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Formulation and Evaluation of Floating *In-Situ* Gel of Clopidogrel

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Abstract: *The characterization peaks of clopidogrel were found in formulation undisturbed indicates the compatibility off the drug used with the excipients of the formulation the monograph off Clopidogrel has melting point range 198 – 200 ^{0}C. The sample of clopidogrel obtained from Dr. MACS Bio Pharma Pvt Ltd, Hyderabad. Indicating the purity of the drug sample. Clarity of all the formulations was found to be satisfactory. The pH of all formulations was found to be satisfactory and lies in the range 1.2 – 4.8. The drug content uniformity data has shown that all the formulations were found to be uniform in drug content in the range of 95.05-99.25%. Gelation study was performed to explain gelling capacity. Gelling capacity of all formulations were designated as + (gel formation takes after few minutes and disperse rapidly), ++ (immediate gel formation, remains un-dispersed for few hours) and +++ (immediate gel formation, the gel was remains for extend time). The results of rheological study of prepared in situ gel confirms as the viscosity decreases with increase in angular velocity. Results indicated that all formulations are having an optimum viscosity and all formulations were pourable at normal conditions. The drug release data obtained for all the formulations were shown in fig 4.5. The cumulative percent drug release of formulation was 99.25±3.5 for formulation 1, for formulations F2 to F6 the values 98.25±2.5, 95.05±2.5, 96.32±1.3, 95.27±0.2 and 98.24±1.5respectively till 12th hour. From the result of drug content, gelation pH, drug content, and drug release studies for all formulation, F5 formulation was selected as best formulation which has shown highest drug release till 12th hour. Hence F5 formulation was chosen for stability studies.*

Key words: *clopidogrel, HPMC, Sodium alginate, Sodium Citrate, Calcium Carbonate.*

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I. Introduction

1.1 NOVEL DRUG DELIVERY SYSTEM:

Novel drug delivery system (NDDS) refers to the approaches, formulations, technologies and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effects. It may involve scientific targeting with in the body, or it might involve facilitating systemic pharmacokinetics.
NDDS is advanced drug delivery system which improves the drug potency, control drug release to give a sustained therapeutic effect, provides greater safety, it is to target a drug specifically to a desired tissue.

1.2 Anatomy and physiology of stomach:

The stomach is the most dilated part of the GIT and is situated between the lower end of the esophagus and the small intestine. Its opening to the duodenum is controlled by pyloric sphincter. The stomach can be divided into four anatomical regions, namely

- Fundus
- Body
- Antrum
- Pylorus

The two major functions of the stomach are:

- To act as a temporary reservoir for ingested food and to deliver it into the duodenum at a controlled rate.
- To reduce the ingested solids to form creamy consistency, known as chyme, by the action of the acid and enzymatic digestion .This enables the better contact of ingested food with the mucous membrane of intestine and there by facilitates absorption.

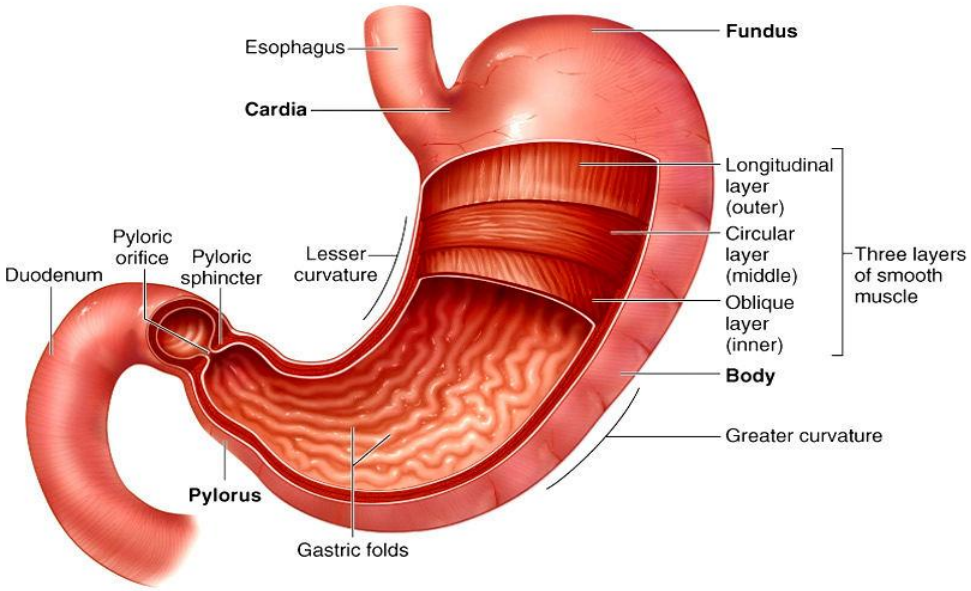

Fig 1.1 Internal structure of stomach

1.2.1 Gastric motility:

Gastric emptying occurs during fasting as well as fed states. During the fasting states an inter digestive series of events takes place, which cycles through stomach and intestine every 2-3 hrs. .This is called the interdigestive myoelectric cycle or migrating myoelectric cycle (MMC), which is further divided into 4 phases by Wilson and Washington.

- Phase 1: Basal phase- lasts for 40-60 min with rare contractions.
- Phase 2: Pre burst phase- lasts for 40-60 min with intermittent action and potential contractions.
- Phase 3: Burst phase- lasts for 4-6 min. It includes intense and regular contractions for short period.
- Phase 4: Lasts for 0-5 min and occurs between phase 3 and 1 of two consecutive cycles.

1.3 Approaches to Gastric retention:

A number of approaches have been used to increase the gastric residence time of a dosage form in stomach by employing a variety of concepts. These includes

1.3.1 Floating systems:

FDDS have bulk density lower than the gastric fluids and thus remain buoyant in the stomach for a prolonged period of time, without affecting the gastric emptying time. While the system is floating on the gastric contents, the drug is released slowly at a desired rate from the system. After the release of the drug, the residual system is emptied from the stomach. These results in an increase in the gastric retention time and better control of fluctuation in plasma drug concentrations. Floating systems can be classified into two distinct categories. They are:

- Effervescent systems
- Non – effervescent systems.

1.3.2 Bio/ Muco- adhesive systems:

Bio-adhesive or muco-adhesive systems are used to localize a delivery device within the lumen and cavity of the body to enhance the drug absorption process in a site-specific manner. The approaches involve the use of bio adhesive polymers that can be adhering to the epithelial surface of the GIT. The proposed mechanism of bio adhesive is the formation of hydrogen and electrostatic bonding at the mucous polymer boundary.

1.3.3 Swelling and expanding systems:

These are the dosage forms, which after swallowing, swell to an extent that prevents their exit from pylorus. As a result, the dosage form is retained in the stomach for a longer period. These systems may be named as "plug type system" since they exhibit the tendency to remain logged at the pyloric sphincter, if that exceed a diameter of approximately 12-28 mm in their expanded state. Such polymeric matrices remain in the gastric cavity for several hours even in the fed state.

A balance between the extent and duration of swelling is maintained by the degree of cross linking between the polymeric chains. A high degree of cross linking retards the swelling ability and maintains its physical integrity for prolonged period.

1.3.4 High density systems:

These systems with a density of about 3 g/cm^3 are retained in the rugae of the stomach and are capable of withstanding its peristaltic movements. A density of 2.6- 2.8 g/cm^3 acts as a threshold value after which system can be retained in the lower part of the stomach. High density formulation include coated pellets. Coating is done by heavy inert materials such as barium sulphate, zinc oxide, titanium dioxide, and iron powder.

1.3.5 Incorporation of passage delaying food agents:

Food excipients like fatty acids eg; salts of myristic acid change and modify the pattern of the stomach to a fed state, thereby decreasing the gastric emptying rate and permitting considerable prolongation of release. The delay in the gastric emptying after meals rich in fats is largely caused by saturated fatty acids with chain length of C_{10}-$C_{14.}$

1.3.6 Ion Exchange resins:

A coated ion exchange resin bead formulation has been shown to have gastric retention properties, which was loaded with bicarbonates. Ion exchange resins are loaded with bicarbonate and a negatively charged drug is bound to the resin. The resultant beads were then encapsulated in a semipermeable membrane to overcome the rapid loss of carbon dioxide. Upon arrival in the acidic environment of the stomach, an exchange of the chloride and bicarbonate ions takes place, as a result of this reaction carbon dioxide was released and trapped in the membrane there by the carrying beads towards the top of gastric content and producing a floating layer of resin beads in contrast to the uncoated beads, which will sink quickly.

1.3.7 Osmotic regulated systems:

It is comprised of an osmotic pressure controlled drug delivery device and an inflatable floating support in a bio erodible capsule. In the stomach the capsule quickly disintegrates to release the intragastric osmotically controlled drug delivery device. The inflatable support inside forms a deformable hollow polymeric bag that contains a liquid that gasifies at body temperature to inflate the bag. The osmotic controlled drug delivery device consists of two components, drug reservoir compartment and osmotically active compartment.

Criteria for selection of drug for GRDDS:

GRDDS are suitable for following types of drug therapy

- Absorption from upper GIT, drugs have a particular site for maximum absorption .eg. Ciprofloxacin, whose maximum absorption is in the stomach only. The absorption of metformin hydrochloride is confined to small intestine only and the conventional sustained release dosage forms may have poor bioavailability since absorption appears to diminish when the dosage forms pass in to large intestine.
- Drugs having low P^{ka} , which remains unionised in stomach for better absorption.
- Drugs having reduced solubility at higher pH eg: Captopril and chlordiazepoxide and the bioavailability of drugs that get degraded in alkaline pH can be increased by formulating gastro-retentive dosage forms.eg: doxifluridine , which degrades in small intestine.
- Local action as it's seems in the treatment of H. Pylori by Amoxicillin and Misoprostol for ulcers.
- To minimize gastric irritation that may be caused by sudden increase of drug concentration in the stomach. Eg: NSAIDS.
- Improves effectiveness of particular drugs .eg: Antibiotics in the colon tend to disturb the micro flora causing the overgrowth of microorganisms like Clostridium difficile causing colitis.

1.4 Factors affecting Gastro retentive system:

The GRT of dosage forms is controlled by several factors such as density and the size of the dosage form, food intake, nature of the food, posture, age, gender, sleep and disease condition of the individual and administration of drugs such as prokinetic agents.

1.4.1 Density of dosage form:

Dosage forms having a density lower than that of the gastric fluid experience floating behaviour and hence gastric retention. A density of < 1.0 gm/cm^{3}is required to exhibit floating property. However, the floating tendency of the dosage form usually decreases as a function of time, as the dosage form gets immersed into the fluid, as a result of the development of hydrodynamic equilibrium.

1.4.2 Size and shape:

Dosage form unit with a diameter of more than 7.5mm are reported to have an increased gastric residence time competed to with those with a diameter of 9.9nm. The dosage form with a shape tetrahedron and ring shape devices with a flexural modulus of 48 and 22.5 KSI are reported to have better GIT at 90-100% retention for 24 hrs compared with other shapes.

1.4.3 Fed or Unfed state:

Under fasting conditions, the GI motility is characterised by periods of strong motor activity at MMC that occurs every 1.5 to 2 hrs. The MMC sweeps undigested material from the stomach and if the timing of administration of the formulation coincides with that of the MMC, the gastric residence time of unit can be expected to be very short. However, in the fed state, MMC is delayed and GRT is considerable longer.

1.4.4 Nature of the meal:

Feeding of indigestible polymers of fatty acid salts can change the motility pattern of the stomach to a fed state, thus decreasing the gastric residence time and prolonging the drug release.

1.4.5 Caloric content:

Gastric residence time can be increased between 4-10 hrs with a meal that is high in proteins and fats.

1.4.6 Frequency of Feed:

The gastric residence time can increase by over 400 min when successive meals are given compared with a single meal due to low frequency of MMC.

1.4.7 Effect of gender, posture and age:

Females showed comparatively shorter mean ambulatory gastric residence time than males and gastric emptying time in women was slower than in men.

The floating and non-floating systems behaved differently. In the upright position, the floating systems floated to the top of the gastric contents and remained or a longer period, shoeing prolonged gastric residence time, but the non-floating units settled to the lower part of the stomach and undergone faster emptying as a result of peristaltic movements, and the floating units remained away from the pylorus.

1.5 Floating drug delivery systems (FDDS);

Based on the mechanism of buoyancy, the two distinctly different technologies have been utilised in the development of FDDS, which are

- Effervescent system
- Non-effervescent system

1.5.1 Effervescent system:

It includes use of gas generating agents, carbonates and other organic acids to produce carbon dioxide, thus reducing the density of the system and making it to float on the gastric fluid. These effervescent systems further classified into two types

- Gas generating systems
- Hydro dynamically balanced system (HBS).

These are formulated by mixing the carbon dioxide generating agents and the drug within the matrix tablet. These have a bulk density lower than the gastric fluids and therefore remains floating in the stomach unflattering the gastric residence time for prolonged period. The drug is slowly released at a desired rate from the system and after the complete release the residual system is expelled from the stomach. This leads to increase in the gastric residence time and better control over fluctuations in plasma drug concentrations.

1.5.2 Non – Effervescent systems:

These systems are based on the mechanism of swelling of polymer or bioadhesion to mucosal layer in GI tract. The most commonly used excipients in these systems are gel forming or highly swellable cellulose type hydrocolloids, polysaccharides and matrix forming materials such as polycarbonates, polyacrylates, polymethacrylates, polystyrenes etc. And bioadhesive polymer such as chitosan and carbopol. The various types of these systems are

Single layer floating tablets:

They are formulated by intimate mixing of drug with a gel forming hydrocolloid, which swells in contact with gastric fluid and maintain bulk density of less than unity. The air trapped by the swollen polymer confers buoyancy to the dosage forms.

Alginate beads:

Multi-unit floating dosage forms were developed from freeze dried calcium alginate. Spherical beads of approximately 2.5 mm diameter can be prepared by dropping a sodium alginate solution into aqueous solution of calcium chloride, causing precipitation of calcium alginate leading to formation of porous system, which can

maintain a floating force for over 12 hrs. These floating beads gave a prolonged residence time of more than 5.5 hrs

1.6 Mechanism of FDDS:

There are several attempts have been made to retain the dosage form in the stomach as to increase the retention time. These attempts includes introducing floating dosage forms i.e., gas generating, swelling/expandable, mucoadhesive, HBS, Gastric emptying delaying devices. FDDS have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. The drug is slowly released from dosage form at desired rate. After releaseof drug, residual system is emptied from the stomach. This results in an increased gastric retention time and reduced fluctuations in plasma drug concentrations. However, besides a minimal gastric content need to allow the proper achievement of the buoyant retention principle, a minimal level of floating force (F) is also required to keep the dosage form buoyant on the surface of the meal. To measure the floating force, a novel apparatus for determination of resultant weight has been reported in the literature. The apparatus works by measuring continuously the force equivalent to F (as a function of time) that is needed to maintain submerged object. The object floats better if F is on the higher positive side. This apparatus helps in optimising FDDS with respect to stability and duration of floating forces produced in order to prevent drawbacks of intragastric buoyant capability variations.

$F = F_{(Buoyancy)} - F_{(Gravity)} = (Df - Ds)\, gv$; where

F = Total vertical force

Df = fluid density

Ds = Object density

v = volume

g = acceleration due to gravity.

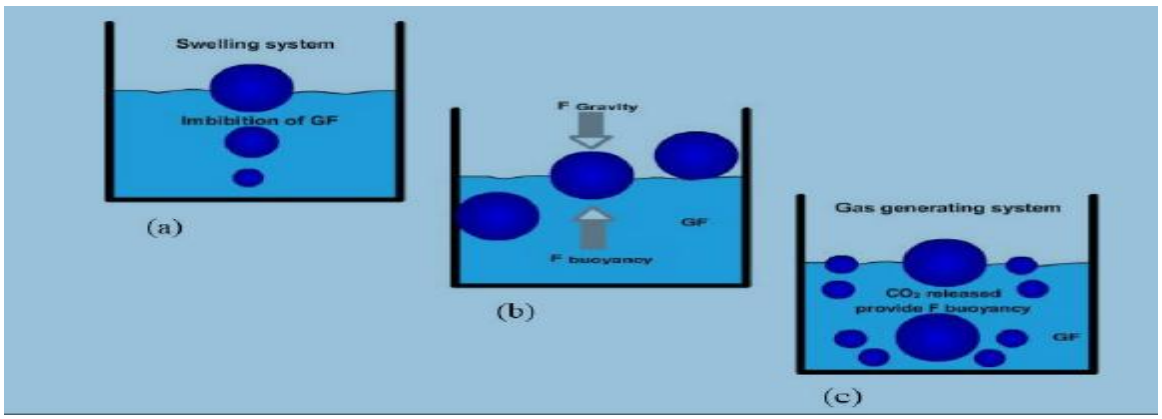

Fig 1.2 Mechanism of floating system

1.7 Advantages and disadvantages of FDDS:

1.7.1 Advantages:

- The gastro retentive systems are advantageous for drug absorbed through the stomach. Eg: Ferrous salts, antacids.
- Acidic substances like aspirin cause irritation on the stomach wall when come in contact with it. Hence HBS formulation may be useful for the administration of aspirin and other similar drugs.
- Administration of prolonged release floating dosage forms, tablets or capsules, will result in dissolution of the drug in the gastric fluid. They dissolve in the gastric fluid would be available for absorption in the small intestine after emptying of the stomach contents. It is therefore expected that a drug will be completely absorbed from floating dosage forms if it retained in the solution form even at the alkaline pH of the intestine.
- The gastro retentive systems are advantageous to drugs meant for local action in the stomach. Eg: Antacids.
- When there is a vigorous intestinal movement and a short transit time as might occur in certain type diarrhea, poor absorption is expected. Under such circumstances it may be advantageous to keep the drug in floating condition in stomach to get a relatively better response.

1.7.2 Disadvantages:

- Floating system is not feasible for those drugs that have solubility or stability problem in GIT.
- These systems require a high level of fluid in the stomach for the drug delivery to float and work efficiently.

- The drugs that are significantly absorbed through the GIT, which undergo significant first pass metabolism, are only desirable candidate.
- Some drugs present in the floating systems causes irritation to gastric mucosa.

1.8 Microspheres:

MICROSPHERES are small spherical particle, with diameters 1µm to 1000µm . There are spherical free flowing particles consisting of proteins or synthetic polymers which are biode-gradable in nature. There are two types of microspheres; microcapsules and micromatrices, which are described as, microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall and micromatrices in which entrapped substance is dispersed throughout the matrix. Microspheres can be manufactured from various natural and synthetic materials. Microspheres play an important role to improve bioavailability of conventional drugs and minimizing side effects.

1.8.1 Ideal characteristics of Microspheres:

- The ability to incorporate reasonably high concentration of the drug.
- Stability of the preparation after synthesis with a clinically acceptable shelf life.
- Controlled particle size and dispersibility in aqueous vehicles for injection.
- Release of active reagent with a good control over a wide time scale.
- Biocompatibility with a controllable biodegradability.
- Susceptibility to chemical modification.

1.8.2 Advantages of Microspheres:

- Particle size reduction for enhancing solubility of the poorly soluble drugs.
- Provide constant and prolonged therapeutic effect.
- Provide constant drug concentration in blood thereby increasing patient compliance.
- Decreased dose and toxicity.
- Protect the drug from enzymatic and photolytic cleavage hence found to be best for drug delivery of proteins.
- Reduce the dosing frequency and thereby improve the patient compliance.
- Better drug utilisation will improve the bioavailability and reduce the incidence or intensity of adverse effects.
- Microsphere morphology allows a controllable variability in degradation and drug release.

1.8.4 Limitations:

Some of the disadvantages were found to be as follows

- The costs of the materials and processing of the controlled release preparation, are substantially higher than those of standard formulation.
- The fate of polymer matrix and its effects on the environment.
- The fate of polymer additives such as plasticizers, stabilizers, antioxidants and fillers.
- Reproducibility is less.
- Process conditions like change in temperature, pH, solvent addition and evaporation/agitation may influence the stability of core particles to be encapsulated.
- The environmental impact of the degradation products of the polymer matrix produced in response to heat, hydrolysis, oxidation, solar radiation or biological agents.

1.9 Types of microspheres:

- Bioadhesive microspheres
- Magnetic microspheres
- Floating microspheres
- Radioactive microspheres
- Polymeric microspheres
- Biodegradable polymeric microspheres
- Synthetic polymeric microspheres

1.9.1 Bioadhesive microspheres:

Adhesion can be defined as sticking of drug to the membrane by using the sticking property of water soluble polymers. Adhesion of drug-drug delivery device to the mucosal membrane such as buccal,rectal, ocular, nasal etc. can be termed as bio adhesion. These kinds of microspheres exhibit a prolonged residence time at the site of application and cause intimate contact with the absorption site and produces better therapeutic action.[4,5]

1.9.2 Magnetic microspheres:

This kind of delivery system is very much important which localises the drug to the disease site. In this larger amount of freely circulating drug can be replaced by smaller amount of magnetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres are chitosan,dextran, etc. The different types of magnetic microspheres

- Therapeutic magnetic microspheres used to deliver chemotherapeutic agent to liver tumour. Drugs like proteins and peptides can also be targeted through this system.
- Diagnostic microspheres , used for imaging liver metastases and also can be used to distinguish bowel loops from other abdominal structures by forming nano size particles supramagnetic iron oxides.[6,7]

1.9. 3 Floating microspheres:

In floating types the bulk density is less than the gastric fluid and so remains buoyant in stomach without affecting gastric emptying rate. The drug is released slowly at desired rate and the system is found to be floating on gastric content and increases gastric residence and increases fluctuations in plasma concentration .Moreover it also reduces chances of dose dumping . It produces prolonged therapeutic effect and therefore reduces dosing frequencies[8, 9, 10]

1.9. 4 Radioactive microspheres:

Radio embolisation therapy microspheres sized 10-30 nm are of larger than the diameter of the capillary and gets tapped in first capillary bed when they come across. They are injected in the arteries that leads them to tumour of interest so all these conditions radioactive microspheres deliver high radiation dose to the targeted areas without damaging the normal surrounding tissues .It differs from drug delivery system, as radioactivity is not released from microspheres but acts as from within a radioisotype typical distance and different kinds of radioactive microspheres are α emitters,β emitters, γ emitters[11,12].

1.9.5 Polymeric microspheres:

The different types of polymeric microspheres can be classified as follows and they are biodegradable polymeric microspheres and synthetic polymeric microspheres.[12]

1.9.5.1 Biodegradable polymeric microspheres:

Natural polymers such as starch are used with the concept that they are biodegradable, biocompatible, and also bioadhesive in nature. Biodegradable polymers prolongs the residence time when contact with mucous membrane due its high degree of swelling property with aqueous medium, results gel formation. The rate and extent of drug release is controlled by concentration of polymer and the release pattern in a sustained manner .The main drawback is, in clinical use drug loading efficacy of biodegradable microspheres is complex and is difficult to control the drug release. However they provide wide range of applications in microsphere based treatment.[13]

1.9.5.2 Synthetic polymeric microspheres:

Synthetic polymeric microspheres are widely used in clinical application, moreover also used as bulking agent, fillers, embolic particles, drug delivery vehicles etc. And proved to be safe and biocompatible but the main disadvantage of these kind of microspheres, are tend to migrate away from injection site and lead to potential risk, embolism, and further organ damage.[14, 15]

1.10 METHODS OF PREPRATION:

- Emulsion solvent evaporation technique.
- Ionic cross linking method.
- Coacervation method.
- Spray drying method.
- Emulsion- solvent diffusion technique.
- Multiple emulsion method.
- Ion gelation method.

1.10.1 Emulsion solvent evaporation technique:

In this technique the drug is dissolved in polymer which was previously dissolved in chloroform and the resulting solution is added to aqueous phase containing 0.2% solution of PVP as emulsifying agent. The above mixture was agitated at 500 rpm, then the drug and polymer was transformed into fine droplet which solidifies into rigid microspheres by solvent evaporation and then collected by filtration and washed with demineralised water and desiccated at room temperature for 24 hrs[16].

1.10.2 Ionic cross linking method:

- Take required quantity of sodium alginate in one beaker and dissolve it in 100ml distilled water.
- Take another beaker add required quantity of HPMC and sodium citrate dissolve in 40ml of water.
- Mix both the solutions place on magnetic stirrer for 20min, for complete swelling of polymer. Add buffer slowly to the 20ml of polymer solution with continuous magnetic stirring forn15 min.
- Specified quantity of calcium carbonate solution is prepared by dissolving it in distilled water. Add to polymer solution with continuous stirring for 10 min. To the final solution if required add preservatives with continuous stirring for 2 to 5 min.

1.10.3 Coacervation method:

- Co-acervation thermal change: Performed by taking weighed amount of ethyl cellulose was dissolved in cyclohexane with vigorous stirring at 80^0C by heating. Then the drug was finely pulverised and added with vigorous stirring on the above solution and phase separation was done by reducing the temperature and using ice bath. Then the above product was washed thrice with cyclohexane and air dried then passed through sieve (no: 40) to obtain individual microspheres.
- Coacervation non solvent addition: Developed by weighed amount of ethyl cellulose was dissolved in toulene containing propyl isobutylene in closed beaker with magnetic stirring for 6 hrs at 500 rpm and the drug is dispersed in it and stirring is continued for 15 min. Then phase separation is done by petroleum benzoin for 5 times with continuous stirring. After that microspheres were washed with n-hexane and air dried for 2 hrs and then in oven at 50^0C for 4 hrs.[17].
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1.10.4 Spray drying technique:

This was used to prepare polymeric blended microsphere loaded with drug. It involves dispersing the core material into liquefied coating material and then spraying the mixture in the environment for solidification of coating followed by rapid evaporation of solvent. Organic solution of PCL and cellulose acetate butyrate (CAB), in different weight ratios and drug was prepared and sprayed in different experimental condition achieving drug loaded microspheres. This is rapid but may loose crystallinity due to fast drying process.

1.10.5 Emulsion-solvent diffusion technique:

The drug polymer mixture was dissolved in a mixture of ethanol and dichloromethane (1:1) and then the mixture was added drop wise to sodium lauryl sulphate (SLS) solution. The solution was stirred with the propeller type agitator at room temperature at 150 rpm for 1 hr. Thus the formed microspheres were washed and dried in desiccator at room temperature which were sieved and collected.

1.10.6 Multiple emulsion method:

Oral controlled release drug delivery of various drugs were prepared by this technique. In the beginning powder drug was dispersed in solution followed by emulsification ethyl cellulose solution in ethyl acetate. The primary emulsion was then emulsified in aqueous medium. Under optimised condition discrete microspheres were formed during this phase.

1.10.7 Ionic- Gelation method:

Alginate/chitosan particulate system of drug was prepared using this technique. Different % (w/v) of drug was added to 2% (w/v) aqueous solution of sodium alginate. In order to get the complete solution stirring is continued and after that it was added to drop wise to a solution of calcium chloride. Microspheres thus formed were kept in original solution for 6 hrs and 24 hrs for internal jellification followed by filtration for separation.[18]

1.11 APPLICATION OF MICROSPHERES:

- Ophthalmic drug delivery
- Oral drug delivery
- Gene delivery
- Nasal drug delivery
- Intratumoral and local drug delivery
- Buccal drug delivery
- Gastrointestinal drug delivery
- Transdermal drug delivery
- Colonic drug delivery
- Vaginal drug delivery
- Targeting by using microparticulate carriers.

1.11.1 Ophthalmic drug delivery:

Microspheres developed using polymer exhibits favorable biological behaviour such as bioadhesion, permeability-enhancing properties and interesting physic-chemical characteristics, which make it a unique material for the design of ocular drug delivery vehicles .Eg. Chitosan, alginate, gelatin.

1.11.2 Oral drug delivery:

The ability of microspheres containing polymer to form films permit its use in the formulation of filming dosing forms, as an alternate to pharmaceutical tablets. The pH sensitivity, coupled with reactivity of the primary amine groups, make microspheres more suitable for oral drug delivery applications. Eg: chitosan, gelatin.

1.11.3 Gene delivery:

Microspheres could be a useful oral gene carrier because of its adhesive and transport properties in the GI tract. Eg: chitosan, gelatin, viral vectors, cationic liposomes, polycation complexes.

1.11.4 Nasal drug delivery:

Polymer based drug delivery systems, such as microspheres, liposomes, and gels have been demonstrated to have good bioadhesive characteristics and swell easily when in contact with the nasal mucosa increasing the bioavailabilty and residence time of the drugs to the nasal route. Eg: starch, dextran, albumin, chitosan.

1.11.5 Intratumoral and local drug delivery:

In order to deliver paclitaxel at the tumour site in therapeutically relevant concentration, polymer films are fabricated. Mixture of drug has promising potential for use in controlled delivery in the oral cavity Eg: chitosan, gelatine, PLGA, chitosan and PCL.

1.11.6 Buccal drug delivery:

Polymer is excellent to be used for buccal delivery because it has muco/bioadhesive properties and can act as an absorption enhancer. Eg: chitosan, sodium alginate.

1.11.7 Gastrointestinal drug delivery:

Polymer granules having internal cavities prepared by de acidification when added to acidic and neutral media are found buoyant and provided a controlled release o drug. Eg: eudragit, ethylcellulose, carbapol BSA, gelatin.

1.11.8 Transdermal drug delivery:

Polymer has good film forming properties. The drug release from the devices is affected by the membrane thickness and crosslinking of the film. Eg: chitosan, alginate, PLGA.

1.11.9 Colonic drug delivery:

Polymer has been used for the specific delivery of insulin to the colon. Eg: chitosan.

1.11.10 Vaginal drug delivery:

Polymer, modified by the introduction of thioglycolic acid to the primary amino groups of the polymer is widely used for the treatment of mycolic infections of the genitourinary tract. Eg: chitosan, gelatin, PLGA.

1.11.11 Targeting by using micro particulate carriers:

Pellets are prepared with the polymer by using the extrusion/sphere ionization technology. Eg: chitosan, microcrystalline cellulose.

II. Material And Methods

3.1. MATERIALS

Clopidogrel was obtained from Dr.MACS Biopharma, Hyderabad, as a gift sample. Sodium alginate, sodium citrate, calcium carbonate and HPMC K100M were purchased from SD Fine Chemicals, Mumbai. All other ingredients used were of analytical reagent grade and double distilled water was used whenever required

Table 3.1: Materials used in the study

Name of chemical	Source
Clopidogrel	Dr. MACS Bio Pharma Pvt Ltd, Hyderabad
HPMC	Lara drugs pvt. Ltd, Hyderabad
Sod. Alginate	Lara drugs pvt. Ltd, Hyderabad
Sod. Citrate	Lara drugs pvt. Ltd, Hyderabad
Calcium carbonate	Lara drugs pvt. Ltd, Hyderabad

Table 3.2: Equipment used in the study

Equipment	Manufacturer
Weighing balance	Vibra HT 220E, Japan
Double beam UV spectrophotometer	Shimadzu 1800, Japan
FTIR spectrophotometer	MB 104; Bruker
Dissolution test apparatus	Lab India Dissolution tester, DISSO 14000
brook field viscometer	DV II+pro, USA

3.2. METHODS

3.2.1. Preformulation Studies

Preformulation may be described as a stage of development during which the physicochemical and biopharmaceutical properties of a drug substance are characterized. It is important part of the drug development process. The information relating to drug development acquired during this phase is used for making critical decisions in subsequent stages of development. A wide variety of information must be generated to develop formulations rationally. Characterization of the drug is a very important step at the preformulation phase of product development followed by studying the properties of the excipients and their compatibility.
The Clopidogrel was tested for the following properties:

3.2.1.1. Determination of melting point:

Melting point of Clopidogrel was determined by melting point test apparatus.

3.2.1.2. Drug-excipient compatibility studies:

Infra-red spectroscopy is widely used in pharmaceutical research. IR spectroscopy is routinely used for compound identification as a fingerprinting tool. IR spectroscopy also has its application in studies of drug - excipient interaction, contaminant analysis etc. IR spectrum with high quality is acquired with the FTIR method. Fourier transformation mathematical operation can resolve the signal captured by detector as a summation of all these signals and in connection with the contribution of each wavelength. Several sampling methods are available for IR spectrum acquisition, such as alkali halide pellet, mineral-oil mull, diffuse reflectance technique and attenuated total reflectance. Each has its advantages and disadvantages.

IR spectrum with high quality is acquired with KBr pellet method. Compatibility study of drug with the excipients was determined by using FTIR. The sample powder of drugs, excipients and mixture of both were subjected to FTIR study. The mixture spectra were compared with that of the original spectra.

3.2.2. Standard Curve of Clopidogrel.

3.2.2.1. Preparation of 0.1N Hydrochloric acid:

8.5 ml of concentrated hydrochloric acid was diluted with distilled water and the volume was made upto 1000ml with distilled water.

3.2.2.2. Preparation of ClopidogrelStandard Stock Solution in 0.1N HCl:

A Standard Solution of Clopidogrel was prepared by dissolving accurately weighed 100 mg of Clopidogrel with little quantity of 0.1N HCl solution, in a 100 ml volumetric flask. The volume was made up to 100 ml with 0.1N HCl, to obtain a stock solution of 1000µg/ml. From the above solution several dilutions are made to obtain 50, 75, 100, 125, 150 µg/ml solutions. The absorbencies of these drug solutions were estimated at λ_{max} 254 nm.

3.2.3. Formulation

Different formulations of Clopidogrel in situ hydrogel were prepared as per the Table 1. Sodium alginate solutions at different concentrations were prepared in half volume of deionized water containing calcium chloride (0.1% w/v) and sodium citrate (0.5% w/v). This solution was heated to 60°C with stirring. After cooling below 40°C, another one-third quantity of deionized water containing HPMC K100M (viscosifying agent) was added with continuous stirring. Further, Clopidogrel, calcium carbonate and sodium citrate were added to above mixture, and final volume was made up to 20 ml with deionized water. The ingredients used in the preparation of formulations were shown in table 3.1.

Table 3.3: Formulations used in the study

Name of the ingredient	Formulations(mg)					
	F1	F2	F3	F4	F5	F6
Clopidogrel.	75	75	75	75	75	75
Sodium Alginate	750	750	1000	1500	1000	1500
Sodium citrate	250	250	250	250	250	250
Calcium carbonate	750	1000	1000	1000	1500	1500
HPMC	200	200	200	200	200	200

Distilled water (ml)	100	100	100	100	100	100

3.2.4. Post formulary Evaluation

The prepared formulations were evaluated for following parameters.

3.2.4.1. Physical appearance and clarity

The developed formulations were inspected visually for clarity in sol and gel form by visual observation in presence of highly illuminated light against a black and white background clarity test apparatus.

3.2.4.2. pH: The pH of the developed formulations was determined using digital pH meter.

3.2.4.3. Drug content uniformity

Each formulated gel was dissolved in 100 ml of 0.1N HCl. The solution was filtered through a cellulose acetate membrane (0.45 µm) and the drug content was determined by UV spectroscopy at a wavelength of 254 nm after suitable dilution with 0.1 N of HCl.

3.2.4.4. *In vitro* gelation studies

The gelling capacity was determined by taking one drop of the preparation in a test tube containing 2 ml of freshly prepared 0.1M HCl at 37±0.5^{o} C and time taken to form gel and the gel to get dissolved was noted.

3.2.4.5. Rheological studies

The study was performed using Brookfield viscometer. Angular velocity was increased gradually from 0.5 to 60 rpm using spindle No. 63. The hierarchy of angular velocity was reversed and the average dial readings were considered to calculate the viscosity. Then the prepared solutions were allowed to gel in 0.1M HCl and then again the viscosity determination was carried out. The temperature was maintained within 37±0.5°C.

3.2.4.6. *In Vitro* Floating Study:

The in vitro floating study was determined with minor modification of the method by using USP dissolution apparatus II (ERWEKA DT 600HH, Germany) having 500 ml of simulated gastric fluid (0.1 N HCl) maintained at 37±0.5^{0}C with a paddle speed of 50 rpm. 10 ml of the prepared in situ gelling formulations were withdrawn with disposable syringe (with removed tip) and added into the dissolution vessel containing simulated gastric fluid. The time the formulation took to emerge on the medium surface (floating lag time) and the time the formulation constantly floated on the dissolution medium surface (duration of floating) were recorded.

3.2.4.7. Floating lag time

The FLT is defined as the time taken by the gel to reach the top from the bottom of the dissolution flask. The FLT of gel was determined by visual inspection using a USP (Type II) dissolution test apparatus containing 900 ml of 0.1N HCl at 37± 0.5°C.

3.2.4.8. Floating duration

The duration of time for which the formulation floats constantly on the surface of the medium is known as the duration of floating. The duration of floating of gels was determined using a dissolution test apparatus USP (Type II) containing 900 ml of 0.1N HCl at 50 rpm at 37°C ± 0.5°C.

3.2.4.9. Gel strength: The gel strength apparatus was fabricated in house using a measuring cylinder of 1.2 cm radius and a bore of 0.1 mm at its base. A needle 2 cm in length was used to which a nylon thread was tied. Test formulation (10 ml) was taken in the cylinder with temporarily sealed bore followed by addition of 50 ml 0.1N HCl for gelation. After gelation, the HCl was drained off by opening bore seal leaving the gel mass formed. The needle was made to rest on to surface of the gel. At the free end of the thread a light weight pan was attached to which the weights were added. The gel strength was reported in terms of weight required to pass the needle probe through the formed gel mass. The gel strength is calculated using this formula:

Gel strength = Mg/a

Where,

M = Weight at which the pass the needle probe through formed gel mass

g = Gravitational force, and

a = Area of surfaces

3.2.4.10. Measurement of drug release rate from gels

The in vitro release of Clopidogrelfrom the gels was measured by using modified USP- II dissolution test apparatus stirrer at 50 rpm. The dissolution medium used was 500 ml of 0.01N HCl and temperature was maintained at 37±0.5°C. 10 ml formulation was drawn up using disposable syringe, the needle was wiped clean and excess formulation was removed from the needle end. 10 ml of in situ gel solution was placed into Petri dish and Petri dish containing formulation was kept in the dissolution vessel containing dissolution medium. At every time interval, a precisely measured sample of the dissolution medium was removed and replenished with pre-warmed (37±0.5°C) fresh medium. The amount of Clopidogrelin each sample was determined by double beam UV Spectrophotometer (Shimadzu Co Ltd, Japan).

3.2.4.11. Accelerated Stabilities Studies

The best formulation was subjected to stability studies at humidity condition at 75±5%, ambient temperature 40±2°C for a period of three months. The samples were collected at periodic interval of 0 days, 30 days, 60 days and 90 days and were evaluated for appearance, content uniformity and in vitro drug release studies.

III. Result

4. EXPERIMENTAL INVESTIGATIONS

The characterization studies on the properties of in situ gels have been performed to investigate whether the in situ gels would be advantageous to the conventional ophthalmic drops. The in situ gel was prepared by varying concentration of using pH sensitive polymer. All the preparations were characterized for various evaluation tests.

4.1. Results of Melting point:

The clopidogrel were subjected to melting point in triplicate and the values shown in table 4.1.

Table 4.1- Melting point of Clopidogrel

Table 4.1: Melting point Trials	**Melting Point (^{O} C)**
1	198.00
2	198.00
3	199.00
Average	**198 ±1.0**

4.2. Results of Drug-excipient compatibility studies:

The FTIR spectrums of pure Clopidogrel and formulation blend were shown in fig 4.1 and 4.2.

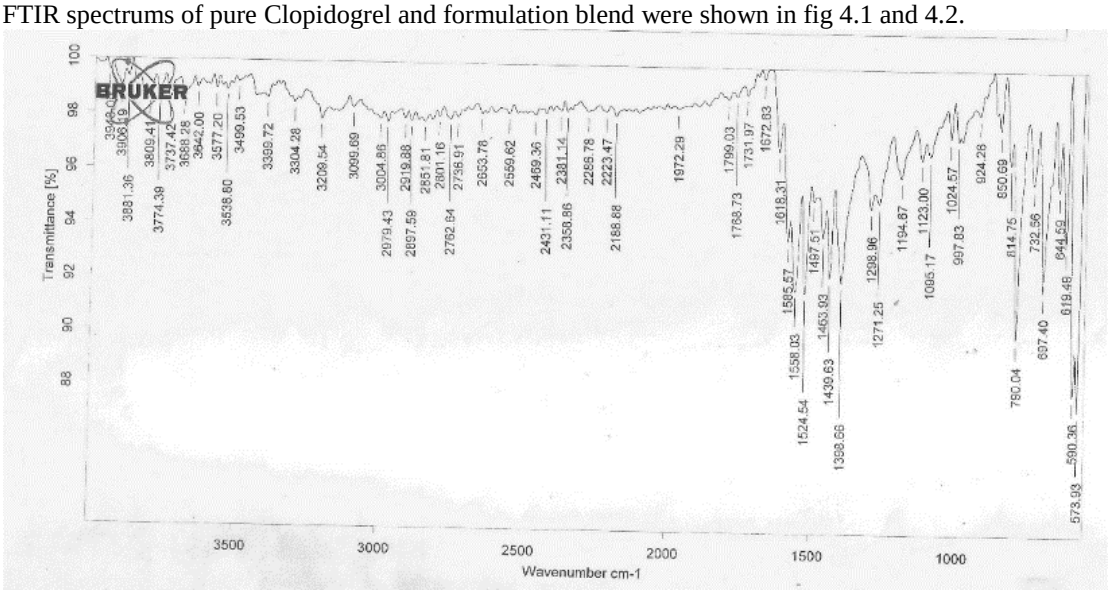

Fig.4.1. FTIR spectra of Clopidogrel pure drug

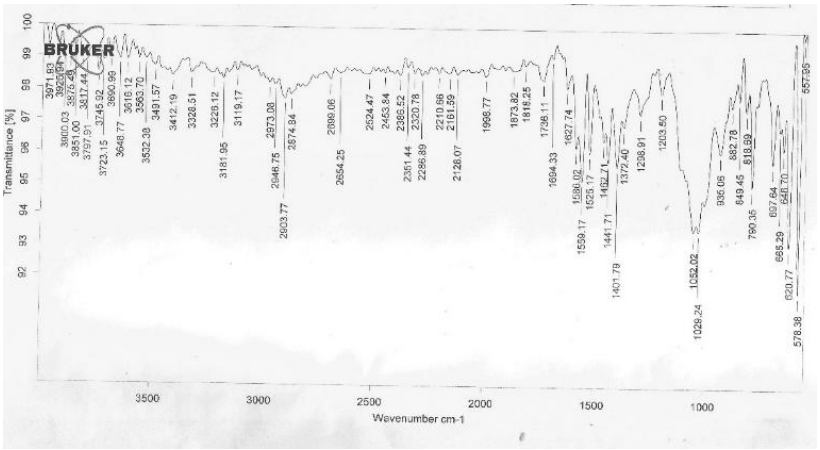

Fig4.2. FTIR spectra of Clopidogrel and excipient blend

4.3. Standard calibration curve of Clopidogrel

Table 4.2: Absorbance of Clopidogrel with different concentrations at 254nm

Concentration (µg/ml)	Absorbance
0	0.000±0.00
50	0.102±0.01
100	0.169±0.01
200	0.296±0.01
300	0.431±0.01
400	0.564±0.02
All values mentioned as mean±SD; Number of trials (N)=3	

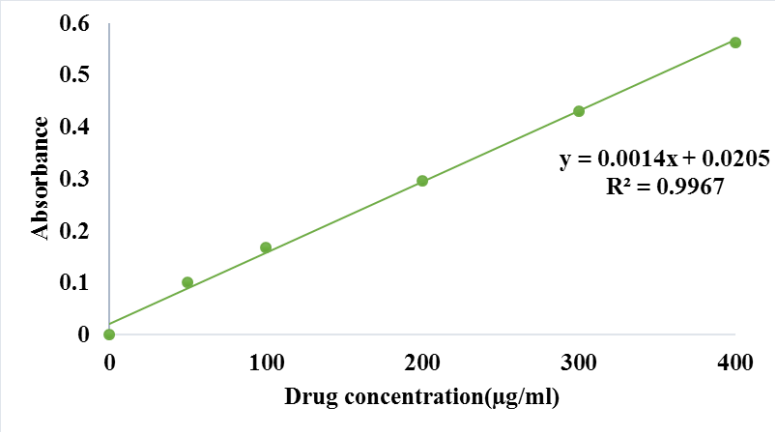

Fig.4.3: standard calibration curve of Clopidogrel.

4.4. Physical appearance and clarity

The prepared gels were in clear and white in colour.

4.5. pH

The prepared gels pH was nearer to neutral and ranged from 1.03±0.1 to 1.54±0.2 (table 4.3).

4.6. Drug content uniformity

The prepared in situ gel found to have uniformity in drug content and ranged from 95.05±2.5-99.25±3.5% (table 4.3).

4.7. *In vitro* gelation studies

Depend on time required for gelation and time of its retention as gel, the prepared in situ gels were categorized as +, ++ and +++. These were shown in table 4.3.

4.8. Rheological studies

The angular velocity and viscosity before and after gelation was tabulated in Tables4.3 and 4.4, the corresponding rheograms are given in Fig 4.3 and 4.4.

4.9. *In Vitro* Floating Study:

Time taken by the formulation for Floating and sustaining on surface of water was determined an d shown in table 4.2.

Table 4.3 *In vitro* gelling capacity

Formulation	Viscosity (cps)	Gelling Capacity	pH	Floating lag time (sec)	Floating time (h)	Drug content (%)
F1	800±2.15	+	1.54±0.2	59±1	=10	99.25±3.5
F2	850±3.26	++	1.39±0.1	59±3	>12	98.25±2.5
F3	900±2.54	+++	1.23±0.2	63±2	>12	95.05±2.5
F4	1200±1.25	+++	1.22±0.1	48±3	>12	96.32±1.3
F5	1150±2.84	+++	1.27±0.1	75±5	>12	98.24±1.5
F6	1050±2.80	+++	1.23±0.3	51±1	>12	95.27±0.2
All values mentioned as mean±SD; Number of trials (N)=3; +Gel after few minutes, dissolved rapidly; ++Gelation immediately, remains for few hours; +++Gelation immediately, remains for extended period.						

Table 4.4 Determination of Viscosity before gelation:

RPM	Formulations (before gelation)					
	F1	F2	F3	F4	F5	F6
10	100±1.2	96±2.5	95±3.5	98±6.2	97±1.2	92±5.2
20	65±2.5	62±2.6	71±1.2	70±2.5	69±3.5	66±0.2
30	62±1.6	60±3.5	62±1.2	65±1.2	56±1.5	55±0.2
40	52±3.5	52±2.8	55±1.2	62±3.2	51±0.3	45±1.2
50	48±2.5	45±2.4	45±0.1	55±1.1	48±0.6	32±0.6
60	35±1.6	32±1.7	32±1.2	51±1.2	35±0.5	25±0.8
All values mentioned as mean±SD; Number of trials (N)=3						

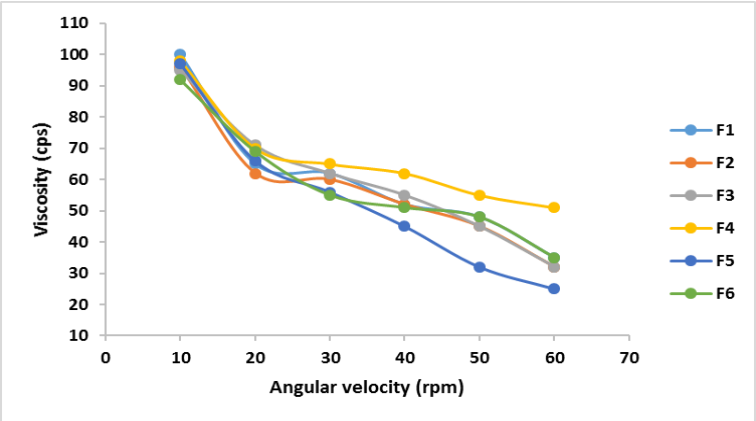

Fig.4.4. Viscosity of formulation before gelation

Table 4.5 Determination of Viscosity after gelation:

RPM	Formulations (after gelation)					
	F1	F2	F3	F4	F5	F6
10	800±2.5	850±2.5	876±2.5	895±2.5	842±3.5	825±2.0
20	621±3.8	745±8.5	721±8.5	701±6.2	705±6.5	703±2.5
30	618±6.5	736±4.9	700±6.5	695±6.2	688±3.2	688±3.2
40	600±1.8	722±4.6	694±5.2	648±3.2	679±2.5	671±5.2
50	585±6.9	714±5.2	685±4.3	635±2.5	655±4.5	652±3.5
60	576±4.8	709±6.2	674±4.7	625±3.2	649±6.2	645±5.2
All values mentioned as mean±SD; Number of trials (N)=3						

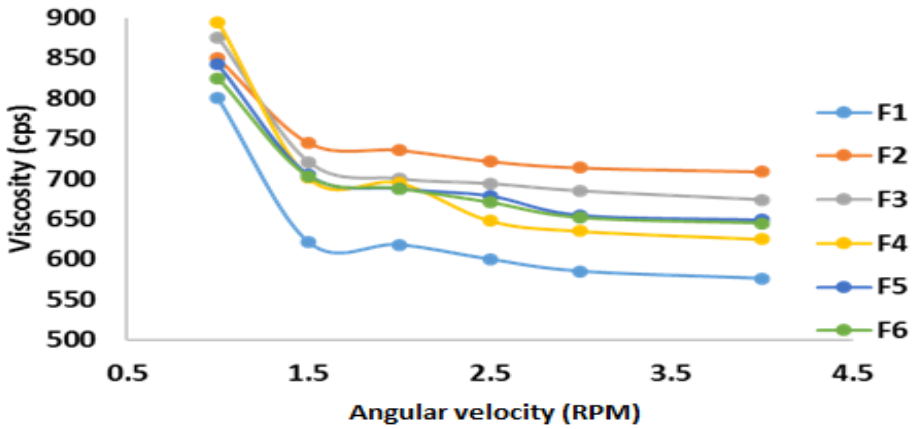

Fig.4.5. Viscosity of formulation after gelation

4.10. *In vitro* drug release studies

The drug release from prepared in situ gels were shown in table 4.5 and fig 4.6.

Table 4.6: *In vitro* drug dissolution profile of prepared formulations

Time (h)	Formulations					
	F1	F2	F3	F4	F5	F6
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.5	23.53±0.2	22.15±0.2	16.61±0.3	19.01±0.2	17.53±0.2	18.92±0.6
1	34.70±1.2	31.68±0.8	22.60±0.2	38.02±0.3	24.46±0.3	25.86±1.2
2	42.55±1.2	40.67±0.9	27.83±1.2	42.01±0.6	29.47±1.2	30.64±0.2
3	49.51±0.5	47.16±1.3	31.23±3.0	45.02±1.2	33.57±1.2	34.99±2.5
4	56.05±0.6	55.07±0.5	35.57±1.3	50.03±1.3	37.24±1.3	45.02±1.2
5	65.87±1.2	64.19±0.3	40.41±0.3	59.05±0.6	42.31±1.2	56.03±1.3
6	77.13±1.5	75.21±1.2	44.11±0.2	65.09±0.3	47.65±1.3	59.08±1.2
7	86.37±1.5	83.68±2.5	49.22±1.2	67.05±0.2	53.24±0.5	63.01±0.2
8	92.00±0.2	91.39±1.6	55.29±2.3	70.05±3.2	60.49±0.3	66.18±0.6
9	95.01±0.5	95.02±1.5	61.39±3.2	75.06±2.0	67.31±1.2	70.02±0.3
10	98.02±0.3	96.02±1.3	68.45±2.2	80.03±1.2	74.64±1.2	72.05±0.5
11	99.05±0.2	97.09±1.2	76.47±1.2	85.96±1.5	82.69±0.3	75.02±0.3
12	99.02±0.6	99.39±1.2	88.03±2.3	93.63±1.2	92.19±1.2	79.03±0.9
All values mentioned as mean±SD; Number of trials (N)=3						

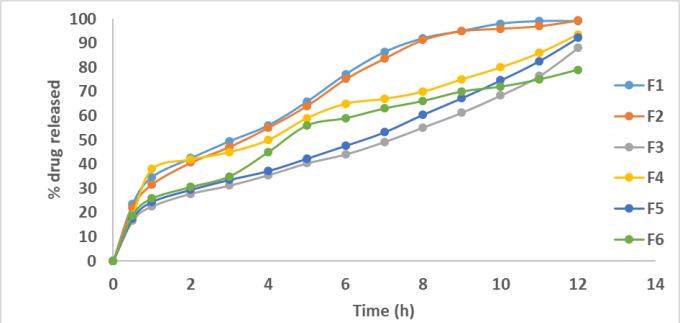

Fig 4.6. *In vitro* drug dissolution profile of prepared formulations

4.11. Accelerated stability studies

The optimized formulation shown the following characteristics before and after accelerated stability studies (table 4.6).

Table 4.7. The parameters before and after stability studies

Stability studies	Parameters					
	Viscosity (cps)	Gelling Capacity	pH	Floating lag time (sec)	Floating time (h)	Drug content (%)
Before	1150±2.80	+++	7.27±0.3	51±1	>12	98.24±1.5
After	1150±3.10	+++	7.27±0.3	51±1	>12	98.20±1.1
All values mentioned as mean±SD; Number of trials (N)=3; +Gel after few minutes, dissolved rapidly; ++Gelation immediately, remains for few hours; +++Gelation immediately, remains for extended period.						

IV. Discussion

Clopidogrel shown its characteristic peaks in both in pure drug and formulation and proved its compatibility with the excipients used.

The melting point of Clopidogrel shown its purity. The prepared in situ gels were clear and neutral in pH. The formulations shown uniformity in drug content. The Gelling capacity of all formulations were satisfactory. The viscosity of the formulations decreases with increase in angular velocity. Results indicated that all formulations are having an optimum viscosity and all formulations were pourable at normal conditions. The drug release from the formulation was extended up to 12 hrs. Formulation F3 and F5 retained its physical characteristics even after stressed storage conditions.

V. Conclusion

In the present work, an attempt was made to develop in situ gelling system of Clopidogrel with pHsensitive polymer. FTIR studies revealed that the drug and excipients were compatible with each other. Preparations were found to be clear, pH and drug content of all the preparations were found within the acceptable ranges. The study revealed that Clopidogrel can be formulated as in situ gels by using HPMC, Sod. Alginate, Sod. Citrate and Calcium carbonate. All formulations showed optimum viscosity and remained in gel form for few hours. They were pourable at normal conditions and viscosity increased after contact with 0.1M HCl. These formulations showed pseudo plastic flow behaviour. Formulation F5 was found to show prolonged drug release for a period of >12 h.

References

[1]. Chein Y.W. Concepts and system design for rate controlled drug delivery in novel drug delivery systems. 2nd edition, New York: Marcel Dekker Inc., 1992; 50: 1-42.
[2]. Aulton, M.E., Pharmaceutics- „The science of dosage form design", Third edition, Churchil Living Stone, 2007; 419-421.
[3]. Remington The Science and Practice of Pharmacy 21st edition, Lippincott Williams & Wilkins, 2006.
[4]. Lachman L, Lieberman HA. Theory and practice of industrial pharmacy, 3rd ed. New Delhi: CBS Publishers & distributors; 2009.
[5]. Manoj G, Rajesh P, Purohit KK and Mehta SC , Floating Drug Delievery System, JCPR 2011; 05:(1) 7-18.
[6]. Nikita D, Floating Drug Delivery System, 2011; 7 (1): 6-20.
[7]. Kumar N, Niranjan SK, Irchhaiya R, Verma V, Kumar V, Novel Approaches of Floating Drug Delivery System: A Review, 2012; 1(4): 2277-7873.
[8]. Uddin M, Rathi PB, Siddiqui AR, Sonawane AR, Gadade DD, Recent Development in Floating Delivery Systems for Gastric Retention of Drugs An Overview, Asian Journal of Biomedical and Pharmaceutical Sciences, 2011; 1 (3), 26-42.
[9]. Praveen N, Mahant S, Deepika S, Floating Systems: A Novel Approach towards Gastroretentive drug delivery systems, International Journal of Pharmacy and Pharmaceutical Sciences, 2010: 2 (3).
[10]. Abhishek , Kapil C, Bharat P, Kumar H, Sonia A, Floating drug delivery systems: A better approach, International Current Pharmaceutical Journal, 2012; 1(5): 110-118.
[11]. Shweta A, Javed A, Alka Ahuja, Roop K. Khar, and Sanjula B, Floating Drug Delivery Systems: A Review, PharmSciTech 2005; 6(3): 47.
[12]. Shahaa SH, Patelb JK, Pundarikakshudua K, Patelc NV, An overview of a gastro-retentive floating drug delivery system, Asian Journal of Pharmaceutical Sciences. 2009; 4 (1): 65-80.
[13]. Sanjay G and Shringi S, Gastroretentive Drug Delivery Systems, Int.J. Pharm., 1990; 207–216.
[14]. Nayak AK, Ruma M, Das B, Gastroretentive drug delivery systems: a review, Asian Journal of Pharmaceutical and Clinical Research, 2010; 3(1).
[15]. Dash S, Murthy PN, Nath L, Chowdhury P. "Kinetic modeling on drug release from controlled drug delivery systems". ActaPoloniae Pharm Drug Res, 2010; 67(3): 217-223.
[16]. Costa P, Manuel J, Lobo S. "Modeling and comparison of dissolution profiles". Eur J Pharm Sciences, 2001; 13: 123-133.
[17]. Shahaa SH, Patelb JK, Pundarikakshudua K, Patelc NV, An overview of a gastro-retentive floating drug delivery system, Asian Journal of Pharmaceutical Sciences, 2009; 4 (1): 65-80.
[18]. Nikita D, Floating Drug Delivery System, 2011; 7 (1): 6-20.
[19]. Kumar MV, Rajat K, Kumar S, Singhai AK, Sharma D, Formulation and Evaluation of Sustaind-Release Floating tablets of Ciprofloxacin, 2012; 3[9].
[20]. Reddy ES, Iqbal MM , Kazim SM , Asad SB and Ibrahim M, Formulation and Evaluation of Sustained Relese Floating tablets of Ciprofloxacin with Hepato-Protectan, International Journal of Pharmaceutical Applications, 2012; 3(1): 289-292.
[21]. Kunihiko I, Tomohiro H, Akie T, Wataru K, Shozo M, Masatake D, Mitsuo T, Ryozo M, David A, In situ gelling pectin formulations for oral drug delivery at high gastric pH, International Journal of Pharmaceutics, 2007; 335(1-2): 90-96.
[22]. Wataru K, Shozo M, Masatake D, Mitsuo T, Ryozo M, David A, Oral sustained delivery of ambroxol from in situ-gelling pectin formulations, International Journal of Pharmaceutics, 2004; 271 (1–2): 233-240.
[23]. Shozo M, Hirotatsu A, Naoko K, Wataru K, David A, In situ-gelling gellan formulations as vehicles for oral drug delivery, Journal of Controlled Release, 1999; 60(1-2); 287-295.
[24]. Shozo M, Wataru K, David A, Oral sustained delivery of theophylline using in-situ gelation of sodium alginate, Journal of Controlled Release, 2000; 67(2-3): 275-280.
[25]. Shozo M, Wataru K, KunihikoI, Yasuhiro K, Mariko F, Masatake D, Mitsuo T, Ryozo M, David A, The effect of taste masking agents on in situ gelling pectin formulations for oral sustained delivery of paracetamol and ambroxol, International Journal of Pharmaceutics, 2005; 297(1-2): 38-49.
[26]. Tofeeq R, Staffan T, Gerhard G, Chitosan in situ gelation for improved drug loading and retention in poloxamer 407 gels, International Journal of Pharmaceutics, 2011; 409(1-2): 19-29.
[27]. Gamal ME, Maghrabya AEE, Gehad AE, Development of liquid oral sustained release formulations of nateglinide: In vitro and in vivo evaluation, Journal of Drug Delivery Science and Technology, 2015; 29: 70-77.

[28]. Rajinikanth PS and Mishra B, Floating in situ gelling system for stomach site-specific delivery of clarithromycin to eradicate H. pylori, Journal of Controlled Release, 2008; 125(1): 33-41.
[29]. Rajinikantha PS, Balasubramaniam J, Mishra B, Development and evaluation of a novel floating in situ gelling system of amoxicillin for eradication of Helicobacter pylori, International Journal of Pharmaceutics,2007; 335(1-2); 114-122.
[30]. Dilesh JS, Rashmi SP, Shagufta K, Famotidine microspheres reconstituted with floating in situ gel for stomach-specific delivery: Preparation and characterization, Journal of Drug Delivery Science and Technology, 2017; 41: 251-259.
[31]. Kunihiko I, Masayuki Y, Akie T, Reina T, Shozo M, Masatake D, Mitsuo T, Ryozo M, David A, In situ gelling xyloglucan/pectin formulations for oral sustained drug delivery, International Journal of Pharmaceutics, 2008; 356(1-2): 95-101.
[32]. Miyazaki S, Kawasaki N, Kubo W, Endo K, Attwood D, Comparison of in situ gelling formulations for the oral delivery of cimetidine, International Journal of Pharmaceutics, 2001; 220(1-2): 161-168.
[33]. Shastri DH, Patel LD, Parikh RK. Studies on In situ hydrogel: A smart way for safe and sustained ocular drug delivery. J Young Pharmacists 2010; 21: 16-20.
[34]. Dasharath M, Divyesh K, and Chhagan N. Patel Formulation and Evaluation of Floating Oral In Situ Gelling System of Amoxicillin, 2011.
[35]. Wataru K, Shozo M, David A, "Oral sustained delivery of paracetamol from in situ-gelling gellan and sodium alginate formulations". International Journal of Pharmaceutics, 2003; 258: 55–64.
[36]. Miyazaki S, "Comparison of in situ gelling formulations for the oral delivery of cimetidine".International Journal of Pharmaceutics, 2001; 220: 161–168.
[37]. Anjali Devi N, Abdul Hadi M, Rajitha P, Sharma JVC, Srinivasa Rao A, Formulation and Evaluation of Floating Controlled Release Tablets of Clopidogrel . using Hydrophilic Matrix system, 2013; 1: 5.
[38]. Vinod KR, Santhosh V, Sandhya S, David Banji, Otiliabanji and Madhusudan rao Y, Gastroretentive Mucoadhesive Clopidogrel . single unit dosage form – A comparative study of two mucoadhesive polymers, 9-2-5.
[39]. Yang FC, Ingram DA, Chen S, Zhu Y, Yuan J, Li X, Yang X, Knowles S, Horn W, Li Y, Zhang S, Yang Y, Vakili ST, Yu M, Burns D, Robertson K, Hutchins G, Parada LF, Clapp DW. "Nf1-dependent tumors require a microenvironment containing Nf1+/-- and c-kit-dependent bone marrow". Cell. 2008; 135 (3): 437–48.
[40]. The price of drugs for chronic myeloid leukemia (CML) is a reflection of the unsustainable prices of cancer drugs: from the perspective of a large group of CML experts", 2013; 121 (22): 4439–42.
[41]. Yin W, Penrod JR, Maclean R, Lakdawalla DN, Philipson T. "Value of survival gains in chronic myeloid leukemia". Am J Manag Care, 2012; 18 (11): 245-49.
[42]. Koroloff N, Boots R, Lipman J, Thomas P, Rickard C, Coyer F. "A randomised controlled study of the efficacy of hypromellose and Lacri-Lube combination versus polyethylene/Cling wrap to prevent corneal epithelial breakdown in the semiconscious intensive care patient". Intensive Care Med. 2004; 30 (6): 1122–6.
[43]. Ali N, Shaista R, Pryia P, Kofi A.,"The Role of Oral Controlled Release Matrix Tablets in Drug Delivery Systems". bioimpact. 2012; 2: 175–87.
[44]. Niazi, Sarfaraz. Handbook of Pharmaceutical Manufacturing Formulations. 2004; 275–276.
[45]. Leid JG, et al. The exopolysaccharide alginate protects Pseudomonas aeruginosa biofilm bacteria from IFN-gamma-mediated macrophage killing. J Immunol. 2005; 175(11): 7512-8.
[46]. Gordon A, Tristan F., SI Chemical Data Book (4th ed.). John Wiley & Sons Australia, Ltd., 2005; 978-81.
[47]. Blount Z.D, Borland C.Z, Lenski R.E., "Historical contingency and the evolution of a key innovation in an experimental population of Escherichia coli. Proceedings of the National Academy of Sciences, 2008; 105 (23): 7899–7906.
[48]. White DL, Dang P, Engler J, Frede A, Zrim S, Osborn M, et al. Functional activity of the oct-1 protein is predictive of long-term outcome in patients with chronic-phase chronic myeloid leukemia treated with clopidogrel . J ClinOncol, 2010; 28(16): 2761-7.
[49]. Peng B, Hayes M, Resta D, Racine-Poon A, Druker BJ, Talpaz M. Pharmacokinetics and Pharmacodynamics of clopidogrel in a phase i trial with chronic myeloid leukemia patients. J ClinOncol, 2004; 22(5): 935-42.
[50]. Shojaei AH, Zhuo SL, "Transbuccal delivery of acyclovir (II): feasibility, system design, and in vitro permeation studies," Journal of Pharmacy & Pharmaceutical Sciences, 1998; 1(2): 66–73.
[51]. Hearnden H, Sankar V, Hull K, "New developments and opportunities in oral mucosal drug delivery for local and systemic disease," Advanced Drug Delivery Reviews, 2012; 64(1): 16–28.
[52]. Perchyonok VT, Shengmiao Z, and Theunis O, "Chitosan and gelation based prititype delivery systems for the treatment of oral mucositis: from materials to performance in vitro," Current Drug Delivery, 2013; 10(2): 144–150.
[53]. Tejas G, Vishal G, Vishal C, and Umesh UA, "Review on novel in-situ polymeric drug delivery system," International Journal of Pharmaceutical Research and Development, 2011; 3(5): 53–59.
[54]. Remunan C, Bretal M, Nunez A, BilaJato JL., Accelerated stability of sustained release tabletprepared with Gelucire. Int J Pharm; 1992; 80(2): 151-159.

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