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RESEARCH ARTICLE

Formulation and Evaluation of Gastroretentive Drug Delivery System of Repaglinide

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ABSTRACT

The concept of dosage form in which medicine is embedded, regardless of its safety and pharmacological effect, is developing successfully. Various scientific technologies have been developed for designing drug delivery systems.Floating microspheres were evaluated for micromeritics properties such as particle size, tapped and true density, and compressibility index and flow properties such as angle of repose. Repaglinide (Prandin) a member of meglitinide class used orally for secretion of insulin was selected for the present investigation. Various materials utilized during preformulation, formulation, characterization, and for other experimental work are given below. Laboratory/ analytical grade chemicals were used during the research work.The goal of the research work was to summarize the principals, mechanisms, and technological approaches of FDDS in general. Introduction part represented the physiological properties and factors influencing absorption and gastric motility in the stomach.

Keywords: Gastroretentive, Repaglinide, Novel drug delivery system

INTRODUCTION

Among the various scientific achievements, use of effective medicinal agents plays a key role for healthy life. The concept of dosage form in which medicine is embedded, regardless of its safety and pharmacological effect, is developing successfully. Various scientific technologies have been developed for designing drug delivery systems. Drug administered through oral route is important and mostly used in view of excellent accessibility, painless dose administration, and patient compliance for non-invasive drug delivery (Motlekar *et al*., 2006). Oral dosage forms possess some disadvantages such as incomplete release of drug, short residence time in the stomach, and non-uniform drug absorption. Nowadays, much attention has been given on modification and transforming oral dosage form to survive in various conditions of gastrointestinal tract (GIT) to overcome these limitations (Hirtz, 1985).

For drug development, selection and formulation of ideal dosage form are an important step, though the relationship between administration of drug and its pharmacological effect is complex parameter. Several intrinsic and extrinsic variables influence drug response within individuals such as product bioavailability (drug absorption rate), pharmacokinetics, and the particular concentration-effect relationship (Martinez *et al*., 2002; Javadzadeh *et al*., 2010).

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Novel drug delivery system (NDDS)

It is evident that the effect of drug substance not only depends on its pharmacological effect but also on the efficacy with which it has delivered at the site of action. Thus, interest on the latter helps in development of various NDDS aiming at increasing performance of drug for maximum activity (Chein, 1992).

Conventional immediate release dosage forms when administered, maintains concentration level of drug in therapeutic range.

Modified release dosage forms can be broadly classified under four categories:

a) Delayed release
b) Site specific release
c) Receptor specific release
d) Sustained release
    1. Controlled release (CR)
    2. Prolonged release.

Sustained release drug delivery system

Sustained release dosage forms are safe and more effective than other dosage forms due to reduced frequency of dosing, prolonged effect, and reduction of side effects. Major advantage of sustained release dosage form is reduced fluctuation in plasma drug concentration and less often administration of medication. Due to which patient compliance is maintained and blood chemistry does not undergo frequent chemical imbalance due to foreign material (Klausner *et al*., 2003; Tang *et al*., 2007). Some bioavailability problems may arise due to variations in gastric emptying process thereby variation during *in vivo* performance may occur (Locatelli *et al*., 2010).

Oral CR drug delivery system (CRDDS)

Desired bioavailability of medicament depends on some factors such as reaching the drug to effective plasma level by consuming less time, maintaining effective concentration of drug plasma level for longer period of time, and avoiding overshoot of drugs which can be rapidly absorbed. The intensity of pharmacological effect of drug is affected by its concentration at site of action, which is correlated with plasma drug concentration. An ideal condition exists when therapeutics index is attained, that

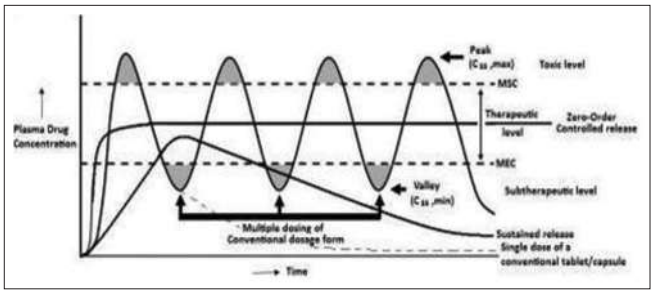


Figure 1.1: Plasma concentration-time profile (Bhramankar *et al*., 2003)

is, drug concentration is maintained between minimum effective and maximum safe level. Invariably, conventional dosage forms lacks in maintaining the therapeutics index to its level best. For maintaining the therapeutic index for longer period of time, repeated drug administration at fixed dosing interval is desired, which causes certain problems as patient noncompliance and large peaks and troughs in drug concentration time curve [Figure 1.1]. CRDDS release the drug in planned, predictable sustained manner and maintain drug concentration at effective level by spatial placement or temporal delivery. Thus, CRDDS have number of advantages such as better patient compliance, minimum fluctuation in plasma level, reduces total intake of drug, reduces drug accumulation, and minimize local and systemic side effects (Bhramankar *et al*., 2003; Sood *et al*., 2003).

The problems associated with delivering a drug by oral route can be overcome by the development of programmable CR dosage forms. Research activities in the development of CRDDS show promising and encouraging results (Yeole *et al*., 2005).

Objective and advantages of CRDDS

The objectives are as follows:

- To reduce dosing frequency and provide constant therapeutic drug level.
- To maintain uniform pharmacological response.
- To reduce total quantity of drug used.
- Increasing patient compliance by avoiding night-time dosing.
- Decreasing local and systemic side effects.
- Reduce drug accumulation and fluctuation in plasma drug concentration.
- Utilize drug having low therapeutic index.

- Increase in bioavailability of some drugs.
- Economical to patients.

Disadvantages of CR drug delivery

- Possibility of dose dumping.
- Potential for accurate dose adjustment and systemic availability reduced.
- Increase first-pass metabolism.
- During serious poisoning or intolerance immediate stoppage of pharmacological action is difficult.
- Depend on the residence time of dosage form in GIT.

Drug substances that are particularly absorbed easily from GIT and possess less life span are removed frequently from blood circulation. Such problem can be solved by developing CR dosage form that will release the medicament slowly into GIT and maintains a fixed concentration of drug for prolonged time (Muthusamy*et al*., 2005). The objective of CRDD is to increase bioavailability and achieving more predictable drug. CRDDS administered orally has some shortcomings such as less gastric retention (GRT) and unpredictable gastric emptying time (Vyas *et al*., 2002). Emptying of formulation from gastric region is an undefined process and ability to increase and control the emptying time is precious asset for dosage form so that it remains in the stomach for prolonged time period than conventional dosage form (Arora *et al*., 2005).

GIT anatomy and dynamics

The position of stomach is situated just below the diaphragm in the upper left portion of abdomen. It acts as most important part which serves primarily as area for mixing and storing, it also secretes various contents such as gastric lipase, pepsinogen, hydrochloric acid, and intrinsic factor. These sections from stomach are normally not permeable for absorbing many substances into the blood with exception of ions, alcohol, water, and certain drugs (aspirin). Stomach is followed by small intestine; region where majority of digestion and absorption takes place as shown in Figure 1.2. Length of small intestine alone provides a large and extended surface area for better digestion and absorption, which too is further increased by the

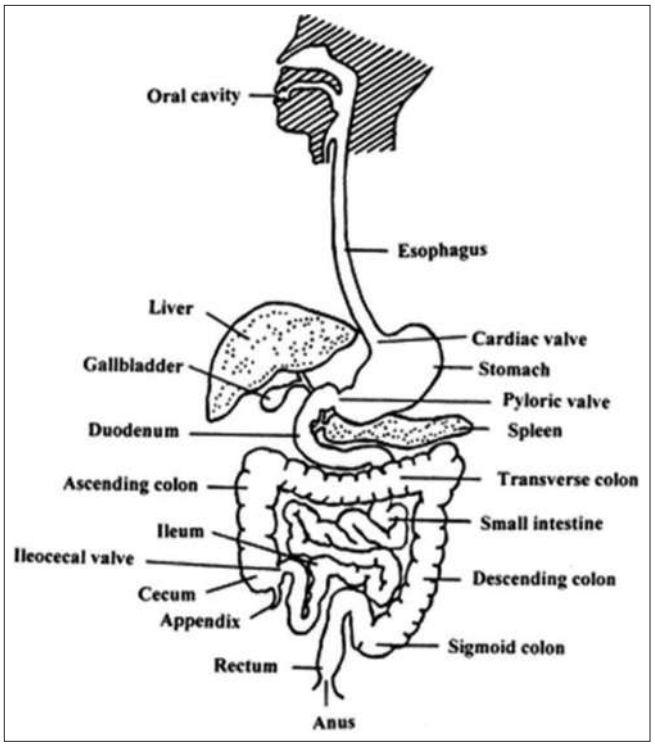


Figure 1.2: Anatomy of the human gastrointestinal tract (Tortora *et al*., 2006)

presence of circular folds, villi, and microvillus. Large intestine is the last part of GIT starting from the ileum and ends with anus having length of 1.5 m and 2.5 inches in diameter mainly for storage of fecal matter and water absorption. Significantly, there is no secretion of enzymes in the mucosa, and the resident bacteria's finally conducts the last stage of digestion (Mc Connell *et al*., 2008).

Anatomy and histology of stomach

The stomach has four main regions: Cardiac, fundus, body, and pylorus. Cardiac area is wide 1–4 cm that guards the esophageal orifice. The rounded portion left to cardiac is fundus. Large central portion of stomach inferior to fundus is body. Pylorus is the part that connects the stomach to duodenum [Figure 1.3]. Pyloric area is about 15% of total gastric mucosal area. It is having two parts: Pyloric antrum and pyloric canal.

Antrum is short and wider proximal chamber whereas canal is narrow tubular passage 3 cm long ends in pyloric sphincter. Food is stored in fundus and body, whereas mixing is done in cardiac. The fundus muscle fiber relaxes to adjust the increased volume during eating and also exerts pressure

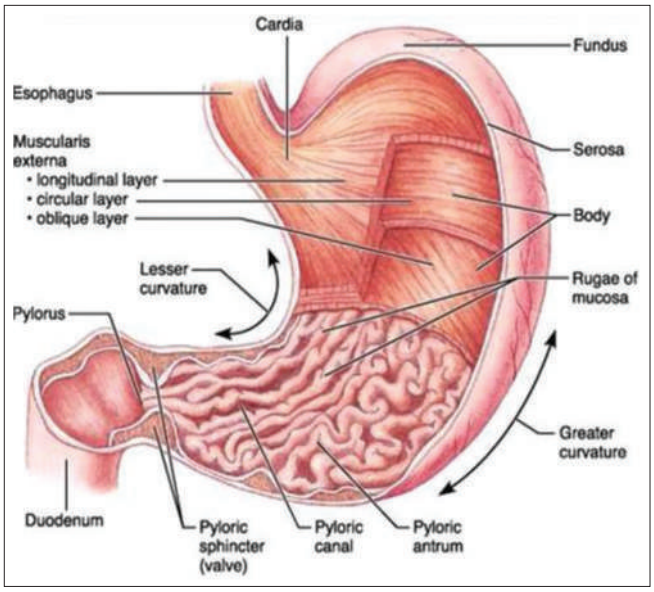


Figure 1.3: Different parts of stomach (Tortora *et al*., 2006)

to pass the contents toward distal region. The diameter of pyloric sphincter is 12.8 mm which helps in passing large particles.

Histological, stomach is formed of four layers with characteristic difference. The outer coat consists of peritoneum followed by muscular coat having three layers: Longitudinal outer layer, circular middle layer, and inner oblique layer. Then, there is layer of submucous followed by layer of muscular and supporting connective tissues. Final is mucous membrane which is having large folds called rugae (Tortora *et al*., 2006). The gastric gland is composed of mainly three types of exocrine glands such as chief cells, mucous neck cells, and parietal cells. Main function of chief cells is to secrete gastric lipase and pepsinogen, whereas parietal cells secretes intrinsic factors and hydrochloric acid. Mucus and bicarbonates are secreted by mucosal cells and neck cells which protects from the adverse effects of hydrochloric acid.

Functions of stomach

Stomach acts as a reservoir for storing food, digesting the food and passes it to small intestine. It also secretes gastric juice containing hydrochloric acid, pepsin, gastric lipase, and certain intrinsic factors. Stomach mixes the food with these gastric secretions to form chyme. Stomach is the part which gives space to orally administered drug to get absorbed and perform its pharmacological effect.

Physiology of stomach

The rate and absorption of drugs are affected by the anatomical and physiological conditions of GIT and should be assessed. For increased absorption and bioavailability of drug as orally administered dosage form, several factors in developing CR formulation have to be considered. Enhanced absorption is one of the parameters for successful development of CR formulation which ensures systematic absorption of drug released (Davis, 2005). Absorption capacity of stomach and colon is far less as compared to small intestine. Drugs showing absorption at a particular region may be due to difference in solubility and stability in various parts of the GIT. Presystemic clearance is another factor. Even if drug absorption is uniform, degradation due to hydrolysis, enzymes, or metabolism by microorganisms and brush border of gut wall will leads to variation in plasma drug concentration. Ideal formulation can be designed by the concept of region specific drug absorption. Regulation of motility and gastric secretion also plays key role in drug absorption. Gastric juice secretion and smooth muscles contraction of stomach wall are controlled by both neural and hormonal mechanism. Three overlapping phases are responsible for gastric secretions summarized as:

Cephalic phase

There is influence of brain on gastric secretion and stimulation of nerves is responsible for it. Before the food reach the stomach its sight, taste, and even thought leads to increase pepsin and acid secretion in the stomach.

Gastric phase

As the food reaches the stomach the gastric phase secretions starts. In the mucosa of pyloric region gastrin hormone is produced and is released when due to presence of food stretching of antrum takes place. Food particularly containing protein, alcohol, and coffee stimulates the gastrin secretion,

which further initiates the secretion of hydrochloric acid and pepsinogen.

Intestinal phase

When gastric acid secretion influences the small intestine. It is referred to as intestinal phase. Intestinal mucosa releases hormone when there is too acidic condition in the duodenum of intestine. The released hormone protects the small intestine by inhibiting the further secretion of acid. All the stimuli discussed above liberates in total about 2–3 l of gastric secretion per day.

Gastric transit and emptying time

For the development of sustained-release dosage forms, stomach may be used as "depot" (Fell, 1996). However, the transit time of drugs and nutrients vary from region to region in GIT. Around 3 h of time is taken by drug formulation or nutrients to transit from stomach to small intestine (Davis *et al*., 1986) and for colon it is 20 h or more (Washington *et al*., 2001). Residence of drug is for short period of time at its site of absorption, more so if it is absorbed in proximal part rather than thoroughly in small intestine. GIT time of formulation is the foremost physiological parameter which obstructs the development of CRDDS which also changes by physiological properties of GIT. Physical form of formulation either in solid or liquid and also the fasted or fed condition of person affects the pattern of GIT and gastric emptying time (Bode *et al*., 2004). Emptying time in the fasting state for liquid is a function of the volume administered, while elimination of indigestible solids will remain as such due to their physical size from the stomach (Bardonnet *et al*., 2006; Khobragade *et al*., 2009).

The pattern of motility of gastric emptying differs markedly during the fasting as well as the fed state. Fasted state is composed of a cyclic process of interdigestive series of electrical events in between the stomach and small intestine for every 2–3 h. Such process occurs in four consecutive phases and is known as interdigestive myoelectric circle or migrating myoelectric complex (MMC) (Etyan *et al*., 2003).

Phase I

Also called as basal phase having no contractions and electrical activity. The secretions last for 40–60 min.

Phase II

The preburst phase remains for 20–40 min with intermittent contraction (Minami *et al*., 1984) and secretion of bile (Gurber *et al*., 1987) in duodenum, whereas gastric mucous discharge occurs.

Phase III

This burst phase remains for 10–20 min with intense and large continuous contractions for short period of time. These waves called as "housekeeping waves" helps in sweeping undigested material to small intestine.

Phase IV

Transitional phase is only for 0–5 min between Phases I and III.

An average duration of 90–120 min is required for completing this cycle. For prolonging the retention of any drug in GIT, the CRDDS should resist housekeeping action of III phase. The type of contraction varies from fasted to fed state called as digestive motility pattern which consists of regular contraction same as in II phase of fasted state. Thus, during the fed state gastric emptying rate slows down due to delayed onset of MMC (Arora *et al*., 2005).

The possibility to conclude exactly the effectiveness and clinical relevance of drug substances which are absorbed primarily by interaction with GIT is difficult. Therefore, designing of a successful dosage form which will work irrelevant to clinical conditions, digestive state and variations in GI motility is desired. One important requirement for better action of oral delivery system is absorption of drug substance in GIT. If a formulation cannot retains at the site of absorption, maintaining a long gastric transit time, then such oral CRDDS shows limited therapeutic application after drug administration.

Gastroretentive drug delivery system (GRDDS)

Various scientific literatures reveal the increased interest of developing gastroretentive dosage form by the industry and academic research groups (Deshpande *et al*., 1996). The suitable approach for attaining predictable and prolonged drug release in GIT is to maintain residence period in the gastric region by holding the delivery system above the absorption window. Thereby developing gastroretentive and sustained release dosage forms which are safe and effective than convention drug delivery systems. These formulations can retain in gastric region for various hours and release the drug in sustained manner. Prolonged GRT helps in improving bioavailability, solubility profile of drugs which are having less solubility in high pH conditions and reduces wastage of drug. It is also applicable for local delivery to stomach and for medicaments having less absorption window in GIT. Rate of drug absorption can be increased by mutual contact of formulation with absorbing membrane. Uniform absorption of drug over the GIT may not be achieved, as the dosage forms may be transported rapidly from the upper region of intestine (more absorptive) to lower region (less absorptive). Absorption of drugs mostly occurs in stomach and small intestine (upper part) (Davis, 2005). New therapeutic possibilities can provide benefit to the patients by developing gastroretentive dosage forms (Arora *et al*., 2005).

Parameters influencing retention

Various parameters which influence the emptying and there by gastric residence of gastroretentive dosage forms are as follows:

Density

Certain GRDDS shows prolonged retention in the stomach due to their floating properties. For a formulation to remain buoyant in the stomach its density must be less than that of gastric content. The density of 1.0 g/ml or less is required to show buoyancy of dosage form. Bulk density is not the only criteria but buoyancy is also studied by measurement of weight and swelling experiments (Gerogiannis *et al*., 1993). However, the magnitude of buoyancy decreases with time due to the development of hydrodynamic equilibrium in the fluid where dosage form is placed (Timmermans *et al*., 1990).

Size of dosage form

Another factor which influences the retention of dosage form is its size. Non-floating dosage form shows high variation in residence time due to small, medium, and large unit size. Smaller units are emptied easily from the stomach during digestive phase, while larger units disappeared during housekeeping waves (Oath *et al*., 1992). Floating units with diameter ≤7.5 mm are having longer retention than non-floating units, whereas both types of units having 9.9 mm diameter shows equivalent retention. Floating units remain buoyant during digestive phase whereas non-floating disappeared due to peristalsis in digestive phase (Timmermans *et al*., 1989).

Presence of food

In fasting state, the gastric emptying time of both floating and non-floating units is shorter (<2 h), which increases after meals to about 4 h. As the gastric emptying depends on the MMC, in fed condition MMC is delayed which increases the retention (Desai *et al*., 1993). During fed condition by single meals GRT is prolonged by 2 h for floating dosage form with floating time (FT) of 5 h, whereas after successions of meals most of the units have FT of 6 h with prolongation of GRT up to 9 h as compared to control. Due to mixing of dosage form with heavy solid food taken the variation in FT and GRT was observed (Iannuccelli *et al*., 1998). Basic drugs probably absorbs better in fed state rather in fasted condition.

Effect of posture

Upright or supine position effects the GRT of floating and non-floating units. Non-floating subject sank rapidly after ingestion and never come back to the surface in upright position where as floating units lie continuously above the gastric content and thereby showing prolonged GRT (Van Gansbeke *et al*., 1991). In upright posture, GRT of only non-floating unit is influenced by size and is increased with increase in the size of the units, whereas in supine posture

the size effects the GRT for both types of the units (Timmermans *et al*., 1994).

Age and sex

Gastric emptying duration in females is slower than that of male in spite of weight, height, and even when hormonal changes take place during menstrual cycle. Aged peoples about 70 years or more show prolonged GRT than younger ones (Mojaverian *et al*., 1988).

Targets for developing gastroretentive delivery system (Garg et al., 2008)

GRDDS can be developed for enhancing the release of suitable drugs in controlled manner for longer period. Several drugs show its highest pharmacological response when they are released in the stomach in controlled manner. This reduces the side effects and frequency of dosing. GRDDS is suitable for certain category of drugs having following characteristics:

- Drugs whose absorption window is narrow in GIT such as riboflavin, furosemide, L-dopa, and Para amino benzoic acid. Some important drugs display low bioavailability as they have absorption window in small intestine.
- Drugs which act locally in the stomach, for example: Misoprostol and antacids.
- Highly unstable and rapidly degradable drug molecules in colonic and intestinal environment, for example: Ranitidine hydrochloride, captopril, and metronidazole.
- Drugs having less solubility at higher values of pH, for example: Verapamil hydrochloride, chlordiazepoxide, and diazepam.
- Drugs substances which disturb the microbes in the colonic region, for example : Amoxicillin trihydrate and certain antibiotics.
- Substances with easy absorption from the GIT.

Advantages of GRDDS (Nayak et al., 2010)

- The various benefits of gastroretentive systems are summarized as follows:
- Increased patient compliance by reducing dosing frequency.
- Increases therapeutic efficacy of drugs having short half-life.
- Achievement of site-specific drug delivery particularly to the stomach.
- Increased bioavailability of drugs.
- Minimizes fluctuation of plasma drug concentration.
- Sustained release of drug can be achieved, there by showing prolonged action.

Limitations of GRDDS (Khan, 2013)

There are certain conditions when GRDDS is not satisfactory such as:

- Drugs which cause gastric irritation and lesions should not be released in the stomachin slow manner, for example: Aspirin and nonsteroidal anti-inflammatory drugs.
- Drugs whose absorption is good throughout the GIT are unsuitable for GRT, for example: Isosorbide dinitrate.
- Drugs with limited acidic solubility will not have good release on GRT.
- Colon specific drug release candidates.
- Drugs having first-pass metabolism and unstable in gastric fluid.
- Due to high variation in gastric emptying time unpredictable bioavailability results.
- Effectiveness of the technique is doubtful due to bioadhesion of drug to the acidic environment and high turnover of mucus.
- Exact buoyancy of the system cannot be predicted as the GRT is influenced by gastric motility, presence of food, and pH around the system.

Approaches to GRT

Varieties of concepts are involved for developments of successful gastroretentive system which can increase the residence time of drug in gastric region are discussed below.

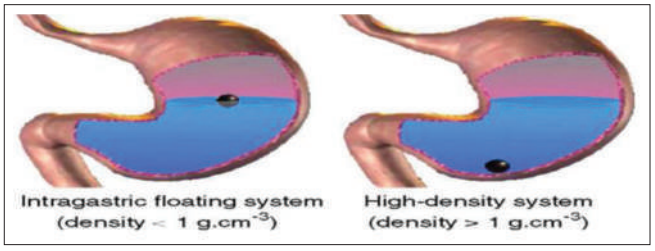


Figure 1.4: Positioning of an intragastric floating system and a high-density system in the stomach (Bardonnet *et al*., 2006)

High-density system

Small pellets which have density above 1.004 g cm^{-3} (density of gastric fluid) will sinks at the bottom of the stomach [Figure 1.4] thereby withstand the peristaltic waves of the stomach walls by getting entrapped in the folds of antrum. These small pellets were prepared by heavy core inert material such as iron powder, zinc oxide, barium sulfate, or titanium dioxide as polymers with density of about 2.5 g cm^{-3} to produce significant increase in gastric residence time (Clarke *et al*., 1993). Good results were reported for ruminants (Rinner *et al*., 1982) but not in human beings and thus no such system is available for use in the market (Garg *et al*., 2003).

Bioadhesive or mucoadhesive system

Bioadhesive or mucoadhesive system adheres to the mucosal surface for long time with the help of mucoadhesive polymers by different mechanism. Several mucoadhesive polymers used to develop such system are polyacrylic acid (Carbopol\polycarbophil), chitosan, (Polymethyl vinyl ether/maleic anhydride copolymers), cholestyramine, Hydroxypropyl methylcellulose (HPMC), sephadex, sodium alginate, polyethylene glycol (PEG), dextran, sucralfate, poly (alkyl cyanoacrylate), and polylactic acid. Different theories to explain process of mucoadhesion includes the electronic theory which suggested that there is an attractive electrostatic force between the bioadhesive material and glycoprotein mucin network. Adsorption theory, on the other hand, suggests that there are Van der Waals forces and hydrogen bonding (secondary forces) which helps in mucoadhesion. Development of intimate contact between bioadhesive polymers with mucus layers is the basis of wetting theory whereas, physical entanglement of mucin strands with flexible polymer chains is proposed by diffusion theory (Huang *et al*., 2000; Vasir *et al*., 2003). The fast production of mucus secreted in the GIT causes difficulty in maintaining the effective mucoadhesion of polymers. Furthermore, the hydrated nature of stomach reduces the polymer efficacy for bioadhesiveness.

Expandable or swelling system

Formulations whose sizes are bigger than the pyloric sphincter can survive in gastric transition of the stomach. However, the formulation must be of such size that could be easily swallowed and should not show any gastric obstruction. Thus, three essential requirements for developing such system are: A small size dosage form for easy oral administration, an expanded gastroretentive form, and finally small size which do not easily evacuate and show characteristic drug release (Klausner *et al*., 2003). Unfoldable or expandable and swellable systems were investigated. Various biodegradable polymers are used for designing such systems. The system consists of a capsule acting as a carrier which contains a compressed part that expands in the stomach. Different geometric forms of bioerodible polymers that can be compressed in carrier such as ring (Caldwell *et al*., 1988), tetrahedron, or planar membrane (4-lobed, discor 4-limbed cross form) (Caldwell *et al*., 1988) are presented in Figure 1.5. Expandable systems show some disadvantages such as: Problem in storage of biodegradable polymers, the mechanical shape memory of unfolding system is short-lived (Klausner *et al*., 2003), costly (Hwang *et al*., 1998), and moreover difficult to industrialize.

Swelling system is also called as plug-type system. This system swells in the presence of gastric fluid by osmotic absorption and prevent to exit from the pylorus. A suitable polymer with appropriate molecular weight is selected which swells and thereby release the drug in sustained manner (Groning *et al*., 1984). Balance

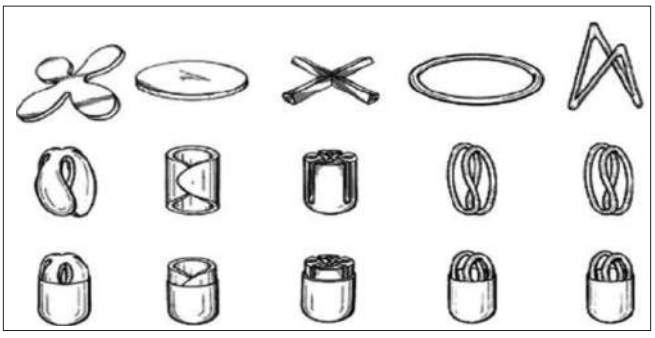


Figure 1.5: Various unfoldable systems of different geometry (Caldwell *et al*., 1988). 4-lobedDisc4-limbedcrossRingTetrahedron

is maintained between swelling and dissolution due to optimum cross-linking in the hydrophilic polymer network.

Magnetic system

In this system for gastroretention an external magnet is placed over the position of the stomach on the abdomen and formulation itself contains a small piece of magnet. This technique was applied in rabbits with formulation containing ultrafine ferrite (Fe_2O_3) as bioadhesive granules. External magnet is used for guiding the formulation from esophagus and retention was achieved for 2 h (Ito *et al*., 1990). However, the positioning of external magnet with a degree of precision is difficult and it also compromise patient compliance.

Superporous hydrogel system

These are also swellable system with separate classification from the conventional system. The average pore size of superporous hydrogel is >100 µm as compared to conventional hydrogel ranging from 10 nm to 10 µm. Conventional hydrogel absorbs water very slowly and requires long time to reach an equilibrium state whereas superporous hydrogel takes a minute to swell to equilibrium size by rapid absorption of water through capillary wetting using number of interconnected open pores (Chen *et al*., 2000). Largely, swell superporous hydrogel is of sufficient mechanical strength to resist the pressure of gastric contraction. A hydrophilic material such as croscarmellose sodium (Ac-Di-Sol®) is needed with formulation for developing such system (Chen *et al*., 2000). Dry and swollen form of superporous hydrogel is shown in Figure 1.6.

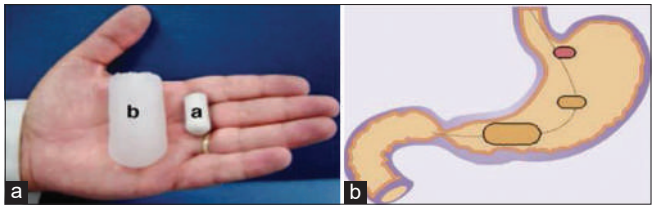


Figure 1.6: (a) Dry and (b) water swollen state of superporous hydrogel (Left side) and schematic transition of superporous hydrogel (Right side) (Shah *et al*., 2003)

Floating system

Floating formulations resides in the stomach for prolonged time due to their density less than that of gastric fluid. These formulations remain buoyant releasing the medicament in control process and finally emptying of residual system form the stomach occurs. In fasting state gastric emptying is rapid and floating system rely on fed state as it retards emptying and provides required liquid to show effective buoyancy (Saito *et al*., 2003).

Ion exchange resin

Drug substances containing negative charge can bind to the ion exchange resin loaded with bicarbonate to increase GRT. To prevent the rapid loss of carbon dioxide, the beads were entrapped in a semi-permeable membrane. Once the formulation reaches the acidic content of stomach exchange of chloride and bicarbonate ions takes place which leads to release of carbon dioxide. The released air (CO_2) is trapped in the membrane and carries the microspheres at the top layer of gastric content, developing a floating layer of resin beads which maintains drug release for long time (Atyabi *et al*., 1996).

Incorporation of passage delaying food agents

The pattern of stomach can be changed and modified to a fed state using food substances such as fatty acids (salts of myristic acid). Thus, prolonged drug release and decrease in gastric emptying rate will achieve. The saturated fatty acids with chain length of C10-C14 present in meals rich in fat, helps in delaying the gastric emptying time. Thus, this technique can be used for increasing GRT of drug.

Floating drug delivery systems (FDDS)

Considering the different approaches for developing GRDDS, floating system is logical and easy to formulate from technological point of view. Such system reduces fluctuations of drug bioavailability as they work independent of variations occurring during gastric emptying process. For supporting, the buoyancy of the system different approaches is followed. FDDS can be classified on the basis of mechanism of floatation as effervescent and non-effervescent systems.

Effervescent system

This system of FDDS consists of swellable polymers along with carbon dioxide (CO_2) or other organic acids (citric and tartaric acid) entrapped in swollen hydrocolloids of formulation which acts as effervescent component. Incorporation of gas within the formulation helps in reducing the density and makes the system to buoyant over the gastric fluid (Krogel *et al*., 1999; Sungthongjeen *et al*., 2008). Carbonates apart from providing buoyancy to the system also provide basic microenvironment for the polymer to convert to gel (Rajab *et al*., 2010). Effervescent system can be further subdivided into two categories:

Gas generating system

In gas generating system drug along with polymer is mixed with gas forming agent and compressed into matrix tablet. Effervescence is produced by the reaction of salts of carbonate or bicarbonate with citric or tartaric acid to produce CO_2 which entrapped in jellified polymer layer thereby decreasing the specific gravity of the formulation and remain buoyant over the gastric fluid. Such matrix tablet can be single, doubled, or multi layered. In multilayer tablets gas forming substances are compressed in layer having drug and hydrocolloid as outer layer to provide sustained release effect. Water gets diffuse through the swellable membrane generates CO_2 neutralization in the effervescent layer. On the other hand, there is lag period due to gas generation reaction that could end in gastric emptying of the dosage form before floatation. Multiparticulate formulation containing number of small discrete units shows more reliable gastric emptying patterns in comparison to formulations containing single unit suffering from "all or none concept" (Sungthongjeen *et al*., 2006).

Volatile liquid/vacuum containing system

Inflatable GRDDS

In such systems liquids such as ether and cyclopentane were incorporated in inflatable chamber which gasify due to body temperature and helps the chamber to float over the stomach. Drug reservoir is loaded in this chamber as an impregnated polymer matrix, encapsulated in gelatin capsule as shown in Figure 1.8. The capsule dissolves in the stomach to release the drug substance along with chamber for prolonged period of time.

Non-effervescent system

Non-effervescent systems do not contain gas forming agents but incorporate one or more cellulose hydrocolloids, poly saccharides as highly swellable and gel forming polymers during formulation. Floating of dosage form occurs due to hydration of polymer in gastric fluid and forming a colloidal gal barrier. Number of approaches in developing non-effervescent FDDS is available.

Hydrodynamically balanced system (HBS)/colloidal gel barrier system

The HBS system is a single-unit dosage form having gel-forming hydrophilic polymer of one or more type. HPMC is basically used apart from hydroxy ethyl cellulose (EC), sodium carboxymethylcellulose, hydroxypropyl cellulose, agar, and carrageenan polymers (Hwang *et al*., 1998). The formulation is composed of a gelatin capsule in which polymer mixed with drug is placed. In the gastric fluid, the capsule rapidly dissolved and flotation is achieved due to hydration and swelling of polymer surface. Drug is released in controlled manner due to formation of a hydrated layer at the surface. Surface hydration and buoyancy are maintained by the penetration of water in the inner layer due to continuous erosion of the surface (Reddy *et al*., 2002) [Figure 1.9]. A barrier of soft gelatin around the dosage form is formed due to hydration which provides a water-impermeable colloid gel barrier on the surface of

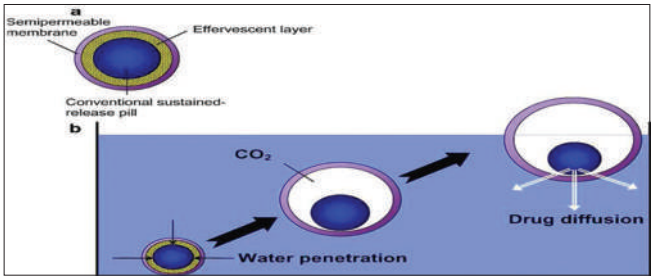


Figure 1.8: Inflatable gastroretentive drug delivery system (Chien YW *et al*., 1992)

the tablets. Drug release rate from HBS is controlled by hydrated gel. HBS system has number of approaches as single and bi-layered tablets. Single layer formulation was simply prepared by mixing gel-forming hydrocolloid polymer with drug. The air entrapped within the swollen polymer makes the system buoyant. Intragastric single and bilayer tablets are shown in Figure 1.10.

Bi-layer tablet contains one immediate release layer which delivers the starting dose and another is sustained release layer which forms colloidal gel barrier on its surface for sustained release of drug (Mayavanshi *et al*., 2008). Multi-layer flexible sheath such as device consists of one self-supporting carrier film which is developed by water insoluble polymer containing barrier film and drug has been developed. Both the films were sealed in a manner such as to entrap minute air pockets that help the laminated films to remain buoyant. New improved HBS system with impermeable polyethylene cylinder, 10–15 min length sealed from both the sides by drug containing hydrophilic polymer (HPMC) has also been designed. Buoyancy is imparted by the entrapment of air in the core of the cylinder (Krogel *et al*., 1999).

Microporous system

Porous materials due to their unique characteristics of stable porous structure, high surface area, changeable pore sizes, and good surface properties comprises new category in delivery system. During the development of FDDS such material plays an important role, especially to improve solubility of drugs having poor water solubility (Ahuja *et al*., 2009; Sher *et al*., 2009). Several substances contain porous structures such as silica, porous ceramic; ethylene vinyl acetate; and titanium dioxide which possess low density than gastric fluid thereby remain floated in the stomach containing drug inside the porous compartment (Streubel *et al*., 2002). The principal is based on the entrapment of drug reservoir into porous compartment having several pores among the walls. These pores were covered moderately using other polymers and entrap air inside the developed system. Direct contact of the system with gastric surface is prevented by sealing of the peripheral walls thereby showing delayed release of drugs. In exposure to gastric medium, the entrapped air would be gradually removed, thereby increases FT and releases the drug in predictable and reproducible manner.

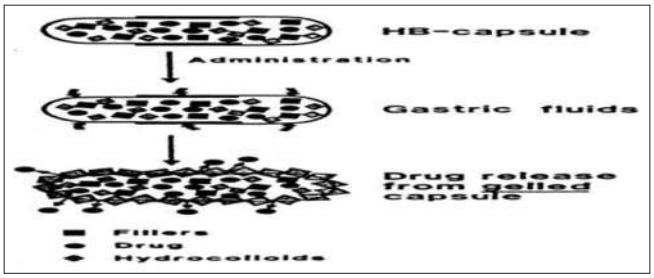


Figure 1.9: Working principal of hydrodynamically balanced system (Bogentoft, 1982)

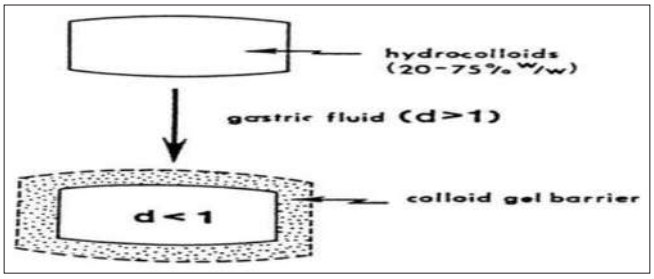


Figure 1.10: Intragastric floating tablet (Chien *et al*., 1992)

Alginate beads

Floating system can be prepared both by synthetic and natural hydrophilic polyionic polymer like alginate. Alginate is mainly used owing to its biocompatible and non-toxic behavior when taken orally. It also possesses protection for the mucous membranes present in the upper GIT. Beads prepared from alginate are spherical in shape with 2.5 mm in diameter and formed by addition of aqueous alginate solution dropwise to solution of calcium and/or other di and polyvalent cations (Javadzadeh *et al*., 2010). Dried alginate beads were administered as multi-unit dosage form using unique vehicle in GIT as early as 1980s due to its pH dependent reswelling property (Murata *et al*., 2000).

Hollow microspheres/microballoon

These are multiple unit floating systems having density <1 g/cm^3 with immediate floating characteristics. The hollow microspheres (microballoons) are spherical shaped empty particles <200 μm size without core (Gholap *et al*., 2010). Drug is dispersed throughout the particle matrix and is released in controlled

manner from microballoon. Solvent evaporation and diffusion methods are generally used to prepare floating microspheres. Polymers such as Carbopol cellulose acetate, calcium alginate, Eudragit, agar, polycarbonate, chitosan, methocil, polyvinyl acetate, and pectin are used for preparation of hollow microspheres (Soppimath *et al*., 2006).

Advantages of FDDS (Babu et al., 1990)

Substances which get absorbed through the stomach such as ferrous salts and antacids when formulated as gastroretentive systems are advantageous as they remain for longer duration at the site of action. Certain acidic substances which cause irritation on the walls of stomach when come in contact with it such as aspirin can be delivered as FDDS.

The dissolution of drug from FDDS will occur in gastric fluid and the system will emptied to small intestine where also some absorption may occur. Thus, even if the drug remains in basic pH of the intestine in the solution form, it will be completely absorbed from floating dosage form.

- Drugs acting locally in the stomach are advantageous to be formulated as gastroretentive systems.
- In certain disease like diarrhea when sever intestinal movement and short transit time occur causes poor absorption of drug therefore to get better response it may be advantageous to keep the drug in floating condition in stomach.
- The problem encountered with CRDDS taken orally such as short gastric residence time can be overcome with these systems.

Disadvantages of FDDS

- Drugs having insolubility and instability in gastric fluid are not suitable for FDDS.
- FDDS needs large concentration of fluid in the stomach for proper floating of drug and show efficient results.
- Drugs that absorbed significantly throughout GIT that shows significant first pass metabolism, cannot be delivered as FDDS as the reduced systemic bioavailability will results due to slow gastric emptying time.
- Some drugs formulated as floating system may cause irritation to gastric mucosa.

Drug release kinetics of floating system

Several formulations as FDDS having different physicochemical properties shows varied drug release pattern. The prime objective of any dosage form is to maintain plasma drug concentration in a target tissue for longer period, which is even more rational for FDDS. Drug release kinetics deals with obtaining one or more parameters which can be used for comparison and relating release data with important parameter such as bioavailability (Wagner, 1969; Barzegar-Jalali *et al*., 2008; Dash *et al*., 2010). Several kinetic models describe the release of drug from the dosage form.

Any qualitative and quantitative variation in a formulation can change *in vitro* release as well as *in vivo* activity of the dosage form. Therefore, to facilitate drug development several tools have been developed that helps in decreasing the need to perform bio-studies (Arifin *et al*., 2006). Even the floating strategies for different FDDS are same; there is not a fixed kinetic model specifically for FDDS (Adibkia *et al*., 2011). Selection of suitable formulation variables for maintaining floating behavior and kinetic model for drug release study should be considered simultaneously to present a successful model fitting for FDDS. In general, it is complicated to evaluate the mode of drug release from FDDS therefore keen observation on physical and chemical properties of dosage form is required.

Types of FDDS studied (Nayak et al., 2010)

Various drugs are formulated in different dosage forms in FDDS which are summarized in Table 1.2.

Marketed products (Mathur et al., 2010)

Some marketed products of gastroretentive systems are summarized in Table 1.3.

Floating microspheres

Floating microspheres are multiple unit dosage form of non-effervescent type drug delivery system. They are also termed as hollow microspheres as they spherically shaped particles without core. These are having size of less than 200 µm and are free flowing powder of synthetic polymer. Drug is uniformly distributed throughout particle matrix

Table 1.1: Features of human GIT (anatomical and physiological)

Section	Length (cm)	Diameter (cm)	pH	Major constituents	Transit time (h)
Oral cavity	15–20	10	5.2–6.8	Amylase, Maltase, Ptyalin, Mucin	Short
Esophagus	25	2.5	5–6	-	Very short
Stomach	20	15	1.2–3.5	Hydrochloric acid, Pepsin, Renin, Lipase, Intrinsic factor	0.25–3
Duodenum	25	5	4.6–6.0	Bile, Trypsin, Chymotrypsin, Amylase, Maltase, Lipase, Nuclease, CYP3A4	1–2
Jejunum	300	5	6.3–7.3	Amylase, Maltase, Lactase, Sucrase, CYP3A5	-
Ileum	300	2.5–5.0	7.6	Lipase, Nuclease, Nucleotidase, Enterokinase	1–10
Cecum	10–30	7	7.5–8	-	Short
Colon	150	5	7.9–8	-	4–20
Rectum	15–19	2.5	7.5–8	-	Variable

GIT: Gastrointestinal tract

Table 1.2: Types of FDDS studied

S. no.	Dosage forms	Name of drugs
1.	Tablets/Pills	Acetaminophen, aspirin, amoxicillin trihydrate, ampicillin, Atenolol, captopril, ciprofloxacin, chlorpheniramine maleate, Cinnarizine, Furosemide, 5-Fluorouracil, Isosorbide mononitrate, diltiazem, isosorbide dinitrate, nimodipine, para amino benzoic acid, piretenide, pentoxyfillin, prednisolone, Quinidine, Verapamil HCl, Riboflavin, Sotalol, Theophylline
2.	Capsules	Chlordiazepoxide HCl, Diazepam, Furosemide, L-Dopa and Benserazide, Misoprostol, Nicardipine, Propranolol HCl, Ursodeoxycholic acid
3.	Powders	Several basic drugs
4.	Films	Drug delivery device
5.	Microspheres	Aspirin, Griseofulvin, P-nitro aniline, Ibuprofen, Ketoprofen, Terfenadine, Tranilast
6.	Granules	Diclofenac Sodium, Indomethacin, Prednisolone

FDDS: Floating drug delivery systems

Table 1.3: Marketed products of gastroretentive drug delivery system

S. no	Brand	Drug	Company	Remark
1	Madopar HBS	Levodopa and benserazide	Roche products, USA	Floating capsule
2	Valrelease	Floating capsule, Diazepam	Hoffmann LaRoche, USA	Floating capsule
3	Topalkan	Floating Antacid, aluminum and magnesium mixture	Pierre fabre Drug, France	Floating liquid alginate preparation
4	Conviron	Ferrous sulfate	Ranbaxy, India	Colloidal gel forming FDDS
5	Cifran OD	Ciprofloxacin	Ranbaxy, India	Gas generating floating tablet
6	Cytotech	Misoprostol	Pharmacea, USA	Bilayer floating capsule

FDDS: Floating drug delivery systems, HBS: Hydrodynamically balanced system

and released in controlled manner for prolonged time (Vyas *et al*., 2002). Variety of polymers was used for formulation of hollow microspheres, recent developments includes polymethylmethacrylate (PMMA), acrylic resins, Eudragit, polyethylene oxide (PEO), polystyrene floatable shell, cellulose acetate, floating balloons of polycarbonate, and Gelucire floating granules (Hilton *et al*., 1992).

Release of drug from microspheres is controlled by hydrated polymer which forms colloidal gel barrier due to penetration of gastric fluid in the formulation. Buoyancy of system is due to entrapment of air by the swollen polymer.

Advantages of floating microspheres (Vyas et al., 2011)

- CR of drug specifically in the stomach can be done.
- Reduced frequency of dose improves patient compliance.
- Short half-life drugs can achieve better therapeutic efficacy through CR.
- Drug release is uniform and without dumping of dose due to single unit dosage form.

- Increases bioavailability and absorption of drug which are soluble only in stomach.
- Avoid irritation of gastric mucosa due to sustained release effect and uniform drug release.
- Drugs having short elimination half-life adhere to the wall of stomach during gastric emptying process.

Disadvantages of floating microspheres (Chawla et al., 2003)

- For enabling the system to float and release drug efficiently, excess amount of fluid is required in the stomach.
- Drugs which are unstable in stomach and are insoluble in gastric fluid are not suitable to be delivered as floating system.
- Drugs having irritant effect on gastric mucosa cannot be formulated as floating microspheres.

Requirement for formulation of hollow microspheres (Vyas et al., 2011)

- Ability to incorporate required concentration of drug.
- Stability and specific density lower than gastric fluid should be maintained.
- Release the drug in controlled manner for prolonged time.
- Should be biocompatible and susceptible to chemical modification.

Method of preparation (Jain, 2002)

For preparation of hollow microspheres, proper choice of polymer is most important for CR of drug. Floating properties can be improved using high molecular weight and less hydrophilic polymers. Different methods for the preparation of floating microspheres include:

Solvent diffusion and evaporation method

Hollow inner cores were made by dissolving the polymer in organic solvent whereas drug is dispersed or dissolved in it. Oil-in-water emulsion is formed by emulsifying the drug and polymer solution into polyvinyl alcohol (PVA) containing aqueous phase. When stable emulsion is formed, the evaporation of organic solvent is achieved by continuous stirring or elevating temperature under pressure. Precipitation of polymer at oil water interphase helps in cavity formation thereby imparting floating characteristic to the hollow microspheres [Figure 1.11]. The prepared formulation is filtered, washed, and air dried.

Spray drying

Solid form of drug is dispersed under high speed homogenization to polymeric solution prepared in suitable volatile organic solvent. Atomization of dispersed phase is done in steam of hot air which helps in preparation of small droplets or fine mist. Solvent evaporation from these droplets leads to formation of microspheres. Cyclone separator is used to separate the microparticles from hot air and solvent if remains are removed by vacuum drying.[1-10]

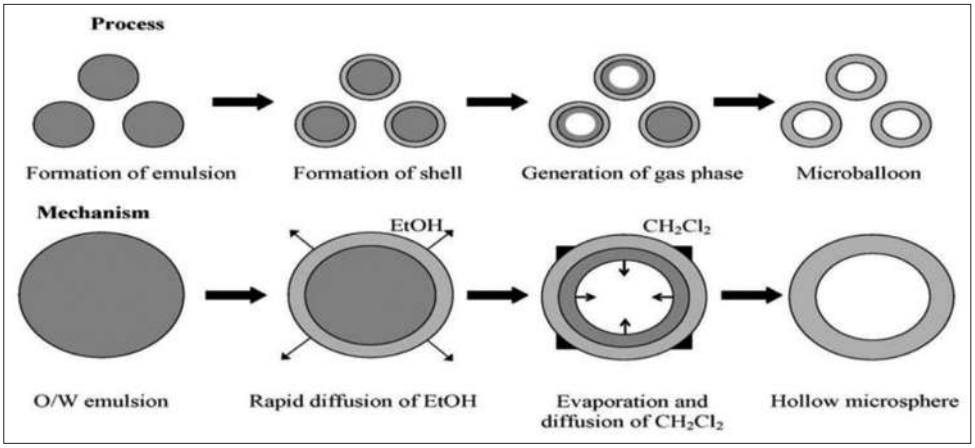


Figure 1.11: Formation of the hollow inner core microspheres by solvent diffusion and evaporation method (Javadzadeh *et al*., 2012)

Evaluation of floating microspheres

Floating microspheres were evaluated for micromeritics properties such as particle size, tapped and true density, and compressibility index and flow properties such as angle of repose. Other important parameter includes surface morphology, *in vitro* buoyancy, drug entrapment efficiency, and drug release. Size of particle and size distribution is determined using optical microscopy. The external features and hollowness of spheres are determined by scanning electron microscope (SEM). Bulk density apparatus is used to measure tapped density and compressibility index. True density and angle of repose are measured by liquid displacement method and fixed funnel method, respectively (Tanwar *et al*., 2007). Floating behavior of hollow microsphere is studied by placing the formulation on simulated gastric fluid (SGF) (pH 1.2) having tween 80 surfactant in a dissolution test apparatus. The solution is stirred continuously and 37°C temperatures throughout the study are maintained. Percent buoyancy was calculated by collecting both types of floating and settled microspheres. 0.1 N HCl as dissolution media was chosen to measure the *in vitro* drug release using dissolution test apparatus.

Diabetes mellitus (DM)

DM commonly occurring oldest and sever non-communicable disease. During the heterogeneous metabolic disorder metabolism of carbohydrate, lipid and protein are altered (Das *et al*., 1996). DM is the major reason of death in various developed countries with the substantial proof to be epidemic in developing and various newly industrialized countries, affecting about 25% of the population (Cline *et al*., 1991). Diabetes results in a condition of sever increase in glucose level (peripheral insulin resistance), glycosuria, polyuria, polydipsia, sudden loss of weight, increase hunger (polyphagia), formation of urinary ketone, and ketoacidosis, etc. (Ronald, 1994). During diabetes several disorders such as absence or deficient (relative or absolute) secretion of insulin occur (Dhawan *et al*., 1996). Hyperglycemia leads to auto-oxidative glycosylation of proteins and glucose auto-oxidation; due to which oxidative stress develop which exaggerates the condition with occurrence of several secondary complications (Baynes, 1991). Number of complications such as retinopathy, nephropathy, neuropathy, amputation, coronary artery disease, myocardial infarction, stroke, hypertension, and hyperlipidemia (UKPDS, 1998) is developed due to DM. Management of the disease is a global issue and needs the discovery of successful treatment. Ideal choice for treatment of DM is use of insulin but due to its frequent administration difficulty occurs, which led to search for new agents to treat it. New agents of modern era include insulin and oral hypoglycemic agents which when administered regularly decreases hyperglycemia (Upadhyay *et al*., 1996).

Indians get diabetes much earlier, that is, at an average of 35 years. About 25–30 million Indians suffer from diabetes, almost half of them are aware of the disease. However, urban population as compared to rural one has a much higher incidence of diabetes. People consuming high cholesterol diet, who are generally obese and leading sedentary lifestyle, or with a family history of diabetes are more prone to diabetes. As per medical science, detail of the disease and its management is not well known till date. Due to this reason, there is a constant zeal all over the world to identify and develop alternative remedies from the so called "Alternative systems" of medicine (Satyavati *et al*., 1989).

Types of DM (Guyton et al., 2006)

Two types of DM are as follows:

- Type–I DM formerly called insulin dependent DM (IDDM).
- Type–II DM (formerly called non-IDDM) NIDDM.

Type–I DM (IDDM)

Type-I diabetes is most prevalent in countries of south East Asia including India (Joel *et al*, 1996). Destruction of pancreatic beta cells in patients is due to viral infections or autoimmune disorder, although heredity also plays a major role. Type-I diabetes emerges very fast over a period of a few

days or weeks, with certain characters such as hyperglycemia, enhanced utilization of fats for formation of cholesterol by the liver and energy production and depletion of the body's proteins. Lack of peripheral glucose uptake leads to increase in blood glucose concentration which further leads to loss of glucose in urine and dehydration. Osmotic diuresis occurs due to loss of glucose in the urine in addition to the direct cellular dehydrating effect of excessive glucose. Classical symptoms of diabetes are polyuria (excessive excretion of urine), increased thirst and intracellular and extracellular dehydration. Increased risk for heart attack, kidney disorder, stroke, ischemia, retinopathy and blindness, and gangrene of the limbs occurs due to increased glucose level. Peripheral neuropathy that is abnormal function of peripheral nerves and autonomic nervous system dysfunction frequently occurs in chronic DM. Peripheral neuropathy results in impaired bladder control, cardiovascular reflexes, decrease sensation in the extremities, or peripheral nerve damage. In addition, hypertension, atherosclerosis, secondary to renal injury, and abnormal lipid metabolism, often develop in patients. Due to which diabetic coma and even death may occur. Abnormal depletion of the body's protein occurs in this disease. Due to which if patient suffer from disease and remain untreated will have rapid weight loss and asthenia (lack of energy) in spite of taking large amounts of food (polyphagia).

Type–II DM (NIDDM)

It is often characterized by late onset, insensitive to insulin and partial insulin deficiency. The prevalence of diabetes, especially NIDDM, is both in developed and developing countries. Type-II diabetes is referred to as insulin resistance, caused by greatly diminished sensitivity of target tissues to the metabolic effects of insulin. Like IDDM this type is also associated with multiple metabolic abnormalities, but increase levels of keto acids are usually not present. Type-II diabetes accounts for 80–90% of all cases of diabetes. Mostly Type-II diabetes occurs after age of 40 years, often between the ages of 50 and 60 years, and the disease develops gradually and thereby referred to as adult-onset diabetes.[11-15]

NIDDM is associated with enhanced concentration of plasma insulin in contrast to Type-I. Such condition arises due to decrease carbohydrate utilization which results in increase blood glucose level. However, even increase concentration of insulin is not sufficient for maintenance of normal glucose regulation due to diminished insulin sensitivity of the peripheral tissues. Due to which slight increase in glucose level occurs after taking carbohydrates in the early phase of the disease. The pancreatic beta cells become exhausted in the later phase of Type-II diabetes and are not able to prepare sufficient insulin to prevent more severe increase in glucose level, particularly after the ingests of a carbohydrate rich meal.

Issues tempting DM

Several factors which induce DM are discussed below:

General factors

The causes of diabetes absolute in IDDM and partially in NIDDM are pancreatic disorder, defect in formation of insulin, destruction of beta cell, decrease insulin sensitivity, genetic defect, and auto-immunity.

Host factors

Age and sex of patient are an important host factor for occurring diabetes. Although diabetes may occur at any age, NIDDM appears in the middle period. Young people also in large number are affected by malnutrition related diabetes. As per the WHO 1980, male-female ratios are equal, but more male diabetic patients are there in Southeast Asia.

Genetic factors

IDDM is not totally a genetic entity but NIDDM is associated with strong genetic component. IDDM is associated with specific human leukocyte antigen (HLA) B8 and B15 as genetic markers and more powerfully with HLA-DR3 and HLA-DR4, whereas, NIDDM is not HLA associated (Todd *et al*., 1987). Some people appear to have cell-

mediated and humoral defective immunity due to which they attack their own insulin producing cells. Obesity in terms of both duration and degree act as a risk factor for NIDDM. Many obese subjects are diabetic as it reduces the number of insulin receptors on target cells, where as in most cases, it produces resistance to the action of insulin. Physical inactivity and/or deficiencies of specific nutrients may also be a reason for diabetes. Obesity appears to play no role in IDDM pathogenesis.[16-20]

Environmental risk factors

Number of environmental factors such as sedentary lifestyle, diet, malnutrition, viral infection, chemical agents, and stress may induce the disorder. Interaction between insulin and its receptors may alter due to lack of exercise and subsequently leads to NIDDM. As per criteria of diet, there is no sound evidence that diabetes is specially associated with high intake of any of the major nutrients. Partial failure of beta-cell function may occur in early infancy and childhood due to malnutrition. Damage to beta-cell may occur in impaired carbohydrate tolerance in kwashiorkor. Excessive intake of alcohol damages the pancreas and liver and by promoting the obesity may increase the risk of diabetes.

Certain viral infections such as rubella, mumps, and human Coxsackie virus B4 may trigger a sequence of events resulting in destruction beta cell in immuno-genetically susceptible persons. Certain condition of trauma and stress and surgery may "bring out" the disease.

Signs and symptoms

Various signs and symptoms of diabetes include:

- Infection of bladder, kidney, skin, or others that are more frequent or heal slowly.
- Fatigue and weight loss.
- Polyphagia (increased hunger).
- Polydipsia (increased thirst).
- Polyuria (increased urination frequency).
- Blurred vision.
- Erectile dysfunction.
- Pain or numbness in feet or hands.

Diagnosis

Characteristics of the disease include recurrent or persistent hyperglycemic and can be diagnosed by observing any one of the following:

- Greater than or equal to 7.0 mmol/L (126 mg/dL) of fasting plasma glucose level.
- Greater than or equal to 11.1 mmol/L (200 mg/dL) of plasma glucose 2 h after a 75 g oral glucose load as in glucose tolerance test.
- Greater than or equal to 6.5% of glycated hemoglobin (Hb A1C).

In the absence of unequivocal increase glucose level positive result must be confirmed by repeating the test listed above on a different day. Measuring fasting glucose level is preferred due to easy measurement and utilization of less time (2 h) commitment of formal glucose tolerance testing with no prognostic advantage over the fasting test. Two fasting glucose measurements above 126 mg/dL confirm diagnosis for DM.

Patient having fasting glucose level from 100 to 125 mg/dL is considered to have impaired fasting glucose. People with glucose level 2 h after a 75 g oral glucose, above 140 mg/dL but not over 200 mg/dL are considered to have impaired glucose tolerance. The probability of latter shows major risk for progression to full-blown DM along with cardiovascular diseases.[21-25]

Treatment for DM

There are several classes of medication available for treatment of the disease. For first-line treatment metformin is generally recommended as reduced mortality rate is successfully reported. In addition, insulin injections can be used alone or given with oral medication. Several medications used to treat diabetes are summarized in Table 1.4.

LITERATURE REVIEW

In the recent literature, gastroretentive drug delivery by flotation mechanism has been proved to be very effective. In the past, various gastroretentive systems of antidiabetic and other agents have been formulated successfully. After thorough survey of literature no article related to microencapsulation

Table 1.4: Drugs recommended for treatment of diabetes

Classification	Drug	Route	Mode of action	Dose
Sulfonylureas	Tolbutamide, Glyburide (Glibenclamide), Glipizide, Glicazide, Glimepiride	Oral	Increases production of insulin	1–2 times a day
Biaguanides	Phenformin, Metformin	Oral	Lowers glucose from digestion	2–3 times a day
AlphaGlucosidase Inhibitors	Acarbose (oligosaccharide of microbial origin), Miglitol (Dextroxynijirimycin Derivative)	Oral	Slows digestion and glucose production	Take before each meal
Thiazolidinedione	Rosiglitazone, Pioglitazone	Oral	Lowers glucose production	Once daily with or without food
Meglitinide	Repaglinide, Nateglinide	Oral	Increase insulin production	5–30 min before meals

of repaglinide with EC and HPMC (various viscosity grades) alone and in combination has been reported. Therefore, it was thought to formulate and characterize various formulations using both the polymers and evaluates the effect of using different viscosity of HPMC on the release on drug.

Reported reviews of literature are as follows:

Patel *et al*., 2006, formulated, optimized, and characterized metformin hydrochloride loaded floating microspheres using EC polymer by non-aqueous emulsification solvent evaporation method. The floating microspheres were analyzed for size of particle, surface morphology, *in vitro* buoyancy, and drug release study. Results revealed that size, size distribution, yield, FT, and release were affected by changing ratio and components during selection of an organic phase. Finally, it was concluded that the developed formulation can be utilized for enhancing release of drug for about 8 h in the stomach, thus improving patient compliance and bioavailability.

Samal *et al*., 2011, prepared floating microspheres of nateglinide utilizing w/o/o emulsification solvent diffusion technique by EC and hydroxypropyl methyl cellulose polymer. Prepared formulations were characterized for optical microscopy, SEM, Fourier Transform Infrared (FTIR) analysis, *in vitro* drug release, and kinetics study by linear regression method. The result shows that with higher polymer concentration particle size increases and drug release decreases.

Pandaya *et al*., 2011, prepared and characterized porous carrier-based (calcium silicate) glipizide floating microspheres using Eudragit S polymer. Various process variables influencing the size, yield, surface morphology, drug loading, and release of microsphere were studied during the formulation. The microspheres show prolonged drug releases (8 h) with buoyancy of about 10 h.

Ghodake *et al*., 2010, developed metformin hydrochloride floating microspheres using HPMC K4M and Eudragit RS 100 polymer and characterized for particle size, FTIR, percent yield, surface morphology, and drug release study. Three months stability of best formulation was assessed at different temperatures. Entrapment efficiency was found in the range 41.14–74.19%. The entrapment was observed to decrease on increasing HPMC and decreasing Eudragit concentration. Highest floating ability was found to be 91.47% for 6 h.

Lian-Dong *et al*., 2010, developed rosiglitazone maleate sustained release floating microspheres. During formulation EC and octadecyl alcohol as carrier were used. The microspheres were investigated for morphology, drug loading, entrapment, release, and pharmacokinetic studies. Increase in EC concentration resulted in decrease of drug release. Healthy male volunteers were selected for bioavailability studies and demonstrated that floating microspheres of drug maintain better plasma concentration than commercial tablet of rosiglitazone.

Jain *et al*., 2005, reported microencapsulation of repaglinide using emulsion solvent diffusion method by calcium silicate (porous carrier) and Eudragit as polymer. The drug entrapment efficiency was good (75 ± 3%). Differential scanning calorimetry (DSC) thermogram suggests that repaglinide was slightly soluble in the selected polymer and distributed throughout the system in amorphous form. Floating ability shows that more than 80% of microspheres floated over 10 h. Statistical data reveal that release of drug at pH 2 and 7.4 was significant ($P < 0.05$) whereas at pH 6.8 was insignificant.

Kamila *et al*., 2009, investigated on rosiglitazone maleate floating microspheres of Eudragit RS 100 formulated by non-aqueous emulsification/solvent evaporation technique. *In vitro* release study and *in vivo* analysis of best preparation were studied. Size of microspheres was in the range of 596.4–813.1 μm. Statistical optimization of release was studied by a {3,3} simplex lattice mixture. It was noted that as the concentration of polymer is increased there is significant decrease in release of drug. Highest regression was obtained from Higuchi model during drug release kinetics studies. Optimized formulation's antidiabetic activity was performed and compared with pure drug on streptozocin-induced male albino rats. The lowering of blood glucose level was slower but remained for longer time up to 12 h as compared to pure drug (4 h).

Rao *et al*., 2009, reported the increment in GRT of rosiglitazone maleate by formulating floating microspheres of EC and hydroxypropyl methyl cellulose polymers utilizing solvent evaporation technique. Optimization of formulation was carried out on the basis of full factorial design. Various process variables such as drug polymer ratio, polymer concentration, and stirring rate were studied and preparations were evaluated for size, FT, entrapment, and drug release. It was observed that the concentration of EC and stirring rate influenced the evaluating parameters.

Chaurasia *et al*., 2007, formulated and *in vitro* characterized rosiglitazone maleate floating microspheres using acrylic polymers. Optical microscopy and SEM studies were performed to study size of particle and morphology respectively. Release of drug was performed at pH 1.2 without enzyme. Microspheres with good entrapment of drug and high yield were reported.

Shilorkar *et al*., 2010, studied pioglitazone hydrochloride loaded EC and HPMC K100M microspheres using 3^2 factorial designs. On the basis of characterized parameters such as size of particle, morphology, percent yield, drug entrapment, and release formulation with EC and HPMC K100M in the ratio of 12:1% at 900 rpm was selected as optimized formulation. The use of two polymers in combination increases the drug release from microspheres.

Nath *et al*., 2009, designed and evaluated metformin hydrochloride floating microcapsules. Cellulose acetate butyrate and Eudragit RL 100 both were used separately and also in combination for formulation of sustained release microspheres. Higher *in vitro* release was observed in phosphate buffer pH 6.8 then at pH 1.2. *In vitro,* data were fitted in different kinetic equations and found to follow Higuchi release than first order and finally zero order.

Salunke *et al*., 2010, prepared multiunit floating micro-carrier of metformin hydrochloride by inotropic gelation method using sodium alginate, HPMC K4M, and EC polymers. Calcium carbonate and sodium bicarbonate were used separately as gas forming agents along with drug. Prepared formulations were characterized for micromeritics properties, shape, size, yield, drug entrapment, and release. Percent yield was found to be 48.3–98.5%, which decreases with increasing polymer and gas forming agents. The results of dissolution data reveal that calcium carbonate presence showed sustained effect and burst release with HPMC K4M whereas EC acted as release retardant.

Ali *et al*., 2007, demonstrated *in vitro* and *in vivo* evaluation of prepared single unit floating metformin capsules using HPMC K4M with various grades of poly ethylene oxide (60 K, WSR 303, and WSR 301). *In vitro* floating and drug release study was completed in citrate phosphate buffer pH 3 (pH of fed state gastric fluid). Effect of different concentrations of EC, liquid paraffin, and cellulose acetate phthalate as release modifiers was also studied. Drug release was observed to follow zero order and Fickian diffusion mechanism. Gamma scintigraphy and pharmacokinetic studies were performed in rabbit.

Jain *et al*., 2009, prepared metformin hydrochloride loaded floating beads of gelucire 43/01 and characterized for size, surface morphology, entrapment, DSC, floatability, and *in vitro* release. Formed beads were hard; spherical in size (3.8–3.9 mm) with porous surface. Entrapment efficiency was not affected by increasing lipid concentration. CR of metformin hydrochloride was observed as compared to sustained release marketed product.

Reddy *et al*., 2011, developed metformin hydrochloride loaded EC microspheres by solvent evaporation method and evaluated for size, yield, entrapment, SEM, FTIR, DSC, and drug release. Results show that size of particle decreases on increasing speed of rotation and volume of processing medium. *In vitro* drug release shows initial burst release due to the presence of particles on the surface, which decreases with increasing EC concentration.

Jain *et al*., 2006, evaluated the *in vivo* performance of optimized floating microsphere of repaglinide prepared from calcium silicate. In this work organ distribution, pharmacokinetics and gamma scintigraphy studies were performed. As compared to available marketed formulation, 6 h prolonged GRT and 3.17 times increased bioavailability of prepared formulation was observed.

Tripathi *et al*., 2011, in his study formulated and evaluated hollow microballoon of glipizide using Eudragit RS100 and HPMC. Successful drug entrapment up to 76.1% was observed which decreases with increasing HPMC content. No remarkable change in the drug content of optimized formulation was observed stored at different temperatures during stability studies.

Dubey *et al*., 2012, formulated metformin hydrochloride floating microspheres using HPMC and Eudragit RS 100 polymers. Anti-hyperglycemic activity on rats was preformed along with stability study. Optimized formulation showed excellent buoyancy, good entrapment with high yield, prolonged release with diffusion mechanism, and maximum reduction (43%) of blood glucose level within 2 h. Result of stability study of optimized formulation for drug content after 90 days was found to be acceptable.

Pandit *et al*., 2013, reported pharmacokinetic and pharmacodynamic parameters of floating microspheres of metformin hydrochloride studied on diabetic rats. Relative bioavailability was increased by 3.5 folds with extended duration of action up to 14.1 h. A non-significant difference for oral glucose tolerance test was observed between control and drug treated animals, analyzed by one-way ANOVA test.

Tekade *et al*., 2013, studied various parameters of evaluation of floating microspheres of nateglinide prepared by varying concentration (0.25–1.75%) of EC. Favorable results of floatability (44–95.4%) and drug release (89.1–98.7%) after 12 h were observed.

Sarode *et al*., 2011, reported variation in release of glipizide from floating microspheres prepared by acryl coat S100, Eudragit, and EC polymers. The yield was more than 60% for microspheres were prepared by all the polymers of which 40% were found to remain floating after 12 h. Highest entrapment and release were observed for acryl coat, followed by Eudragit and EC.

Tanwar *et al*., 2007, developed verapamil hydrochloride loaded cellulose acetate, acryocoat S100 and Eudragit S100 floating microparticles using solvent diffusion-evaporation technique. Maximum % yield (70.51%) and entrapment efficiency of prepared formulation were with cellulose acetate polymer. Release rate of drug was influenced by polymer and was reported to be in the order cellulose acetate >acryl coat S100 >Eudragit S100. Higher regression values were obtained for zero-order equation. The *in vivo* buoyancy of barium sulfate loaded optimized formulation of cellulose acetate was studied by X-ray photography on the stomach of beagle dog, which showed buoyancy of microspheres for about 3.2 h over gastric fluid.

Jain *et al*., 2006, in their study prepared calcium silicate (carrier based) floating microspheres of orlistat by Eudragit S polymer. Characterization followed by scintigraphy study of best formulation was performed and compared with non-floating formulation. Release rate of microspheres without carrier was observed to be more as compared to calcium silicate based microspheres. Highest regression was observed for Higuchi matrix and Peppas-Korsmeyers model. Gamma scintigraphy studies showed higher retention of 6 h for floating than 2 h for non-floating microspheres. Increased t_{max}, AUC, and elimination half-life of optimized formulation as compared to marketed preparation were observed during pharmacokinetic study.

El-Kamel *et al*., 2001, formulated and evaluated ketoprofen loaded floating microspheres. Formulations with various concentrations of Eudragit S100 and RL grades were prepared by emulsion solvent diffusion technique.

Maiti *et al*., 2009, designed alginate facilitated w/o/w emulsion solvent evaporation technique for formulation of EC loaded floating microspheres of fluconazole. Increase in polymer concentration during formulation increases the % yield, size, and entrapment of drug whereas the release rate was observed to decrease. X-ray diffraction (X-RD) and DSC results showed that drug was entrapped in amorphous form. Optimized formulation showed good *in vitro* antifungal activity against *Candida albicans* fungus.

Badve *et al*., 2007, prepared hollow beads of calcium pectinate loaded with diclofenac sodium by the process of evolution of carbon dioxide during cross linking in acidic medium. Beads were characterized for size, floatability, interaction studies, SEM, porosity, drug release, and scintigraphy studies. Gamma scintigraphy studies of optimized formulation on rabbit showed residence of floating beads for 6 h which is more as compared to non-floating beads (2 h).

Joseph *et al*., 2002, formulated piroxicam loaded microspheres of polycarbonate by solvent evaporation technique. Release of drug showed no burst effect and was found to be less in SGF as compared to intestinal fluid. Healthy rabbits were selected for performing *in vivo* evaluation of free drug, drug encapsulated microspheres, and formulation with loading dose. Highest concentration C_{max} (4.3 µg/ml) for microspheres with loading dose in 8 h was observed.

El-Gibaly, 2002, compared the release of melatonin from floating and non-floating microspheres formulated by ionic reaction of chitosan with surfactant sodium dioctyl sulfosuccinate (DOS) which is negatively charged and sodium tripolyphosphate, respectively. Influence of time of cross linking, ratio of drug/polymer, and concentration of DOS and chitosan were studied. The influence of cross-linking time factor on release of drug showed no significant effect in floating microspheres whereas decreased rate was observed for non-floating formulations. Higher regression values were obtained for zero-order kinetics, representing time-dependent release pattern.

Satoa *et al*., 2003, investigated the effect of temperature during formulation on cavity formation and buoyancy of floating microspheres. Author also studied the entrapment efficiency of five drugs (aspirin, salicylic acid, ethoxybenzamide, indomethacin, and riboflavin) with different water solubility. Hollow microspheres were developed by emulsion solvent diffusion technique using polymer Eudragit S100 and monostearin as wall membrane-reinforcing agent. The appropriate temperature was observed to be 40°C for the ideal production of hollow buoyant microspheres. Decrease buoyancy accompanied with increased porosity was observed with increasing concentration of riboflavin. Good entrapment (54%) even at low distribution coefficient of riboflavin was observed.

Patil *et al*., 2009, reported the retention and release of floating acyclovir microspheres formulated by emulsion solvent diffusion process using EC of different viscosities. Prepared formulations were characterized for size, entrapment, floating ability, and release. Physical state of acyclovir and polymer was determined by SEM and X-RD. Increase in drug release with increasing plasticizer (triethyl citrate) concentration from 10 to 20% was observed. EC (50 cps) showed better release and floating ability than 100 cps.

Sungthongjeen *et al*., 2006, designed theophylline loaded core pellets coated with sodium bicarbonate as inner effervescent layer and Eudragit RL 30D, RS 30D, and NE 30D as outer polymeric membrane prepared by extrusion-spheronization process. Formulation with Eudragit RL 30D as polymeric membranes floated successfully with 80% drug release, which successively decreased as the concentration of polymer coating increases from 0 to 10%.

Iannuccelli *et al*., 1998, prepared and evaluated a multiple-unit air compartment system consist of calcium alginate core separated by an air compartment from calcium alginate with or without PVA membrane. Author studied SEM, buoyancy, artificial gastric juice uptake and effect of different concentration and molecular weight of PVA on floating ability. Permeation rate was observed and was found to be highest for 5% PVA (10,000).

Stops *et al*., 2006, prepared and *in vivo* analyzed calcium alginate beads (radio-labeled) of riboflavin under fasting state administered either with an aqueous solution of citric acid or water. Gastro-

retention data were obtained by gamma scintigraphy and are better for formulation administered with citric acid.

Tang *et al*., 2007, synthesized multi-unit floating gel beads of ibuprofen, niacinamide and metoclopramide HCl (different hydrophilicity) with calcium alginate and sunflower oil using emulsification/gelation process.

Jain *et al*., 2009, prepared famotidine loaded microspheres of acrycoat S100 and cellulose acetate polymers. The effect of rate of stirring and concentration of polymer on size of particle and release of drug was studied. Microspheres were characterized for micromeritics, entrapment, buoyancy, drug release, kinetic study, and *in vivo* floating ability.

Shaji *et al*., 2009, had designed and optimized gastroretentive dosage form of famotidine using PMMA. Prepared formulations were successfully characterized and found to follow either zero-order or Higuchi kinetics release.

Veda Hari *et al*., 2010, had prepared and evaluated nevirapine loaded alginate beads by inotropic gelation method using calcium carbonate as gas forming agent and HPMC polymer. Increase in concentration of gas forming agent resulted in decreased drug loading where by floatability and drug release increases.

Choi *et al*., 2002, described the effect of calcium carbonate and sodium bicarbonate as gas forming agent on release of riboflavin from alginate beads. Results of *in vitro* data shows extended release of riboflavin with calcium carbonate beads.

Ganesan *et al*., 2013, prepared and evaluated floating hollow microspheres of telmisartan using HPMC and Eudragit RS100 polymers. Effect of solvent composition, polymer, and temperature was investigated. Overall decrease of entrapment and yield was observed with increasing amount of dichloromethane. 2:1 ethanol: dichloromethane ratio and 35–40°C temperature were optimum for preparation of microballoon.

Stops *et al*., 2006, had studied the purpose of citric acid to increase the gastroretention of beads of calcium alginate in fed condition on healthy human volunteers. Gamma scintigraphy study was performed for monitoring of radio labeled beads administered with water or citric acid. Author reported prolonged retention of formulation taken with citric acid than without citric acid.

Reddy *et al*., 2010, developed and characterized cyclobenzaprine hydrochloride loaded EC microspheres.

Najmuddin *et al*., 2010, developed floating microspheres of ketoprofen by utilizing Eudragit S and L100 polymers. Best results of micromeritics properties, % yield, and entrapment with sustained release of drug were observed for formulation with 1:2 drug:polymer ratios.

Iannuccelli *et al*., 1998, performed *in vivo* study of control release and floating system at three different conditions, that is, fasted, fed state after meal, and after a succession of meals. The floating units composed of a core of calcium alginate which is separated by calcium alginate: PVA membrane as an air compartment. X-ray studies were performed for studying GRT data. All floating units floats up to 5 h in fed state unlike control release which sinks at the same time, where by prolongs GRT by 2 h. In fed state after successive intake of food, buoyancy of 6 h with prolonged GRT of 9 h was observed for floating unit as compared to control unit.

Garg *et al*., 2010, prepared gastroretentive microspheres of silymarin using HPMC:EC and Eudragit S100: RL polymers. The Higuchi kinetics and non-Fickian release mechanism from microspheres were studied and reported.

Gattani *et al*., 2008, developed floating microspheres of diltiazem hydrochloride by emulsification solvent evaporation method with two different polymers EC and Eudragit RS100.

Kouchak *et al*., 2007, formulated floating microspheres of theophylline using EC and dibutyl phthalate. Formulations were physically characterized for shape, size, buoyancy, drug loading, and release study.

Sonar *et al*., 2007, developed rosiglitazone maleate floating-bioadhesive and bilayer tablets by sodium bicarbonate and HPMC. Various parameters such as buoyancy lag-time, drug release, detachment force, and swelling ability were successfully measured. Gamma scintigraphy study was performed to evaluate floating ability of the optimized batch.

Sudharshini *et al*., 2010, designed floating tablets of glipizide by solvent casting sintering technique with HPMC K4M and K15M polymers. More than

22 h of FT and less than 5 min of floating lag time were observed for the tablets. *In vitro* drug release follows Peppas model with non-Fickian diffusion.

Mallikarjun *et al*., 2009, had prepared glipizide loaded floating tablets with sodium carbonate as gas-forming agent and HPMC K4M and K15M polymers. Results showed uniformity of weight, appreciable drug content, appreciable floating, and CR of glipizide. Longer duration of buoyancy was observed for tablets prepared by HPMC K15M.

Khan *et al*., 2012, developed and compared release of gliclazide from tablets formulated by various viscosity grades of hypromellose. HPMC K4M, K100M, and K100 of CR and Direct compression (DC) grades were used for preparing tablets by DC method. Higher release was observed for higher viscosity grade HPMC and improved release of DC than CR grade was observed.

Kshirsagar *et al*., 2009, had studied use of two viscosity grades of HPMC (K15M and K100) with or without Carbopol for formulation of metformin hydrochloride floating tablets. Drug release data of optimized batch prepared by HPMC K15M and Carbopol were fitted to different kinetic models. Highest release was reported for Korsmeyer and Peppas model.

Raju *et al*., 2010, developed metformin hydrochloride floating tablets using HPMCK100M, Carbopol 934P and sodium bicarbonate by wet granulation method. Formulation prepared from Carbopol shows least floating lag time (2 min 34 s) and 91.2% drug release at the end of the study. Diffusion CR of drug which follows first-order kinetics was observed during the study.

Silvabalan *et al*., 2011, prepared and evaluated glipizide loaded floating tablets using HPMC, methylcellulose, and EC polymers. Various parameters such ass weight variation, hardness, friability, drug content, buoyancy, and release were determined.

Patel *et al*., 2010, prepared and evaluated bioadhesive-floating tablets of glipizide using HPMC/chitosan or Carbopol 934/PMA along with citric acid and sodium bicarbonate as effervescent agents. Carbopol 934 shows more bioadhesion than chitosan. Author reported formulation with 20% HPMC - 80% chitosan or 80% Carbopol 20% PMA to be optimum for bioadhesion and for prolonged drug release.

Jamzad *et al*., 2006, developed glipizide tablets with swellable HPMC and erodible PEO polymers by DC technique. Author focused on statistical analysis of release data along with hydration, erosion studies, textural analysis, and swelling behavior. Replacement of high molecular weight HPMC K100M with K15M increases drug release by 90%. Higher rate of swelling and hydration with strong texture of HPMC than PEO was reported. Glipizide tablets found to follow zero-order release kinetics during the study.

Agarwal *et al*., 2010, prepared floating tablets of cinnarizine hydrochloride using different viscosity grades HPMC (K100 LV, K4M, K15M, and K100 MCR) and sodium bicarbonate or calcium carbonate as gas forming agent. Drug release, kinetics, and bioavailability of best formulation were performed. Decrease in drug release was observed on increasing viscosity of polymer. Slowest release was reported with formulation containing HPMC K100 MCR. Sodium bicarbonate shows better release characteristics than calcium carbonate. Non-Fickian diffusion was observed from all the prepared formulations. Bioavailability of optimized formulation was compared with cinnarizine suspension and prolonged plasma drug concentration was observed.

Fassihi *et al*., 2000, in his study compared the buoyancy and release of matrix tablet of verapamil hydrochloride using USP dissolution apparatus I and II with additional effect of single ring or double mesh device below the paddle of apparatus. It was reported that dissolution profile with apparatus I along with single and double mesh devices have insignificant variation during initial release profile (5–6 h). Author suggested the use of double mesh device for the study of true kinetics of sticking and floating delivery system.

Shah *et al*., 2004, in his work studied the release of chlorpheniramine maleate (polar) and diazepam (non-polar) from glycerol monooleate matrix. Effect of PEG 4000, PEG 10000, and stearic acid on floatability and release was also studied. Gamma scintigraphy studies revealed the retention of matrix in the stomach for 5–6 h. Surge in loading of polar drug, increases water content and is higher for chlorpheniramine maleate. The

presence of stearic acid retards the release of both the drugs.

Jain *et al*., 2010, utilized statistical optimization technique and prepared ranitidine hydrochloride loaded floating tablets. The formulations were prepared by 3^2 factorial design, with two independent formulation variables (ratio of HPMC 100 KM: Xanthan gum and concentration of Aerosil) and four dependent variables (drug release, $T_{50\%}$, floating lag time, and hardness). Drug release mechanism shows non-Fickian transport. An increase in release of drug and hardness was observed with increase in polymer and Aerosil concentration.

Rajinikanth *et al*., 2008, developed floating *in situ* gelling system of clarithromycin using gellan and calcium carbonate. Sucralfate was additionally added to suppress the degradation of drug at low pH. Polymerase Chain Reaction and microbial culture techniques were used to compare the *Helicobacter pylori* clearance efficacy on infected Mongolian gerbils. Author reported the prolonged retention of drug and increased stability contributes better for eradication of *H. pylori*.

Campos-Aldrete *et al*., 1997, reported the release of metronidazole from floating tablets prepared from various viscosity grades and particle size of HPMC. Different viscosity grades HPMC 15, 860, 5000, 20,000, and 30,000 cps and particle sizes of 163, 213, 335, and 505 pm were selected for the study. The author reported that on increasing HPMC viscosity, burst effect increases whereas release rate of drug decreases. Increase of particle size leads to gel barrier formation thereby, increasing burst effect and release rate.

Mastiholimath *et al*., 2008, prepared ranitidine hydrochloride loaded EC microspheres for treatment of ulcer. Free flowing properties of prepared microspheres with high entrapment efficiency (96%) were reported. Improvement in bioavailability was observed during *in vivo* analysis on rabbits. Author reported effective delivery of drug with improved oral bioavailability.

Sudhamani *et al*., 2010, developed EC microspheres of ibuprofen by solvent evaporation technique. Best *in vitro* release profile was observed for formulation containing 1:2 ratios of drug and polymer over a period of 8 h.

Rao *et al*., 2005, formulated and evaluated microspheres of zidovudine using EC polymer. Double emulsification (w/o/o) solvent diffusion technique was used to prepare microspheres using span 80 as surfactant. Free flowing, spherical, and smooth surface microspheres were formed with 41–55% entrapment efficiency.

Shrivastav *et al*., 2005, developed successful preparation of cimetidine loaded floating microspheres using EC and HPMC. During the study, effect of stirring rate, concentration of polymer, composition of solvent, and media used for dissolution on the size and drug release of microspheres was the major concern. Increase in particle size with decreased drug release was observed at higher polymer concentration.

Karthikeyan *et al*., 2010, prepared floating microspheres of cefpodoxime proxetil by non-aqueous solvent evaporation technique with different ratios of HPMC K4M and EC. Results of evaluation revealed that formulation with 1:2 ratios of EC and HPMC K4M is quite effective.

Singh *et al*., 2010, formulated and characterized microsphere of famotidine using EC and HPMC as control release polymers. Results of parameters used for characterization were satisfactory and best results were obtained for microsphere prepared with ratio of 1:6 of HPMC: EC.

Goyal *et al*., 2006, estimated repaglinide in tablet formulation using visible spectrophotometric methods.

Dhole *et al*., 2012, compared Ultraviolet (UV) spectrophotometric and High Performance Liquid Chromatography (HPLC) method for the determination of repaglinide in tablets.

Baravaliya *et al*., 2013, developed analytical method of repaglinide in bulk and single component formulation.

Fouad *et al*., 2014, developed and validated chromatographic and spectroscopic methods for simultaneous determination of repaglinide and metformin HCl in single dosage form.

Sharma *et al*., 2011, studied simultaneous spectrophotometric estimation of repaglinide in pharmaceutical dosage form using *Indigo carmine*.

Makwana *et al*., 2012, simultaneously estimated pharmaceutical dosage form of metformin and repaglinide by difference spectrophotometric method.

Xavier *et al*., 2013, developed and validated two stability-indicating UV-spectrophotometric methods for estimation of repaglinide in bulk and dosage forms.

Patel *et al*., 2015, developed and validated method for simultaneous estimation of metformin HCl and repaglinide by spectrophotometer in bilayer tablet.

Prameela *et al*., 2009, determined repaglinide in pharmaceutical formulations by reversed-phase HPLC (RP-HPLC) method.

Sharma *et al*., 2011, developed stability indicating RP-HPLC method to determine repaglinide in dosage form and perform its validation.

Tiwary *et al*., 2010 perform *in vitro* analysis of repaglinide by spectrofluorimetric and HPLC method.

Jirovsky *et al*., 2010, determines repaglinide in human plasma by HPLC using dual channel coulometric detector.

OBJECTIVE OF RESEARCH WORK

Oral CR delivery systems were developed to provide the drug in predictable time period there by increases the efficacy, reduce side effects, and helps in increasing drug's bioavailability [Table-1.1]. Apart from various routes of drug administration's the safest, simple and suitable are the oral route due to several advantages such as cost effectiveness, ease of administration, and compatible with patient. The drawbacks of conventional dosage forms can be overcome by advancing techniques which have led to the development of CRDDS. Such new approaches help in revolutionize medication which could provide several therapeutic benefits.

Drugs having short half-life and prime absorption from the GIT removes quickly from the blood circulation and thus required repeated dosing. To overcome such problems, formulation of oral CRDDS is emphasized. CR formulations impart slow release of drug into the GIT and maintain a fixed therapeutic concentration of drug for longer period of time in the serum.

Tremendous work is being carried out for the treatment of diabetes, by formulating antidiabetic drug loaded system, in spite of this the major problem occurs in ensuring proper drug delivery for better absorption, compliance concerning its delivery and enhanced bioavailability. Gastric region is the site where gastroretentive systems can remain for various hours and thus significantly increase the GRT of drugs. Gastroretentive systems have successfully progressed as an effective means for increasing bioavailability and control release of many drug substances. Literature reveals several reported approaches such as mucoadhesion, floatation, expansion, modified shape systems, sedimentation, or using simultaneous administration of pharmacological agents which decreases gastric emptying time.

For drugs having poor bioavailability FDDS offers a number of applications. Formulation containing drug retains at absorption site, thereby increases the bioavailability of medicament by its continuous release for longer period of time. It is particularly advantageous for chemical entities which either act or primarily absorbed in the stomach while remain unstable in the intestine or colon, having poor solubility in alkaline pH or having narrow absorption window.

The purpose of the present research work is to formulate and evaluate formulation which will deliver the drug in controlled manner thus leads to reduce dosing frequency, shows better absorption, decreases side effects, improves systemic bioavailability, and maintains a constant therapeutic plasma level. Present research work is envisaged to develop and to evaluate floating microspheres of repaglinide as model entity to increase GRT using EC and HPMC as polymer.

Plan of work

Proposed methodology during the tenure of the research work:

- Literature survey.
- Selection of drug and polymers.
- Preformulation studies.

Optimization of formulation variables during the production of batches.

- Polymer ratio
- Stirring rate
- Concentration of drug
- Concentration of emulsifier.

Preparation of optimized microsphere formulation batches.

Characterization of drug loaded formulations.
Micrometric properties

- Morphology (SEM)
- Percentage yield
- Entrapment efficiency
- Percent buoyancy
- *In vitro* release of drug or kinetic studies.

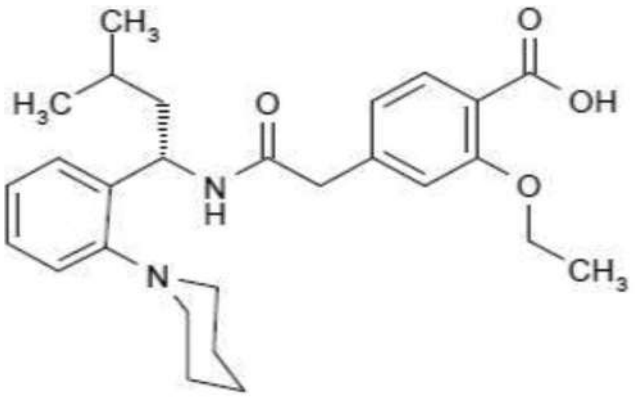
Stability study.
Compilation and analysis of data's, interpretation of results, and final conclusion.

DRUG AND POLYMERS PROFILE

Repaglinide (Prandin) a member of meglitinide class used orally for secretion of insulin was selected for the present investigation. Repaglinide is indicated only in type II DM as an alternative to sulfonylurea's, or to supplement metformin/ long acting insulin. It should be avoided in the liver disease. Repaglinide is the first derivative of Meglitinide group developed to normalize the elevated glucose level after meals. It represents fast onset of action with short lasting insulin release.

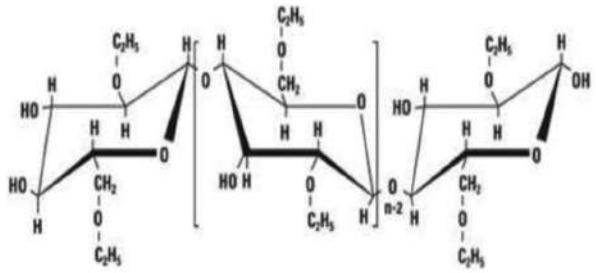
Repaglinide – drug profile (NovoNordisk; Drugs.com)

Chemical structure



IUPAC name	S(+) 2-ethoxy-4 (2((3-methyl-l-(2-(l-piperidinyl) phenyl)-butyl) amino)-2-oxoethyl) benzoic acid.
Molecular weight	$C_{27}H_{36}N_{204}$
Molecular Formula	452.6 g/mol
Color	Ranging from white to off-white
Solubility	Slightly soluble in water, soluble in PBS pH 6.8, 0.1 N HCl, 0.1N NaOH, alcohols, acetonitrile, chloroform.
Melting point: 129–130.2°C	
PKa	3.9 (in acidic) and 6.0 (in amine)
Category	New derivative of oral hypoglycemic drug, of meglitinide class. It is a carbamoylmethyl benzoic acid.
Description	Repaglinide comes under the category of organic compounds known as phenylpiperidine. It is a phenylpiperidine skeleton in which piperidine ring is bound to a phenyl group. Repaglinide is specifically used as monotherapy to control meal related (prandial) glucose fluctuation in patients having Type II diabetes.
Dosage and Administration	Repaglinide do not have fixed dosage schedule to fight against Type II diabetes. Periodical monitoring of blood glucose is necessary to know the minimum effective dose of drug. For observing, the long-term response of patient toward therapy value of glycosylated hemoglobin levels is important. Doses of repaglinide should be administered within 15 min of eating food whereas time period can change from immediately intake of food as long as about 30 min before taking the food.
Starting Dose	The starting dose should be 0.5 mg for untreated patients and 1 or 2 mg for patients taking other hypoglycemic agents along with each meal, respectively.
Dose Adjustment	By observing level of fasting blood glucose level (70–130 mg/Dl), adjustments in dosing are done. For patients with satisfactory fasting glucose level (70–130 mg/dL) but inadequate overall glycemic control (HbA1c) postprandial glucose level is tested. The pre-prandial dose of drug should be doubled up to 4 mg with every intake of food until satisfactory level of blood glucose is achieved. The recommended dose range is 0.5–4 mg with meals. It can be dosed pre-prandial up to 4 times a day in accordance with varying pattern of meal taken by the patient. The maximum dose of 16 mg is recommended per day.
Combination Therapy	If monotherapy of repaglinide results in inadequate glycemic control, thiazolidinedione, or metformin can be added. The combination therapy dose is similar as that for monotherapy. To calculate the minimum dose required to achieve the desired pharmacological response dose of each drug must be adjusted carefully otherwise incidence of hypoglycemic episodes may increase.
Available dosage forms	Tablets are available as unscored, biconvex tablets with 0.5, 1 and 2 mg strengths having white, yellow and peach color, respectively, to indicate strengths. Symbol of Novo Nordisk (APIs) is embossed on every tablet.
Bioavailability	56%
Half-life	1 h
Metabolism	Drug administered by oral or iv route is completely metabolized through oxidative biotransformation and glucuronic acid conjugation process. The aromatic amine (Ml), oxidized dicarboxylic acid (M2), and acyl glucuronide (M7) are the major metabolites. For N- dealkylation of drug to M2 and the further oxidation to Ml particularly 2C8 and 3A4 of cytochrome P-450 (CYP) enzymes were used. No reduction in glucose level is contributed by any of the metabolites of the drug.
Excretion	Biliary-fecal route. Within 96 h after oral dosing approximately 90% is eliminated in the feces (<2% as unchanged drug) and about 8% in the urine. In the form of unchanged drug in the urine about 0.1% of the dose is cleared. The major metabolite (M2) accounted for 60% of the administered dose.
Plasma protein Binding	Repaglinide is highly protein bound about>98% particularly-acid glycoprotein.

Polymers profile

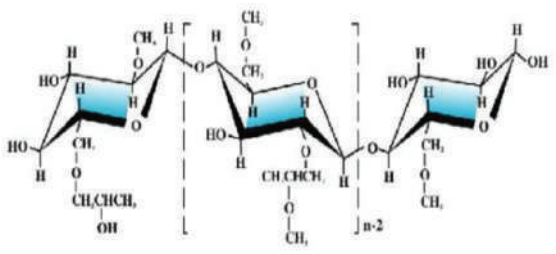


3.2.1 Ethyl cellulose (IP 1996)	
Synonym	Ethocel, Aquacoat ECD, Aqualon E462, Surelease. Cellulose ethyl ether.
Chemical name	Ethocel, Aquacoat ECD, Aqualon E462, Surelease.
Empirical formula	Cellulose ethyl ether.
Category	Pharmaceutical aid
Description	A white to light tan powder almost odorless.
Solubility	Soluble in alcohol, chlorinated solvents, and natural oils whereas practically insoluble in water glycerin and propylene glycol

Physical properties	
Bulk density	0.3–0.4 g/cm^3
Specific Gravity	1.12–1.15 g/cm^3
Softening point	158–162°C
Refractive index	1.47

Incompatibility	Incompatibility with paraffin wax and microcrystalline.
Safety	Ethyl cellulose is non-toxic, non-irritating and non-allergenic.
Storage	Stored between 7 and 32°C in dry area away from heat in a well close container.
Stability	It is resistant to salt solution and to both dilute and concentrated alkali. It is more sensitive to acidic material and undergoes oxidative degradation at increased temperature.
Handling Precautions	Fine particles of ethyl cellulose must be prevented from reaching potentially explosive levels in air. Repaglinide is combustible and may cause irritation to eyes.
Labelling	The label states the apparent viscosity in mPas of a 2.0% w/v solution and its ethoxy content.

Hydroxypropyl methyl cellulose (Lin et al., 1986)



Synonym	Methyl hydroxypropyl cellulose, propylene glycol ether of methyl cellulose, hypromellose, methocel.
Chemical Name	Cellulose, 2-Hyrdoxypropylmethyl ether.
Empirical Formula	$C_{56}H_{108}O_{30}$
Structural Formula	

Category	USP: Suspending and or viscosity increasing agent, tablet binder, coating agent. BP: Viscosity increasing agent, adhesive anhydrous ointment ingredient. Others: Film former, emulsion stabilizer
Description	A white or yellowish white powder; almost odorless and hygroscopic on drying.
Grades	Various grades of different viscosity are available, differentiated by a number which indicates the apparent viscosity of a solution (2% w/v) in mPas measured at 20°C. For example: HPMC K100 (100cps) and HPMCK4M (4000cps).
Solubility	In cold water it is soluble forming a viscous colloidal solution, insoluble in alcohol, ether and chloroform whereas soluble in mixtures of methanol and methylene chloride.
Physical properties	
Softening point	170–180°C
Melting point	Brown at 200°C and chares at 230°C.
Pharmacopeia specification	19–24%
Methoxyl content	7–12%
Hydroxypropyl content	20 ppm max
Heavy metal	1.5%
Residue on ignition	10% max
Loss on drying	
Sulphated ash	
Safety	It is non-toxic, non-irritant but excessive oral consumption causes laxative effect.

Storage	Stored in a well close container protected from moisture.
Stability	Stable between pH 3–11 and viscosity of HPMC solution reduced with increase in temperature.
Handling	HPMC dust causes irritation to eyes hence safety of eyes is recommended. Generation of large quantity of dust must be avoided to reduce the risk of explosion.
Labeling	The label states the apparent viscosity of a 2.0% w/v solution in mPas.

EXPERIMENTAL PROCEDURE

Introduction

For designing a dosage form with consistent and controlled residence in the stomach, an important strategic consideration is selection of suitable excipient .[Table-5.1]. Floating properties of dosage

forms are anticipated to be improved using high molecular weight and less hydrophilic polymers. Non-effervescent systems can be developed using number of polymers including swellable and gel forming cellulose derivative hydrocolloids (e.g., HPMC, hydroxyethyl cellulose, and high substituted hydroxypropyl cellulose), chitosan or Carbopol derivatives, polysaccharides, and matrix forming polymers (e.g., polycarbonate, polyacrylate, polymetacrylate, and polystyrene). For creation of a suitable dosage form excipients and active pharmaceutical ingredient (APIs) is mixed to for powder blends (Dorozynski *et al*., 2004, Khattar *et al*., 1990).

In course of drug release, the outer layer of the formulation gets hydrated and swell as it come in contact with the gastric fluid due to diffusion of the aqueous medium inside the delivery system from gastric region. The average density of the preparation decreases as the air entraps in the gel forming gelatinous layer. A relative integrity of shape and bulk density less than that of the medium can be maintained by the structure. The diffusion of medium into the preparation decreases as the thickness of the swollen layer increases (Ju *et al*., 1995). During the process, active ingredient from one layer to another is dissolved and is released through diffusion controlled mechanism. Thus, coherent gel structure is created which acts as reservoir for the sustained release of the API (Sheth *et al*., 1984). The first hydrated swollen layer gets dissolved during hydration then further layers get hydrated after its detachment. This technology is the key of HBS™ and several commercially available products work on the basis of this system. Technologically non-effervescent floating dosage forms are of following types: Microporous systems, alginate beads, and hollow microspheres. During development of multiple-unit floating system alginate beads and hollow microspheres plays significant role. Microballoons another terminology of microspheres are prepared by dissolving suitable polymer(s) in organic phase in which drug is either dispersed or dissolved, followed by emulsification in aqueous solution containing surfactant(s). Organic solvent is removed by evaporation and an emulsion of O/W type is prepared. A cavity is formed by precipitation of polymer onto the surface of remaining drops resulting in formation of hollow spheres.

Among several polymers used for formulation of FDDS, EC, and HPMC have number of applications. Floating microspheres using EC and HPMC recently have received much attention (Mastiholimath *et al*., 2008; Vaghani *et al*., 2010) for delivering drug in controlled manner in the stomach. EC is non-polar dissolves in several organic solvents, biocompatible, non-toxic, non-biodegradable, and non-irritant cellulose derivative (Das *et al*., 2006). EC can clearly slow down the rate of drug release rate and is especially effective for water-soluble drugs.

Among cellulose ether derivatives, HPMC is commonly used in the formulation of CR dosage forms. HPMC hydrates in water, forming a gel layer at the matrix periphery. Release of drug from HPMC is controlled by combination of diffusion through and erosion of gel. HPMC has a number of advantages such as non-toxic, relatively inexpensive, it can be directly compressed into matrices and the many grades available helps in the ability to tailor desired drug release profiles (Mitchell *et al*., 1990).

HPMC being very versatile release agents and its non-ionic property helps in minimizing problem of interaction with several acidic, basic, or other electrolytic systems. The polymer works successfully with several drugs having variable solubility and at various levels of doses (Lin *et al*., 1986). After studying, the properties of EC and HPMC such as entrapment efficiency and holding capacity EC and different viscosity grades of HPMC were selected to prepare sustained release microspheres. While using HPMC matrix system, release of drug was greatly affected by type of polymer, ratio of polymer used in preparation, and viscosity of polymer (Vueba *et al*., 2004).

In the present study, EC and HPMC (5, 100, and 4000 cps) microspheres alone and in combinations were used for preparation of microspheres using solvent evaporation technique and perform its evaluation.

Preparation of Rapeglinide (RG) loaded EC floating microspheres

Floating microspheres were prepared by slightly modifying solvent diffusion-evaporation method

(Kawashima *et al*., 1992). EC and 0.1% of PEG (as surfactant) both were dissolved in 1:1 mixture of ethanol and dichloromethane at room temperature. Drug was dispersed to this polymeric solution. The slurry was slowly introduced into 80 ml of water containing PVA emulsifier (0.46% w/v). The system was stirred using propeller agitator for about 1 h to evaporate the organic solvent. Microspheres prepared were washed properly 3–4 times with distilled water, dried at room temperature for about 1 h, and finally kept in desiccators containing fused calcium chloride. Compositions of different formulations prepared are shown in Table 6.1.

Effect of process variables on microspheres

For optimization of drug-loaded floating microspheres of EC following process variables were studied:

- Effect of varying polymer ratio.
- Effect of varying drug concentration.
- Effect of varying emulsifier concentration.
- Effect of varying stirring rate.

Characterization of EC floating microspheres

Micromeritics properties (Aulton, 2002, Jain et al., 2006, Hanna, 1990)

The prepared microspheres were characterized for micromeritics properties such as particle size, bulk density, tapped density, and angle of repose.

a. Particle size: Optical microscope was used to determine the particle size of prepared microspheres. In distilled water dried microspheres were dispersed and suitably placed on a glass slide. Using stage micrometer, the number of divisions of the eye piece was counted. 200 microspheres were randomly selected and there mean particle diameter was measured using calibrated ocular micrometer. Using Edmundson's equation (Rawat *et al*., 2007) average particle size was determined.

$$D_{mean} = \frac{\Sigma Nd}{\Sigma N} \quad (2)$$

Where, n= number of microspheres counted; d=mean size.

a. Bulk density: The mass of powder divided by bulk volume is termed as bulk density. Bulk volume is the true volume and it includes the void space among the microspheres. Accurately weighed 25 g of samples were filled in a 50 graduated cylinder and reported for the unsettled level as bulk volume (Vb). Using the formula given below bulk density is calculated and its unit is g/cm^3

$$\text{Bulk Density (BD)} = M/V \quad (3)$$

Where, M = mass of powder taken (g) and Vb= bulk Volume (cm^3)

b. Tapped density: The samples were weighed accurately to 25 g and filled in a 50 ml graduated cylinder and report the tapped volume (Vt). Tapped density tester was used and 100 drops per minute mechanical tapping of the cylinder was done. Constant volume was observed and reported as tapped volume Vt (cm^3). Using the formula given below tapped density was calculated in g/cm^3.

$$\text{Tapped density} = \frac{M}{V_t} \quad (4)$$

Table 6.1: Formulation code and composition of EC microspheres

Batch code	Amount of EC (mg)	Concentration of emulsifying agent (%)	Stirring rate (RPM)	Amount of drug (mg)
E1	10	0.46	900	10
E2	20	0.46	900	10
E3	30	0.46	900	10
E4	20	0.46	600	10
E5	20	0.46	1200	10
E6	20	0.66	900	10
E7	20	0.86	900	10
E8	20	0.46	900	20
E9	20	0.46	900	30

EC: Ethyl cellulose

c. Carr's Index or Compressibility Index (Raut *et al*., 2013)

Carr's index also called as compressibility index is an indication of compressibility of powder. The bulk density and tapped density of free-flowing powder are close in value; thus, the value of Carr index will also be small. Whereas for poor-flowing powder difference between two densities is greater due to greater interparticle interactions thereby, the value of Carr index will also be larger.

$$\text{Carr's index} = \frac{\text{Tapped density-Bulk density}}{\text{Tapped density}} \times 100 \quad (5)$$

According to Carr's index flow properties of the powder is mentioned below

Carr's index %	Flow
5–15	Excellent
12–16	Good
18–21	Fair to passable
23–25	Poor
33–38	Very poor
>40	Very very poor

d. Hausner Ratio

Ratio of tapped density to bulk density is also a measure of flow properties and is termed as Hausner ratio.

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad (6)$$

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

e. Angle of repose

The maximum angle formed between the surface and the horizontal plane is termed as angle of repose. Fixed funnel method was used to determine the resistance to particle flow of prepared microspheres. Funnel height was kept in such a manner that its tip just touches the heap of the blend. On a stand a glass funnel is placed with the support of ring over a glass plate. With the help of lower thumb the orifice of the funnel is blocked and an average 100 g of microspheres was placed into the funnel. Angle of repose is determined by formula mentioned below from the pile which is formed by flow of particles as the thumb is removed from the orifice.

$$\tan \Theta = h/r$$

$$\Theta = \tan^{-1}(h/r) \quad (7)$$

The results of micromeritics properties of EC formulations are reported in Table 6.3.

Angle of repose	Flowability
<25	Excellent
25–30	Good
30–40	Passable
>40	Very poor

Batch code	Mean particle size (μm)	Bulk density	Tapped density	Carr's index (%)
E1	187.12±7.2	0.71±0.01	0.80±0.05	11.25
E2	200.01±6.1	0.73±0.09	0.85±0.03	14.11
E3	234.21±10.2	0.74±0.04	0.87±0.04	14.94
E4	243.41±9.1	0.75±0.03	0.89±0.06	15.73
E5	188.17±4.6	0.70±0.02	0.82±0.02	14.63
E6	185.29±9.0	0.72±0.02	0.84±0.01	14.28
E7	174.41±11.4	0.71±0.02	0.81±0.05	12.34
E8	235.32±5.1	0.73±0.14	0.89±0.04	17.97
E9	240.42±3.9	0.77±0.21	0.88±0.05	12.50

All values are represented as mean±SD (n=3)

Morphological study using SEM

SEM was used to study the morphology of prepared microspheres which helps in correlating characteristics features at surface of the samples. SEM is better than light microscope as higher resolution maximum up to 10–20 nm was obtained as compared to 200–300 nm from light microscope. SEM studies were carried out using Jeol JSM-1600, Tokyo, Japan. Prepared microspheres were lightly sprinkled on a double adhesive tape which is fixed to aluminum stubs. A thin layer of gold about 300°A was vacuum coated using a sputter coater and samples were randomly scanned and photographs were taken. The SEM images obtained are shown in Figure 6.1.

FTIR spectral analysis

Investigation of any sample by FTIR confirms the chemical integrity between drug and polymer used in formulation. Bruker (Lab India), Germany FTIR Spectrometer was used to obtained the spectra. Scanning range of 400–4000 cm^{-1} with resolution of 1 cm^{-1} was selected. Sample was prepared by mixing 1 mg of formulation with 300 mg of dried powder of potassium bromide (FTIR grade) which is then uniformly spread in the die and compressed

under vacuum at a pressure under 10 ton. Prepared disk was mounted in the holder in the FTIR spectrophotometer and spectra were recorded. The spectrum is shown in Figure 6.2. In the spectra positions and relative intensities of the absorption bands obtained for the pure drug, place bo and RG loaded microspheres were compared and reported in Table 6.5

Percentage yield (Singhal et al., 2011)

The prepared microspheres of all the batches were accurately weighed. The percentage yield of floating formulations was calculated using following formula:

$$\% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of polymer and drug}} \times 100 \quad (8)$$

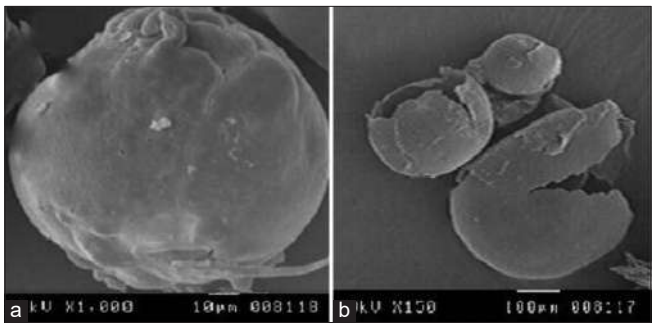


Figure 6.1: Scanning electron microscope images: (a) Spherical shaped ethyl cellulose microsphere and (b) ruptured surface showing hollow nature of microspheres

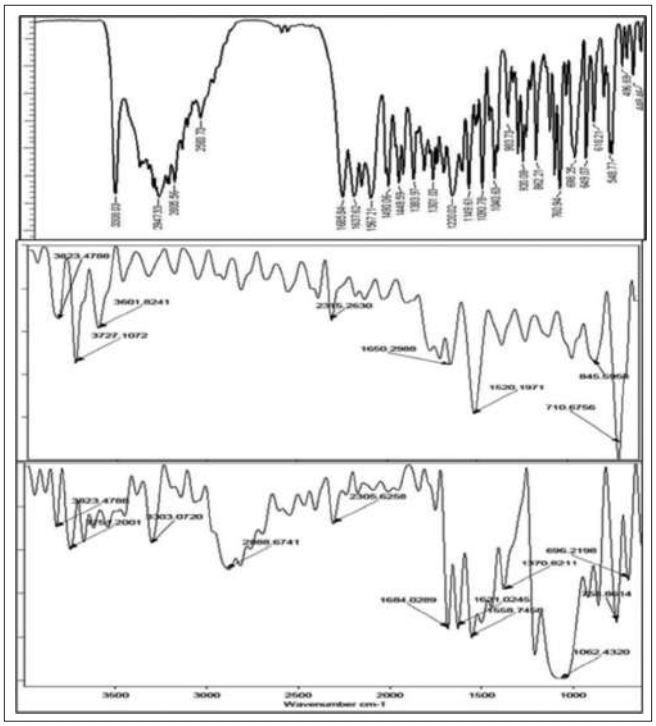


Figure 6.2: Fourier transform infrared spectrum of (a) repaglinide, (b) placebo ethyl cellulose microspheres, and (c) drug-loaded microspheres

Drug entrapment efficiency

The floating microspheres containing 50 mg of drug from each batch were weighed accurately and crushed. The powdered microspheres were placed in ethanol (10 ml). After 12 h, solution was filtered using Whatman filter paper no. 44. After proper dilution, the absorbance of the sample was recorded at 245 nm using UV spectrophotometer and entrapment of drug was estimated using the formula given below.

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \times 100 \quad (9)$$

In vitro buoyancy study

In vitro buoyancy was determined by placing 50 mg of formulation in 100 ml of SGF (pH 1.2) containing Tween 20 (0.02 w/v%) stirred at 100 rpm using a magnetic stirrer. Layer of floating microspheres was separated from the microspheres which were settled down by filtration after 12 h. Both the obtained particles were dried and separately weighed. Using the formula given below, buoyancy of microspheres was determined.

$$\text{Buoyancy (\%)} = W_f/(W_f+W_S) \times 100 \quad (10)$$

Where Wf and Ws are the respective weights of the floated and settled microparticles.

The results of percent yield, drug entrapment efficiency, and percent buoyancy for all the batches are reported in Table 6.4

In vitro drug release study

Paddle type dissolution apparatus having six stations (Veego, VDA-6DR, USP Std) was used to determine release of drug from formulation. Floating microspheres equivalent to 16 mg of drug was kept in 0.1 N HCl containing Tween 20 (0.02 w/v%). Temperature was maintained at 37 ± 0.5°C with 100 rpm speed of rotation. During the study, sink condition was maintained. 1 ml sample was withdrawn at 30 min time interval, passed through 5 µm membrane filter,

and analyzed spectrophotometrically at 245 nm. The cumulative percent drug release was calculated using standard calibration curve.

Drug release kinetics

Nearly, first-order drug release profile was observed for swollen hydrophilic matrix systems. For such systems, the dissolution of the drug available at the surface shows high release rate initially followed by rapid decline in rate due to swelling and consequent increasing of the dissolution path-length of the matrix (Narasimhan *et al*., 1997). Optimized formulation's release data were fitted to various mathematical models to reveal the release mechanism from the microspheres (Martin *et al*., 2001). All curve fitting, simulation, and plotting were performed using commercially available Microsoft Excel solver and regression coefficient (r^2) values were calculated. The mathematical models utilized for the study includes:

Zero-order release kinetics

In a zero-order process, the rate of diffusion is constant. The differential rate law would take the form Rate= k. This characteristic indicates that the process progresses at the same speed regardless of the concentration of the substance present until the substance is completely consumed. It is represented by following equation:

$$Q_t=Q_o+K_o t \quad (11)$$

Where

Q_t = Amount of drug dissolved in time t

Q_o = Initial amount of drug in solution

K_o = Zero-order release constant.

First-order release kinetics

In a first-order process, the rate of diffusion is directly proportional to concentration of drug. The rate law follows Rate = k [A], where k is a rate constant whose units vary depending on the rate order and [A] is the concentration of substance "A." The first-order law shows the progression of physical process and consumption of contained concentration, the diffusion rate decreases with the drop in molecular concentration. It is represented by following equation:

$$\text{Log } Q_t=\log Q_o+K_1 t/2.303 \quad (12)$$

Where Qt = Amount of drug released in time t

Q_o = Initial amount of drug in solution

K_1 = First-order release constant

First-order graph is the plot of log % drug release versus time.

Higuchi model

Higuchi developed several theoretical models to study the release of water soluble and low soluble drugs incorporated inside solid or semisolid support. Thus, equation obtained for drug particles dispersed in a uniform matrix behaving as the diffusion medium. The Higuchi equation is:

$$Q_t=K_H \times t_{1/2} \quad (13)$$

Where Qt = amount of drug released in time t

K_H= Higuchi diffusion constant

Higuchi graph is the plot of % cumulative drug release versus square root of time.

Table 6.4: Percent buoyancy, entrapment efficiency, and yield of EC microspheres

Batch code	Buoyancy (%)	Drug entrapment (%)	Yield (%)
E1	81.31±1.1	63.28±2.4	68.54±4.6
E2	80.41±2.4	68.03±2.2	76.15±2.5
E3	78.67±2.2	72.32±4.1	79.81±3.6
E4	84.31±3.2	65.47±2.2	72.42±2.3
E5	71.81±2.4	60.21±5.1	68.47±5.4
E6	77.12±3.3	62.45±1.5	74.12±2.3
E7	76.53±1.1	58.63±1.7	72.46±1.2
E8	76.24±3.2	71.47±2.2	76.94±3.2
E9	73.48±4.0	73.16±3.3	78.43±2.5

All values are represented as mean±SD (*n*=3). EC: Ethyl cellulose

Table 6.5: Results of FTIR spectra of RG and RG-loaded microspheres

S. no.	System	N-H (cm^{-1})	C-H (cm^{-1})	C=O (cm^{-1})	N-H bending
1.	Drug (RG)	3308.03	2947.33	1685.84	1637.62
2.	RG-loaded microspheres	3303.07	2888.67	1684.02	1631.02

FTIR: Fourier transform infrared, RG: Repaglinide

Peppas exponential equation

To study this model, release data are fitted to the following equation:

$$M_t/M=K.t^n \quad (14)$$

M_t/M= Fraction of drug release

K = Release constant

t= Drug release time and n = Diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form.

It is represented as the plot of log % drug release versus log time (Costa *et al*., 2001).

Results and discussion of EC microspheres

Preparation and optimization of EC microspheres

Solvent diffusion evaporation technique was successfully used to prepare floating microspheres of EC. During optimization different ratios of drug and polymer were studied by varying stirring speed, concentration of drug, and emulsifier for determination of qualitative and quantitative characteristics of floating microspheres. Drug and EC were dissolved in solution containing equal volume of ethanol and dichloromethane forming organic phase and were finally dispersed into PVA containing aqueous phase. As the organic phase is added to external aqueous phase, it gets partitioned into the two phases and results in complete precipitation of polymer around the drug particle. Continuous stirring of the aqueous phase helps in proper evaporation of solvent and formation of microspheres.

SEM analysis

SEM images shown below indicated that the microparticles prepared were discrete, perfectly good sphere having smooth and dense outer surface. Number of pores and inter-granular spaces is present in the surface of microspheres. The ruptured surface showing hollow nature of microspheres from the interior which helps them to remain buoyant on the GIT fluid [Figure 6.1b].

FTIR analysis

The FTIR spectra of drug-loaded EC microsphere clearly reveal the presence of characteristic peaks which were not present in the spectra of placebo microsphere (without drug). The positions of characteristic peaks present in drug and in repaglinide loaded microspheres are compiled in Table 6.5. The results show that neither EC nor the process of formulation affects the stability of drug [Figure 6.2].

Effect of experimental variables

Micromeritics properties

Results show that the mean particle size of the microspheres significantly increases (187 ± 7.2–234 ± 10.2 μm) with increase in EC concentration. On increasing the concentration of polymer, the viscosity of the medium increases thereby increases the interfacial tension. Larger particles were formed due to increased shearing efficiency at higher viscosities. Increase in stirring speed from 600 to 1200 rpm decreases particle size. Carr's index for all the formulation was found to be in range of 11.25–17.97% which indicates the flow to be excellent too good, while the results for Hausner ratio followed the same trend ranging between 1.12 and 1.21 indicating good flow ability of microspheres. The angle of repose was observed to be <40° implies non-aggregated nature of microspheres and hence excellent flow property [Table 6.3].

Percent buoyancy, yield, and entrapment efficiency

Determination of buoyancy is the most important parameter during optimization of formulation. Change in concentration of drug, EC, emulsifier, and speed of rotation shows prominent effect in parameters characterized during the study.

The buoyancy of microspheres slightly decreases with increase in concentration of EC (81.3–78.6%). This may be due to increase in particle density as concentration of polymer increases resulting in reduction in the porosity; thus, decreasing the buoyancy. As the stirring speed is increased from 600 to 1200 the buoyancy of microparticles decreases sharply from 84.1 to 71.8%.

Increase in concentration of emulsifier does not have much effect on floating property (decreases to lesser extent). On increasing, the concentration of drug during formulation from 10 to 30 mg decreases buoyancy from 80.4 to 73.8%. This may

be due to decrease porosity and hydrophobicity of the system as the concentration of EC is reduced.

Entrapment efficiency was good for all the prepared formulations (58.6–73.1%). The high entrapment was obtained owing to poor water solubility of RG. Drug loading has noteworthy effect on particle size distribution of formulations. Larger particles were formed due to high loading of drug. On increasing concentration of polymer resulted in increasing drug loading capacity (63.2–72.3%). The entrapment efficiency was increased when stirring speed was increased from 600 to 900 rpm. However, on further increase in stirring speed to 1200 rpm, the entrapment was observed to decrease. This can be attributed to reduced particle size at higher stirring speed.

As the concentration of EC and stirring speed (600–900) was increased, the yield of microspheres also increases from 68.5 to 79.8% and 72.4 to 76.1%, respectively. However, further increase in speed to 1200 rpm results in decrease in yield. Increase in the concentration of emulsifier (0.46–0.86%) shows no noteworthy effect on yield of microspheres. However, slight increase was observed with increasing concentration of drug from 10 to 30 mg. The effect of stirring rate and emulsifier concentration on various parameters is shown in Figures 6.3 and 6.4, respectively.

Thus, on the basis of obtained results, it is concluded that formulation E2 with 80.2% buoyancy, 68.0% drug entrapment, and 76% yield is the most satisfactory among all the formulations.

Analysis of drug release

An analysis of drug release studies reveals "no initial burst effect" in EC formulations; indicates homogenous distribution of drug [Table 6.6]. The release rate of RG decreases from 68.2 to 60.2% as the EC concentration was increased in formulation E1 to E3. As the presence of drug closer to the surface for release is decreased due to in polymer concentration. The release of drug is not very high, only 70.2% RG was the maximum release, due to hydrophobic characteristics of EC. Drug release is also less during initial hours of study as the solubility of EC in gastric fluid is poor. Figure 6.5 shows the CR of drug from all the formulations.

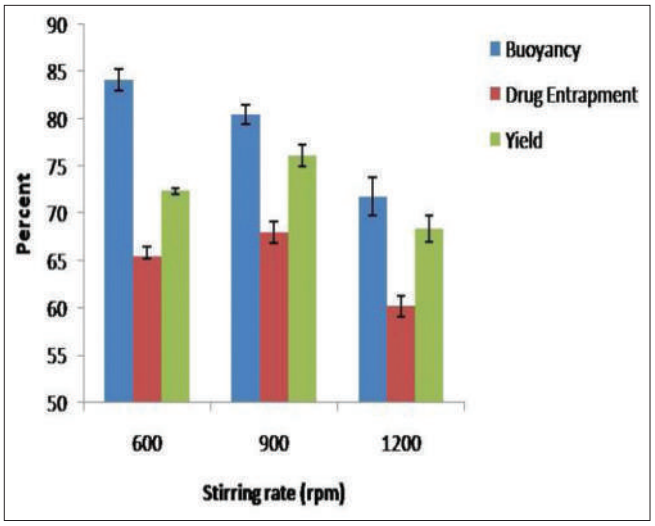


Figure 6.3: Effect of stirring rate on various optimization parameters

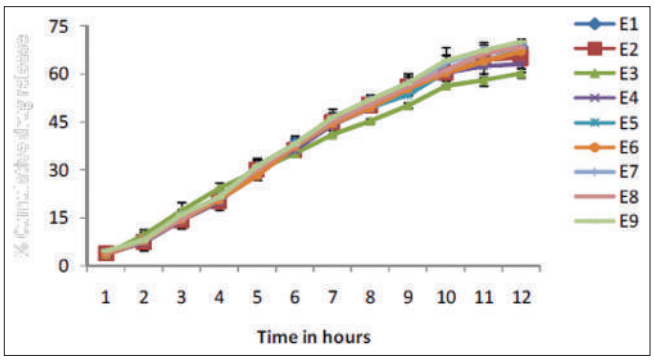


Figure 6.5: Percent cumulative drug release of ethyl cellulose microspheres

Slight increase in rate of release of RG was observed with increase in stirring speed from 600 to 1200 rpm. This may be due to reduced size of the particle with increasing stirring speed, thus exposing large surface area in the medium for drug release. A reduction in particle size is also observed with increasing concentration of emulsifier from 0.46 to 0.86%. Thereby release of drug is increased from 65.1 to 69.6%. Increase in drug concentration does not significantly influence the release of drug from the formulation.

Kinetic analysis of release

Release data produced were substituted in different equations of zero, first-order, Higuchi, and Peppas model. The results were interpreted based on the values of regression coefficients obtained. The result in Table 6.7 shows that *in vitro* release follows first-

order kinetics, followed by Peppas model. Study by Peppas equation helps to explain the release mechanism. Value of slope (*n*) was calculated and was found to be 0.89 suggesting anomalous diffusion which is the coupling of diffusion and erosion mechanism, indicating that rate of drug release is governed by more than one process. Thus, it is concluded that drug release follows first-order, diffusion, and erosion mechanism.

Preparation of RG-loaded EC and HPMC floating microspheres

Floating microspheres were prepared by same procedure as discussed in preparation of rapeglinide loaded EC floating microspheres. Different viscosity grades of HPMC (5, 100, and 4000 cps) were used along with EC to prepare three different types of formulations. Summary of various formulations prepared are presented in Tables 6.8-6.10. The same process variables as discussed in effect of process variables on microspheres were studied for optimization of microspheres.

Characterization of microspheres

Characterization and evaluation of microspheres were performed similarly as mentioned in characterization of EC floating microspheres. Results of micromeritics properties of prepared microspheres with different viscosity grades of HPMC are shown individually in Tables 6.11-6.13. The morphological study was performed as described in morphological study using SEM and SEM images of different viscosity grades of HPMC are shown in Figures 6.9-6.11.

FTIR spectral analysis of RG, placebo microspheres and RG-loaded microspheres were evaluated and comparison of spectrum is presented in Figures 6.12-6.14 for formulations prepared with 5, 100, and 4000 cps viscosity grade HPMC, respectively.

The microspheres were characterized for percent buoyancy, entrapment of drug, and yield as detailed in percentage yield–drug entrapment efficiency. The results of all the above parameters are summarized in Tables 6.14-6.16 for three different formulations containing HPMC of different viscosity grades 5, 100, and 4000 cps, respectively.

Drug release (*in vitro*) of all the formulations was studied similarly as discussed in *in vitro* drug release study. The drug release is reported in Tables 6.17-6.19.

Result and discussion of EC: HPMC (5, 100, and 4000 cps)

Preparation of EC: HPMC microspheres

Microspheres were successfully prepared using solvent-diffusion evaporation method using EC and HPMC (5, 100, and 4000 cps). Formulations

Table 6.6: Results of *in vitro* drug release from EC microspheres

Time (h)	Mean % drug released								
	E1	E2	E3	E4	E5	E6	E7	E8	E9
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	4.1±0.2	3.7±0.2	3.7±0.8	3.7±0.5	3.9±0.8	3.8±0.9	4.4±0.8	4.3±0.8	4.6±0.2
2	7.9±0.9	7.4±0.8	9.4±1.9	7.4±0.9	7.6±2.9	7.8±0.4	7.9±0.3	7.8±0.9	7.9±0.5
3	14.8±0.7	14.1±1.2	17.1±2.6	14.1±1.6	14.5±3.0	14.5±0.3	15.8±0.6	14.8±2.1	15.9±0.5
4	21.4±1.5	20.3±0.4	24.1±1.8	20.1±2.8	20.7±2.4	20.7±0.7	21.4±1.3	21.4±1.1	21.4±2.6
5	30.2±1.8	30.5±0.4	29.8±0.5	30±0.7	28.4±0.8	28.2±1.3	31.2±2.4	30.2±1.6	31.2±1.8
6	37.8±2.1	36.2±0.9	35.2±0.7	36.2±0.6	37.1±0.4	37.2±2.4	38.2±1.8	37.8±1.5	38.2±2.4
7	45.4±1.9	45.1±1.2	41±0.1	44.9±0.2	44.5±0.2	44.5±2.3	45.9±1.9	45.2±0.7	46.4±2.6
8	50.9±0.3	50.3±0.7	45.3±0.6	49.3±0.9	49.5±0.6	49.5±1.4	51.5±1.4	50.5±1.6	52±1.5
9	56.1±0.8	56.1±0.6	50.1±0.8	55.1±1.8	53.4±0.1	55.4±0.2	57.1±2.9	56.1±1.8	57.1±2.0
10	61.3±0.7	60.3±0.3	56.3±0.2	60.3±2.6	60.7±0.8	60.3±0.7	63.3±2.6	61.3±2.5	64.3±4.0
11	66.4±0.3	64.4±1.1	58.1±1.9	62.4±3.5	64±0.4	64±0.8	67.4±2.4	66.4±2.6	67.4±0.3
12	68.2±0.8	65.1±1.2	60.2±1.4	63.1±2.6	67.5±0.9	67.1±2.8	69.6±0.5	68.3±2.0	70.2±0.6

EC: Ethyl cellulose

Table 6.7: r^2 of release of E2 formulation using different kinetic models

Formulation code	Zero-order	First-order	Higuchi's	Peppa's
E2	0.9797	0.9899	0.9812	0.9888

Table 6.8: Formulation and optimization of EC: HPMC (5 cps) microsphere

Ratio of EC: HPMC	Concentration of emulsifying agent (%)	Stirring rate (rpm)	Amount of drug (mg)
1:1	0.46	900	10
1:2	0.46	900	10
1:3	0.46	900	10
1:2	0.46	600	10
1:2	0.46	1200	10
1:2	0.66	900	10
1:2	0.86	900	10
1:2	0.46	900	20
1:2	0.46	900	30

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

were prepared by varying concentration of HPMC with fixed ratio of EC to reveal the influence of increasing concentration of polymer on various parameters. Microspheres were formed by pouring solution of polymer and drug prepared in ethanol and dichloromethane, into aqueous solution containing PVA. Layer of polymer get precipitated around dichloromethane droplets due to rapid distribution of ethanol into external aqueous phase. Entrapped dichloromethane evaporates thereby helps in building small cavities within the floating microspheres (Jain *et al*., 2005). Thus, smooth surfaced and spherical shaped microsphere were formed that can float over gastric fluid.

SEM analysis

SEM images of optimized microspheres prepared from three viscosity grades of HPMC (5, 100, and 4000 cps) are shown in Figures 6.9-6.11, respectively. The prepared microspheres were distinct, absolutely good spherical geometry with porous outer skin and smooth surface. The surface of microspheres contains small cavity which may be due to solvent evaporation during drying process and inter-granular spaces whereas hollow nature of

Table 6.9: Formulation and optimization of EC: HPMC (100cps) microspheres

Batch code	Ratio of EC: HPMC	Concentration of emulsifying agent (%)	Stirring rate (rpm)	Amount of drug (mg)
B1	1:1	0.46	900	10
B2	1:2	0.46	900	10
B3	1:3	0.46	900	10
B4	1:2	0.46	600	10
B5	1:2	0.46	1200	10
B6	1:2	0.66	900	10
B7	1:2	0.86	900	10
B8	1:2	0.46	900	20
B9	1:2	0.46	900	30

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

Table 6.10: Formulation and optimization of EC: HPMC (4000cps) microspheres

Batch code	Ratio of EC: HPMC	Concentration of emulsifying agent (%)	Stirring rate (rpm)	Amount of drug (mg)
C1	1:1	0.46	900	10
C2	1:2	0.46	900	10
C3	1:3	0.46	900	10
C4	1:2	0.46	600	10
C5	1:2	0.46	1200	10
C6	1:2	0.66	900	10
C7	1:2	0.86	900	10
C8	1:2	0.46	900	20
C9	1:2	0.46	900	30

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

microspheres contribute to floating characteristics. The smooth surface of microspheres reveals the homogeneity of drug and polymers. Dense and smooth outer surface with distinct pores on the surface of microsphere was observed.

FTIR analysis

Spectra obtained from FTIR spectrophotometer for drug, placebo microspheres, and drug-loaded microspheres prepared by different viscosity grades of HPMC were obtained and characteristic peaks were observed [Figures 6.12-6.14]. Spectra of microspheres containing drug reveal the presence of characteristic peaks (–CH and –C=O stretching at 2994 and 1645 cm^{-1}) which were not present in the spectra of placebo microsphere (without drug) indicting proper drug loading in the microspheres. The characteristic peaks of drug appear within the

Table 6.11: Results of micromeritics properties of EC: HPMC (5 cps) microspheres

Batch code	Mean particle size (μm)	Bulk density	Tapped density	Carr's index	Hausner's ratio	Angle of repose
A1	191.12±1.0	0.72±0.07	0.84±0.18	14.28	1.16	31.7±3º
A2	208.27±2.7	0.73±0.06	0.85±0.26	14.11	1.16	27.1±4º
A3	224.74±3.0	0.75±0.27	0.88±0.02	14.77	1.17	33.6±7º
A4	211.34±1.0	0.74±0.47	0.86±0.07	13.95	1.16	35.4±2º
A5	181.54±1.4	0.70±0.02	0.82±0.11	14.63	1.17	33.3±4º
A6	194.94±5.8	0.73±0.18	0.86±0.16	15.11	1.17	35.2±6º
A7	182.37±5.2	0.74±0.10	0.86±0.24	13.90	1.16	35.1±8º
A8	238.18±6.5	0.73±0.01	0.88±0.54	17.04	1.20	38.2±9º
A9	244.61±9.4	0.74±0.12	0.88±0.98	15.90	1.18	38.1±6º

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

Table 6.12: Results of micromeritics properties of EC: HPMC (100cps) microspheres

Batch code	Mean particle size (μm)	Bulk density	Tapped density	Carr's Index%	Hausner ratio	Angle of repose
B1	221.41±3.2	0.75±0.06	0.87±0.23	13.79	1.16	31.6±3°
B2	234.15±5.9	0.73±0.32	0.88±0.48	17.04	1.20	36.7±1°
B3	250.34±8.6	0.70±0.78	0.86±0.39	18.60	1.22	37.3±4 °
B4	258.52±6.3	0.70±0.35	0.85±0.10	17.64	1.21	39.7±1°
B5	221.30±4.2	0.74±0.70	0.88±0.80	15.90	1.18	29.4±2°
B6	243.12±3.5	0.69±0.54	0.90±0.64	23.33	1.30	34.8±2°
B7	229.84±1.5	0.72±0.62	0.88±0.81	18.18	1.22	29.9±9°
B8	248.48±8.3	0.71±0.84	0.85±0.41	16.47	1.19	36.1±5°
B9	252.91±2.8	0.69±0.12	0.86±0.98	19.76	1.24	38.8±3°

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

Table 6.13: Results of micromeritics properties of EC: HPMC (4000 cps) microspheres

Batch code	Mean particle size (μm)	Bulk density	Tapped density	Carr's Index%	Hausner ratio	Angle of repose
C1	324.34±1.2	0.68±0.24	0.84±0.12	19.04	1.23	31.8±3°
C2	330.32±2.0	0.67±0.48	0.85±0.47	21.17	1.26	35.5±2°
C3	349.07±2.2	0.66±0.54	0.84±0.34	21.42	1.27	37.3±7 °
C4	345.48±7.2	0.67±0.31	0.83±0.25	19.27	1.23	37.8±4°
C5	312.82±2.1	0.70±0.36	0.87±0.21	19.54	1.24	36.2±4°
C6	321.37±1.5	0.68±0.41	0.86±0.38	20.93	1.26	37.9±7°
C7	314.74±2.3	0.69±0.48	0.87±0.35	20.68	1.26	35.7±3°
C8	348.37±3.5	0.65±0.21	0.84±0.65	22.61	1.29	38.1±5°
C9	359.64±2.7	0.64±0.45	0.86±0.31	25.58	1.34	37.6±5°

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

range in the spectra of microspheres indicating no chemical changes appear during formulation. As compared to the spectra of pure drug and polymers, some peak broadening and shifting of bands in functional group region appeared on interaction of drug with polymer. However, the spectra indicate that the stability of drug is not affected by the polymers or by the method of preparation.

The plain drug (RG) shows characteristic peaks at 3308, 2947, 1685, and 1637 cm^{-1} indicated the –NH, -CH, C=O stretching, and –NH bending vibrations, respectively. The characteristic bands of RG such as –CH and –C=O stretching vibrations appeared at 2994 and 1645 cm^{-1}, respectively, in microspheres containing drug. Thus, no chemical change during formulation of microspheres in the drug was observed.

The plain drug shows characteristic peaks at 3308, 2947, 1685, and 1637 cm^{-1} indicated the

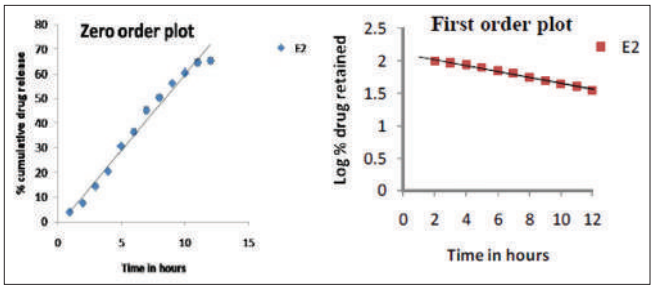


Figure 6.6: Zero- and first-order plot of E2 formation

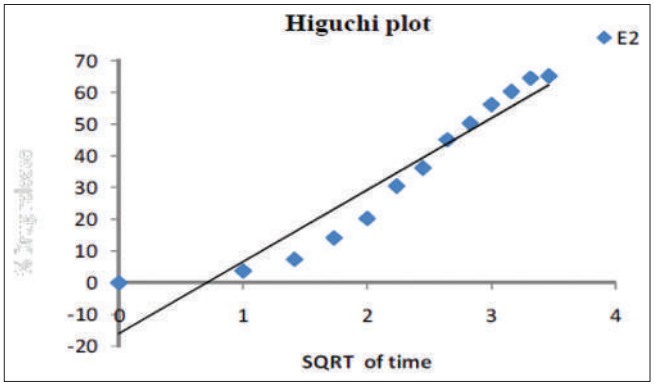


Figure 6.7: Higuchi plot of E2 formulation

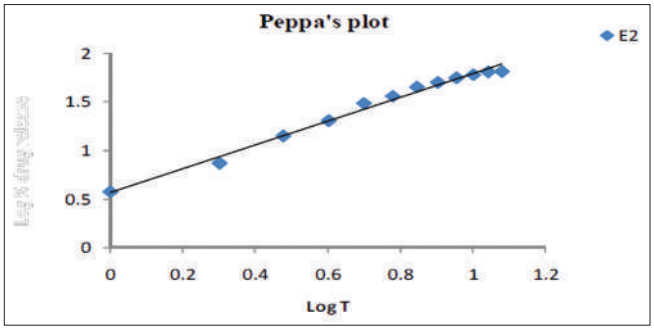


Figure 6.8: Peppa's plot of E2 formulation

–NH, -CH, C=O stretching, and –NH bending vibrations, respectively. The characteristic bands of repaglinide such as –CH stretching and –NH bending vibrations appeared at 2974 and 1608 cm^{-1} in microspheres having drug with no alteration. Thereby indicating drug polymer compatibility during the formation of microspheres.

The plain drug (RG) shows characteristic peak of –CH and –NH stretching vibrations at 2947 and 3308 cm^{-1}, respectively. Similar peaks were observed in spectra of drug loaded microsphere at 2941 and 3351 cm^{-1}, respectively, without any change. This implies no chemical interaction and proper drug loading in the prepared formulation.

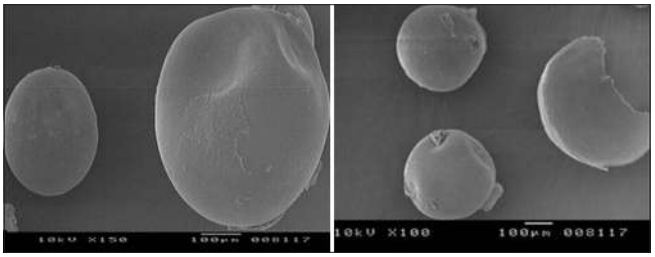


Figure 6.9: Scanning electron microscope images of Ethyl cellulose: Hydroxypropyl methylcellulose (5 cps) microspheres

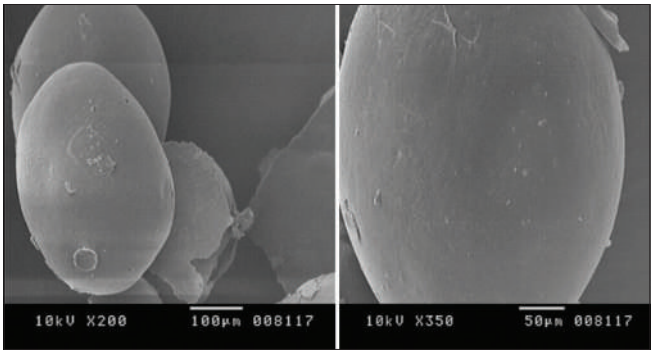


Figure 6.10: Scanning electron microscope images of Ethyl cellulose: Hydroxypropyl methylcellulose (100 cps) microspheres

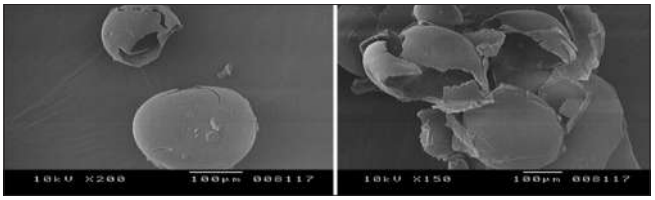


Figure 6.11: Scanning electron microscope images of Ethyl cellulose: Hydroxypropyl methylcellulose (4000 cps) microspheres

Effect of experimental variables

Micromeritics properties

Different viscosity grades of HPMC were used to demonstrate the effect of viscosity on particle size. Results of micromeritics properties are shown in Tables 6.11-6.13 for formulation containing 5, 100, and 4000 cps, respectively. The mean size of particles of prepared formulation increases with increasing concentration and viscosity of HPMC at the same ratio. It could be postulated that increased frequency of collisions occurs due to higher concentration of polymer, resulting in partially formed particles and thereby increased size of the microspheres (Jeffery *et al*., 1991). The microspheres prepared were absolutely spherical, free-flowing without forming aggregates. The particle size ranges from 181.54

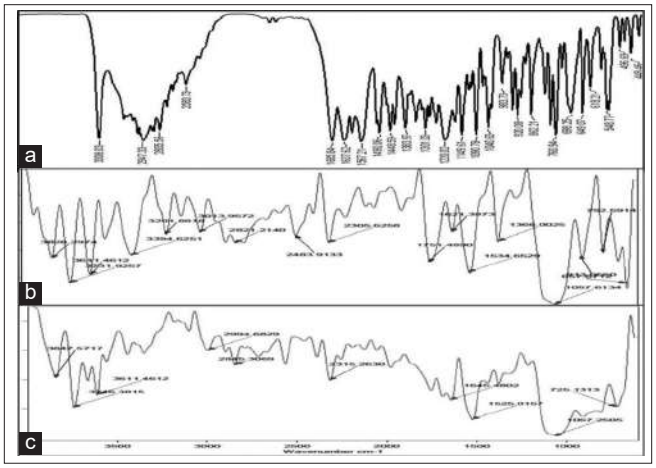


Figure 6.12: Comparison of Fourier transform infrared spectrum of (a) Rapeglinide, (b) Placebo Ethyl cellulose: Hydroxypropyl methylcellulose (EC: HPMC) (5 cps) microsphere, and (c) drug-loaded EC: HPMC (5cps) microsphere

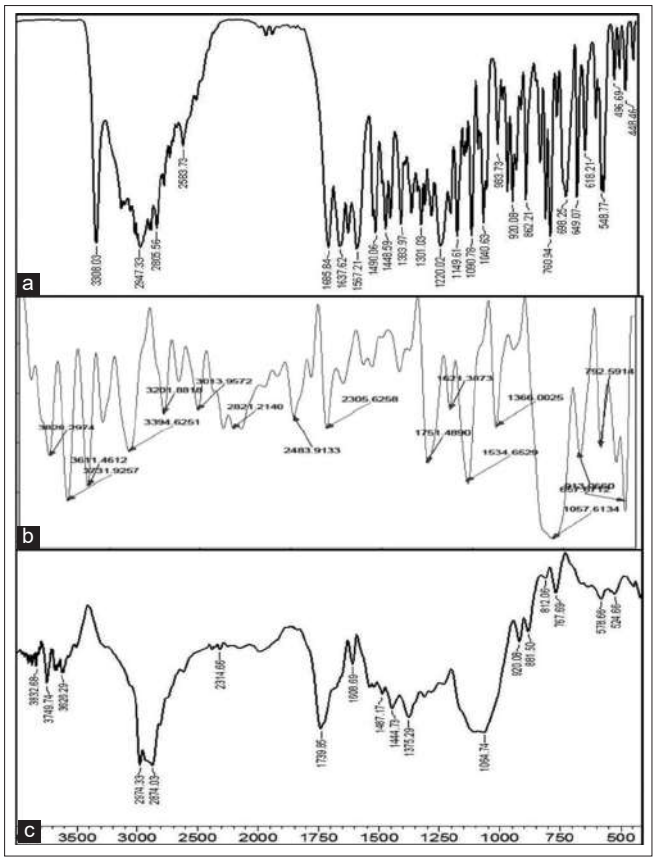


Figure 6.13: Comparison of Fourier transform infrared spectrum of (a) repaglinide, (b) placebo Ethyl cellulose: Hydroxypropyl methylcellulose (EC: HPMC) (100 cps) microsphere and (c) Drug loaded EC: HPMC (100 cps) microsphere

to 244.61 µm, 221.3 to 258.52 µm, and 312.82 to 359.64 for microspheres prepared by HPMC (5, 100, and 4000 cps), respectively. Increase in

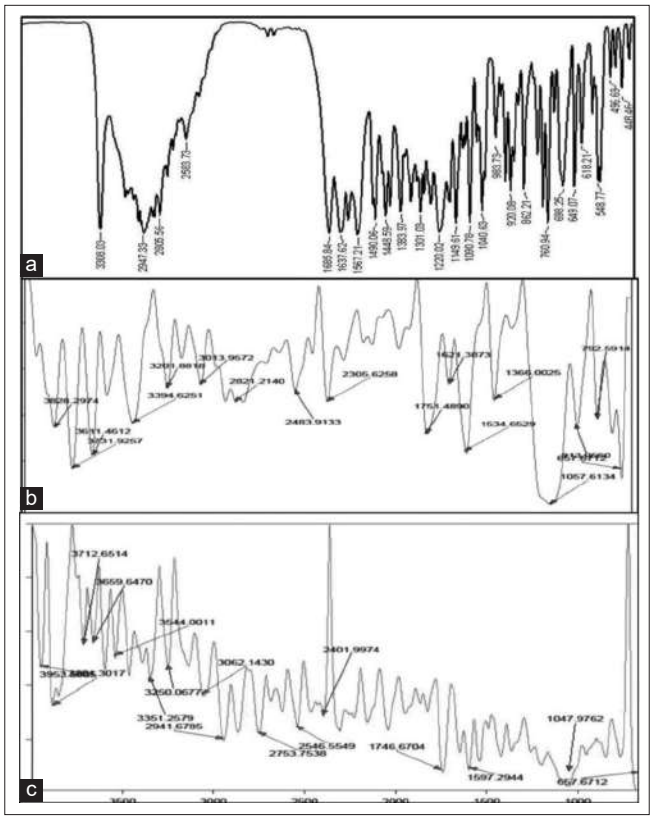


Figure 6.14: Comparison of Fourier transform infrared spectrum of (a) repaglinide, (b) placebo Ethyl cellulose: Hydroxypropyl methylcellulose (EC: HPMC) (4000 cps) microsphere and (c) Drug loaded EC: HPMC (4000 cps) microsphere

HPMC concentration increases the size of particles and largest size microspheres with HPMC 4000 cps as compared to those prepared with HPMC of 5 and 100 cps. This is due to increasing viscosity in a constant volume of solvent, thereby causing increment in drop size of emulsion and results in increasing particle size (Fu *et al*., 2005).

Bulk and tapped density were observed to increase with increase in particle size and more dense particles were obtained in batch C (EC: HPMC 4000 cps). Carr's index for formulations of batch A, B, and C (HPMC 5, 100, and 4000 cps) varies from 13.95 to 17.04%, 13.79 to 23.33%, and 19.04 to 25.58%, respectively. Thus, excellent to good property microspheres of batch A, whereas good and fair to passable type spheres of batch B and C were prepared. Results for Hausner ratio of all the three batches ranged between 1.16 and 1.34 indicating free flowability of microspheres. The non-aggregated nature of prepared floating microspheres is showed by good flow characteristics and as expressed by

Table 6.14: Percent buoyancy, entrapment efficiency, and yield of EC: HPMC (5 cps) microspheres

Batch code	Buoyancy (%)	Drug entrapment (%)	Yield (%)
A1	82.21±1.6	68.50±0.1	72.38±1.1
A2	84.13±0.4	71.63±0.5	70.46±0.1
A3	87.31±0.4	74.13±1.4	67.53±0.2
A4	87.37±0.5	66.74±0.3	65.84±0.5
A5	78.43±0.1	62.63±0.2	64.28±0.7
A6	79.64±0.2	66.55±0.3	67.34±0.5
A7	74.22±0.3	60.13±0.6	65.67±0.3
A8	78.13±0.3	73.41±0.4	68.43±0.2
A9	73.45±0.2	75.66±0.3	66.84±0.4

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

Table 6.15: Percent buoyancy, entrapment efficiency, and yield of EC: HPMC (100 cps) microspheres

Batch code	Buoyancy (%)	Drug entrapment (%)	Yield (%)
B1	75.30±0.2	70.36±0.8	70.24±0.1
B2	80.21±0.7	73.57±0.4	67.47±0.4
B3	83.14±0.4	75.52±0.2	65.65±1.2
B4	84.26±0.7	69.12±0.3	64.88±0.5
B5	74.82±0.3	64.34±0.6	62.63±0.2
B6	77.91±0.4	66.25±0.8	66.27±0.3
B7	75.33±0.1	62.38±0.2	64.81±0.1
B8	74.37±0.2	70.15±0.1	65.40±0.2
B9	70.61±0.9	67.35±0.2	63.43±0.8

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

Table 6.16: Percent buoyancy, entrapment efficiency, and yield of EC: HPMC (4000 cps) microspheres

Batch code	Buoyancy (%)	Drug entrapment (%)	Yield (%)
C1	70.11±0.2	65.32±0.8	64.38±0.8
C2	73.12±0.7	68.44±0.4	62.87±0.7
C3	76.28±0.4	70.14±0.2	60.14±0.2
C4	75.14±0.6	64.15±0.3	59.26±0.5
C5	67.26±0.3	62.18±0.6	56.14±0.4
C6	70.18±0.6	63.17±0.8	60.47±0.7
C7	68.27±0.2	58.28±0.2	58.38±0.4
C8	68.41±0.8	65.37±0.1	59.24±0.7
C9	63.18±0.7	61.18±0.2	57.14±0.9

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

angle of repose <40° (Hanna, 1990) [Tables 6.11-6.13].

Influence of increasing concentration of emulsifier on particle size of prepared formulation was also observed. When the concentration of emulsifier was low (0.46%), small emulsion droplets were not stable which results in formation of larger size microspheres than those prepared with higher emulsifier concentration (Blanco-Prieto *et al*., 1994) in all three types of formulations. This may be accredited to more PVA molecules overlaid the surface of droplets; which prevents coalescence of droplets resulting in formation of small droplets of emulsion. After evaporation of solvent from emulsion droplets microspheres were formed; thus, their size was similar to the size of droplet of emulsion formed (Lakshmanna *et al*., 2009). Effect of stirring rate was also studied and as it increases from 600 to 1200 rpm there is decrease in size of particles in all the three batches.

Percent buoyancy, entrapment efficiency, and yield

The buoyancy was studied for 12 h and was in the range of 73.4–87.3, 70.6–84.2, and 63.1–76.2% for microspheres prepared with HPMC 5, 100, and 4000 cps, respectively. Concentration and different ratio of polymers used during formulation effects the floating of microspheres (Streubel *et al*., 2006). Floating of microspheres was continuous and percent buoyancy was found to increase with increasing amount of polymer in all three types of formulations. Formulations prepared by combination of EC and HPMC show increase in buoyancy to some extent. As HPMC is water soluble, it forms hydrated layer due to which several pores are generated on the surface of microspheres. Air occupies these pores thereby increasing buoyancy (Karthikeyan *et al*., 2008). Further, when the viscosity of HPMC is increased from 5 to 100 and 4000 there is decrease in buoyancy. The increase in viscosity increases the relative density and overall weight of particles, which moves the system more downward. As the stirring speed is increased there is decrease in buoyancy of microspheres, this is due to formation of smaller size microspheres. Increase in concentration of drug from 10 to 30 mg, decreases the buoyancy from 84.1 to 73.4, 80.2 to 70.6, and 73.1 to 63.1% for microspheres prepared from 5, 100, and 4000 cps HPMC, respectively, due to reduction in hydration (as the volume of polymer reduces). The amount of PVA as an emulsifier decreases percent buoyancy slightly (lesser extent). The decrease may be caused due to tightening of polymeric network, which leads to shrinkage of microspheres.

Table 6.17: Results of *in vitro* release from EC: HPMC (5 cps) microspheres

Time (h)	Mean % drug released								
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	11.1±0.2	10.1±0.4	9.2±0.4	6.2±0.8	13.8±1.1	12.1±5.0	13.2±3.0	11±2.3	12±0.3
2	20.1±0.8	18.1±0.8	17.3±2.1	12.8±1.6	20.6±2.0	20.7±2.3	22.5±1.1	20±0.3	20.5±1.3
3	32.2±1.1	30.2±1.3	28.3±1.6	23.6±0.9	32.5±1.3	31.8±3.2	34.5±0.2	31.2±1.3	32.2±2.5
4	43.3±0.5	41.3±1.4	37.4±1.1	35.3±4.0	48.6±0.6	43.5±3.3	47.3±0.9	43.3±0.1	43.8±5.0
5	52.1±1.6	50.1±0.2	45.5±0.6	46.8±1.2	56.4±0.8	52±1.3	57.4±1.2	52.1±1.6	52.5±4.2
6	61.4±0.3	59.4±0.1	58.2±0.8	55.4±0.3	63.1±1.1	61.2±0.3	65.4±0.6	61.4±2.3	61.4±2.3
7	67.5±0.4	67.5±0.6	65.2±0.3	62.1±5.1	72.8±0.4	67.5±0.8	72.5±4.3	67.5±3.5	67.5±2.1
8	74.1±0.7	72.1±0.9	69.7±1.8	69.8±2.6	76.5±0.6	74.5±0.6	78.3±3.5	74.1±1.2	74.1±1.4
9	80.1±0.6	79.5±1.1	74.2±4.3	74.5±1.3	81.6±0.5	79.6±1.2	82.3±2.3	80.1±2.4	80.1±0.2
10	82.7±0.3	81.7±0.7	76.1±2.3	76.3±2.2	83.4±4.0	82.4±2.1	84.4±3.2	82.4±3.2	82.4±0.5
11	83.7±1.3	82.6±1.5	77.4±1.2	78.4±0.3	84.8±2.6	83.8±3.0	86.3±3.8	83.5±1.0	84.5±0.9
12	84.8±1.2	83.2±1.6	78.4±0.6	80.5±0.4	86.4±3.2	85.3±3.3	87.1±2.1	84.5±0.2	85.8±0.7

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

Table 6.18: Result of *in vitro* release from EC: HPMC (100 cps) microspheres

Time (h)	Mean % drug released								
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	5.7±1.2	5.1±0.2	4.2±0.1	4.4±0.3	7.1±2.1	5.3±0.5	7.6±0.9	5.4±3.1	5.3±0.2
2	10.8±0.2	9.2±0.9	6.8±0.8	7.3±0.9	13.8±0.6	9.6±0.7	13.9±5.2	9.4±4.2	9.6±0.9
3	21.9±3.1	17.4±1.5	11.1±3.4	11.5±0.4	25.2±0.8	17.2±3.6	23.1±0.6	16.8±0.9	17.3±3.1
4	29.2±0.9	28.1±4.3	18.4±1.5	18.6±3.1	34.4±2.3	28.5±1.3	39.1±0.8	26.8±1.5	28.5±2.1
5	38.9±0.5	39.6±2.3	27.1±0.6	27.1±0.6	41.2±0.9	38.8±2.3	47.2±0.7	38.3±1.3	38.8±0.4
6	48.8±0.4	48.6±0.6	38.3±0.7	38.3±2.5	50.2±3.5	48.6±0.5	54.3±1.3	48.1±0.5	48.6±1.3
7	55.5±1.3	55.9±0.8	48.4±2.2	48.4±4.6	58.1±4.3	56.4±0.9	64.8±2.4	56.4±0.3	57.1±1.8
8	63.3±4.3	65.4±2.3	55.3±1.9	55.3±2.3	65.1±2.3	65.4±3.1	68.7±0.3	64.4±2.9	65.2±2.6
9	70.4±2.9	70.5±3.3	61.0±2.0	63.1±0.8	71.2±0.8	70.3±3.8	72.6±0.8	69.7±3.4	71.1±0.7
10	72.1±3.4	71.1±0.7	65.1±2.1	67.2±1.1	74.1±2.9	71.1±0.2	75.1±0.1	71.1±2.5	73.7±1.1
11	74.8±1.1	73.7±5.2	68.0±0.5	69.1±3.4	76.3±3.4	73.7±0.7	77.3±2.3	73.7±1.7	75.2±2.1
12	75.2±2.3	74.1±0.4	70.2±0.5	70.8±2.1	77.4±2.9	75.2±0.3	78.9±1.1	74.2±2.6	76.3±0.8

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

Entrapment efficiency was calculated for all the batches and was found to be in the range of 60.1–74.6, 62.3–75.5, and 61.1–70.1%, respectively, for three different types of formulations prepared. When concentration of polymer is increased in the internal phase, there is increase in drug loading. Which results from increased viscosity of internal phase thus decreases the migration of drug in aqueous phase (Das and Das 1998). On comparing the entrapment of formulations prepared from various grades of HPMC, it is seen that entrapment increases from 5 to 100 cps but gradually decrease with formulation containing 4000 cps HPMC. This may be due to less space available for drug as the overall viscosity of the system increases. Entrapment efficiency increases slightly on increasing speed of rotation from 600 to 900 rpm, whereas it decreases with further increase to 1200 rpm. This may be due to formation of smaller size microspheres at higher speed of rotation. The increased stress generated in the emulsion with an increase in speed of mechanical agitation tends to break the droplets of emulsion and thus resulted in small particles. Smaller emulsion droplets formed facilitates diffusion of drug out of the microspheres before they harden and ensured lower drug entrapment efficiency (Alex *et al*., 1990). Furthermore,

Table 6.19: *In vitro* release of repaglinide from EC: HPMC (4000 cps) microspheres

Time (h)	Mean % drug released								
	C1	C2	C3	C4	C5	C6	C7	C8	C9
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	4.8±0.4	4.1±0.4	3.7±2.3	3.5±2.4	4.9±0.7	4.8±0.1	5.1±0.6	3.7±0.4	4.8±0.2
2	7.8±2.1	7.9±0.9	7.4±1.2	8.0±1.3	8.2±1.5	8.3±0.9	9.2±1.5	7.2±1.2	7.3±1.2
3	14±0.6	14.8±1.2	14.1±0.6	14.1±3.6	15.5±2.6	14.8±5.2	17.4±3.2	13.3±2.3	14.8±0.7
4	22±0.8	21.4±3.1	20.1±0.8	20.1±1.2	25.1±1.7	22.6±4.8	28.5±2.8	20.4±3.3	21.6±0.6
5	33±1.6	30.2±0.4	30.6±2.4	29.3±1.1	35.3±0.5	32.1±2.3	38.8±0.8	28.5±0.4	32.1±2.3
6	39±3.6	37.8±0.8	36.2±3.6	38.1±4.3	40.2±1.4	40±0.6	48.6±1.5	39.8±1.2	38.3±0.5
7	49.4±4.5	45.4±0.9	45.2±2.9	45.5±0.9	51.5±4.6	49.4±1.8	55.9±4.2	47.4±0.6	48.4±0.7
8	57.3±2.3	50.9±3.5	50.3±1.7	50.3±0.5	60.2±0.9	56.3±3.1	63.4±2.9	55.9±0.7	55.3±1.2
9	63±0.6	56.1±1.6	56.1±0.9	55.2±3.1	65.4±2.5	63.1±0.2	69.3±0.3	60.7±2.1	63.1±3.4
10	67±0.8	61.3±1.8	60.3±0.4	60.3±0.4	68.3±0.6	67.4±0.7	71.6±0.8	63.3±0.5	67.4±0.8
11	69.5±1.0	66.4±0.7	62.4±0.3	62.8±0.9	70.8±0.9	68.9±1.1	72.4±1.4	65.5±1.1	68.5±0.1
12	71.2±0.4	68.9±0.9	64.4±0.1	64.3±0.4	72.4±1.2	70.1±2.3	73.2±0.9	66.8±0.6	70.8±0.9

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

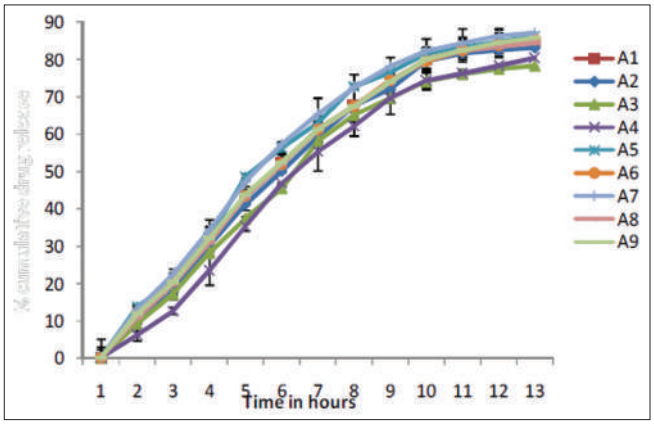


Figure 6.15: *In vitro* release of drug from Ethyl cellulose: Hydroxypropyl methylcellulose (5 cps) microspheres

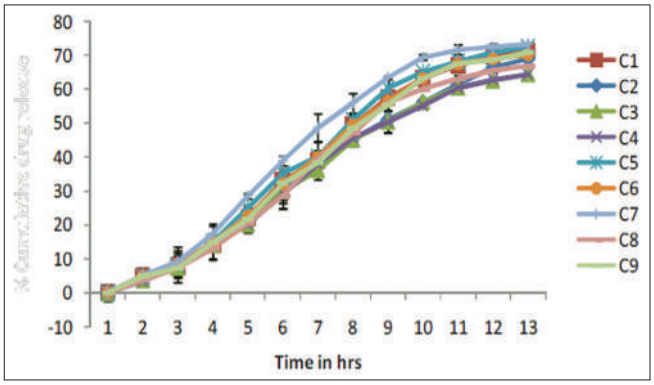


Figure 6.17: % Cumulative drug release of Ethyl cellulose: Hydroxypropyl methylcellulose (4000 cps) microspheres

from surface of small particles loss of drug is more during washing compared to larger particle surface. Increase in emulsifier concentration from 0.46 to 0.86 decreases entrapment of drug may be due to enhance ethanol and dichloromethane miscibility (processing medium), and extraction of more quantity of drug into it (Jain *et al*., 2015). Entrapment efficiency also increases with increasing drug concentration due to greater free space available in microsphere for drug. However, further increase makes constant size particles there by limiting the free space available for drug.

Size of particle and entrapment efficiency is based on several factors such as concentration of emulsifier, stirring rate, and time (Bansal *et al*., 2014). Increase in polymer concentration will affect the equilibrium maintained among these factors, whereas it also decreases the drug release. Percent yield was decreased on increasing concentration of HPMC (72.3–67.5, 70.2–65.6, and 64.3–60.1%) for 5, 100, and 4000 cps HPMC, respectively. This may be due to migration of HPMC into continuous phase forming agglomerates accompanied with sticking of polymer to the stirring blade and surface of beaker. No prominent effect of increasing concentration of emulsifier and drug was observed on yield.

Analysis of drug release

Drug release from microspheres was estimated in SGF for 12 h. None of the formulation showed burst effect during release, indicating homogenous distribution of drug, which may

also be attributed to less solubility of drug in the medium. Release of drug decreases with increasing polymer concentration from 1 to 3% and was found to be 84.8 to 78.4%, 75.2 to 70.2%, and 71.2 to 64.4% decrease for 5, 100, and 4000 cps grades HPMC, respectively. As the rate of release of drug depends on the presence of drug closer to surface, which decreases with increasing polymer concentration there by decreases the amount of uncoated drug (Behera *et al*., 2008). Furthermore, smaller hollow spheres are prepared at low polymer concentration and shows faster drug release by providing large surface area to dissolution medium.

Microspheres prepared at higher shear rates shows faster drug release. As the speed of rotation is increased from 600 to 1200 rpm, the release of drug also increases from 80.5 to 86.4, 70.8 to 77.4, and 64.3 to 72.4% from formulations prepared with 5, 100, and 4000 cps grade HPMC, respectively. As the size of the particles is reduced due to increase in stirring rate, thereby larger surface area is exposed in dissolution medium for drug release (Singh *et al*., 2010)

The increase in emulsifier concentration does not significantly affect release of drug. As the emulsifier concentration is increased from 0.46 to 0.86% slight increase in release of drug was observed, which may be due to formation of smaller size microspheres (Behera *et al*., 2008). Increase in concentration of drug shows no considerable effect on release profile of drug.

The release rate was found to be highest for A7, B7, and C7 formulations, but their entrapment, buoyancy, and percent yield values were less as compared to A2, B2, and C2 formulations. Based on the overall results of characterization parameters A2, B2 and C2 were selected as optimized formulation for comparing release kinetics.

On comparing the cumulative release of optimized formulation prepared by various viscosity grades HPMC, it was seen that low viscosity HPMC showed good release as compared to higher viscosity HPMC [Figure 6.18]. Drug release was observed in the order 83.2 >74.18 >68.9% for A2, B2, and C2 formulations, respectively. As the density of polymer matrix is increased due to increased viscosity, diffusional path-length also increases thereby decreases the drug release pattern from polymer matrix (Ganesan *et al*., 2013). Swelling rate of particles with viscous HPMC was slower as compared to less viscous grade HPMC.

As compared to formulation containing EC alone EC: HPMC formulations had shown better release of drug. It was concluded that use of HPMC helps in formation of gel layer around its core by absorption of fluid due to its high swellability and thus attributes for CR drug delivery.

Release kinetics study

The release data obtained from optimized formulations from each batch prepared by three viscosity grades of HPMC (5, 100, and 4000 cps) were substituted to different mathematical models such as zero-order, first-order, Higuchi, and Peppas kinetic models. Highest regression was obtained for first-order kinetics (Bhosale *et al*., 2012), followed by Higuchi and Peppas model for A2 formulation. Similarly, B2 and C2 formulations also followed first-order kinetics, followed by Peppa's and Higuchi model [Table 6.20]. When mechanism of release is not exactly known or probability of more than one phenomenon was expected Peppa's model is widely used. Value of slope (n) was calculated and could be used to characterize different release mechanism (data shown below). Peppa's equation is given as:$\% R = K t^n$

or $$\text{Log } \% R = \log K + \log t \qquad (15)$$

Where R = drug release, k = constant, n= slope and t= time

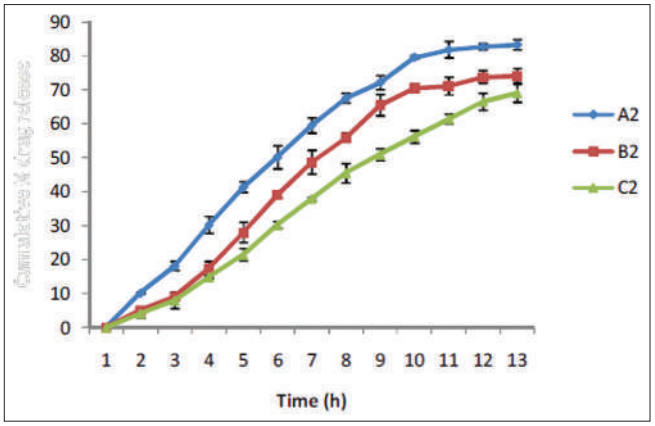


Figure 6.18: Comparison of drug release of optimized A2, B2, and C2 formulations

In case of Fickian diffusion, release of drug mainly depends on the diffusion across the matrix. Case II release generally refers to the polymer relaxation.

"*n*"	Mechanism
>0.5	Fickian diffusion (Higuchi matrix)
0.5<*n* < 1	Non Fickian Diffusion or anomalous release
<1	Case II Transport

Value of "*n*" for all the three formulations was found to be <0.89 which indicates non-Fickian (anomalous) case, drug is released by combination of diffusion and relaxation of polymer. Zero, First-order plot, Higuchi, and Peppa's plot of optimized formulations of different viscosity grades HPMC formulations are shown in Figures 6.19-6.22, respectively.

PREFORMULATION AND ANALYTICAL STUDIES

Introduction

Preformulation studies are the prime phase in the rational development of dosage form from drug molecule (Carstensen *et al*., 2002; Fiese *et al*., 1986). It can be defined as study of physicochemical properties of drug alone and in combination with excipients. During the study, the physicochemical properties and compatibility with other excipients that could affect the performance of drug and development of an effective dosage form were studied. These investigations primarily confirm that there are no significant barriers which influence the manufacturing of product and formulation design (Guy *et al*., 1987). Preformulation studies summarize the following information's.

- To study physical and chemical properties of the medicament.
- To determine its kinetic release rate profile.
- To study drug excipient compatibility.

Table 6.20: Correlation coefficient (r^2) of A2, B2, and C2 formulations by different kinetic models

Formulation code	Zero-order	First-order	Higuchi's	Peppa's
A2	0.9392	0.9828	0.9808	0.9796
B2	0.9479	0.9744	0.9708	0.9729
C2	0.9740	0.9819	0.9786	0.9803

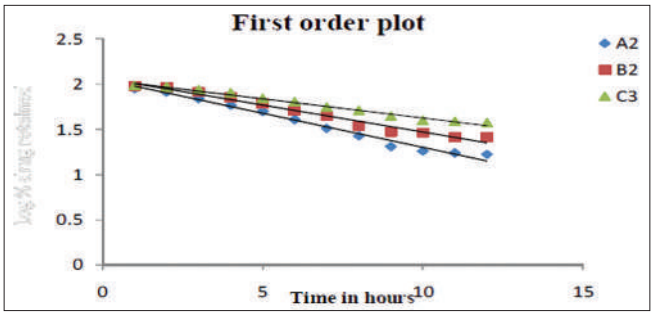


Figure 6.19: First-order plot of A2, B2, and C2 formulations

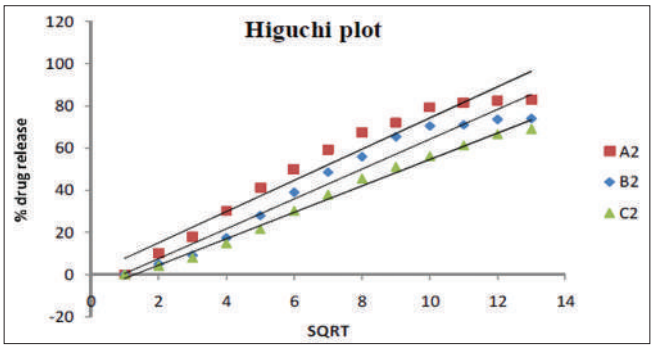


Figure 6.20: Higuchi order plot of A2, B2, and C2 formulations

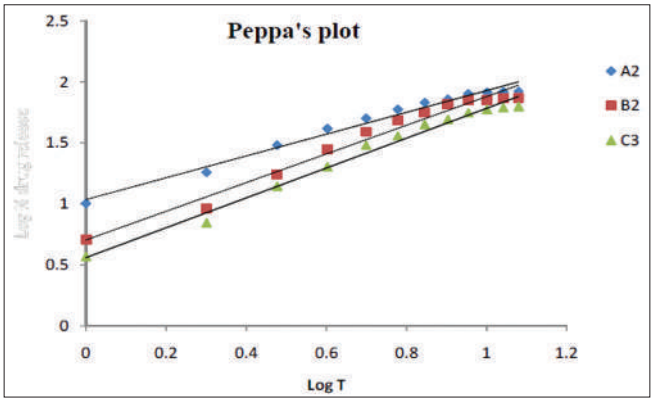


Figure 6.21: Peppa's order plot of A2, B2, and C2 formulations

- To determine degradation process.
- Toxicity determination by analyzing different formulations of same compounds.

These studies are also needful for selection of most suitable container-closure and development of analytical method. During this stage, drug of choice and polymers was standardized as per specifications of respective pharmacopeias. Some excipients not official in standard pharmacopeias can be standardized by manufacturer's specifications (Sarfaraz *et al*., 2010). The summarized data

obtained after preformulation studies helps in providing necessary groundwork for successful formulation.

Materials and Equipments

Various materials utilized during preformulation, formulation, characterization, and for other experimental work are given below. Laboratory/ analytical grade chemicals were used during the research work [Table- 7.6].

Physicochemical Properties (Aulton 1996, Sahitya *et al*., 2013)

The physical characterization of procured drug sample of repaglinide and polymers was determined as follows:

Organoleptic properties

Drug is characterized for its color, odor, and taste results were reported utilizing descriptive terminology.

Melting point

Melting point apparatus (Lab-Hosp Corporation, Mumbai) was used to determine the melting point of drug by open capillary method.

Solubility

The solubility of drug and polymers was determined in different polar and non-polar solvents. Fixed quantity of drug and polymers subsequently was added separately to a series of 10 ml solvents in test tubes at room temperature till particles get solubilizes. These test tubes containing solutions were vortexed and kept for 24 h. The observations are recorded as per I.P. 1985.

Partition coefficient

Partition coefficient of repaglinide was examined in n-Octanol: water system. 5 mg of drug was placed in a separating funnel having 10 ml of octanol and distilled water each. The apparatus was shaked for 2–3 h on rotatory shaker for equilibration. The concentration of drug in Octanol was estimated spectrophotometrically by preparing calibration curve in octanol. The partition coefficient of drug in phases was calculated as:

$$\text{The Partition Coefficient K} = \frac{\text{Amount of drug in organic layer}}{\text{Amount of drug in aqueous layer}} \quad (7)$$

Determination of wavelength (λ_{max})

10 mg of repaglinide was weighed accurately and placed in 10 ml volumetric flask to which 10 ml of methanol was added. 1 ml of the solution from the above stock was taken into a separate volumetric flask to which 10 ml of methanol was added. Same procedure was again followed with this sub-stock to prepare a solution of 10 µg/ml. This solution was then scanned between 200 and 400 nm in UV-Visible spectrophotometer to determine the absorption maxima λ_{max}). Result of UV scan is shown in Figure 5.1.

FTIR spectral analysis

To generate FTIR spectra base line correction of FTIR spectrophotometer (Shimadzu 8400, Japan) was done by taking IR grade potassium bromide previously dried at 40–50°C. A known amount of drug was thoroughly mixed with potassium bromide and compressed in a hydraulic press under 10 ton pressures to form pellet and was scanned from 4000 to 400 cm^{-1}. The spectra of other polymers were also obtained using same procedure. Band frequencies obtained are reported in Table 5.4-5.7.

Compatibility studies of drug and polymer

Compatibility of drug with different polymers was studied by the following ways:

Physical observant

Using a glass mortar 100 mg of drug is mixed with different polymers and was triturated for 15 min separately. The mixture was packed in closed vials using butter paper and placed in accelerated environmental conditions (40°C ± 75% RH). Any physical change was observed visually after every week for 4 weeks. The results are tabulated in Table 5.8.

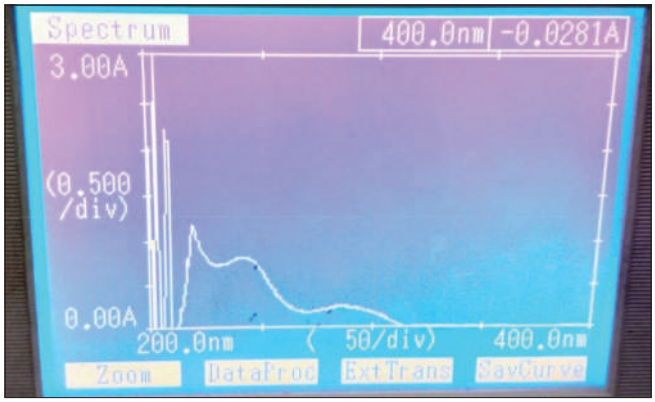


Figure 5.1: Ultraviolet spectra of repaglinide

Using FTIR

Drug polymer compatibility was studied by FTIR spectroscopy. Equal amount of drug with polymers separately was thoroughly mixed with potassium bromide and compressed in a hydraulic press fewer than 10 ton pressures to form pellet and was scanned from 4000 to 400 cm^{-1}. The FTIR spectra's of combination of both were compared with individual spectra of pure drug and polymers.

RESULTS AND DISCUSSION

Organoleptic properties

By visual observation RG is white or almost off white, crystalline, and odorless powder. EC is white odorless powder. HPMC of different grades were white powder almost odorless.

Melting point

RG is having melting point in the range of 128–131°C.

Solubility

The solubility of drug and excipients was determined and reported below in Tables 5.2 and 5.3, respectively.

Partition coefficient

Drug was found to have partition coefficient 4.1.

Determination of λmax

The absorption maximum was observed to be 245 nm as shown in Figure 5.1

FTIR spectral analysis

FTIR spectra of RG and polymers (EC, HPMC, HPMC K100, and HPMC K4 M) were obtained and shown in Figures 5.2-5.6.

The observed peaks were identified and were similar to reported reference FTIR spectra (Prajapati *et al*., 2011). Characteristic peaks are summarized in Table 5.4.

The FTIR spectra were identified and were similar to reported reference spectra (Lakshmi *et al*., 2013, Patel *et al*., 2011), for different polymers (EC, HPMC, HPMC K100, and HPMC K4 M) are shown in Table 5.5-5.7.

Drug polymer compatibility studies

Physical observation

Any change in color, formation of lumps or gas and liquefaction was observed during the study. Results of physical observations are shown in Table 5.8. During the study, no mark able variation in the physical properties of drug and polymers was seen. Thus, no physical interaction between the drug and polymers resulted.

Using FTIR

Physicochemical compatibility was also studied by FTIR analysis. The characteristic peaks observed in spectra of physical admixture of drug and polymers were almost similar to the peaks of drug alone. There was no major shift in the peaks of repaglinide along with appearance of any additional peaks was observed. Thus, the probabilities of chemical interaction were nullified. The FTIR spectra are shown in Figures 5.7-5.9. The comparative data are reported in Table 5.9.

CONCLUSION

On the basis of physicochemical characteristics drug and polymers was selected. Drug and polymers were identified by physical characterization and FTIR analysis. Compatibility of drug-polymer was studied by physical observation and FTIR analysis. No sign of interactions between drugs with any of the polymer was observed during the study. Thus, selected drug and polymers were suitable for formulation of floating microspheres.

Table 5.1: Various materials and equipments used with detail of their source

S. no.	Material	Source
1	Repaglinide	Gift sample from Torrent Pharmaceuticals, Ahmadabad, India.
2	Ethyl cellulose	HiMedia Chemicals, India.
3	Hydroxypropyl methyl cellulose	HiMedia Chemicals, India.
4	Polyvinyl alcohol (PVA)	SD fine chemicals Mumbai, India.
5	Ethanol	SD fine chemicals Mumbai, India.
6	Dichloromethane	SD fine chemicals Mumbai, India.
7	Distilled water	Prepared in laboratory by distillation Assembly
8	Polyethylene glycol	Sun Chem, India
9	Calcium chloride	Sun Chem, India
10	Concentrated Hydrochloric Acid	Merck (India) Limited, Mumbai
11	Chloroform	CDH Pvt Ltd., New Delhi
12	Methanol	CDH Pvt Ltd., New Delhi
13	Acetonitrile	CDH Pvt Ltd., New Delhi
14	Ethanol	CDH Pvt Ltd., New Delhi
15	Benzene	CDH Pvt Ltd., New Delhi
16	Sodium Hydroxide pellets	CDH Pvt Ltd., New Delhi
17	Octanol	CDH Pvt Ltd., New Delhi
18	Calcium chloride	CDH Pvt Ltd., New Delhi
19	Petroleum ether	HiMedia Chemicals, India
20	Potassium Dihydrogen orthophosphate	CDH Pvt Ltd., New Delhi
21	Di-potassium Hydrogen Phosphate anhydrous	CDH Pvt Ltd., New Delhi
22	Alloxan	CDH Pvt Ltd., New Delhi
23	Glassware	Borosil Ltd, Mumbai
24	Digital balance	Jyoti Scientific Industry, Gwalior, India
25	Digital pH meter	Elico India, Ahmedabad, India.
26	Magnetic Stirrer	Remi, Scientific Equipments, Mumbai, India
27	Mechanical Stirrer	Remi, Scientific Equipments, Mumbai, India
28	Hot Air Oven	P.S.M Industries, Bangalore, India
29	UV–Visible Spectrophotometer Shimadzu UV_1601, Japan	Shimadzu-1700, Japan
30	FTIR 200 Spectrometer	Shimadzu 8400, Japan
31	FTIR Spectrometer	Bruker (Lab India), Germany
32	Programmable environmental test chambers	ACMAS Technologies (p) LTD, Sonepat, India.
33	Heating mental, thermostat	Jyoti Scientific Ind, Gwalior, India11
34	Scanning Electron Microscopy	Jeol JSM-1600, Tokyo, Japan
35	Photomicrography SLR Camera	Cosina Co, Ltd Japan
36	Dissolution apparatus	Veego, VDA-6DR, USP Std
37	Double Distillation Unit	Mono Quartz Distillation Unit, Borosil
38	Desiccators	Borosil Ltd, Mumbai
39	Light Microscope	Lab India Pvt. Ltd.

Table 5.2: Solubility of repaglinide

S. no.	Solvents	Solubility
1	Water	+
2	Methanol	+++++
3	ACN	+++++
4	Benzene	++++
5	Chloroform	++++
6	Dichloromethane	+++++
7	0.1 N HCl	+++++
8	0.1 N NaOH	++++
9	Phos. Buffer pH 6.8	+++++

Table 5.3: Solubility of polymers

Solvents	EC	HPMC	HPMC K100	HPMC K4M
Water	-	++++	++++	++++
Methanol	++++	++	++	++
CAN	++++	++	++	++
Benzene	++++	-	-	-
Chloroform	++++	-	-	-
Dichloromethane	-	-	-	-
Petroleum Ether	-	-	-	-

(-) practically insoluble, (+) very slightly soluble, (++) sparingly soluble, (+++) soluble, (++++) freely soluble, (+++++) very soluble, EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

Table 5.4: Important band frequencies in FTIR spectra of repaglinide

S. no.	Groups	Band frequencies obtained (cm^{-1})
1.	N-H stretching vibration	3308.03
2.	C-H stretching vibration	2947.33
3.	C=O stretching	1685.84
4.	N-H bending	1637.62
5.	CH3 stretching vibration	1220.02

FTIR: Fourier transform infrared

Table 5.5: Important band frequencies in FTIR spectra of EC polymer

S. no.	Groups	Band frequencies obtained (cm^{-1})
1.	O-H stretching vibration	3481
2.	C-H stretching vibration	2929

FTIR: Fourier transform infrared, EC: Ethyl cellulose

Analytical technique

Experimental

UV-visible spectrophotometer (Shimadzu-1700) having 2 nm spectral bandwidth, wavelength accuracy of ±0.5 nm and a pair of 1.00 cm

matched quartz cells was used for analytical determinations.

Preparation of stock and sub-stock solutions

10 mg of repaglinide was weighed and dissolved in 10 ml of 0.1 N HCl to prepare stock solution of 1000 µg/ml. 1ml of above solution was diluted with 9 ml solvent to form sub-stock of 100 µg/ml.

Preparation of calibration curves

From the prepared sub-stock solution, different aliquots were withdrawn using calibrated graduated pipette into a series of volumetric flasks and diluted up to10 ml with 0.1 N HCl resulting in solutions of concentration ranging from 2 to 20 µg/ml respectively. Calibration standards were analyzed at λ_{max} 245 nm by spectrophotometer [Table 5.10] taking 0.1 N HCl as blank. Above procedure was repeated 3 times. Standard curve was plotted between absorbance and concentration as shown in Figure 5.10. Slope, intercept, and coefficient of regression were calculated.

Regression equation y = 0.0165 x – 0.0065; correlation coefficient r^2 = 0.9990

Table 5.6: Important band frequencies in FTIR spectra of HPMC polymer

S. No	Groups	Band frequencies (cm^{-1})
1.	O-H stretching vibration, broad band denotes the superposition of stretching for two types of OH groups, free at 3585 and self-associated group through 3444.	3480
2.	C-H stretching vibration	2973
3.	C-O Stretching vibration of six membered cyclic ring	1665–640
4.	C-O-C and symmetric bending of methoxy group (cyclic anhydride)	1400–1350
5.	Ethereal C-O-C group stretching vibration	1166–1000
6.	Rocking mode of CH^2 group	846

HPMC: Hydroxypropyl methylcellulose, FTIR: Fourier transform infrared

Validation of method

Reproducibility

Six individual samples of RG were weighed and reproducibility of the method was analyzed. The percent relative standard deviation (RSD) of the reproducibility was found to be <0.5%.

Interference

Interference of the polymer (EC and three viscosity grades of HPMC) in the above method was determined by testing their effects individually. Weighed quantity of drug and polymers was mixed thoroughly in 1:1 ratio. Powder equivalent to 20 mg of drug was weighed and assayed by the developed method. Calibration curve was used to determine the RG content [Figure 5.11] and the results are given in Table 5.10.

Results

Concentration range of 2–20 µg/ml obeyed Beer's law in the developed method. Reproducibility of the method was ensured by observing low RSD (<0.44) values. Results of interference study shown

Table 5.7: Important band frequencies in FTIR spectra of two grades of HPMC polymer

Polymer	O-H str vibration	C-H str vibration	C-O Str vibration	C-O-C and symm bending methoxy	Etheral C-O-C of group stretching
HPMC K4 M	3484	2931	1644	1450–1330	1192–1000
HPMCK100	3258	2932	1644	1456–1300	1062

HPMC: Hydroxypropyl methylcellulose, FTIR: Fourier transform infrared

Table 5.8: Results of physical observation during compatibility study

S. no.	API and polymers	Ratio	1st week RH	2nd week 40°C/75% RH	3rd week 40°C/75% RH	4th week 40°C/75% RH
1.	RG	1	No change	No change	No change	No change
2.	RG+EC	1:1	No change	No change	No change	No change
3.	RG+HPMC 5	1:1	No change	No change	No change	No change
4.	RG+HPMC K4M	1:1	No change	No change	No change	No change

HPMC: Hydroxypropyl methylcellulose, EC: Ethyl cellulose, RG: Rapeglinide

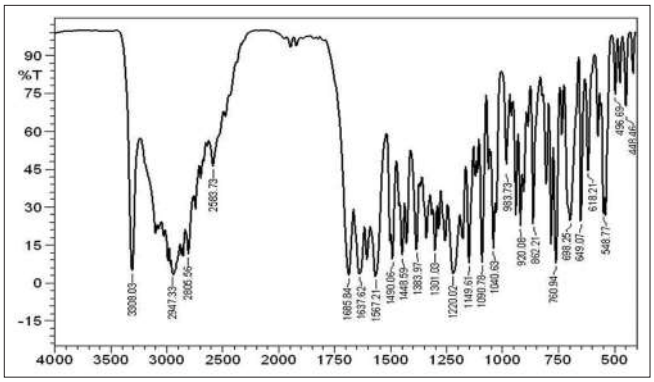


Figure 5.2: Fourier transform infrared spectra of repaglinide

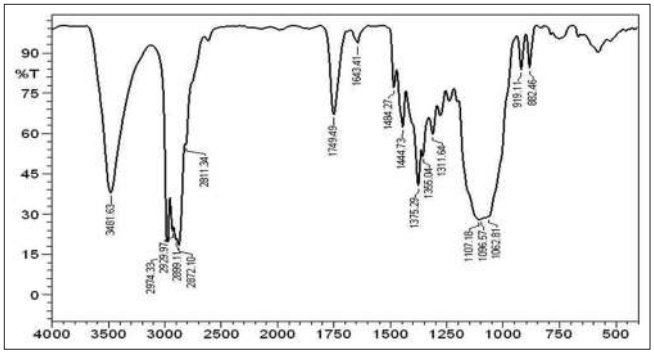


Figure 5.3: Fourier transform infrared spectra of ethyl cellulose

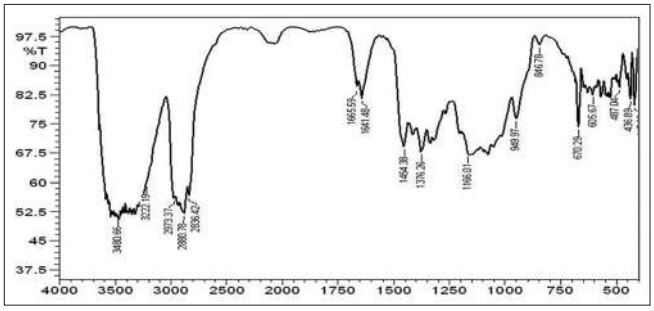


Figure 5.4: Fourier transform infrared spectra of hydroxypropyl methylcellulose (5 cps)

in Table 5.11 indicate no excipients used during the study interference in the method of estimation. More than 97.40% recovery was observed. Thus, the developed method for the estimation of RG in *in vitro* dissolution studies and from other products was found to be appropriate.

ACCELERATED STABILITY STUDIES

Introduction

Scientific success and commercial launching of any drug product solely depends on the drug

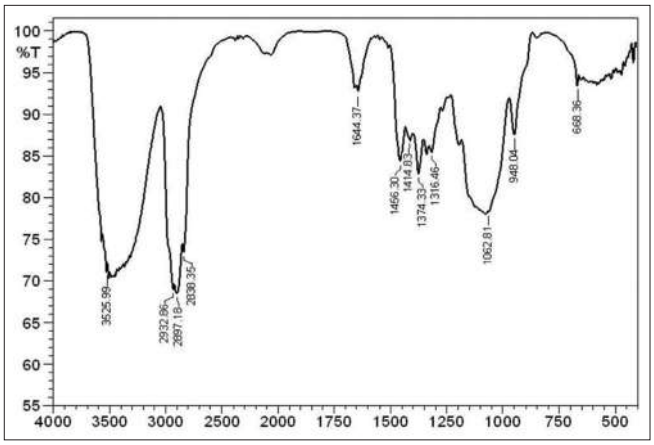


Figure 5.5: Fourier transform infrared spectra of hydroxypropyl methylcellulose K100 (100 cps)

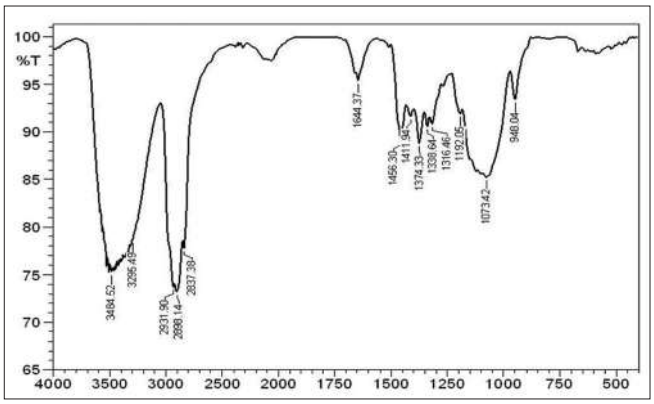


Figure 5.6: Fourier transform infrared spectra of hydroxypropyl methylcellulose K4 M (4000 cps)

development process. Pharmaceutical analysis and stability studies are the key steps of the developmental stage which are necessary to assure and to estimate the identity, potency, and purity of raw material as well as finished products (Singh *et al*., 2000).

Pharmaceutical product stability can be defined as, the ability of specific formulation in a prescribed closure or container to maintain its physicochemical, microbiological, toxicological, protective, and effective integrity (Kommanaboyina *et al*., 1999). Thus, the factors that affect the quality of the drug substance or formulation such as environmental factor which is also used to determine shelf life are studied thoroughly. Predetermined storage conditions and proper instructions regarding labeling of a product are very important during stability studies for regulatory approval of formulation (Singh, 2000). Stability testing is a complex process which involves a variety of factors during which drug

Table 5.9: Characteristic peaks of repaglinide alone and in combination with polymers

S. no.	System	N-H (cm^{-1})	C-H (cm^{-1})	C=O (cm^{-1})	CH3 (cm^{-1})
1.	Repaglinide (RG)	3308	2947	1685	1220
2.	RG-HPMC	3308	2935	1686	1218
3.	RG-HPMC K100	3308	2935	1686	1218
4.	RG-HPMC K4M	3308	2935	1686	1218
5.	RG-EC	3308	2934	1687	1218

RG: Repaglinide, HPMC: Hydroxypropyl methylcellulose

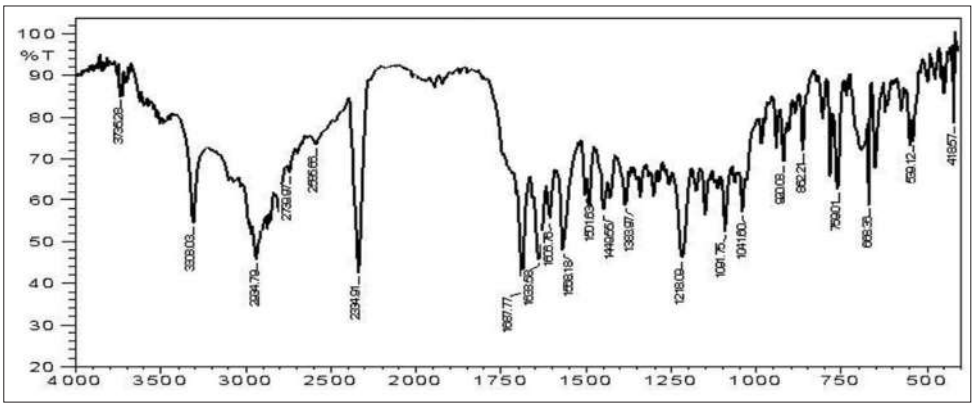


Figure 5.7: Fourier transform infrared spectra of physical mixture of Repaglinide + Ethyl cellulose

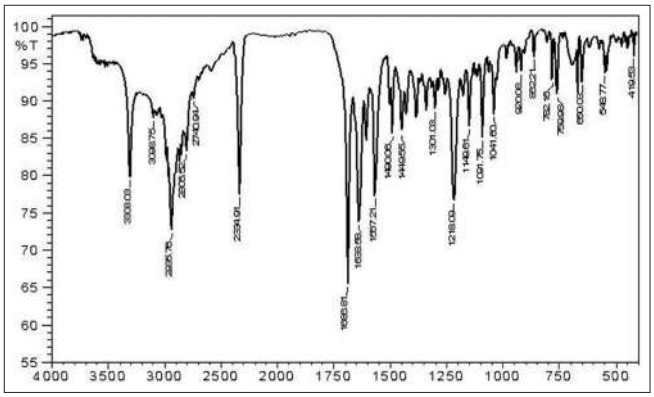


Figure 5.8: Fourier transform infrared spectra of physical mixture of Repaglinide + Hydroxypropyl methylcellulose 5 cps

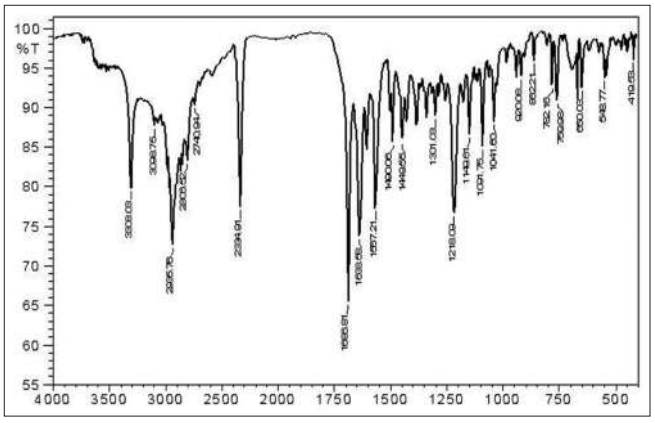


Figure 5.9: Fourier transform infrared spectra of physical mixture of Repaglinide + Hydroxypropyl methylcellulose K100

formulation can undergo variation in consistency, appearance, uniformity of content, particle shape and size, moisture contents, pH, package integrity thus, and influencing its stability. During the period of stability study product may undergo various chemical reactions such as reduction, oxidation, racemization, and results in production of the degraded product, loss of excipient and change in API and pharmacological potency (Carstensen *et al*., 1993).

A successful dosage form should be stable with regard to its physical, chemical, therapeutic, toxicological, and specially drug release characteristics. Stability of drug is its ability to remain in the prescribed container and maintain its identity under the influence of different environment factors such as temperature, light, and humidity (Kulkarni *et al*., 2004). In different countries, various regulatory authorities have made provisions for submission of data generated during stability studies by the manufacturers to assure the formulation of product which is stable to be available for use of patients. Various guidelines issued by International Conference on Hormonisation (ICH) and the WHO for smooth and planned conduction of stability studies and must

Table 5.10: Calibration curve of NG in 0.1 N HCl

S. no.	Vol. of stock Sol. (µg/ml)	Vol. of diluents (ml)	Conc. (µg/ml)	Absorbance at 225 nm	RSD (%)
1	0.2	9.8	2	0.028±0.014	0.32
2	0.4	9.6	4	0.058±0.005	0.28
3	0.6	9.4	6	0.090±0.007	0.21
4	0.8	9.2	8	0.122±0.011	0.11
5	1.0	9.0	10	0.156±0.006	0.14
6	1.2	8.8	12	0.190±0.008	0.35
7	1.4	8.6	14	0.222±0.013	0.24
8	1.6	8.4	16	0.258±0.004	0.10
9	1.8	8.2	18	0.292±0.009	0.27
10	2.0	8.0	20	0.329±0.015	0.44

RSD: Relative standard deviation

Table 5.11: Results of interference studies

S. no	Material	Quantity of Repaglinide added (mg)	Quantity estimated (mg)	Percent estimated (recovery)
1.	EC	20	19.75	98.75
2.	HPMC	20	19.48	97.40
3.	HPMC K100	20	19.53	97.65
4.	HPMC K4M	20	19.69	98.45

EC: Ethyl cellulose, HPMC: Hydroxypropyl methylcellulose

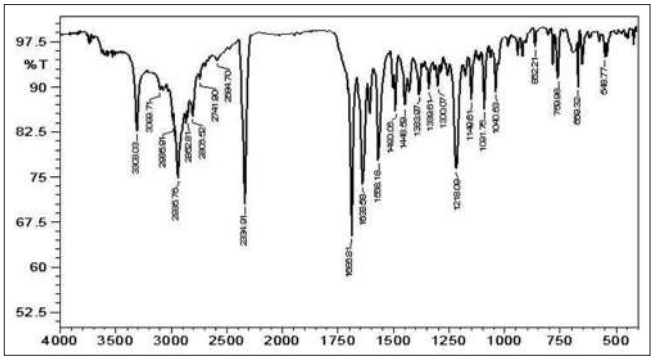


Figure 5.10: Fourier transform infrared spectra of physical mixture of Repaglinide + Hydroxypropyl methylcellulose K4M

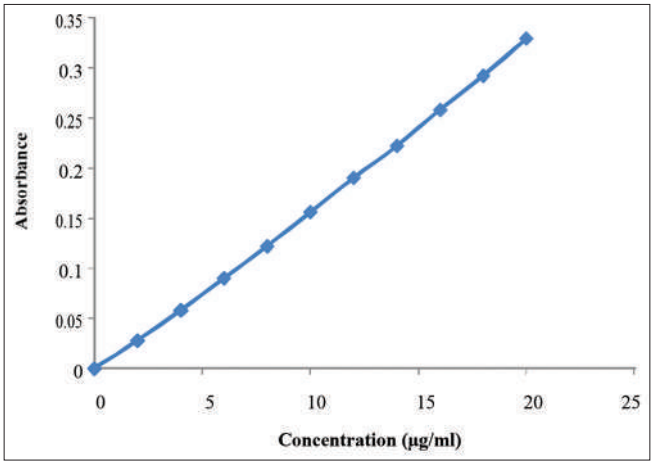


Figure 5.11: Calibration curve of rapeglinide in 0.1N HCl

be followed strictly for quality results (ICH 2003, WHO 2004). The selection of storage conditions solely depends on the climatic zone where the formulation is intended to be marketed or proposed to be filed for regulatory approval. ICH, CPMP, and the WHO gives the general recommendations on the storage conditions. Various guidelines provided by ICH and WHO regarding storage of drug product are summarized in Table 7.1.

If long-term studies are conducted at 25 ± 2°C/60 ± 5% RH and at any time within 6 months if some noticeable changes occur, additional testing should be conducted at the intermediate storage condition for evaluating significant change. During such condition all long-term tests, unless otherwise justified should be included at the intermediate storage condition. When such testing is required due to appearance of significant changes, a minimum of four set of study for 12 months is recommended at different time interval that is 0, 6, 9, and 12 months. The stability testing of selected optimized formulation (A2) at normal and accelerated condition was performed as per ICH and WHO guidelines. In the present study, storage condition of normal (25 ± 2°C/60 ± 5% RH), freezing (5–8°C/65 ± 5% RH), and for accelerated testing oven temperature (40 ± 2°C/75 ± 5% RH) for 6 months was selected.

Experimental

Optimized formulation (A2) was placed separately in amber colored screw capped borosilicate glass

container and kept at normal room temperature (25 ± 2°C/60 ± 5% RH), freezing temperature (5–8°C/65 ± 5% RH), and oven temperature (40 ± 2°C/75 ± 5% RH), respectively, for 6 months using programmable environmental test chambers. After every 2 months, the stored formulations were tested for several parameters such as physical appearance, SEM, % buoyancy, % residual drug content, and drug release as per methods described earlier in chapter 6. The observations of physical appearance and size are reported in Table 7.2.

SEM studies were carried out as reported in morphological study using SEM and images of formulations stored at different conditions are shown in Figures 7.1 and 7.2.

Percent buoyancy and residual drug content of microspheres stored at different temperature were calculated and reported in Tables 7.3 and 7.4, respectively.

Drug release was studied after every 2 months and reported in Table 7.5. Figure 7.4 shows the histogram plot of % drug release verses time for formulations stored at different condition. At the end of the study, 12 h releases of stored formulations are taken summarized in Table 7.7. Release data of formulations at different temperature were compared by plotting graph between cumulative % drug release and time [Figure 7.5].

RESULTS AND DISCUSSION

Stability of optimized formulation was carried out at three different temperatures (5–8°C, 25 ± 2°C, and 40 ± 2°C) and evaluated for physical appearance, SEM, % buoyancy, % residual drug content, and drug release after every 2 months for 6 months. Physical appearance, showed no prominent variation and change in color. Slight change in size of microspheres from initial day was observed at end of 6 months for the samples stored at refrigeration and oven temperature, respectively. Little increment in particles size of microspheres stored in refrigerated conditions was observed which might be due to aggregation of particles. Formulation stored at high temperature shows no aggregation of microspheres as the probability of melting of polymer is negligible due to their high melting point. The particle size slightly decreases at oven temperature may be due to the evaporation of residual amount of organic solvent at higher temperature from the microspheres (Tyagi *et al*., 2014). During surface morphology SEM images indicates the retention of spherical shape of microspheres, there was no sign of any morphological transformation like development of cracks or rupturing of surface of stored formulations at different temperatures [Figure 7.1].

The floating capacity was retained as the % buoyancy was not changed much (<5%) for the stored formulation at three different conditions. The % buoyancy of formulation was 84.36 ± 0.9% on the initial day which changes to 82.85 ± 0.3, 84.02 ± 0.4, and 82.91 ± 0.5% for increasing order of temperature selected during the study (n = 3). The histogram was plotted between % buoyancy and time for formulations stored at different temperatures and the results were almost similar

Table 7.1: Storage conditions for stability of drug products

Intended storage condition	Stability test method	ICH test temperature and humidity (Period in months)	WHO test temperature and humidity (Period in months)
Room temperature	Long term	25±2°C/60±5% RH (12)	25±2°C/60±5%RH or 30±2°C/65±5%RH 30±2°C/75±5%RH (12)
	Intermediate	30±2°C/65±5% RH (6)	30±2°C/65±5%RH (6) 40±2°C/75±5%RH (6)
	Accelerated	40±2°C/75±5% RH (6)	
Refrigerated	Long term	5°C/ambient (12)	5±3°C
	Accelerated	25±2°C/60±5%RH (6)	25±2°C/60±5%RH or 30±2°C/65±5%RH
Freezer	Long term	−20°C/ambient (12)	−20±5°C

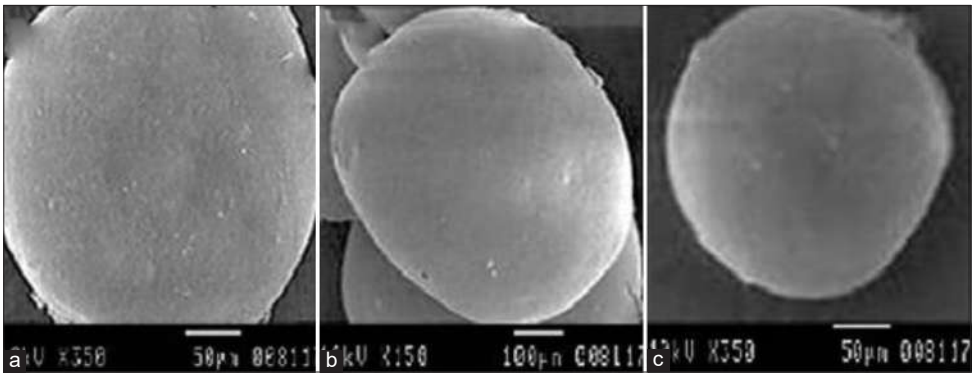


Figure 7.1: Scanning electron microscope images of microspheres: (a) Freezing (b) room and (c) oven temperature

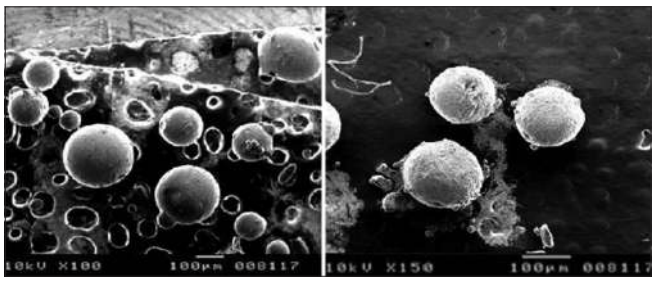


Figure 7.2: Scanning electron microscope images of microspheres at freezing and oven temperature

to initial day of storage [Figure 8.3]. Thus, sample stored at room temperature shows maximum buoyancy.

Percent residual drug content was determined and the initial drug content of all the formulations was taken 100%. No prominent effect from initial day was observed when graph between percent drug content and time was plotted [Figure 8.3]. All data's are represented as mean ± SD (n = 3). A non-significant loss of drug was found in sample stored at 40 ± 2°C as compared to 5–8°C and 25 ± 2°C which may be due to slight loss of integrity of the system (Gawde *et al*., 2012).

Drug release was studied and histogram between percent drug release and time was plotted for different formulations, showing no characteristic change [Figure 8.4]. At the end of study 12 h release of stored formulation was taken and compared by plotting graph between cumulative % drug release and time [Figure 8.5]. There was no change in release rate at 5–8°C and 25 ± 2°C temperature. The release is slightly increased (84.5 ± 2.1–86.00 ± 2.1) for formulation kept at 40 ± 2°C temperature as compared to initial day release which might be due to formation of more pores in the microspheres due to evaporation of organic solvent from the surface. Results of stability indicate that the drug loaded floating microsphere was stable at all conditions but most stable at room temperature. The storage conditions employed do not have drastic effect on microspheres integrity. Stability of product is thus, justified by observing the results of physical and chemical stableness of formulation. Finally, it could be concluded that EC and HPMC microspheres of RG are a suitable delivery system for prolonged activity having significant stability

Table 7.2: Physical appreance of formulation during stability study after 6 months

S. no	Condition	Color change	Mean particle size (μm)	
			Initial day of storage	After 6 months
1.	Room temperature	No change	208.27±2.7	208.14±1.1
2.	Freezing temperature	No change	208.27±2.7	211.51±1.5
3.	Oven temperature	No change	208.27±2.7	204.36±06

Table 7.3: Percent buoyancy of formulation during stability study

Time interval in months	% buoyancy		
	5–8°C/65% RH	25°C/60% RH	40°C/75% RH

Table 7.4: Percent buoyancy drug content of formulation during stability study

Time interval in months	% buoyancy		
	5–8°C/65% RH	25°C/60% RH	40°C/75% RH
0	84.36±0.9	84.36±0.8	84.36±0.6
2	84.01±0.7	84.30±0.2	84.14±0.8
4	83.64±0.5	84.25±0.6	83.74±0.4
6	82.85±0.3	84.02±0.4	82.91±1.5

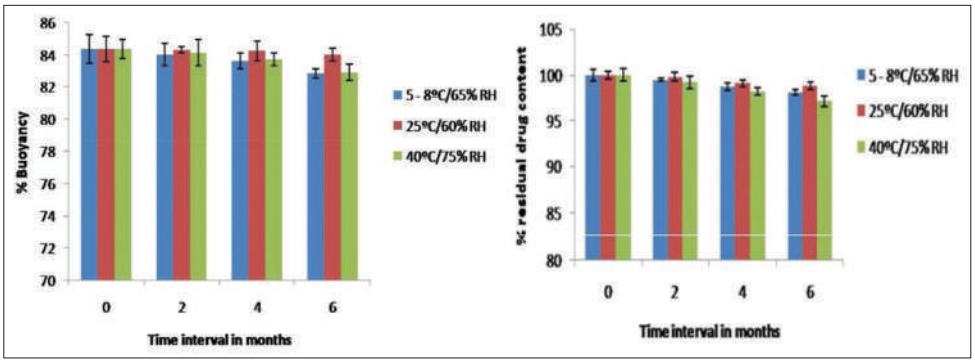


Figure 7.3: Histogram of percent buoyancy and residual drug content

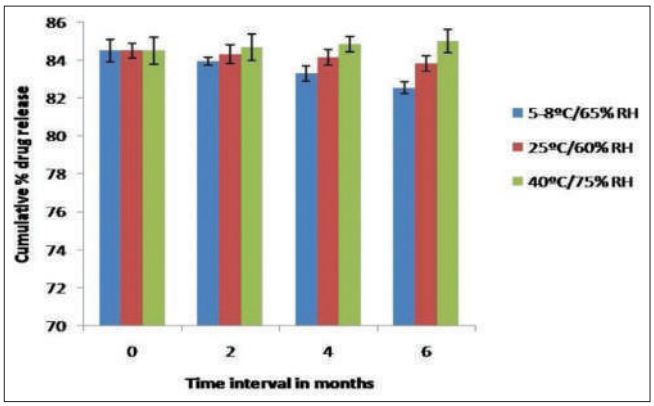


Figure 7.4: Histogram of percent drug release of formulations at different time interval

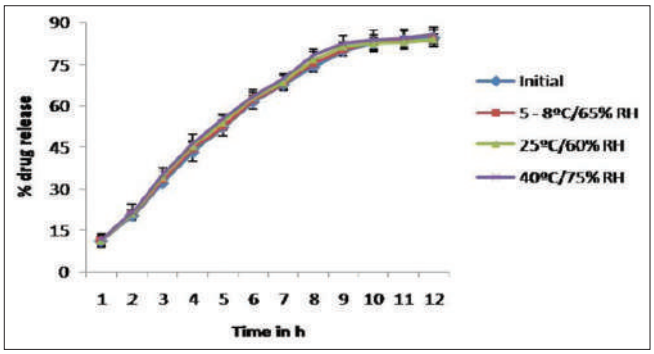


Figure 7.5: Percent cumulative drug release after 6 months storage at different temperatures for 12 h

without losing its therapeutic activity when stored at different temperatures.

SUMMARY

Among the various developed CR formulations GRDDS are one of the unique systems and are getting considerable attention in the field of designing delivery system. The classical advantage of gastroretentive system is to assure

Table 7.5: Percent residual drug content of formulation during stability study

Time interval in months	% Residual drug content		
	5–8°C/65% RH	25°C/60% RH	40°C/75% RH
0	100.0±0.6	100.0±0.4	100.0±0.7
2	99.51±0.2	99.81±0.5	99.23±0.7
4	98.73±0.4	99.11±0.4	98.21±0.4
6	98.14±0.3	98.87±0.4	97.10±0.6

Table 7.6: Percent drug release of formulation during stability study

Time interval in weeks	Cumulative percent drug release		
	5–8°C/65% RH	25°C/60% RH	40°C/75% RH
0	84.51 ± 0.6	84.51 ± 0.4	84.51 ± 0.7
2	83.94 ± 0.2	84.31 ± 0.5	84.68 ± 0.7
4	83.29 ± 0.4	84.16 ± 0.4	84.84 ± 0.4
6	82.54 ± 0.3	83.82 ± 0.4	86.00 ± 0.6

that physiological conditions such as GRT time will work in favor of the developed system. The disadvantage associated with oral sustained release systems is that it will not remain in the absorption site till the complete release of active medicament is achieved. To overcome such drawbacks gastroretentive system works efficiently by increasing retention of system at site of absorption. To prolong GRT number of GRDDS has been developed over the past two decades. Floating systems are more popular in comparison to other GRDDS as they do not impose any adverse effect on GI motility.

Due to low density of floating formulations; they remain buoyant in the gastric fluid for long duration thereby releasing drug in sustained manner. The attractive properties of floating microspheres, give

Table 7.7: Percent drug release of formulations during stability study

Time in h	% Cumulative drug release			
	Initial	5–8°C/65% RH	25°C/60% RH	40°C/75% RH
1	11.1±2.1	11.2±0.1	11.5±1.2	11.5±2.3
2	20.1±0.2	20.7±0.3	20.7±1.5	21.6±3.1
3	32.2±0.7	33.2±0.4	34.2±0.3	35.2±2.5
4	43.3±3.2	44.4±0.5	45.4±1.7	46.3±3.5
5	52.1±2.9	52.4±0.3	53.9±2.6	55.6±1.2
6	61.4±2.4	62.1±0.5	63.0±1.8	63.4±2.4
7	67.5±1.5	67.8±0.7	68.2±2.6	69.8±1.7
8	74.1±2.1	75.4±0.3	76.8±3.0	78.7±1.8
9	80.1±1.9	80.4±0.02	81.5±1.4	82.5±2.8
10	82.7±2.9	83.1±0.01	82.6±2.1	83.7±3.4
11	83.7±3.1	83.1±0.05	82.9±1.8	84.3±3.1
12	84.5±3.0	83.8±0.04	83.9±1.7	85.8±2.4

them a unique success in formulation development. Suitable selection of drug and excipient is required for development of effective formulation. Since every drug cannot be delivered effectively by such system. Drugs which primarily absorbed in the stomach not remain stable in the colonic or intestinal environment, whose solubility at alkaline pH is poor and having narrow absorption window are delivered through floating system. Excipients should be compatible, that is, should not affect the physical and therapeutic efficacy of the drug. Natural or synthetic polymers were used which can modify the release profile of drug.

HBS consists of drug encapsulated within gel-forming hydrocolloids which remain buoyant over gastric fluid. Floating provides prolonged GRT and increases quantity of drug reaching site of absorption in solution form where it gets easily absorbed. System consists of one or more highly soluble gel-forming cellulose derivatives hydrocolloid such as hydroxypropyl cellulose, hydroxyethyl cellulose, HPMC, polysaccharides and matrix-forming polymer such as polycarbophil, polyacrylate, and polystyrene. These hydrocolloids in contact with gastric fluid get hydrates forming gel barrier around its surface and releases the drug in controlled manner.

From the past years, tablet and capsule type conventional dosage forms were utilized for treating Type-II DM. Administration of such dosage forms required frequent dosing to reach and maintain drug concentration within the therapeutic effective range during medication. This leads to fluctuated level of drug in plasma, poor efficiency and consequently undesirable toxicity effects are resulted. Several imposed factors such as repeated administration of drug and unpredictable absorption leads to the use of CRDDS. The advantages of a CRDDS over a conventional dosage forms include reduced frequency of dosing thereby improved patient convenience, decrease fluctuation in steady-state to maintain control over disease condition, decreases local or systemic side effects intensity, and utilization of drug to the maximum to avoid its frequent administration.

The aim of present investigation is to develop, characterize, and evaluate floating microspheres of RG an antidiabetic drug used for treating type II DM. The plasma half-life of RG is about 1 h. Due to short half-life and prime absorption from the GIT, it is quickly eliminated from blood circulation and thus required frequent dosing. Several side effects emerge due to repeat dosing of RG. Thus, the research work was mainly emphasis on development of floating microspheres to overcome such problems. EC and HPMC were employed as control release and swellable polymers for effective release of drug. An attempt is thus, made to microencapsulate RG by solvent evaporation technique with a view to prevent the gastric side effects and to achieve control release of drug. Floating microspheres of EC alone and in combination with HPMC of different grades (5, 100, and 4000 cps) have been developed which shows good *in vitro* buoyancy and release in gastric fluid. Drug was stable, slightly water soluble whereas soluble in acetonitrile, methanol, dichloromethane, and chloroform. Several UV spectrophotometric, colorimetric, and chromatographic methods were reviewed in the literature for identification and determination of RG in biological fluids and in various dosage forms. In the present study, RG was estimated by UV spectroscopy due to easiness and feasibility to study number of samples. For *in vitro,* assessment in 0.1 N HCl 247 nm was selected where maximum absorption of RG was observed. Beer-Lambert's law was obeyed from 0.2 to 2 µg/ml

concentration range. The value of r^2 was calculated to as 0.9990 which indicates positive correlation. Low RSD (<0.44) values ensured reproducibility of the method during validation of the method.

During preformulation studies organoleptic properties, melting point, solubility, partition coefficient, UV, and IR analysis of drug and polymers were performed. The drug and polymers solubility were determined in various solvents as per IP. FTIR spectra of RG, EC, and different grades of HPMC were having peaks almost similar to their reported reference FTIR peaks. Compatibility of drug and polymers was studies by visible physical observations for any change in color, formation of lump or gas, and liquefaction. Physical properties of drug and polymers show no significant variation indicating no interaction. FTIR analysis is also utilized to study the physical and chemical compatibility of drug with polymers. The chief characteristic peaks of drug were mostly similar to the peaks obtained in the mixture of drug with EC and various viscosity grades of HPMC. No interaction revealed as no significant change and major shifts in the characteristic peaks of drug was observed. Similarly, no chemical interaction between RG and polymers was revealed as no additional peaks appeared in any of the spectra studied.

The placebo and drug-loaded EC and EC:HPMC floating microspheres were formulated by solvent diffusion-evaporation method with slight change (Kawashima *et al*.,1992). The RG-loaded microspheres (E1-E9) of EC (A1-A9, B1-B9, and C1-C9) of EC: HPMC (5, 100, and 4000 cps), respectively, were prepared using various process variables such as polymer ratio, stirring speed, drug, and emulsifier concentration. The effect of process variables was observed for different characterized parameters of formulations such as flow behavior, SEM, FTIR, *in vitro* buoyancy, percent yield, drug entrapment, and drug release studies.[23-35]

Microscopic data of SEM indicated that the RG-loaded microspheres of different polymers were discrete, spherical shaped, free flowing, and uniform. The ruptured surface showing hollow nature of EC microspheres from the interior which helps them to remain buoyant over gastric fluid. The surface of the microsphere prepared from EC: HPMC 5, 100, and 4000 rpm was dense and smooth, with distinct pores on the surface. The smooth surface of microspheres reveals the homogeneity of drug and polymers distribution.

FTIR spectra of drug, placebo, and drug-loaded microspheres prepared from all the four types of polymers were studied and any change in the functional group frequency of microspheres was compared with frequency obtained in spectra of drug. Obtained results show that the stability of drug is not affected by the polymers or method of preparation as FTIR spectra of individual batch of microspheres did not show any band shift, broadening, or appearance of additional peaks.

The effect of process variables selected was studied and its affect on parameters used for characterization of floating microspheres were observed. The size of particles increases on increasing concentration of EC and HPMC and viscosity at the same ratio. The outcomes of micromeritics properties studied show excellent flow ability and non-aggregated nature of all the formulations prepared from different polymers. Yield and drug entrapment increases with increase in EC concentration, whereas increase in speed of rotation first increases than decrease the same. Increasing speed of rotation, polymer and drug concentrations resulted in decreased buoyancy in all the categories of prepared microspheres. Effect of increase in concentration and viscosity of HPMC polymer resulted in decreased production of microspheres. When the stirring speed and concentration of drug is enhanced the floating capacity of the microspheres prepared from all viscosity grades HPMC decreases, whereas entrapment efficiency increases on increasing HPMC concentration and viscosity.

The experimental condition of release study was quite similar to the physiological requirement. Release was observed in 0.1 N HCl for 12 h. Release rate of drug decreases with increasing EC and HPMC concentration. Drug release increases for microspheres of all the four batches on increasing the speed of rotation from 600 to 1200 rpm. Release of drug for the optimized formulations of three viscosity grades HPMC was compared and

was more for formulation prepared by HPMC 5cps as compared to other high viscosity 100 and 4000 cps HPMC.

The *in vitro* release data of optimized formulation (E2, A2, B2, and C2) of all the batches were fitted to various mathematical models to study the release kinetics. Selected optimized formulations showed highest correlation coefficient for first rather than zero-order release equation. Thus, all the RG microspheres were found to follow first-order kinetics for drug release. However, this model fails to explain drug release mechanism.

Therefore, Korsmeyer–Peppas equation was also studied and value of "n" was obtained which best describe the release mechanism. Optimized EC microspheres find to follow first-order kinetics having $r^2 = 0.989$, followed by Peppas equation whose $r^2 = 0.988$. Finally, it was concluded that drug is released by first-order, diffusion, and erosion mechanism.

Optimized A2 formulation of EC: HPMC 5cps shows highest regression for first-order kinetics, followed by Higuchi and Peppas model. Similarly, B2 and C2 formulations of EC: HPMC 100 and 4000 cps also resulted in highest regression for first-order kinetics, followed by Peppa's and Higuchi model. Values of "*n*" for all the optimized formulations were found to be <0.89.

All the parameters characterized were found to be satisfactory and best for A2 formulation (EC: HPMC 5cps 1:2); therefore, its stability studies were performed.

The aim of stability study is to indicate that how the quality of an API or finished product changes with time under changing environmental conditions such as temperature, light, and humidity. ICH has given guidelines regarding storage conditions to estimate shelf-life for drug product. The stability of optimized formulation (A2) at normal and accelerated condition was performed according to ICH and WHO guidelines. Best formulation (A2) was placed separately in amber colored screw capped borosilicate glass container and kept at normal (25 ± 2°C/60 ± 5% RH), freezing (5–8°C/65 ± 5% RH), and oven temperature (40 ± 2°C/75 ± 5% RH), respectively, for 6 months period using programmable environmental test chambers.

After every 2 months, the stored preparations were evaluated for several parameters such as physical appearance, SEM, % buoyancy, % residual drug content, and drug release. Physical appearance showed no significant variation and change in color whereas insignificant change in particle size was observed. SEM images indicate the retention of spherical shape of microspheres, with no sign of morphological transformation on the formulations stored at different temperatures. The floating capacity was retained as the % buoyancy was not changed much (<5%) for the stored formulations at three different conditions. Percent residual drug content was determined and a non-significant loss of drug was observed in sample stored at 40 ± 2°C as compared to temperature conditions.

Drug release was studied and plot of histogram between % drug release and time for different formulations were prepared, which shows no variations compared to the initial day of the study in the release data. At the end of study 12 h releases of stored formulations was taken which did not reveal any significant change in drug release. Thus, it may be concluded that EC and HPMC 5 cps microspheres of RG are a suitable delivery system for prolonged activity having significant stability without losing its therapeutic activity when stored at different temperatures.

CONCLUSION

The goal of the research work was to summarize the principals, mechanisms, and technological approaches of FDDS in general. Introduction part represented the physiological properties and factors influencing absorption and gastric motility in the stomach. It has been mentioned that in developing FDDS, role of physiological parameters, and biopharmaceutical evaluation have more significance. These will influence the selection of API, polymers, and the utilized technologies.

Literature reports have shown that shape or size of the formulation can have considerable influence on the buoyancy and dissolution profile of floating formulations.

Experimental target of the performed work was to design and develop floating microspheres containing

significant buoyancy and dissolution properties. Four types of formulations of EC, EC: HPMC 5, 100, and 4000 cps were developed, optimized, and sequentially evaluated. Prepared formulations were suitably sized and had best *in vitro* buoyancy. Drug entrapment and product yield were high for all the formulations. Proportion of drug–polymer, speed of rotation, and emulsifier concentration affects the shape, size, and other evaluated parameters during the study. Short half-life thereby fast elimination of RG makes it advantageous to be delivered through gastroretentive system.

The cellulose polymers EC and different viscosity grades of HPMC were used to develop systems which will modify the release of RG. Due to prolonged residence time at absorption site, enhanced bioavailability can be attained. Optimized formulations show satisfactory drug release up to 12 h. The observed release mechanism from microspheres was diffusion and erosion controlled. The optimized formulation during stability studies does not show any variation in physicochemical properties of microspheres.

FUTURE SCOPE

Floating dosage form offers various future potential as evident from several recent publications. Among the recently used clinical drugs several narrow absorption window drugs may benefit from compounding into a FDDS. Replacing parenteral administration of drugs to oral pharmacotherapy would substantially improve treatment. It may be believed that it can be possible with FDDS. The investigations can be concentrated on the concept of design of novel polymers according to clinical and pharmaceutical need.

The present work contributes significantly in the field of GRDDS through floating technique. This system is a starting footstep to fight with DM. However, some recommendation and extension in the research work done are required to be fulfilled such as:

- To prove the efficacy and enhanced bioavailability of developed antidiabetic formulations, *in vitro* and *in vivo* correlation has to be established.
- Rapid analytical methods such as HPLC and LC-MS can be developed for assessment of bioavailability.
- Preclinical and clinical trials should perform for the use of formulation in human volunteers.

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