. The polymers with a large number of ionizable groupsare known as polyelectrolytes. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups. The most of anionic pH-sensitive polymers are based on PAA (Carbopol®, carbomer) or its derivatives. Likewise polyvinylacetal diethylaminoacetate (AEA) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition. Drug formulated in liquid solutions have several limitations,including limited bioavailability and propensity to be easily removed by tear fluid.

Analytical method for evaluation of insitu gels

Determination of Drug Content

Accurately, 10 mL of formulation from different batches was measured and transferred to 100 mL volumetric flask. To this 50-70 mL of 0.1 N HCl was added and sonicated for 30 min. Volume was adjusted to 100 mL. Complete dispersion of contents was ensured visually and the dispersion was filtered using Whatman Filter Paper. From this solution, 10 mL of sample was withdrawn and diluted to 100 mL with 0.1 N HCl. Contents of metronidazole was measured at maximum absorbance at 278 nm using UV-Visible Spectrophotometer.

pH Measurement

In-situ solution formulation pH measure by using calibrated digital pH meter at room temperature.

In-Vitro Gelling Capacity

Evalution for gelling capacity can be measure by visualization method. In that method coloured solution of different formulations were prepared. In-situ gelling formation was measured into 5ml of gelation solution (0.1 N HCL) in 15ml borosilicate glass tube at 37 ±1in situ formulation was added in such a way that tip of pipette touch to gelation solution and solution release slowly. during that time stiffness of gel and time duration to remain as such as a gel.color was added for the visualization purpose. In-situ gelling capacity was categorized in three class based on gelation time and time period at they remain as such. (+) gel after few minutes, dispersed rapidly (++) gelation immediate, remain for 12hr. (+++) gelation immediate, remain for more than 12hr.

In Floating Lag Time

In this parameter 10ml of in-situ formulation was added into the 900ml dissolution vessel containing 0.1N HCL at 37 c.the time the formulation took to emerge on medium surface (floating lag time) and the time formulation constantly floated on surface of dissolution medium (duration of floating).

In-Vitro Drug Release

The drug release was measured using USP dissolution apparatus I (basket covered with muslin cloth) at 50rpm. The speed of apparatus was maintain as slow as possible to avoid breaking of gelation formation and maintain mild agitation conditions to believe to exist in-vivo condition. 900 ml dissolution medium (0.1N HCL) at 37 ±1 c temperature. To that 5ml dissolution medium was pipette out at 1,2,4,6,8,10 and 12hour interval. And measured absorbance at particular wavelength of drug using uv-spectrophotometer.

Measurement of Water Uptake

The water uptake of selected formulation was determined by simple method. In this study 40ml of in situ gel formed in 40ml 0.1N HCL from all the formulation formed gel was separated and excess 0.1 NHCL was removed bytissue paper. Before transfer gel formulation to water initial weight was taken and then added to 10ml water after every 30 min water was decant and weight the gel formulation. The data was calculated and reported.

Fourier transform infra red spectroscopy (FT-IR): The FTIR spectra of the prepared formulations were recorded over the range of 400 - 4000 cm-1 by KBr pellet method using FT-IR spectrophotometer. The compatibility between the drug and the polymers were compared by FT-IR spectra.

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CONCLUSION

Gastroretentive dosage forms prolong the gastro retention by targeting site-specific drug release in upper part of GIT. It helps to extend the duration of drug release and improve bioavailability of drugs that have narrow therapeutic window and prolong dosing interval and increase compliance of the patient. The effective understanding of several physiological functions aids in achieving gastric retention. The micro organisms are highly sensitive to many antibiotics which eradicate high concentration of antibiotics to maintain within gastric mucosa for a prolonged time period.[50] The time when the drug is taken is an important parameter to find out gastric retention time. The drug delivery system must remain for a sufficient time in the stomach, which is not compatible with normal physiology. The gastroretentive drug delivery systems (high density, floating, expandable or unfoldable or swelling, superporous, bioadhesive, magnetic systems etc. having it merits and demerits. The major advantage of this formulation is enhanced bioavailability and controlled delivery of drug. The impact of GIT physiology on drug delivery ensure the development of an increasing number of drug delivery system to optimize drug delivery of molecules exhibiting regional variability in drug absorption mechanisms. The novel drug delivery technologies will ensure the development of gastro retentive drug delivery to optimize the delivery of molecules that exhibit absorption window, low bioavailability and more first pass metabolism. The effective selection of correct drug combinations, excipients helps to design appropriate formulations to drug release in targeted site.

REFERENCES

1. Wilson CG, Washington N. The stomach: its role in oral drug delivery. In: Rubinstein, MH, editors. Physiological Pharmaceutical: Biological barriers to drug absorption. Chichester, U.K.: Ellis Horwood, 1989; 47-70.
2. Streubel A, Siepmann J, Bodmeier R. Drug delivery to the upper small intestine window using Gastroretentive technologies. Curr Opin Pharmacol, 2006; 6: 501-8.
3. Larhed AW, Artursson P, Grasjo J, Bjork K. Diffusion of drugs in native and purified gastrointestinal mucus. J Pharm Sci, 1997; 86(6): 660-65.
4. Dubernet C. Syste`mes a` liberation gastrique prolonge`e. In: Falson-Rieg F, Faivre V, Pirot F, editors. Novelles formes me`dicamenteuses. Editions Me`dicales Internationales. Editions TEC and DOC. Cachan, 2004; 119-33.
5. Arrora S, Ali J, Khar RK, Baboota S. Floatng drug delivery systems: A review. AAPS Pharm Sci Tech, 2005; 6(3): 372-90.

6. El-Kamel AH, Sokar MS, Al Gamal SS, Naggar VF. Preparation and evaluation of ketoprofen floating oral delivery system. Int J Parm, 2001; 220: 13-21.
7. Garg S, Sharma S. Gastroretentive drug delivery systems. Business Briefing: Pharmatech, 2003; 160-66.
8. Khosla R, Feely LC, Davis SS. Gastrointestinal transit of non-disintegrating tablets in fed subjects. Int J Pharm, 1989; 53: 107-17.
9. Mojaverian P, Vlasses PH, Kellner PE, Rocci Jr ML. Effects of gender, posture and age on gastric residence time of an indigestible solid: Pharmaceutical considerations. Pharm Res, 1988; 10: 639-44.
10. Vyas SP, Khar RK. Gastroretentive systems. In: Controlled drug Delivery. Vallabh Prakashan, Delhi, India, 2006; 197-217.
11. Streubel A, Siepmann J, Bodmeier R. Floating matrix tablets based on low density foam powder: effects of formulation and processing parameters on drug release. Eur J Pharm Sci., 2003; 18: 37-45.
12. Sato Y, Kawashima Y, Takenchi H, Yamamoto H. Physicochemical properties to determine the buoyancy of hollow microspheres (microballoons) prepared by the emulsion solvent diffusion method. Eur J Pharm Biopharm, 2003; 55: 297-304.
13. Talukdar R, Fassihi R. Gastroretentive delivery systems: hollow beads. Drug Dev Ind Pharm, 2004; 30: 405-12.
14. Hilton AK, Deasy PB. In vitro and in vivo evaluation of an oral sustained release floating dosage form of amoxicillin trihydrate. Int J Pharm, 1992; 86: 79-88.
15. Seth PR, Tossounian J. The hydrodynamically balanced system, a novel drug delivery system for oral use. Drug Dev Ind Pharm, 1984; 10: 313-39.
16. Hwang SJ, Park H, Park K. Gastroretentive delivery systems. Crit Rev Ther Drug Carrier Syst, 1998; 15(3): 243-84.
17. Reddy LH, Murthy RS. Floating dosage system in drug delivery. Crit Rev Ther Drug Carrier Syst, 2002; 19(6): 553-85.
18. Huang Y, Leobandung W, Foss A, Peppas NA. Molecular aspects of muco- and bioadhesion: tethered structures and site-specific surfaces. J Control Release, 2000; 65(1-2): 63-71.
19. Klusner EA, Lavy E, Friedman M, Hoffman A. Expandable gasrtroretentive dosage forms. J Control Release, 2003; 90(2): 143-62.

20. Klusner EA, Lavy E, Stepensley D, Friedman M, Hoffman A. Novel gasrtroretentive dosage form: evaluation of gastroretentivity and its effect on riboflavin absorption in dogs. Pharm Res, 2002; 19: 1516-23.
21. Caldwell LJ, Gardner CR, Cargill RC. Drug delivery device which can be retained in the stomach for controlled period of time. US Patent 473 5804. April 5, 1988.
22. Klusner EA, Eyal S, Lavy E, Friedman M, Hoffman A. Novel levodopa gasrtroretentive dosage form: in vivo evaluation in dogs. J Control Release, 2003; 88: 117-26.
23. Dixit N. Floating drug delivery system. Journal of Current Pharmaceutical Research, 2011; 7(1): 6 - 20.
24. Streubel A, Siepmann J, Bodmeier R. Floating matrix tablets based on low density foam powder: effects of formulation and processing parameters on drug release. European Journal of Pharmaceutical Sciences, 2003; 18: 37 - 45.
25. Khan R. Gastro retentive Drug Delivery System –A Review. Int J Phar m Bio Sci, 2013; 4(2): 630 - 646.
26. Sharma S and Pawar A. Low density multiparticulate system for pulsatile release of meloxicam. Int J Pharm, 2006; 313: 150-158.
27. Sangekar, S., Evaluation of effect of food and specific gravity of the tablets on gastric retention time. Int.J.Pharm, 1985; 35: 34-53.
28. Bardonnet PL, Faivre V, Pugh WJ, Piffaretti JC and Falson F. Gastroretentive dosage forms: overview and special case of helicobactor pylori. J Control Release, 2006; 111: 1-18.
29. Whitehead L, Fell JT and Collett JH. Development of a gastroretentive dosage form. Eur J Pharm Sci., 1996; 4: 182.
30. Klusner EA, Lavy E, Friedman M, Hoffman A. Expandable gasrtroretentive dosage forms. J Control Release, 2003; 90(2): 143-62.
31. Klusner EA, Lavy E, Stepensley D, Friedman M, Hoffman A. Novel gasrtroretentive dosage form: evaluation of gastroretentivity and its effect on riboflavin absorption in dogs. Pharm Res, 2002; 19: 1516-23.
32. Abubakar O, Nur Jun S, Zhang. Recent progress in sustained: controlled oral delivery of captopril: an overview. Int J Pharm, 2000; 139- 146.
33. Chen J, Blevins WE, Park H, Park K. Gastric retention of superporous hydrogel composites. J Control Release, 2000; 64(1-3): 39 - 51.
34. Ahmad, N., Alam, M. A., Ahmad, R., Umar, S., Jalees Ahmad, F. Improvement of oral efficacy of Irinotecan through biodegradable polymeric nanoparticles through in vitro and

in vivo investigations. J. Microencapsul, 2018; 35(4): 327–343. doi: 10.1080/02652048.2018.1485755.

35. Albenberg, L. G., Wu, G. D. Diet and the intestinal microbiome: associations, functions, and implications for health and disease. Gastroenterology, 2014; 146(6): 1564–1572.
36. Al-Gousous, J., Tsume, Y., Fu, M., Salem, I. I., Langguth, P. Unpredictable Performance of pH-Dependent Coatings Accentuates the Need for Improved Predictive in Vitro Test Systems. Mol. Pharm, 2017; 14(12): 4209–4219.
37. Ali, H., Weigmann, B., Neurath, M. F., Collnot, E. M., Windbergs, M., Lehr, C. M. Budesonide loaded nanoparticles with pH-sensitive coating for improved mucosal targeting in mouse models of inflammatory bowel diseases. J. Control Release, 2014; 183: 167–177.
38. Yu, M., Yang, Y., Zhu, C., Guo, S., Gan, Y. Advances in the transepithelial transport of nanoparticles. Drug Discovery Today, 2016; 21(7): 1155–1161.
39. Yu, X., Wen, T., Cao, P., Shan, L., Li, L. Alginate-chitosan coated layered double hydroxide nanocomposites for enhanced oral vaccine delivery. J. Colloid Interface Sci., 2019; 556: 258–265.
40. Zema, L., Maroni, A., Foppoli, A., Palugan, L., Sangalli, M. E., Gazzaniga, A. Different HPMC viscosity grades as coating agents for an oral time and/or site-controlled delivery system: an investigation into the mechanisms governing drug release. J. Pharm. Sci., 2007; 96(6): 1527–1536.
41. Zhang, N., Ping, Q. N., Huang, G. H., Xu, W. F. Investigation of lectin-modified insulin liposomes as carriers for oral administration. Int. J. Pharm, 2005; 294(1-2): 247–259.
42. Zhang, N., Ping, Q., Huang, G., Xu, W., Cheng, Y., Han, X. Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin. Int. J. Pharm, 2006; 327(1-2): 153–159.
43. Zhang, Z., Huang, Y., Gao, F., Bu, H., Gu, W., Li, Y. Daidzein-phospholipid complex loaded lipid nanocarriers improved oral absorption: in vitro characteristics and in vivo behavior in rats. Nanoscale, 2011; 3(4): 1780–1787.
44. Zhang, Z., Huang, J., Jiang, S., Liu, Z., Gu, W., Yu, H., et al. Porous starch based self-assembled nano-delivery system improves the oral absorption of lipophilic drug. Int. J. Pharm, 2013; 444(1-2): 162–168.
45. Zhang, J., Tang, C., Yin, C. Galactosylated trimethyl chitosan-cysteine nanoparticles loaded with Map4k4 siRNA for targeting activated macrophages. Biomaterials, 2013; 34(14): 3667–3677.

46. Zhang, J., Zhu, X., Jin, Y., Shan, W., Huang, Y. Mechanism study of cellular uptake and tight junction opening mediated by goblet cell-specific trimethyl chitosan nanoparticles. Mol. Pharm, 2014; 11(5): 1520–1532.
47. Zhang, X., Qi, J., Lu, Y., He, W., Li, X., Wu, W. Biotinylated liposomes as potential carriers for the oral delivery of insulin. Nanomedicine, 2014; 10(1): 167–176.
48. Zhang, S., Langer, R., Traverso, G. Nanoparticulate Drug Delivery Systems Targeting Inflammation for Treatment of Inflammatory Bowel Disease. Nano Today, 2017; 16: 82–96.
49. Kuksal, A. Formulation and In Vitro, In Vivo Evaluation of Extended- release Matrix Tablet of Zidovudine. AAPS Pharm Sci Tech, 2006; 7: E1-E9.
50. Prabhu, P., Harish, N. M., Gulzar, A. M., Yadav, B. Formulation and In Vitro Evaluation of Gastric Oral Floating Tablets of Glipizide. Indian J Pharm Educ Res., 2008; 42: 174-8.