PhD Thesis entitled

STUDIES IN DESIGN AND DEVELOPMENT OF STOMACH SPECIFIC DRUG DELIVERY SYSTEM USING VARIOUS APPROACHES

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2010

Certificate

I hereby certify that Prof. Jayant R. Chavda has completed his thesis for doctoral degree on the topic "**Studies in design and development of stomach specific drug delivery system using various approaches**"

I further certify that the work done by him is of his own and original and tends to the general advancement of knowledge. For the thesis that he is submitting, he has not been conferred any diploma or degree or distinction by either this university or other university according to the best of my knowledge.

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DECLARATION

I hereby declare that the thesis entitled "**Studies in design and development of stomach specific drug delivery system using various approaches**" is a bonafide and genuine research work carried out by me, under the guidance of Dr. Jayvadan Patel, Professor of Pharmaceutics and Principal, Nootan Pharmacy College, Visnagar, Gujarat, India. The results presented this dissertation is original and has not been submitted in part or full any degree and diploma to any university.

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Jayant R.Chvada

DEDICATED TO
MY BELOVED FAMILY

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Aim of present investigation

1 Aim of present investigation

Historically, oral drug administration has been the predominant route for drug delivery. During the past two decades, numerous oral delivery systems have been developed to act as drug reservoirs from which the active substance can be released over a defined period of time at a predetermined and controlled rate. From a pharmacokinetic point of view, the ideal sustained and controlled release dosage form should be comparable with an intravenous infusion, which supplies continuously the amount of drug needed to maintain constant plasma levels once the steady state is reached.

Although some important applications, including oral administration of peptide and protein drugs, can be used to prepare colonic drug delivery systems, targeting drugs to the colon by the oral route. More often, drug absorption is unsatisfactory and highly variable among and between individuals, despite excellent in vitro release patterns. The reasons for this are essentially physiological and usually affected by the gastrointestinal (GI) transit of the form, especially its gastric residence time (GRT), which appears to be one of the major causes of the overall transit time variability.

Over the past three decades, the pursuit and exploration of devices designed to be retained in the upper part of the GI tract has advanced consistently in terms of technology and diversity, encompassing a variety of systems and devices such as floating systems, raft systems, expanding systems, swelling systems, bioadhesive systems and low-density systems.

Stomach specific (gastricretention) will provide advantages such as the delivery of drugs with narrow absorption windows in the small intestinal region. Also, longer residence time in the stomach could be advantageous for local action in the upper part of the small intestine, for example treatment of peptic ulcer disease.

Furthermore, improved bioavailability is expected for drugs that are absorbed readily upon release in the GI tract. These drugs can be delivered ideally by slow release from the stomach. Many drugs categorized as once-a-day delivery have been demonstrated to have suboptimal absorption due to dependence on the transit time of the dosage form, making traditional extended release development challenging. Therefore, a system designed for

longer gastric retention will extend the time within which drug absorption can occur in the small intestine.

Optimization techniques have been applied in the present study to systemically study the influence of process variables on the formulation of dosage forms. These designs provide an effective means for studying the effect of various parameters on the dependent variables. Thus, factorial designs were applied to optimize the formulation and development of mucoadhesive microspheres and in situ gel. In vivo studies of the formulations were also conducted to ascertain the effect of the designed dosage forms in vivo.

The present study was aimed at the development of stomach specific drug delivery systems using various approaches like mucoadhesive or floating. Mucoadhesive microspheres of amoxicillin, in situ gelling system of famotidine and mucoadhesive/floating tablets of glipizide were prepared. Different formulation variables were studied and optimized to achieve the desired mucoadhesive or floating properties and release profiles. The stability of the formulations was evaluated after 3 months of storage at accelerated stability conditions.

Chapter 1.1

Introduction to Stomach Specific drug delivery systems

1.1 Stomach Specific drug delivery systems

1.1.1 Introduction

The design of oral control drug delivery systems (DDS) should be primarily aimed to achieve more predictable and increased bioavailability [1]. Approximately 505 of the drug available in the market are oral DDS and these systems have more advantages due to patients acceptance and ease of administration. Nowadays most of the pharmaceutical scientist is involved in developing the ideal DDS. This ideal system should have advantage of single dose for the whole duration of treatment and it should deliver the active drug directly at the specific site. Scientists have succeeded to develop a system and it encourages the scientists to develop control release systems. Control release implies the predictability and reproducibility to control the drug release, drug concentration in target tissue and optimization of the therapeutic effect of a drug by controlling its release in the body with lower and less frequent dose [2,3].

Under certain circumstances prolonging the gastric retention of a delivery system is desirable for achieving greater therapeutic benefit of the drug substances. For example, drugs that are absorbed in the proximal part of the gastrointestinal tract [4],and the drugs that are less soluble or are degraded by the alkaline pH may benefit from the prolong gastric retention [5,6]. In addition, for local and sustained drug delivery to the stomach and the proximal small intestine to treat certain conditions, prolonging gastric retention of the therapeutic moiety may offer numerous advantages including improved bioavailibility, therapeutic efficacy and possible reduction of the dose size [7,8]. It has been suggested that prolong local availability of antibacterial agents may augment their effectiveness in treating *H.Pylori* related peptic ulcers.Gastroretentive Drug delivery systems (GRDDS)[9-14], however are not suitable for drugs that may cause gastric lesions,e.g., Non-steroidal anti-inflammatory agents.

1.1.2 Basic physiology of the gastrointestinal tract

The complex anatomy and physiology of the GIT, including variations in acidity, bile salts, enzyme content, and the mucosal absorptive surface, significantly influence the release, dissolution, and absorption of orally administered dosage

forms. Two distinct patterns of gastrointestinal (GI) motility and secretion exist, corresponding to the fasted and fed states. As a result, the BA of orally administered drugs will vary depending on the state of feeding. The fasted state is associated with various cyclic events, commonly referred to as the *migrating motor complex* (MMC), which regulates GI motility patterns. The MMC is organized into alternating cycles of activity and quiescence and can be subdivided into basal (Phase I), preburst (Phase II), and burst (Phase III) intervals (Figure 1.1.1) [1]. Phase I, the quiescent period, lasts from 30 to 60 min and is characterized by a lack of secretory, electrical, and contractile activity. Phase II exhibits intermittent action for 20–40 min during which contractile motions increase in frequency and size. Bile enters the duodenum during this phase, whereas gastric mucus discharge occurs during the latter part of Phase II and throughout Phase III. Phase III is characterized by intense, large, and regular contractions, termed *housekeeper waves*, that sweep off undigested food and last 10–20 min. Phase IV is the transition period of 0–5 min between Phases III and I. This series of electrical events originates in the foregut and continues to the terminal ileum in the fasted state, repeating every 2–3 hrs. Feeding sets off a continuous pattern of spike potentials and contractions called *postprandial motility*.

The particular phase during which a dosage form is administered influences the performance of peroral CRDDS and GRDDS. When CRDDS are administered in the fasted state, the MMC may be in any of its phases, which can significantly influence the total gastric residence time (GRT) and transit time in the GIT. This assumes even more significance for drugs that have an absorption window because it will affect the amount of time the dosage form spends in the region preceding and around the window. The less time spent in that region, the lower the degree of absorption. Therefore, the design of GRDDS should take into consideration the resistance of the dosage form to gastric emptying during Phase III of the MMC in the fasted state and also to continuous gastric emptying through the pyloric sphincter in the fed state. This means that GRDDS must be functional

quickly after administration and able to resist the onslaught of physiological events for the required period of time.

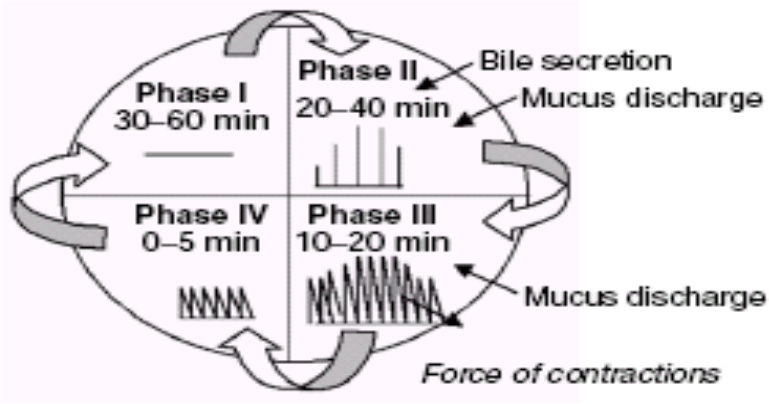

Figure 1.1.1: ***Motility patterns of the GIT in fasted state***

1.1.3 Gastric emptying and problems

It is well recognized that the stomach may be used as a depot for Sustained release dosage forms, both in human and veterinary applications, stomach is anatomically divided in to three parts: Fundus, body and pylorus [15].

The proximal stomach made up of the fundus and body region serves as a reservoir for ingested materials, while the distal region (antrum) is the major site for the mixing motion, acting as a pump to accomplish gastric emptying. The process of the gastric emptying occurs both during fasting and fed stages.

Scintigraphy study involving measurement of gastric emptying rates in healthy human subject have revealed that an orally administered Controlled release dosage form is mainly subjected to two physiological adversities [15],

a) The short GRT (Gastric Residence Time)
b) Variable (unpredictable) GET (Gastric Emptying Time)

Yet another major adversity encountered through the oral route is the first pass effect, which leads to reduce systematic availability of a large number of a drug. These problems can be exacerbated by alteration in the gastric emptying that occur due to factors such as age, race, sex and disease states, as they may seriously affect the release of a drug from DDS. It is therefore desirable to have a

Controlled release product that exhibits an extended, GI residence and a drug release profile independent of patients' related variables.

1.1.4 Potential drug candidates for stomach specific drug delivery systems

1. Drugs those are locally active in the stomach e.g. misroprostol, antacids etc.
2. Drugs that have narrow absorption window in gastrointestinal tract (GIT) e.g. l-dopa, paraaminobenzoic acid, furosemide, riboflavin etc.
3. Drugs those are unstable in the intestinal or colonic environment e.g. captopril, ranitidine HCl, metronidazole.
4. Drugs that disturb normal colonic microbes e.g. antibiotics against Helicobacter pylori.
5. Drugs that exhibit low solubility at high pH values e.g. diazepam, chlordiazepoxide, verapamil HCl.

1.1.5 Drugs those are unsuitable for stomach specific drug delivery systems

1. Drugs that have very limited acid solubility e.g. phenytoin etc.
2. Drugs that suffer instability in the gastric environment e.g. erythromycin etc.
3. Drugs intended for selective release in the colon e.g. 5- amino salicylic acid and corticosteroids etc.

1.1.6 Approaches to gastric retention/ stomach specific delivery

Various approaches have been paused to increase the duration of oral dosage form in the stomach, including floating systems, swelling and expanding system, modified shape system, high density systems and other delayed gastric emptying devices. (Magnetic systems, super porous –biodegradable hydrogel systems).

1.1.6.1 High density (sinking) system or non- floating drug delivery system

This approach involves formulation of dosage forms with the density that must exceed density of normal stomach content (~ 1.004 gm/cm^3). These formulations are prepared by coating drug on a heavy core or mixed with inert materials such as iron powder, barium sulphate, zinc oxide and titanium oxide etc. The materials increase density by up to 1.5- 2.4 gm/cm^3. A density close to 2.5 gm/cm^3 seems

necessary for significant prolongation of gastric residence time. But, effectiveness of this system in human beings was not observed and no system has been marketed [16].

1.1.6.2 Bioadhesive or mucoadhesive drug delivery systems

Bioadhesive drug delivery systems are used as a delivery device within the human to enhance drug absorption in a site-specific manner. In this approach, bio adhesive polymers are used and they can adhere to the epithelial surface in the stomach. Thus, they improve the prolongation of gastric retention. The basis of adhesion in that a dosage form can stick to the mucosal surface by different mechanism.

These mechanisms are:

1. The wetting theory, which is based on the ability of bioadhesive polymers to spread and develop intimate contact with the mucous layers.
2. The diffusion theory, which proposes physical entanglement of mucin strands the flexible polymer chains, or an interpenetration of mucin strands into the porous structure of the polymer substrate.
3. The absorption theory, suggests that bioadhesion is due to secondary forces such as Vander Waal forces and hydrogen bonding.
4. The electron theory, which proposes attractive electrostatic forces between the glycoprotein mucin net work and the bio adhesive material.

Materials commonly used for bioadhesion are poly acrylic acid, chitosan, cholestyramine, sodium alginate, hydroxypropyl methylcellulose (HPMC), sucralfate, tragacanth, dextrin, polyethylene glycol (PEG) and polylactic acids etc. Even though some of these polymers are effective at producing bioadhesive, it is very difficult to maintain it effectively because of the rapid turnover of mucus in the gastrointestinal tract (GIT).

1.1.6.3 Expandable, unfoldable and swellable systems

A dosage form in the stomach will withstand gastric transit if it bigger than pyloric sphincter. However, the dosage form must be small enough to be swallowed, and must not cause gastric obstruction either singly or by accumulation. Thus,

their configurations are required to develop an expandable system to prolong gastric retention time (GRT):

1. a small configuration for oral intake,
2. an expanded gastroretentive form, and
3. a final small form enabling evacuation following drug release from the device.

Thus, gastroretentivity is improved by the combination of substantial dimension with high rigidity of dosage form to withstand peristalsis and mechanical contractility of the stomach. Unfoldable and swellable systems have been investigated and recently tried to develop an effective gastroretentive drug delivery. Unfoldable systems are made of biodegradable polymers. They are available in different geometric forms like tetrahedron, ring or planner membrane (4 - label disc or 4 - limbed cross form) of bioerodible polymer compressed within a capsule which extends in the stomach. Swellable systems are also retained in the gastrointestinal tract (GIT) due to their mechanical properties. The swelling is usually results from osmotic absorption of water and the dosage form is small enough to be swallowed by the gastric fluid (Figure 1.1.2). Expandable systems have some drawbacks like problematical storage of much easily hydrolysable, biodegradable polymers relatively short-lived mechanical shape memory for the unfolding system most difficult to industrialize and not cost effective. Again, permanent retention of rigid, large single-unit expandable drug delivery dosage forms may cause brief obstruction, intestinal adhesion and gastropathy[17].

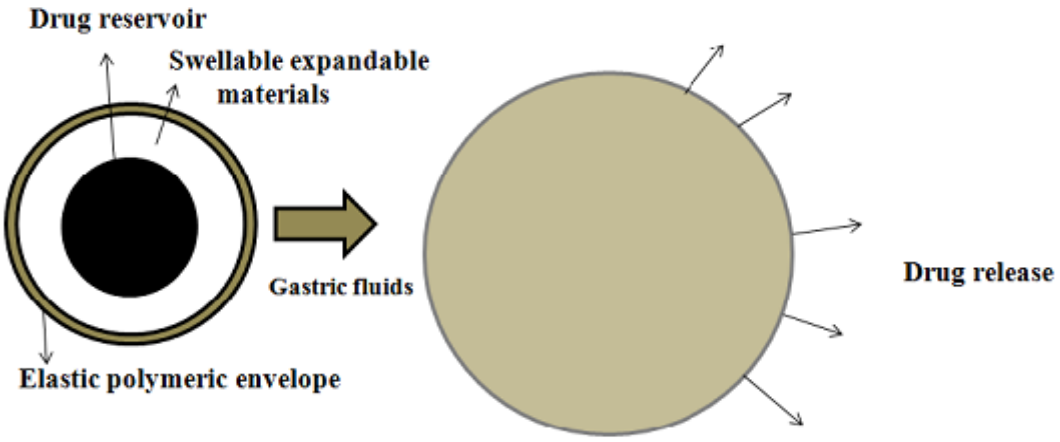

Figure 1.1.2: ***Drug release from swellable systems***

1.1.6.4 Super porous hydrogel systems

These swellable systems differ sufficiently from the conventional types to warrant separate classification. In this approach to improve gastric retention time (GRT) super porous hydrogels of average pore size >100 micro miter, swell to equilibrium size within a minute due to rapid water uptake by capillary wetting through numerous interconnected open pores. They swell to a large size (swelling ratio: 100 or more) and are intended to have sufficient mechanical strength to withstand pressure by gastric contraction. This is advised by co-formulation of hydrophilic particulate material [17].

1.1.6.5 Magnetic systems

This approach to enhance the gastric retention time (GRT) is based on the simple principle that the dosage form contains a small internal magnet, and a magnet placed on the abdomen over the position of the stomach. Although magnetic system seems to work, the external magnet must be positioned with a degree of precision that might compromise patient compliance.

1.1.6.6 Ion exchange resins

Ion exchange resins are loaded with bicarbonate, and a negatively charged drug is bound to the resin. Resultant beads are then encapsulated in a semi-permeable membrane to overcome the rapid loss of carbon dioxide. Upon arrival in the acidic environment of the stomach, an exchange of chloride and bicarbonate ions takes place. As a result of this reaction, carbon dioxide is

released and trapped in the membrane thereby carrying beads towards the top of the gastric contents and producing a floating layer of resin beads – in contrast to uncoated beads, which sink quickly.

1.1.6.7 Raft systems

Raft systems incorporate alginate gel solution (e.g. sodium alginate solution containing carbonates or bicarbonates) that upon reaction with gastric fluid, swell and form a viscous cohesive gel containing entrapped carbon dioxide bubbles, enabling floatation of the drug delivery system. Because raft-forming systems (Figure 1.1.3) produce a layer on the top of the gastric fluids, they are often used for gastroesophageal reflux treatment, as with Liquid Gaviscon (GlaxoSmithKline).

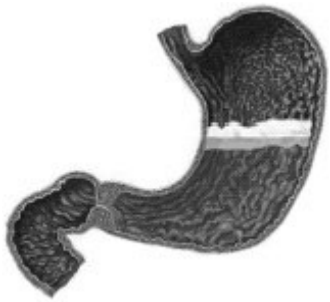

Figure 1.1.3: ***Schematic representation of the barrier created by a raft-forming system***

Other delayed gastric emptying approaches of interest include sham feeding of digestible polymers or fatty acid salts that charges the motility pattern, of the stomach to a fed stage thereby decressing the gastric emptying rate and permitting considerable prolongation of the drug release. But some of this has certain drawbacks, which could limit their uses described in the following Table 1.1.1 [19].

Table 1.1.1: Drawback associated with different types of GRDDS [19]	
Formulations	**Drawback**
Incorporation of passage delaying food excipient such as fatty acids	Affect the emptying mechanism of the entire content
Bio adhesive drug delivery systems	a. Adhesive is non specific b. Efficiency is limited by the possible interaction with food.
Biodegradable and non biodegradable (swelling) formulation in which the size and shape retain in the dosage form.	Present the hazard of permanent retention and might lead to serious life threatening effects if multiple dosing is predicted.

1.1.6.8 Floating drug delivery systems

Floating systems, first described by Davis in 1968, have bulk density lower than that of the gastric fluid, and thus remain buoyant in stomach for a prolong period. This results in an increase in the GRT and a better control of fluctuations in the plasma drug concentrations. Floating system can be effervescent or Non effervescent in nature.

1.1.6.8 .1 Effervescent systems

1.1.6.8 .1 .1 Volatile liquid containing systems

The GRT of a drug delivery system can be sustained by incorporating an inflatable chamber, which contains a liquid e.g. ether, cyclopentane, that gasifies at body temperature to cause the inflatation of the chamber in the stomach. The device may also consist of a bioerodible plug made up of PVA, Polyethylene, etc. that gradually dissolves causing the inflatable chamber to release gas and collapse after a predetermined time to permit the spontaneous ejection of the inflatable systems from the stomach [20].

1.1.6.8 .1 .2 Gas-generating systems

These buoyant delivery systems utilize effervescent reactions between carbonate/bicarbonate salts and citric/tartaric acid to liberate CO_2, which gets

entrapped in the gellified hydrocolloid layer of the systems thus decreasing its specific gravity and making it to float over chime [1,18]. How the dosage form float is shown in the following figure (Figure 1.1.4) [21].

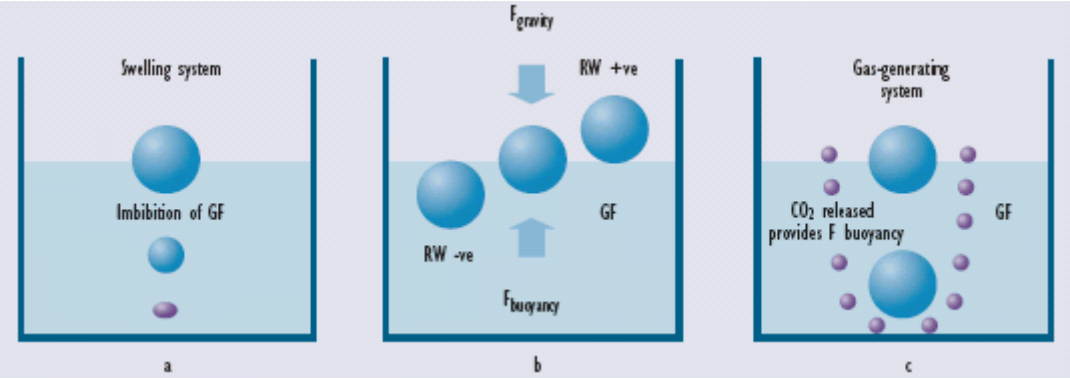

Figure 1.1.4: ***The mechanism of floating systems***

1.1.6.8 .2 Non-effervescent systems

1.1.6.8 .2.1 Colloidal gel barrier systems

Hydrodymamically balance system (HBS^{TM}) was first design by Sheth and Tossounian in 1975.Such systems contains drug with gel forming hydrocolloids meant to remain buoyant on stomach contents. This system incorporate a high level of one or more gel forming highly swellable cellulose type hydrocolloids.e.g.HEC, HPMC, NaCMC, Polysacchacarides and matrix forming polymer such as polycarbophil, polyacrylates and polystyrene, incorporated either in tablets or in capsule. On coming in contact with gastric fluid, the hydrocolloid in the system hydrates and forms a colloidal gel barrier around the gel surface. The air trapped by the swollen polymer maintains a density less than unity and confers buoyancy to these dosage forms [22].

1.1.6.8 .2.2 Microporous compartment system

This technology is based on the encapsulation of drug reservoir inside a microporous compartment with aperture along its top and bottom wall. The peripheral walls of the drug reservoir compartment are completely sealed to prevent any direct contact of the gastric mucosal surface with the undissolved drug. In stomach the floatation chamber containing entrapped air causes the delivery system to float over the gastric contents. Gastric fluid enters through the

apertures, dissolves the drug, and carries the dissolve drug for continuous transport across the intestine for absorption.

1.1.6.8 .2.3 Alginate beads

Multiple unit floating dosage forms have been developed from freeze-dried calcium alginate. Spherical beads of approximately 2.5 mm in diameter can be prepared by dropping a sodium alginate solution in to aqueous solutions of calcium chloride, causing precipitation of calcium alginate. The beads are then separated snap and frozen in liquid nitrogen and freeze dried at -40°C for 24 hrs, leading to the formation of porous system, which can maintain a floating force over 12 hrs [22].

1.1.6.8 .2.4 Hollow microspheres

Hollow microspheres (microballoons), loaded with ibuprofen in their outer polymer shells were prepared by a novel emulsion-solvent diffusion method. The ehanol: dichloromethane solution of the drug and enteric acrylic polymers was poured in to an agitated aqueous solution of PVA that was thermally controlled at 40°C.The gas phase generated in dispersed polymer droplet by evaporation of dichloromethane formed in internal cavity in microspheres of the polymer with drug. The microballoons floated continuously over the surface of acidic dissolution media containing surfactant for greater than 12 hrs *in-vitro* [22].

1.1.7 Factors affecting gastric retention

1.1.7.1 Density

Density of the dosage form should be less than the gastric contents (1.004gm/mL).

1.1.7.2 Size and shape

Dosage form unit with a diameter of more than 7.5 mm are reported to have an increased GRT compared to those with a diameter of 9.9 mm. The dosage form with a shape tetrahedron and ring shape devices with a flexural modulus of 48 and 22.5 kilopond per square inch (KSI) are reported to have better GIT ≅ 90 to 100 % retention at 24 hrs compared with other shapes [1,23].

1.1.7.3 Fed or unfed state

Under fasting conditions, the GI motility is characterized by periods of strong motor activity or the migrating myoelectric complexes (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 GRT of the unit can be expected to be very short. However, in the fed state, MMC is delayed and GRT is considerably longer [18].

1.1.7.4 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 emptying rate and prolonging the drug release [14].

1.1.7.5 Caloric content

GRT can be increased between 4 to 10 hrs with a meal that is high in proteins and fats.

1.1.7.6 Frequency of feed

The GRT can increase by over 400 min when successive meals are given compared with a single meal due to the low frequency of MMC [22].

1.1.7.7 Gender

Mean ambulatory GRT in meals (3.4 ± 0.4 hrs) is less compared with their age and race-matched female counterparts (4.6± 1.2 hrs), regardless of the weight, height and body surface.

1.1.7.8 Age

Elderly people, especially those over 70 years have a significantly longer GRT [23].

1.1.7.9 Posture

GRT can very between supine and upright ambulatory states of the patients [24].

1.1.7.10 Concomitant drug administration

Anticholinergic like atropine and propentheline opiates like codeine and prokinetic agents like metoclopramide and cisapride.

1.1.8 Formulation of stomach specific dosage form

Following types of the ingredients can be incorporated in to HBS dosage form in addition to drugs [23,25].

- Hydrocolloids
- Inert fatty materials
- Release rate accelerants
- Release rate retardant
- Buoyancy increasing agents
- Miscellaneous

1.1.8.1 Hydrocolloids

Suitable hydrocolloids are synthethics, anionic or non ionic like hydrophilic gumes, modified cellulose derivatives. E.g. accasia, pectin, agar, alginates, gelatin, casein, bentonite, veegum, MC, HPC, HEC, and Na CMC can be used. The hydrocolloids must hydrate in acidic medium i.e. gastric fluid is having pH 1.2. Although the bulk density of the formulation may initially be more than one, but when gastric fluid is enter in the system, it should be hydrodynamically balanced to have a bulk density of less than one to assure buoyancy.

1.1.8.2 Inert fatty materials

Edible, pharmaceutical inert fatty material, having a specific gravity less than one can be added to the formulation to decrease the hydrophilic property of formulation and hence increases the buoyancy. Example: Purified grades of beeswax, fatty acids, long chain alcohols, glycerides, and minaral oils can be used. Such materials may be present from about 5-75 % by weight.

1.1.8.3 Release rate accelerant

The release rate of the medicament from the formulation can be modified by including excipient like lactose and/or mannitol. These may be present from about 5-60% by weight.

1.1.8.4 Release rate retardant

Insoluble substances such as dicalcium phosphate, talc, magnesium strearete decresesd the solubility and hence retard the release of medicaments. Such, materials may be present about 5-60 % by weight.

1.1.8.5 Buoyancy increasing agents

Materials like ethyl cellulose, which has bulk density less than one, can be used for enhancing the buoyancy of the formulation. It may be added up to 80 % by weight.

1.1.8.6 Miscellaneous

Pharmaceutically acceptable adjuvant like preservatives, stabilizers, and lubricants can be incorporates in the dosage forms as per the requirements. They do not adversely affect the hydrodynamic balance of the systems.

1.1.9 Evaluation of stomach specific systems

Various parameters need to be evaluated for their effects on gastric residence time of different formulations. These parameters can be categorised into the following classes:

- Galenic: diametral size ('cut-off size'), resultant weight flexibility and density of matrices.
- Control: floating time, dissolution, specific gravity, content uniformity, and hardness and friability (of tablets).
- Geometric: shape.
- Physiological: age, sex, posture, food and bioadhesion.

1.1.9.1 Bio/mucoadhesive systems

1.1.9.1 .1 Bioadhesive strength

Bioadhesive strength of a polymer can be determined by measuring the force required to separate a polymer specimen sandwiched between layers of either an artificial (e.g. cellophane) or a biological (e.g. rabbit stomach tissue) membrane. This force can be measured by using a modified precision balance or an auto mated texture analyzer.

1.1.9.1 .2 In-vivo evaluation

The effects of the mode of riboflavin-5-phosphate administration on the resulting mean drug plasma concentrations and cumulative amounts of riboflavin absorbed in dogs were studied. In contrast, with both a non-gastroretentive control formulation (multilayer film without rigid frame; 5.0 × 2.5 mm) and an oral solution, which resulted in shorter time periods with elevated riboflavin concentrations, the gastroretentive device (multilayer film with rigid frame; 5.0 × 2.5 mm) produced elevated plasma drug concentrations for at least 48 hrs. The absolute bioavailabilities were 17.1 ± 3.5%, 3.9 ± 0.4% and 3.9 ± 1% for the gastroretentive dosage form, control formulation and oral solution, respectively.

1.1.9.2 Magnetic systems

1.1.9.2 .1 In-vitro dissolution study

In-vitro release experiments were carried out according to the US Pharmacopeia (USP) XXIII Paddle method at 37^{o}C and 100 rpm, using 0.01 N HCl as dissolution medium. The measured values were continuously recorded using an IBM compatible AT (Advanced Technology) computer (Friedrich, Munster, Germany). During the release experiments, the magnetic tablets were located at the wall of the release vessel 5 cm under the surface of the liquid, using an external magnet. The distance between the external magnet and the magnetic depot tablet was 8cm.The release from the tablet is directly proportional to the distance between the external magnet and tablet.

1.1.9.2 .2 In-vivo evaluation

Groning et al developed a method for determining the gastrointestinal transit of magnetic dosage forms of acyclovir under the influence of an extracorporeal magnet, using a pH telemetering capsule (Heidelberg capsule). Small magnets were attached to the capsule and administered to humans. *In-vivo* human studies showed that, in the presence of an extracorporeal magnet, the plasma concentrations of aciclovir were significantly higher after 7, 8, 10 and 12 hrs. Furthermore, the mean area under the plasma concentration-time curve from zero to 24 hrs (AUC 0–24) was ≈2800 ng • h/mL with the external magnet and ≈1600 ng • h/mL without the external magnet.

1.1.9.3 Swelling and expanding systems

1.1.9.3 .1 Water uptake study

The swelling of the polymers can be measured by their ability to absorb water and swell. Water uptake studies of the formulation (tablet or granules) are performed using USP dissolution apparatus II. The medium used is usually distilled water or 0.1 N HCl (900 mL) rotated at 50 rpm, and maintained at 37±0.5℃ through-out the study. After a selected time interval, the formulation is withdrawn, blotted to remove excess water, and weighed. Swelling characteristics of the tablets expressed in terms of water uptake (WU) are calculated as (equation 1):

$$WU\ (\%) = \frac{\text{swollen weight- initial weight}}{\text{initial weight}} \times 100$$

In-vitro dissolution studies in swelling and expanding systems are usually carried out by a modified dissolution method, as in the case of FDDS.

1.1.9.4 Floating drug delivery systems

1.1.9.4.1 In-vitro floating time determination

Floating time is determined by using the USP disintegration apparatus containing 900mL of 0.1 N HCl solution as a testing medium maintained at 37±0.5ºC. The time required to float different dosage forms is noted as floating (or buoyancy) lag time, and floating duration of the dosage form is determined by visual observation.

1.1.9.4.2 In-vitro dissolution study

Dissolution tests are performed using USP dissolution apparatus. Samples are withdrawn periodically from the dissolution medium; replenished with the same volume of fresh medium at sampling time points. Recent methodology as described in the USP XXIII states “the dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is started. A small, loose piece of nonreactive material such as not more than a few turns of a wire helix may be attached to the dosage units that would otherwise float”. However, standard dissolution methods based on the USP or British Pharmacopoeia (BP) have been shown to be poor predictors of *in-vitro* performance for floating dosage forms.

Pillay and Fassihi investigated the application of a helical wire sinker to the swellable floating system containing theophylline (a sparingly water-soluble drug). They observed that the procedure tends to inhibit the three-dimensional swelling process of the dosage form, and consequently drug release from the formulation was suppressed. Based on their observations, the researchers proposed an alternative method in which the floatable delivery system was fully submerged under a ring/mesh assembly. The results showed a significant increase in drug release (20%). In addition, the proposed method was found to provide reproducible hydrodynamic conditions and consistent release profiles. However, in the case of a swellable floating system, which contained diltiazem (a highly water-soluble drug), the researchers did not find any difference in release between the proposed method and the USP method. These findings led to the conclusion that drug release from swellable floating systems depends on full surface exposure, unhindered swelling, and the drug solubility in water.

1.1.9.4.3Physiological parameters

Age, sex, posture, food, bioadhesion, health of subject and GIT condition [22,26].

1.1.9.4.4 Galenic parameter

Diametrical size, flexibility and density of matrices [26].

1.1.9.4.5 Geometric parameter

Shape

1.1.9.4.6 Control parameter

Floating time, specific gravity, dissolution, content uniformity, hardness and friability [26].

1.1.9.4.7 Specific gravity

Specific Gravity of the floating system can be determined by the displacement method using benzene as a displacing medium [18].

1.1.9.4.8 Resultant weight

The *in-vitro* measuring apparatus has been conceived to determine the real floating capabilities of buoyant dosage forms as a function of time. It operates by fource equivalent to the fource F required to keep the object totally submerged in the fluid. This fource determines the resultant weight of the object when

immersed and may be used to quantify its floating or nonfloating capabilities. The magnitude and direction of the fource and the resultant weight corresponds to the Victoria sum of buoyancy (F_{buoy}) and gravity (F_{grav}) forces acting on the objects as shown in the equations:

$$F = F_{buoy} - F_{grav}$$

$$F = dfgV - d_fgV = (d_f - d_s)\, gv$$

$$F = (d_f - M/V)\, gV$$

In which the F is total vertical fource (resultant weight of the object), g is the acceleration due to gravity, df is the fluid density, ds is the object density is the object mass and V is the volume of the object [25].

1.1.10 Types of floating dosage forms

- New floating bilayer compressed matrices
- New multiple unit oral floating dosage form
- Sustained release intrsgastric floating granules
- Floatable asymmetric configuration drug delivery system
- Floating non compressed sustained release tablets
- Microballoons

1.1.10.1 New floating bilayer compressed matrices [27]

One of the tablet layers mainly contains the carbon dioxide generating blend and a hydrodynamic polymer. The carbon dioxide being entrapped in the gasified hydrocolloid as liberated by the action of the gastric medium produces the upward motion of the tablet and maintains its buoyancy. The outer layer is hydrophilic matrix and contained the dug which is release in the prolong and controlled way.

Advantages

- Double layer matrix tablet shows a more homogenous behavior with regard to erosion and is less sensitive to the GI peristaltism and the formulation of the matrix dosage form with two distinct layers allows the separate regulation of the floating capabilities and the drug release kinetics.

- Consequently this type of sustained release matrix could be advantageously used for conveying drugs which are sufficiently stable and soluble in acidic media, better reabsorbed in the proximal or middle portion of the GI tract, requiring a sustained release period to improve the bioavalability of poorly soluble products in non acid media or aiming to produce a local and specific effect in the stomach.

1.1.10.2 New multiple unit oral floating dosage forms [28]

The Gastric Emptying Time in the humans is in fed state from 1-6 hrs has been reported. Accordingly when a sustained release dosage form was administered orally, sufficient bioavalability and prolongation of the effective plasma level occasionally could not be obtained especially for drug having a limited absorption site in the intestinal tract. Recently some studies have been reported prolongation of GET (Gastric Emptying Time) of certain preparations, such as the floating dosage systems and bioadhesive systems.

However, as most of the floating dosage systems were single unit preparations, it was possible that a single unit type might be transited in to the small intestine in a short time, irrespective floating ability. A Multiple type of oral floating dosage systems has been prepared in order to prolong the GET of the preparation.

The system was composed of the sustained release pills containing the drug and the double layer surrounding the pills. Inner layer was an effervescent layer containing both sodium bicarbonate and the outer layer was swellable membrane was devided in to two sub layers to avoid direct contact between sodium bicarbonate and tartaric acid in the outer one.

Advantages

- Preparation process of the floating dosage systems is easy and simple. Moreover, conventional sustained release pills, such as matrix type or barrier membrane type, can be used as the central seeds of the system.
- The floating dosage system is compact before immersion n water, the system has higher density compared with other floating systems and is easy to handle.

1.1.10.3 Sustained release floating granules [29]

Drug granules, which remain in the stomach, comprise core- pharmaceutically effective ingredients coated with expansive films. Drug used was Dextromethorphan HCl (20%). Granules are developed based on chitosan of different buoyancy, both in acidic and neutral fluids, and gave the sustained release of prednisolone. The release rate of indomethacine from chitosan granules was compared with that of conventional commercial indomethacine capsules. Furthermore, enhancing the mixing ratio of drug and chitosan can control the release rate.

In case of conventional capsule, the plasma concentration reach the maximum level one-hour after administration, while in case of granules with a 1:2 mixing of drug and chitosan, the chitosan produced a sustained plateau level of the drug.

1.1.10.4 New self-correcting floatable asymmetric configuration drug delivery systems [30]

Apart from encountered difficulties in pulsatile delivery system design, the most challenging controlled drug delivery in the last two decades among pharmaceutical scientists has been design of the systems that would provide zero order kinetics for total drug release with no lag time or burst effect over a prolong period.

Features

- The system is design in such a manner that it floats, thus being likely to prolong gastric residence time *in-vivo*, resulting in longer total transit time within the GIT environment with maximum absorptive capacity and consequently greater bioavalability.
- These particular characteristics would be applicable to drugs, which have pH dependent solubilities, a narrow window of absorption and are absorbed by active transport from either the proximal or distal portion of the small intestine.
- Complete dissolution of the whole system.
- Oral drug release.
- Absences of both burst and lag time.

- Ease of manufacturing because direct compression technology in this case.

Advantages

- One major advantage of these asymmetric configuration delivery systems is that the duration of the drug release and the release pattern could be easily toilered by adjusting the amount or composition of each layer, which offers a greater degree of flexibility to formulation scientists.
- The absence of the real burst effect which is usually seen with matrix type delivery system is highly significant.
- Drug release from these systems may not be affected by changes in pH of the GIT and *in-vivo* situation.
- One feature of these swelling hydrophilic matrices is their low density and the ease with which the system can be easily trapped, adding to floating behavior after exposure to dissolution medium delay gastric emptying of stomach.
- Zero order kinetic is achievable.
- Drug is totally released but always in a controlled manner.

1.1.10.5 Floating noncompressed sustained release tablets [31]

Sustained release noncompressed tablets having a network of multitude air holes and passage there in a density of less than one and capable of floating on gastric juice *in-vivo* comprises a matrix containing

- Gelling agents (0.5-0.4%)
- Inert oil (10-20%)
- Therapeutic agent (50-75%)
- Water up to 100%

Example by an adding an agar solution to a theophyllin oil mixture at 70 °C with vigorous stirring to get O/W emulsion, which was poured into a tablet mould, allowed to cool and gel removed from the mould and air dried. The average diameter of the tablet was 0.70 mm.

1.1.10.6 Microballoons

Multiple units floating system which can be distributed widely through out the GIT providing a possibility of achieving a longer lasting and more reliable release of drugs, has been brought. To achieve this goal a novel method to prepare floating microspheres loaded with drug was developed as a modification of the emulsion solvent diffusion method for the preparation of the polymeric microsponge for a controlled drug delivery system. This microsphere was termed as "microballoons" due to its characteristic internal hollow structure and excellent floatability *in-vitro*. The method of preparation of the microballoons is described earlier in the article[12 32].

Parameters mainly affect the microballoons preparations

1. Temperature
2. Concentration of PVA in aqueous solution
3. Agitation speed
4. Ethanol dichloromethane ratio

Advantages [33]

- More predictive drug release kinetic
- Less chances of localized mucosal damage
- Larger margin of safety against dosage form failure.e.g.air compartment multiple unit system for gastric retention

1.1.11 Advantages of stomach specific drug delivery systems [23, 34]

1. The bioavailability of therapeutic agents can be significantly enhanced especially for those which get metabolized in the upper GIT by this gastroretentive drug delivery approach in comparison to the administration of nongastroretentive drug delivery. There are several different factors related to absorption and transit of the drug in the gastrointestinal tract (GIT) that act concomitantly to influence the magnitude of drug absorption.
2. For drugs with relatively short half life, sustained release may result in a flip- flop pharmacokinetics and also enable reduced frequency of dosing with improved patient compliance.

3. They also have an advantage over their conventional system as it can be used to overcome the adversities of the gastric retention time (GRT) as well as the gastric emptying time (GET). As these systems are expected to remain buoyant on the gastric fluid without affecting the intrinsic rate of employing because their bulk density is lower than that of the gastric fluids.
4. Gastroretentive drug delivery can produce prolong and sustain release of drugs from dosage forms which avail local therapy in the stomach and small intestine. Hence they are useful in the treatment of disorders related to stomach and small intestine.
5. The controlled, slow delivery of drug form gastroretentive dosage form provides sufficient local action at the diseased site, thus minimizing or eliminating systemic exposure of drugs. This site-specific drug delivery reduces undesirable effects of side effects.
6. Gastroretentive dosage forms minimize the fluctuation of drug concentrations and effects. Therefore, concentration dependent adverse effects that are associated with peak concentrations can be presented. This feature is of special importance for drug with a narrow therapeutic index.
7. Gastroretentive drug delivery can minimize the counter activity of the body leading to higher drug efficiency.
8. Reduction of fluctuation in drug concentration makes it possible to obtain improved selectivity in receptor activation.
9. The sustained mode of drug release from Gastroretentive doses form enables extension of the time over a critical concentration and thus enhances the pharmacological effects and improves the chemical outcomes.

1.1.12 Limitations/disadvantages

- These systems require a high level of fluid in the stomach for drug delivery to float and work efficiently-coat, water.
- Not suitable for drugs that have solubility or stability problem in GIT.

- Drugs such as nifedipine which is well absorbed along the entire GIT and which undergoes first pass metabolism, may not be desirable.
- Drugs which are irritant to Gastric mucosa is also not desirable or suitable[1].
- The drug substances that are unstable in the acidic environment of the stomach are not suitable candidates to be incorporated in the systems [1].
- The dosage form should be administered with a full glass of water (200-250 mL) [3].
- These systems do not offer significant advantages over the conventional dosage forms for drugs, which are absorbed through out the gastrointestinal tract.

1.1.13 Application of floating drug delivery system [5]

- Recent study indicated that the administration of Diltiazem floating tablets twice a day might be more effective compared to normal tablets in controlling the Blood pressure of hypertensive patients.
- Modapar® HBS containing l-dopa and Benserazide, here the drug was absorbed over a period of 6-8 hrs and maintained substantial plasma concentration for Parkinsonian patients. Cytotech®- containing Misoprostol, a synthetic prostaglandin –EL analogue, for prevention of gastric ulcer caused by non-steroidal anti-inflammatory drugs (NSAIDS).
- As it provides high concentration of drug within gastric mucosa, it is used to eradicate *H.pylori* (a causative organism for chronic gastritis and peptic ulcers).
- 5-fluorouracil has been successfully evaluated in the patients with stomach neoplasm.
- Developing HBS dosage form for tacrin provide better delivery systems and reduced its GI side effects.
- Treatment of gastric and duodenal ulcer.

1.1.14 Future potential

- Floating dosage form offers various future potential as evident from several recent publications. The reduced fluctuations in the plasma level of drug results from delayed gastric emptying.
- Drugs that have poor bioavailibility because of their limited absorption to the upper gastrointestinal tract can be delivered efficiently thereby maximizing their absorption and improving their absolute bioavalability.
- Buoyant delivery system considered as a beneficial strategy for the treatment of gastric and duodenal cancers.
- The floating concept can also be utilized in the development of various anti-reflux formulations.
- Developing a controlled release system for the drugs, which are potential to treat the Parkinson's disease.
- To explore the eradication of Halico-bector pylori by using the narrow spectrum antibodies.

Table 1.1.2: Drugs explored in stomach specific dosage forms[13, 35]

Types of dosage forms	Drugs explored in stomach specific dosage forms
Microspheres	Aspirin, Griseofulvin, P-nitro aniline, Ibuprofen, Terfenadine, Tranilast
Granules	Diclofenac Sodium, Indomethacin, Prednisolone
Films	Cinnarizine
Powders	Several Basic Drugs
Capsules	Chlordiazepoxide HCl, Diazepam, Furocemide, l-dopa and Benserazide, Misoprostol, Propranolol HCl
Tablets/Pills	Acetaminophen, Aspirin, Amoxycillin, Ampicillin, Atenolol, Chlorpheniramine maleate, Cinnarizine, 5-Fluorouracil, Isosorbide mononitrate, Diltiazem, Isosorbide dinitrate, Piretenide, Quinidine, Varapamil HCl, Riboflavin, Sotalol, Theophylline

Table 1.1.3: Commercial stomach specific floating formulations [3,36-38]		
Name	**Type and drug**	**Remarks**
MadoparHBS® (PropalHBS)	Floating capsule, Levodopa and benserazide	Floating CR capsules
Valrelease® [34]	Floating capsule, Diazepam	Floating Capsules
Topalkan®	Floating Antacid, aluminum and magnesium mixture	Effervescent floating liquid alginate preparation
Amalgate Float Coat® [35]	Floating antacid Floating gel	Floating dosage form
Conviron	Ferrous sulphate	Colloidal gel forming FDDS
Cifran OD®	Ciprofloxacine (1 gm)	Gas generating floating form
Cytotech®	Misoprostol (100 mcg/200 mcg)	Bilayer floating capsule
Liquid Gaviscone®	Mixture of alginate	Suppress gastro esophageal reflux and alleviate the heart burn

1.1.15 Stability studies

The success of an effective formulation can be evaluated only through stability studies. The purpose of stability testing is to obtain a stable product, which assures its safety and efficacy up to the end of shelf life at defined storage conditions and pack profile. The stability studies of different GRDDS are usually carried out as per the International Conference on Harmonisation guidelines.

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Chapter 1.2

Introduction to polymers

1.2 Introduction to polymers

1.2.1 Introduction to chitosan

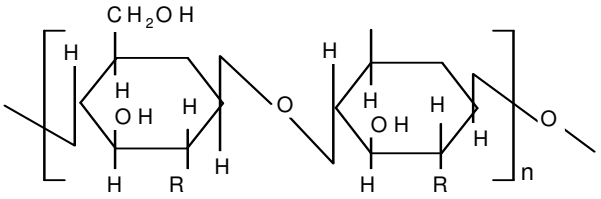
Chitosan are biodegradable, high molecular weight cationic polysaccharides. Industrially they are produced from chitin, the world's second most abundant biopolymer, by deactivation process involving alkaline hydrolysis. The term chitosan refers to a family of polymers, individually characterized by their ratio of acetylated to deactivated units and molecular weight, both parameters being equally responsible for the properties of the polymer. Chitosan has been used for a range of applications as diverse as for water purification, as a food ingredient and as a pharmaceutical excipient. Braconnot[1] first described chitin in 1811. A good deal of fundamental research on chitin occurred in the next century and a half but most of the information available today had been obtained since 1950. Chitin is the major polysaccharide of the shells of crustacean and exoskeletons of insects. It is also found in the cell walls of many fungi, yeast and algae. Chitosan was discovered by Rouget[1] in 1859 and was prepared by Hope Seylar [1]. Chitosan is deactivated chitin derivative. It is found naturally in fungal cell walls but can also be produced by alkaline treatment of chitin.

1.2.1.1 Preparation of chitosan [2]

Chitin is treated for 1 or 2 hrs in 47% sodium hydroxide solution in nickel crucible at 60 ^{o}C under nitrogen atmosphere. The chitosan obtained by alkali treatment is washed to neutrality, the deactivation being about 80% or less by the first alkali treatment. The chitosan after washing in water is treated again in the alkaline solution twice or more to obtain chitosan which has the deactivation of 90-05 % for even further deactivation, thread like chitosan is again subjected to alkali treatment.

1.2.1.2 Structure and properties of chitosan [2]

Chitosan is (1-4)-2-amino-2-deoxy-B-D glucon. It has similar structural characteristics as that of glucosaminoglycans. It is tough, biodegradable and nontoxic.



R = -NH$_2$- Chitosan

Chitin, poly-B-(1-4) linked N acetyl –D- glucosamine is a highly hydrophobic material that is insoluble in water and most ordinary solvents. This property of chitin restricts it use to application that do not require solubilization of the polymer. Considering chitosan as a weak base, a certain minimum amount of acid is required to transform the glucosamine units into the positively charged, water soluble form. At neutral ph most chitosan molecules will lose their charge and precipitate from solution. Chitosan is soluble in dilute organic acids like formic, acetic, propionic, oxalic, malonic, succinic, adipic, lactic, pyruvic, malic, tartaric and citric.

Chitosan is also soluble in dilute nitric and hydrochloric acids, marginally soluble in 0.5% phosphoric acid and insoluble in sulfuric acid at room temperature. Formic acid is the best solvent, overall good solutions being obtained in aqueous systems containing 0.2 to 100% of this acid. Acetic acid has been selected as the standard solvent for solution property measurement. Chitosan readily dissolves is 3:1 glycerol water when the mixture contains 1% acetic acid, resulting in clear colorless and very viscous solution.

Solutions of Chitosan in 10% w/v aqueous oxalic acid show thermo reversible gel property. A solution containing more than 7 % chitosan will gel in less than a day and 3% solution will gel in about 3 weeks. The chitosan films were cross-linked by glutaraldehyde vapors in a closed chamber for 24 hrs at ambient temperature. This process was done to retard the chitosan degradation rate. The decrease in degradation rate of cross linked chitosan was probably due to the retarded hydrolysis of Schiff's bases induced by the glutaraldehyde cross linked of chitosan's amino groups.

Chitosan a linear polyelectrolyte at acidic pH, is soluble in variety of acids and interacts with polyanionic counterions. It forms gels with a number of multivalent anions and also with glutaraldehyde. It has a high charge density i.e. one charge per glucosamine unit. Since many minerals carry negative charges, the positive charge of chitosan interacts strongly with negative surfaces. Chitosan is a linear polyamine where amino groups are readily available for chemical reactions and salt formation with acids. The important characteristics of chitosan are its molecular weight, viscosity, deacetylation degree (DA) crystallinity index, number of monomeric units (n), water retention value, pk_a and energy of hydration.

1.2.1.3 Pharmaceutical requirements of chitosan

Particle size < 30 µm, density between 1.35 and 1.40 g/cc, ph 6.5-7.5, insoluble in water, and partially soluble in acids. Chitosan can also be characterized in terms of its quality, intrinsic properties and physical forms. The quality characteristics of chitosan are levels of heavy metals and proteins, pyrogenicity and degree of deacetylation are the intrinsic properties.

1.2.1.4 Biological and chemical properties of chitosan

Biocompatibility (e.g. Nontoxic, biodegradable, natural), bioactivity, wound healing acceleration, reduced blood cholesterol levels, and immune system stimulant effect. Biomedical properties biological activity and biodegradation of chitosan are stated by Knapczyk et al [3]. Muzzarell[4] gives the chemical behavior of chitosan and modified chitosan. Sanford[5] summarized the chemical and biological properties of chitosan that relate to applications. Tables 1.2.1 and 1.2.2.

Table 1.2.1: Chemical properties of chitosan [5]

Cationic polyamine
High charge density at pH < 6.5
Adheres to negatively charged surfaces
Forms gels with poly anions
High molecular weight linear polyelectrolyte
Viscosity, high to low
Chelates certain transitional metal

Amiable to chemical modification reactive amino / hydroxyl groups

Table 1.2.2: Biological properties of chitosan [5]

Biocompatibility
Natural Polymer
Biodegradable to normal body constituents
Safe and non – toxic
Haemostatic
Bacteriostatic
Fungistatic
Spermicidal
Anticarcinogen
Anticholesteremic

1.2.1.5 Mucoadhesive properties of chitosan

Lehr et al.[6] first evaluated mucoadhesive properties of chitosan. A number of characteristics are necessary for mucoadhesion (a) strong hydrogen bonding groups (-OH, -COOH), (b) strong anionic charges, (c) high molecular weight, (d) sufficient chain flexibility, and (e) surface energy properties favoring spreading on to mucus. However, chitosan is a poly-cationic polymer and does not have any anionic charge. Instead, a positively charged hydrogel is formed in acidic environment that could develop additional molecular attractive forces by electrostatic interactions with negatively charged mucosal surfaces or the negatively charged sialic acid groups of the mucus network. High molecular weight chitosan gave the best mucoadhesive properties.

1.2.1.6 Toxicological studies of chitosan

In-vivo toxicity tests indicated that chitosan is non toxic, inert and sterilized films was free of pyrogens. LD 50 and oral toxicity levels of chitosan were estimated in both rats and mice. Lack of cute oral toxicity to chitosan was noticed as evidenced by an oral LD 50, 10g/ kg in mice. Acute systemic toxicity tests in mice did not show any significant toxic effects of chitosan.

1.2.1.7 Preparation of chitosan microspheres [1]

Chitosan microspheres can prepare by following methods.

1.2.1.7.1 Ionotropic gelation

In this method, chitosan solutions in acetic acid are prepared and extruded drop wise through a needle into different concentrations of aqueous solutions of magnetically stirred tripolyphosphate. The beads are removed from the counter ion solution by filtration, washed with distilled water, dried by an air jet and further air dried at ambient temperature.

1.2.1.7.2 Extrusion- spheromization

In this method ingredients are mixed with chitosan and the wet mass is passed through an extruder. The cylindrical extrudate obtained is immediately processed in a spheronizer. The beads are collected and dried in a hot air oven at 40 ^{o}C for 12 hrs.

1.2.1.7.3 Solvent evaporation technique

Chitosan dissolved in an aqueous acetic acid solution. The solution is added to toluene and sonicated to form a w/o emulsion. Glutaraldehyde solution in toluene is added to the emulsion and stirred at room temperature to give cross-linked microspheres. The suspension is centrifuged. Following evaporation of the solvent, the microspheres are separated, washed with distilled water and dried.

Li et al. [7] modified the solvent evaporation method and named it “Dry-in-Oil” method. Here system is warmed to 50 ^{o}C and the pressure is reduced. When the solvent is evaporated completely the microspheres are separated, washed with sodium hydroxide solution, distilled water and diethyl ether and dried.

1.2.1.7.4 Multiple emulsion method

Multiphase emulsions are also prepared by the solvent evaporation technique by a three step emulsification process.

Aqueous drug solution and oil phase containing emulsion stabilizers are combined to give water-in-oil emulsion (step 1).

Later the w/o emulsion is dispersed in the polymer solution (step 2).

The solvent is evaporated under reduced pressure (step 3).

1.2.1.7.5 Spray drying method

Chitosan microspheres are prepared by using a spray drier apparatus. Microspheres have been prepared from solutions of different concentrations of chitosan in glacial acetic acid/water/acetone.

1.2.1.7.6 Precipitation / coacervation method

Berthold et al [8] prepared chitosan microspheres by a novel precipitation method using sodium sulphate as precipitant. In this method chitosan is dissolved in acetic acid containing polysorbate 80. A solution of sodium sulphate is added dropwise during stirring and ultrasonication. The formation of microspheres is indicted by turbidity. The formed microspheres are purified by centrifugation and resuspended in dematerialized water. This method avoided the use of organic solvents and glutaraldehyde for preparation of chitosan microparticles with high loading capacity and sustained release effect.

Chitosan microparticles can also be prepared by complex coacervation. Sodium alginate, sodium carboxymethylcellulose, kappa-carregeenan and sodium poly-acrylic acid are used in the complex coacervation procedure with chitosan. Here the microparticles are formed by interionic interaction between oppositely charged polymers. Formulation of coacervate capsules of chitosan-alginate and chitosan- kappa-carregeenan is carried out by extruding either an aqueous solution of kappa-carregeenan in a solution of sodium alginate through a hand-operated syringe into potassium chloride or calcium chloride solution. The counterion solution consisted of chitin. The obtained capsules were agitated to harden in the counterion solution before washing and drying.

1.2.1.7.7 Coating by chitosan

In this method, previously formed microparticles are coated with chitosan. HAS microspheres are prepared and added to various concentrations of chitosan-acetic acid solutions and mixed; the chitosan treated microspheres are filtered and dried.

1.2.2 Introduction to alginate

Alginate is a naturally occurring biopolymer that is finding increasing applications in the biotechnology industry. Alginate has been used successfully for many years in the food and beverage industry as a thickening agent, a gelling agent and a colloidal stabilizer. Alginate also has several unique properties that have enabled it to be used as a matrix for the entrapment and/or delivery of a variety of proteins and cells. These properties include: (i) a relatively inert aqueous environment within the matrix; (ii) a mild room temperature encapsulation process free of organic solvents; (iii) a high gel porosity which allows for high diffusion rates of macromolecules; (iv) the ability to control this porosity with simple coating procedures and (v) dissolution and biodegradation of the system under normal physiological conditions.

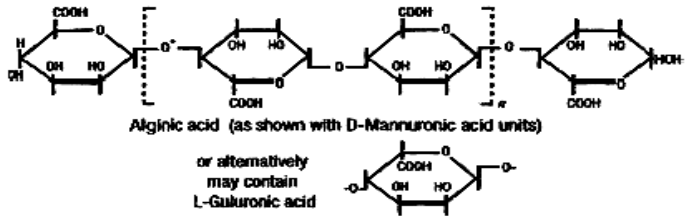
1.2.2.1 Sources of alginate

Commercial alginates are extracted primarily from three species of brown algae (kelp). These include Laminaria hyperborea, Ascophyllum nodosum, and Macrocystis pyrifera. Other sources include Laminaria japonica, Eclonia maxima, Lesonia negrescens and Sargassum species [9]. In all of these algae, alginate is the primary polysaccharide present and it may comprise up to 40% of the dry weight [10].

1.2.2.2 Extraction and preparation

To commercially prepare alginates, the algae is mechanically harvested and dried before further processing except for M. pyrifera which is processed when wet. Alginate is then extracted from dried and milled algal material after treatment with dilute mineral acid to remove or degrade associated neutral homopolysaccharides such as laminarin and fucoidin. Concurrently, the alkaline earth cations are exchanged for H^+. The alginate is then converted from the insoluble protonated form to the soluble sodium salt by addition of sodium carbonate at a pH below 10. After extraction, the alginate can be further purified and then converted to either a salt or acid [10].

1.2.2.3 Chemical structure



1.2.2.4 Microbead preparation

There are three widely-known methods used to prepare alginate microbeads that are less than 0.2 mm in diameter; atomization, emulsification and coacervation. The most commonly used technique is an atomization or spraying method using an extrusion device with a small orifice. A general overview of alginate microbead preparation is as follows. Solutions containing the alginate and protein, as described above in the preparation of large beads, are well mixed and loaded into a syringe mounted on a syringe pump. The mixture of alginate and protein solution is then delivered through an atomization device with a defined diameter (~1 mm) orifice at the tip. Much smaller diameter orifices can be used but may run the risk of orifice clogging/plugging by the high viscosity alginate solution. The sizes of these beads can be controlled by either the pressure of the infusing nitrogen gas, the flow-rate of the syringe pump or the distance between the orifice and the surface of the cross-linking solution. Fine droplets of sodium alginate and protein solution will form the microbeads when cross-linked with the divalent solution. Outer coatings of poly-L-lysine and alginate can then be performed. The second method of microbead preparation involves protein encapsulation by an oil-in-water emulsification technique. Complex coacervation of oppositely charged polyelectrolytes has been commonly used as a method for preparing microbeads. Complex coacervation between alginic acid, gelatin[11], chitosan[12], and albumin[13] has been reported. In the alginate–chitosan system, the complex is formed by spraying a sodium alginate solution into the chitosan solution. The resultant alginate–chitosan microbeads are mechanically strong and stable over a wide pH range. With the alginate–albumin system,

coacervation is found to be limited compared to other polypeptide–polysaccharide systems due to the high viscosity of the albumin–alginic acid complex and a propensity to precipitate. The optimum conditions for maximum coacervate yield are a pH of 3.9, an ionic strength of 1 mM and a 0.15% w/v total polyion concentration.

1.2.2.5 Physical properties

The functional and physical properties of cation cross-linked alginate beads are dependent on the composition, sequential structure, and molecular size of the polymers. The flexibility of the alginate polymers in solution increases in the order MG>MM>GG (G=α-L-guluronic acid; M= β-D-mannuronic acid). Beads with the lowest shrinkage, highest mechanical strength, highest porosity, and best stability towards monovalent cations are made from alginate with an α-Lguluronic acid content greater than 70% and an average length of the α-L-guluronic acid blocks higher than 15. These polymers are called "high G" alginates and for molecular weights higher than 2.4×10^5, the gel strength is independent of the molecular weight. For lower molecular weight alginates however, there is a certain critical molecular weight below which the gel forming properties of alginates are reduced. While a gel made from a high α-L-guluronic acid alginate may be rigid and brittle, gels produced from alginates with a low α-L-guluronic acid content are more elastic. Alginate forms stable gels over the temperature range of 0-100℃, although the modulus of rigidity of the gels decreases with an increase in temperature. The gels can be prepared in either hot or cold water.

1.2.2.6 Chemical reactivity

Although the microenvironment in an alginate bead can be relatively inert to protein drugs and cells (alginate beads typically contain up to 95% water) a positively charged protein can potentially compete with calcium ions for available carboxylic acid sites on the alginate. This has been observed with small drugs by several investigators[14-15] and has been shown to result in protein inactivation in the case of the protein transforming growth factor-beta (TGF- β_1).

1.2.2.7 Porosity and macromolecular diffusion

Proteins encapsulated in alginate matrices are released by two mechanisms: (i) diffusion of the protein through the pores of the polymer network and (ii) degradation of the polymer network. Analysis of calcium alginate gels microbeads by electron microscopy has shown that the pore size ranges from 5 to 200 nm in diameter [16]. In a different approach the porosity was determined by packing alginate beads in a column and recording the exclusion volumes for macromolecular standards [17]. A cut off value of 12–16 nm was determined which is smaller than the pore size distribution obtained by electron microscopy. Diffusion of small molecules such as glucose and ethanol is unaffected by the alginate matrix while diffusion of larger proteins from the gels has been shown to be dependent on their molecular weight. The diffusion of several proteins from alginate beads has been reported including immunoglobulin G (IgG)[18-19], fibrinogen[18] and insulin. Increasing the concentration of alginate in the beads decreases the rate of diffusion of the proteins from the gel.

1.2.2.8 Chemical stability/degradation

Degradation of a Ca^{2+} cross-linked alginate gel can occur by removal of the Ca^{2+} ions. This can be accomplished by the use of a chelating agent such as ethylene glycol-bis (β -aminoethyl ether)-N,N,N′,N′-tetraocetic acid (EGTA), lactate, citrate and phosphate or by a high concentration of ions such as Na^{+} or Mg^{2+} . As Ca^{2+} ions are removed, the cross-linking in the gel decreases and the gels are destabilized. This can lead to leakage of entrapped material and solubilization of the high molecular weight alginate polymers. Alginate gels will also degrade and precipitate in a 0.1 M phosphate buffer solution and will completely dissolve in 0.1 M sodium citrate at pH 7.8. If Ca^{2+} is used in the cross-linking solution and phosphate is used as the dissolution medium, the dissolution medium will turn turbid due to the Ca^{2+} dissociating from the polymer network and forming calcium phosphate precipitate.

1.2.2.9 Biological properties

1.2.2.9.1 Immunogenicity [20]

There are many factors involved in determining the successful application of polymers as drug delivery carriers in humans, with polymer biocompatibility or/and immunogenicity being two of the more important issues. There are numerous reports addressing the fibrotic reaction of implanted alginates. Alginates can be readily purchased in several different grades namely, ultra pure, food or research grade.

1.2.2.9.2 Bioadhesion

Alginate possesses, among other features, a bioadhesive property which could serve as a potential advantage in mucosal drug delivery. The term bioadhesion can be generally defined as the adhesion or contact between two surfaces, with one being a biological substratum[21]. If one of the surfaces involved is a mucosal layer, the term mucoadhesion is then used [22]. Studies have shown that polymers with charge density can serve as good mucoadhesive agents [23-26]. Peppas and colleagues believed that mucoadhesion is achieved by chain penetration across a polymer–mucosa interface [27-28]. It has been reported that polyanion polymers are more effective bioadhesives than polycation polymers or nonionic polymers. Alginate, with its carboxyl end groups, is classified as an anionic mucoadhesive polymer. Alginate mucoadhesion studies, conducted by Chickering et al., were performed with a tensile testing apparatus in which the adhesive forces between different polymers and living intestinal epithelium were evaluated [24-25]. The intestinal epithelium used in these experiments was from excised rat jejunum. In brief, individual polymer beads were placed on an inverted jejunum. The force required to detach the beads from the jejunum's surface was recorded and compared with the values obtained from other types of polymer beads. These studies showed that alginate has the highest mucoadhesive strength when compared to polymers such as polystyrene, chitosan, carboxymethylcellulose and poly(lactic acid). Mucoadhesive drug delivery systems work by increasing the drug residence time at the site of activity or resorption. This mucoadhesive feature of alginate may aid in its utility as a potential delivery vehicle for drugs to

mucosal tissues such as the gastrointestinal tract or the nasopharynx. The adherence of these microbeads to the mucosal tissues localizes the drug and delays the protein transit time, therefore potentially improving the overall drug effectiveness and bioavailability.

1.2.2.10 Microsphere and liposome encapsulation

Alginate gels have been used to encapsulate other delivery systems including microspheres and liposomes. Ethylcellulose microspheres were dispersed into an aqueous solution of sodium alginate which was subsequently dropped into a $CaCl_2$ solution [29]. The authors suggested that the beads could potentially be useful as an oral delivery system for micro-or nanoparticles. Liposomes that contained the model proteins BSA or horse-radish peroxidase were incorporated into alginate spheres with a diameter of 500-800 µm [30-31]. Prior to their entrapment, the liposomes were coated with either phospholipase C, D, or A_2. The alginate microbeads that contained the liposomes remain stable at 10℃. Upon heating to 37℃, release of the protein is triggered by the enzymatic degradation of the phospholipids by the phospholipases. By selecting the appropriate phospholipase the duration of protein release could be controlled.

1.2.3 Introduction to hydroxypropyl methyl cellulose [32]

1.2.3.1 Nonproprietary names

BP/USP: Hypromellose

PhEur: Hypromellosum

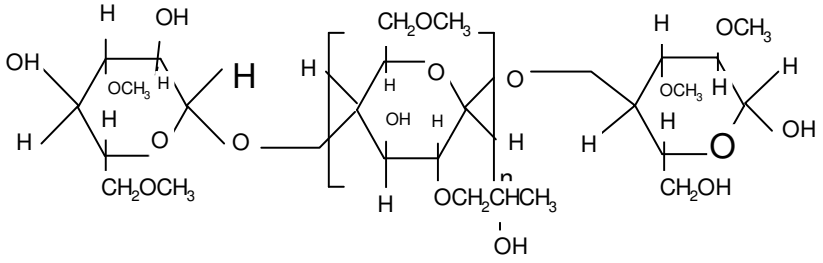
1.2.3.2 Synonyms

Methyl hydroxypropyl cellulose, propylene glycol ether of methyl cellulose, methyl cellulose propylene glycol ether, methocel, HPMC.

1.2.3.3 Chemical names

Cellulose, 2-hydroxy propyl methyl ether.

1.2.3.4 Structural formula



1.2.3.5 Functional category

Suspending and /or viscosity increasing agent, tablet binder, coating agent, Viscosity increasing agent, adhesive anhydrous ointment ingredient, film former, emulsion stabilizer, rate-controlling polymer for sustain release.

1.2.3.6 Method of manufacture

A purified form of cellulose fibers obtained from cotton linters or wood pulp, are treated with caustic (sodium hydroxide) solution. The alkali cellulose thus obtained is in turn treated with methyl chloride and propylene oxide to provide methylhydroxypropyl ethers of cellulose. The fibrous reaction product is then purified and ground to a fine, uniform powder or granules.

1.2.3.7 Description

An odorless, tasteless, white or creamy-white fibrous or granular powder.

1.2.3.8 Applications in pharmaceutical formulation or technology [33-34]

Hypromellose is widely used in oral and topical pharmaceutical formulations. In oral products, primarily used as a tablet binder, in film-coating and as an extended-release tablet matrix. Concentrations between 2 % and 5 % w/w may

be used as a binder in either wet or dry granulation. Depending upon the viscosity grade, concentrations of 2-20 % w/w are used for film-forming solutions to film-coat tablets. Hypromellose is also used as a suspending and thickening agent in topical formulations, particularly ophthalmic preparations. Concentrations between 0.45-0.1 % w/w may be added as a thinking agent to vehicles for eye drops and artificial tear solutions. It is also used as an emulsifier, suspending agent, and stabilizing agent in topical gels and ointments. It is used as an adhesive in plastic bandages and wetting agent for hard contact lenses.

1.2.3.9 Typical properties [35]

Acidity/ alkalinity: pH = 5.5-8.0 for a 1 % w/w aqueous solution.

Autoignition temperature: 360 ^{o}C

Density (bulk): 0.341 g/cm^3

Density (tapped): 0.557 g/cm^3

Density (true): 1.326 g/cm^3

Melting point: browns at 190-200 ^{o}C, chars at 225-230 ^{o}C, glass transition temperature is 170-180 ^{o}C.

Moisture content: hypromellose absorb moisture from the atmosphere, the water absorbed depending upon the initial moisture content and temperature and relative humidity of the surrounding air.

Specific gravity: approximately 1.3

1.2.3.10 Solubility

Soluble in cold water, forming a viscous colloidal solution; in soluble in alcohol, ether and chloroform, but soluble in mixtures of methyl alcohol and methylene chloride. Certain grades are soluble in aqueous acetone, mixtures of methylene chloride and isopropyl alcohol and other organic solvents.

1.2.3.11 Stability and storage conditions

Very stable in dry condition. Solutions are stable at pH 3-11. Aqueous solution is liable to be affected by microorganisms when used as a viscosity-increasing agent in ophthalmic solutions and anti-microbial agent. Hypromellose powder should be store in a well-closed container, cool place and dry place.

1.2.3.12 Incompatibilities

Hypromellose is incompatible with some oxidizing agents. Since it is nonionic, hypromellose will not complex with metallic salts or ionic organics to form insoluble precipitates.

1.2.4 Introduction to carbopol [32]

1.2.4.1 Nonproprietary name

Carbopol-934P, Carbomer, Carbomera.

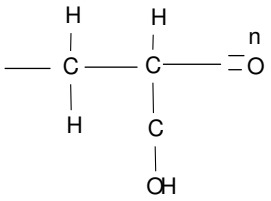
1.2.4.2 Synonyms

Carboxy polymethylene; carboxyvinyl polymer; acrylic acid polymer, carbopol.

1.2.4.3 Chemical name

Carboxy polymethylene.

1.2.4.4 Structural formula



1.2.4.5 Method of manufacture

A synthetic, high molecular weight, cross-linked polymer of acrylic acid co polymerized with approximately 0.75-2.0 % w/w of polyalkylsucrose. The end product contains 56-68% carboxylic acid groups.

1.2.4.6 Description

A white, fluffy, acidic, hygroscopic powder with a slight characteristic odor.

1.2.4.7 Functional category

Bioadhesive, suspending and/or viscosity-increasing agent, release-modifying agent, tablet binder.

1.2.4.8 Typical properties [35]

Carbopol is soluble in water, alcohol and glycerin. Agents that can neutralize carbopol include sodium hydroxide; potassium hydroxide; sodium bicarbonate; borax; amino acids; polar organic amines.

Specific gravity: 1.41

Density (bulk): 5 g/cm^3

Density (tapped): 1.4 g/cm^3

Viscosity (0.2%): 20.5-54.5 poise and (0.5%): 305-394 poise.

Acidity/ alkalinity: pH = 2.7-3.5 for a 0.5 % w/v aqueous dispersion, pH = 2.5-3.0 for a 1 % w/v aqueous dispersion.

Glass transition temperature: 100-105 ^{o}C.

Moisture content: normal water content is up to 2 % w/w. Carbomers are hydroscopic and typical equilibrium moisture content at 25 ^{o}C and 50 % relative humidity is 8-10 % w/w.

1.2.4.9 Applications in pharmaceutical formulation or technology [36-39]

Carbomers are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity-increasing agents. Formulations include creams, gels and ointments for use in ophthalmic, rectal and topical preparations. Carbomer having low residuals only of ethyl acetate, such as carbomer 971P or 974P, may be used in oral preparations, in suspensions, tablets or sustain release tablet formulation. Carbomer resins have also been investigated in the preparation of sustained-release matrix beads as enzyme inhibitors of intestinal proteases in peptide containing dosage forms, as a bioadhesive for a cervical patch and for intranaslly administratered microspheres and magnetic granules for site specific drug delivery to the esophagus.

1.2.4.10 Stability and storage conditions

Dry powder forms of carbopol do not support the growth of molds and fungi; however, microorganisms grow well in unpreserved aqueous dispersions. Dispersions maintain their viscosity on storage during prolonged periods at room temperature or elevated temperature when stored away from light or with the addition of an antioxidant. Store in an airtight or well-closed container.

1.2.4.11 Incompatibilities

Carbopol is incompatible with phenol, cationic polymers, strong acids and high concentrations of electrolytes, and is discolored by resorcinol. Exposure to light causes oxidation, which is reflected in a decrease in viscosity.

1.2.4.12 Safety

Acute oral doses of carbopol-934P to rats, mice and guinea pigs produce LD_{50} values of 4.3, 4.6 and 2.5 g/kg, respectively. In dogs, no fatalities were noted with doses as high as 8g/kg. No primary irritation or any evidence of sensitivity or allergic reaction in humans following topical application of dispersions containing

carbopol-934P has been observed. Carbopol-934P in contact with the eye is very irritating.

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Chapter 1.3
Introduction
to
Drugs

1.3 Introduction to drugs

1.3.1 Introduction to famotidine

1.3.1.1 Therapeutic class

Long-acting histamine H_2-receptor antagonist.

1.3.1.2 Chemical name

3-[2-[(aminoiminomethyl) amino] -4-thiazolyl] methyl] thio]-N- (aminosulfonyl) propanimidamide.

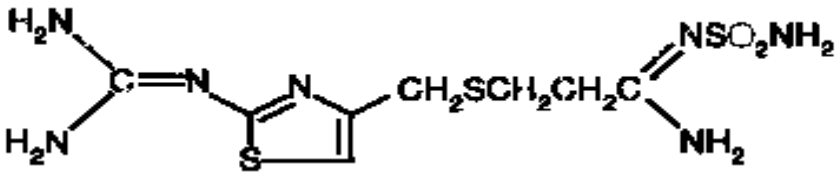
1.3.1.3 Empirical formula

$CH_5N_7O_2S_3$

1.3.1.4 Molecular weight

337.43

1.3.1.5 Structural formula



1.3.1.6 Dose

20 mg twice and 40 mg once a day.

1.3.1.7 Description

Famotidine is a white to pale yellow nonhygroscopic crystalline substance. It is very slightly soluble in water and practically insoluble in ethanol, acetone, ethyl acetate, ethyl ether and acetone. It is freely soluble in glacial acetic acid.

1.3.1.8 Indications

- Duodenal ulcer (4o mg daily by mouth twice daily for 6 to 12 weeks)
- Benign gastric ulcer
- Hypersecretory conditions such as Zollinger-Ellison syndrome (20 mg every 6 hrs)
- Prevention of relapses of duodenal ulceration
- Prevention of relapses of benign gastric ulcer
- Symptomatic relief of gastro-esophageal reflux disease (40 mg twice daily for 6 to 12 weeks)

- Healing of esophageal erosion or ulceration associated with gastro-esophageal reflux disease Prevention of relapses of symptoms and erosions or ulcerations associated with GERD

1.3.1.9 Pharmaceutical precautions

Store in a dry place below 30℃.

1.3.1.10 Medicine classification

Prescription Medicine.

1.3.1.11 Stability

Famotidine at a concentration of 2 mg per mL, diluted with glucose 5%, sodium chloride 0.9%, or sterile water was stable in PVP syringes stored at 4°C for 14 days [1]. It is also stable at room temperature for a 5 days as a 0.2 mg per mL. When stored at -20°C in polypropylene syringes it was stable for 3 weeks when diluted with glucose 5%, and for 8 weeks when diluted with sodium chloride 0.9% or sterile water. The stability of famotidine in a range of parenteral nutrition solutions containing amino acids, glucose lipids, electrolytes, vitamins and trace elements has been investigated [2-4]. In the system tested famotidine was stable for up to 74 hrs at room temperature.

1.3.1.12 Pharmacokoinetic parameters of famotidine [5,6]

Table 1.3.1.1 Pharmacokoinetic parameters of famotidine

Pharmacokoinetic Parameters	**Values**
Bioavailibility (%)	40-45
Time to peak plasma concentration (hrs)	1-3
Peak plasma concentration (μg/mL)	0.076-0.1
Half-life (hrs)	2.5-3.5
Protein binding (%)	15-20
Volume of distribution (L/kg)	1.1-1.4
Elimination (%)	25-30(Oral), 65-70 (IV)
-Urine unchanged (Oral, IV)	30-35
-Metabolized	25-30

1.3.1.13 Contraindications

Hypersensitivity to any component of these products. Cross sensitivity in this class of compounds has been observed. Therefore, famotidine should not be administered to patients with a history of hypersensitivity to other H2-receptor antagonists [7,8].

1.3.1.14 Overdosage

There is no experience to date with overdosage. Patients with Zollinger-Ellison Syndrome have tolerated doses up to 800mg/day for more than a year without development of significant side effects. The usual measures to remove unabsorbed material from the gastro-intestinal tract, clinical monitoring, and supportive therapy should be employed.

1.3.1.15 Adverse effects

Famotidine has been demonstrated to be generally well tolerated. Headache, dizziness, constipation and diarrhea have been reported rarely. Other adverse experiences reported even less frequently included dry mouth, nausea and/or vomiting, abdominal discomfort or distension, anorexia, fatigue, rash, pruritus and urticaria, liver enzyme abnormalities, cholestatic jaundice, anaphylaxis, angioedema, arthralgia, muscle cramps, reversible psychic disturbances including depression, anxiety disorders, agitation, confusion and hallucinations. Toxic epidermal necrolysis has been reported very rarely with H2-receptor antagonists. In addition to the above adverse effects, A-V block has been reported very rarely with H2-receptor antagonists administered intravenously [9-12].

1.3.1.16 Official preparations

USP 23: Famotidine Tablets [12]

1.3.2 Introduction to glipizide

1.3.2.1 Chemical name

1-cyclohexyl-3-[[p-(2-(5-ethylpyrazinecarcoxamido) ethyl] phenyl]sulfonyl]urea.

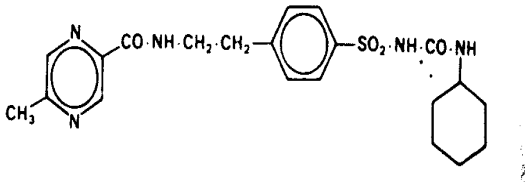
1.3.2.2 Chemical formula

$C_{21}H_{27}N_5O_4S$

1.3.2.3 Molecular weight

445.54

1.3.2.4 Chemical structure



1.3.2.5 Category

Antidiabetic

1.3.2.6 Description

A white powder.

1.3.2.7 Solubility

Practically insoluble in water and ethanol; soluble in chloroform, dimethylformamide, and in dilute solution of alkali hydroxides, sparingly soluble in acetone.

1.3.2.8 Pharmacokinetics

Glipizide is completed and rapidly absorbed ensuring prompt and constant activity. Peak plasma concentrations are attained within 1.5 to 2.0 hrs after a single oral dose. The half-life of elimination ranges from 2 to 3 hrs. The drug is excreted in the urine as virtually inactive metabolites. When taken before each meal, glynase controls post-prandial hyperglycaemia without the risk of delayed episodes of hypoglycaemial [13].

1.3.2.9 Mechanism of action

The primary mode of action of glipizide in experimental animals appears to be the stimulation of insulin secretion from the beta cells of pancreatic islets tissue and is thus dependent on functioning beta cells in the pancreatic islets. In human

glipizide appears to lower the blood glucose acutely by stimulating the release of insulin from the pancreas, an effect dependent upon functioning beta cells in the pancreatic islet. In man stimulation of insulin secretion by glipizide in response to a meal is undoubtedly of major importance. Fasting insulin levels are not elevated even on long term glipizide administration, but the post-prandial insulin response continues to be enhanced after at least six months of treatment. The insulinotopic response to a meal occurs within 30 min after an oral dose of glipizide in diabetic patients, but elevated insulin levels do not persist beyond the time of the meal challenge. Extrapancreatic effects may play a part in the mechanism of action of oral sulfonylurea hypoglycemic drugs. Beginning 2 to 3 hrs after the administration of glipizide sustained release, plasma concentrations of glipizide gradually rise reaching to a maximum concentration within 3 to 8 hrs after dosing. With subsequent once daily dosing of glipizide sustained release, effective plasma concentrations are maintained throughout 24 hrs period with fewer peaks to trough fluctuations. In view of the time required to reach an optimal concentration in plasma, drug may be more effective when given 30 min before eating. Drug in plasma 98.3 % bound to plasma protein especially with albumin. Drug is metabolized in liver, and the metabolites are excreted in the urine. Less than 5 % drug excreted unchanged in urine [14].

1.3.2.10 Indication and dosage

Management of Type 2 diabetes (Non Insuline Dependent Diabetes mellitus) where diet control alone is not effective in controlling the hyperglycemia. Dosage should be adapted to patients individually, on basis of periodic tests of glycosuria and blood sugar. The maximum daily dose should not exceed 10 mg.

1.3.2.11 Contraindications

Like other sulfonylurea, glipizide is contraindicated in: Insulin dependent diabetes mellitus, diabetic-keto-acidosis, diabetic coma, pregnancy, subjects with severely impaired kidney or liver function, adrenal insufficiency and cases of confirmed individual hypersensitivity to the drug. In latent diabetes or prediabetic states, the use of sulfonylurea is not advisable.

1.3.2.12 Interaction

The hypoglycemic actions of sulfonylurea may be potentiated by certain drugs including nonsteriodal anti-inflammatory drugs and other drugs that are highly protein bound salicylates, sulphonamides, and chloramphenicol. When such drugs are administered to a patient receiving Glipizide, the patient should be observed for hypoglycemia.

1.3.2.13 Side effect

Hypoglycemia, gastrointestinal disturbances, allergic reactions including erythema urticaria.

1.3.2.14 Precaution

Patients should be instructed to closely follow their physician's prescription as regards diet, dosage and schedule for taking the drug, and should be taught to recognize promptly the early symptoms of hypoglycemia, that generally are headache, irritability, sleep disorders, tremor and heavy sweating, so they can contact a doctor in good time.

1.3.2 Introduction to amoxicillin

1.3.2.1 Chemical name

2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

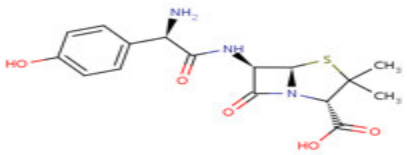
1.3.2.2 Chemical formula

$C_{16}H_{19}N_3O_5S$

1.3.2.3 Molecular weight

365.40

1.3.2.4 Chemical structure



1.3.2.5 Category

Anti-Bacterial Agents

1.3.2.6 Description

A broad-spectrum semisynthetic antibiotic similar to ampicillin except that its resistance to gastric acid permits higher serum levels with oral administration.

1.3.2.7 Solubility in water

3430 mg/L

1.3.2.8 Indication

For the treatment of infections of the ear, nose, and throat, the genitourinary tract, the skin and skin structure, and the lower respiratory tract due to susceptible (only b-lactamase-negative) strains of *Streptococcus* spp. (a- and b-hemolytic strains only), *S. pneumoniae*, *Staphylococcus* spp., *H. influenzae*, *E. coli*, *P. mirabilis*, or *E. faecalis*. Also for the treatment of acute, uncomplicated gonorrhea (ano-genital and urethral infections) due to *N. gonorrhoeae* (males and females).

1.3.2.9 Pharmacology

Amoxicillin is a moderate-spectrum antibiotic active against a wide range of Gram-positive, and a limited range of Gram-negative organisms. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other beta-lactam antibiotics. Amoxicillin is susceptible to degradation by β-lactamase-producing bacteria, and so may be given with clavulanic acid to increase its susceptability. The incidence of β-lactamase-producing resistant organisms, including *E. coli*, appears to be increasing. Amoxicillin is sometimes combined with clavulanic acid, a β-lactamase inhibitor, to increase the spectrum of action against Gram-negative organisms, and to overcome bacterial antibiotic resistance mediated through β-lactamase production.

1.3.2.10 Mechanism of action

Amoxicillin binds to penicillin-binding protein 1A (PBP-1A) located inside the bacterial cell well. Penicillins acylate the penicillin-sensitive transpeptidase C-terminal domain by opening the lactam ring. This inactivation of the enzyme prevents the formation of a cross-link of two linear peptidoglycan strands, inhibiting the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that amoxicllin interferes with an autolysin inhibitor.

1.3.2.11 Absorption

Rapidly absorbed after oral administration.

1.3.2.12 Toxicity

Serious toxicity is unlikely following large doses of amoxicillin. Acute ingestion of large doses of amoxicillin may cause nausea, vomiting, diarrhea and abdominal pain. Acute oliguric renal failure and hematuria may occur following large doses.

1.3.2.13 Side effect

All medicines may cause side effects, but many people have no, or minor side effects. Check with your doctor if any of these most common side effects persist or become bothersome when using amoxicillin:

Diarrhea; nausea; vomiting.

Seek medical attention right away if any of these severe side effects occur when using amoxicillin:

Severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); bloody stools; confusion; dark urine; fever, chills, or persistent sore throat; red, swollen, blistered, or peeling skin; seizures; severe diarrhea; stomach pain or cramps; unusual bruising or bleeding; vaginal discharge or irritation; yellowing of the skin or eyes.

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Chapter 2

Review of work done

2.1 Review of work done on stomach specific drug delivery systems

Sheth et al [1] developed sustained release hydrodynamically balanced capsules which, upon contact with gastric fluid acquired and maintained a bulk density of less than one and remained buoyant in the fluid and remained so until substantially all of the active ingredients are released. The percent Chordiazepoxide release from capsules in to simulated gastric fluid (pH 1.2) after 1,2,3.7 hrs was 39,61,…100 % respectively.

Du Quing et al [2] formulated multiple unit floating sustained release granules of aminophyllin and evaluated. They have reported that increasing quantity of cetyl alcohol and octadecanol could increases the granules floating capability *in- vitro*. Increased concentration of ethyl cellulose delayed the drug release rate.

Stochwell et al [3] formulated and evaluated a floating gel system. Buoyancy was achieved by carbon dioxide gas and its subsequent entrappement in to gel network. Sodium alginate, which undergoes gelation in acidic conditions and in the presence of calcium, was used. It was evaluated *in-vitro* as sustained release floating gel system.

Igani et al [4] formulated dosage form with specific density less than one in the form of double layer sustained release compressed hydrophilic matrix to achieve a reproducible floatation of a tablet. Carbon dioxide was trapped in to gelled hydrocolloids. The gastric retention of HBS dosage form was found to be significantly more than that of the non-floating dosage form.

Shimpi S et al [5] prepared Gelucire 43/01 for the design of multi-unit floating systems of a highly water-soluble drug diltiazem HCl. Diltiazem HCl-Gelucire 43/01 granules were prepared by melt granulation technique. The granules were evaluated for *in-vitro* and *in-vivo* floating ability, surface topography, and *in-vitro* drug release. Aging effect on storage was evaluated using scanning electron microscopy, hot stage polarizing microscopy (HSPM), differential scanning calorimetry (DSC), and *in-vitro* drug release. Granules were retained in stomach at least for 6 hrs. Approximately 65% to 80% drug was released over 6 hrs with initial fast release from the surface. Surface topography, HSPM, DSC study of the aged samples showed phase transformation of Gelucire. The phase

transformation also caused significant increase in drug release. In conclusion, hydrophobic lipid, Gelucire 43/01, can be considered as an effective carrier for design of a multi-unit floating drug delivery system of highly water-soluble drugs such as diltiazem HCl.

Zia et al [6] optimized Sotalol floating and bioadhesive extended release tablet formulation which posses a unique combination of flotation and bioadhesion for prolong residence in the stomach. A new factor factorial design was employed to optimize the tablet formulation contaoining 240 mg Sotalol HCl, the ratio of NaCMC to HPMC and the ratio of EC to Crosspovidone.The dependent variable was dissolution, bioadhesive capability, tablet disintegration and required compression fource for producing 6 kg hardness tablets.

Tossounian et al [7] investigated the *in-vivo* and *in-vitro* characterization of hydrodynamically balanced dosage forms. *In-vivo* visualization was done by using blood level time profiles for diazepam and chlordiazepoxide HBS dosage forms.

Ichiwaka et al [8] prepared floating granules containing 20% Dextromethorphan HCl, coated with sodium bicarbonate –HPC-Ethyl alcohol mixture and a vinyl acetate, shellac, HPMC phthalate, acetylmonoglyceride, calcium stearate and ethanol mixture. The granules floated in acetate buffer solution in 14-15 min after immersion.

Sangekar et al [9] investigated the effect of food and specific gravity on the gastric retention time of floating and non-floating tablet formulations using gama scintigraphy in humans. No correlation was found between gastric residence time and specific gravity of the dosage form.

Nakamichi K et al [10] prepared a floating dosage form composed of nicardipine hydrochloride (NH) and hydroxypropylmethylcellulose acetate succinate (enteric polymer) was prepared using a twin-screw extruder. By adjusting the position of the high-pressure screw elements in the immediate vicinity of die outlet, and by controlling the barrel temperature, he was able to prepare a puffed dosage form with very small and uniform pores. It was found that the porosity and pore diameter could be controlled by the varying amount of calcium phosphate

dihydrate. In the shaking test, the puffed dosage form was found to have excellent floating ability and mechanical strength in acid solution (JP First Fluid, pH 1.2). The dissolution profile of NH was controlled by the amount of wheat starch. In the dissolution test using JP Second Fluid (pH 6.8), rapid dissolution of NH and loss of buoyancy were observed

Fabregas et al [11] formulated long acting antacid compositions with floating properties. It contained a substance soluble in water at neutral pH. The formulation resulted in gastric regularization for epigastric pain and nausea.

Mazer et al [12] observed intragastric behavior and absorption kinetic of normal and floating modified release capsule of iseradipine under fasted and fed conditions. Presence or absence of food rather than buoyancy was the principal determinant of the gastric residence time of the capsule. The drug release and absorption were more by the intragastric interaction with the lipid phase of the meal.

Inouye,Y et al [13] prepared buoyant sustained release granules of Prednisolone using 'H' or 'L' grades of chitosan. The granules were immediately buoyant in both acidic and neutral fluids. Sustained drug absorption from these preparations was noticed in beagle dogs.

Kawashima et al [14] prepared hollow microspheres (microballoons) loaded with drug in their outer polymer shell by a novel emulsion solvent diffusion method. The ehanol: dichloromethane solution of drug (ibuprofen) and an acrylic polymer were poured that were thermally controlled at 40°C.The gas phase generated in the dispersed polymer droplet by the evaporation of the dichloromethane formed and the internal cavity in the microballoons of the polymer. The flowability and packability of the resultant microballons were characterized as an entire property and the drug release rate were drastically reduced depending on the polymer concentration at pH 6.8.

Franz et al [15] prepared sustained release bilayer buoyant floating dosage form containing Misoprostol, one layer is a drug release layer and other is buoyant or floating layer. The dosage form provided extended gastric retention so that the entire drug is released in the stomach over an extended period. The floating layer

included a polymer i.e.HPMC, which has a property of gelling and which on contact with gastric fluids, hydrates and forms a gelatinous barrier. This dosage form is buoyant on gastric fluid for up to approximately 13 hrs.

Desai et al [16] developed a noncompressed controlled release floating tablets of **Thyophylline** using agar and minaral oil. Tablets were made by dispersing a drug /minaral oil mixture in warm agar solution, the resultant mixture was poured in to tablet moulds which on cooling and air-drying formed a floatable CR tablets. The light mineral oil was essential for the floating property of the tablet since relatively high amount of drug (75%) and low amount of agar (2%) were used into formulation.

Baumgartener et al [17] prepared floating matrix tablets with high dose of freely soluble drugs. Tablets containing HPMC, drug and different additives are compressed. Tablet composition and mechanical strength have greater influence on the floating properties and drug release. With the incorporation of gas generating agent, besides optimum floating time of 30 sec and duration of floating > 8 hr, the drug release was also increased.

Whitehead et al [18] prepared floating alginate beads from alginate solution containing either dissolved or suspending Amoxycillin.The beads were produce by a drop-wise addition of the alginate into calcium chloride solution, followed by removal of the gel beads and freeze drying. Drug release study shows that the beads prepared with the drug in solution provided some sustained release characters and these were improved by the addition of amylase. The beads retained their buoyancy were amylase and amoxicilline were incorporated.

Nur Abubakr O et al [19] prepared captopril floating and/or bio adhesive tablets using two grades of HPMC (400 and 15000 cps.).He compared two conventional tablets; release from floating tablets was apparently prolonged. A 24 hrs controlled release dosage form for captopril was achieved. Tablet hardness was found determining factor with regard to buoyancy of the tablets.

Shoufeng et al [20] illustrated statistical experimental design and data analysis using response surface methodology. A central composite box-Wilson design for the controls release of calcium was used with three formulation variables like

HPMC loading, Citric acid loading and magnesium stearate loading. Sustained release floating delivery of calcium with increased bioavalability was achieved.

Farouk et al [21] developed a programmable controlled release drug delivery system. The device in the form of a non digestible oral capsule was design to utilized an automatically operated geometric obstruction that keeps the device floating in the stomach and prevent it from passing through the remainder of the GIT. Different viscosity grades of HPMC were used as a model eroding matrices. Zero-order release could be maintained for period ranging between 5 to 20 days before the geometric obstruction was triggered off.

Talwar et al [22] prepared gastroretentive oral drug delivery system structurally comprised of highly porous matrix having a drug, gas generating component, sugar, release controlling agent and optionally spheronising agents. The pharmaceutical formulation either in the form of pellets, beads, granules or capsules was retained in the stomach while selectively delivering the drug at gastric level or upper part of small intestine for extended period of time.

Joseph et al [23] prepared a floating type dosage form (FDF) of piroxicam in hollow polycarbonate (PC) microspheres capable of floating on simulated gastric and intestinal fluids was prepared by a solvent evaporation technique. Incorporation efficiencies of over 95% were achieved for the encapsulation. *In-vitro* release of piroxicam from PC microspheres into simulated gastric fluid at 37°C showed no significant burst effect. The amount released increased with time for about 8 h after which very little was found to be released up to 24 hrs. In intestinal fluid, the release was faster and continuous and at high drug pay loads, the cumulative release reached above 90% in about 8 hrs. *In-vivo* evaluation of different dosage forms of piroxicam such as free drug, drug-encapsulated microspheres and microspheres along with a loading dose of free drug in rabbits showed multiple peaking in the plasma concentration-time curve suggesting enterohepatic recirculation of the drug.

Patel et al [24] developed freeze dried chitosan polyethylene oxide hydrogel for the site-specific antibiotic release in the stomach. The freeze dried PEO matrix swollen extensively as compared to air-dried hydrogels. The freeze dried

chitosan PEO could be useful for localized delivery of antibiotic in the acidic environment of the gastric fluid.

Atybi et al [25] studied bicarbonate loaded bicarbonate ion exchange resin beads coated with semipermeable membrane. The beads exhibited prolong gastric recidence due to floating. In, addition to bicarbonate, a model drug theophyllin has also been loaded on to the resin. This system gives a controlled release of drug by coating and has potential application as a control release gastric retentive system.

Yang [26] developed an asymmetric three-layered tablet. The outer layer consisted of gas generating system. The other outer layer was similar but devoid of gas generating element. The function of these layers was to provide the necessary buoyancy and control the passage of the fluid in to the drug containing layer. Zero-order release of theophylline *in-vitro* was possible for 16 hrs with buoyancy maintained through out the period.

Timmerman et al [27] optimized floating and non floating hydrophilic matrix capsule *in-vitro* with regard to their buoyancy or non buoyancy capabilities and their diametric sine evaluation with time. The GRT prolongation is obtained with floating dosage form compared to non floating dosage forms.

Sheth et al [28] published a patent for hydrodynamically balance system. This unit consisting of capsule formulation consisting drug, hydrocilloid and other excipients. After emersion in other fluid, the capsule dissolves and hydrocolloid forms a hydrated boundary layer. That gives the formulation floating properties. The drug is subsequently released through this layer is by diffusion.

Wei et al [29] formulated a new kind of two-layer floating tablet for gastric retention (TFTGR) with cisapride as a model drug was developed. The *in-vitro* drug release was determined, and the resultant buoyancy and the time-buoyancy curve were plotted. Because of the sodium bicarbonate added to the floating layer, when immersed in simulated gastric fluid the tablet expands and raises to the surface, where the drug is gradually released. The drug release of this kind of two-layer dosage was controlled by the amount of HPMC in the drug-loading layer. Generally the more HPMC, the slower the drug releases. Because

cisapride has graeter solubility in SGF than SIF, in vitro drug dissolution in SGF is faster than in SIF.

Soppimath et al [30] prepared hollow microspheres of cellulose acetate loaded with four cardiovascular drugs (Nifedipine, Nicardipine HCl, Varapamil HCl and Dypiridamole) were prepared by a novel solvent diffusion-evaporation method. The O/W emulsion prepared in an aqueous solution of 0.05% poly (vinyl alcohol) medium with ethyl acetate, a water-soluble and less toxic solvent, was used as a dispersing solvent. The yield of the microspheres was up to 80%. The microspheres had smooth surfaces, with free floating and good packing properties. Scanning Electron Microscopy (SEM) confirmed their hollow structures, with sizes in the range of 350-489 mm. The microspheres were tended to float over the gastric media of more than 12 hrs.

Jayvadan et al [31] formulated and systematically evaluate *in-vitro* and *in-vivo* performances of mucoadhesive microspheres of glipizide. Glipizide microspheres containing chitosan were prepared by simple emulsification phase separation technique using glutaraldehyde as a cross-linking agent. Results of preliminary trials indicate that volume of cross-linking agent, time for cross-linking, polymer-to-drug ratio, and speed of rotation affected characteristics of microspheres. Microspheres were discrete, spherical, and free flowing. The microspheres exhibited good mucoadhesive property in the *in-vitro* wash-off test and also showed a high percentage drug entrapment efficiency. A 3^2 full factorial design was employed to study the effect of independent variables, polymer-to-drug ratio (X_1), and stirring speed (X_2) on dependent variables percentage mucoadhesion, t_{80}, drug entrapment efficiency, and swelling index. The best batch exhibited a high drug entrapment efficiency of 75% and a swelling index of 1.42; percentage mucoadhesion after 1 hr was 78%. The drug release was also sustained for more than 12 hrs. The polymer-to-drug ratio had a more significant effect on the dependent variables. *In-vivo* testing of the mucoadhesive microspheres to albino Wistar rats demonstrated significant hypoglycemic effect of glipizide.

Jayvadan et al [32] formulated and systematically evaluate *in-vitro* and *in-vivo* performances of mucoadhesive amoxicillin microspheres for the potential use of

treating gastric and duodenal ulcers, which were associated with *Helicobacter pylori*. Amoxicillin mucoadhesive microspheres containing chitosan as mucoadhesive polymer were prepared by simple emulsification phase separation technique using glutaraldehyde as a cross-linking agent. Results of preliminary trials indicate that volume of cross-linking agent, time for cross-linking, polymer-to-drug ratio, and speed of rotation affected characteristics of microspheres. Microspheres were discrete, spherical, free flowing and also showed high percentage drug entrapment efficiency. *In-vitro* mucoadhesive test showed that amoxicillin mucoadhesive microspheres adhered more strongly to gastric mucous layer and could retain in gastrointestinal tract for an extended period of time. A 3^2 full factorial design was employed to study the effect of independent variables, polymer-to-drug ratio (X_1), and stirring speed (X_2) on dependent variables i.e. percentage mucoadhesion, t80, drug entrapment efficiency, particle size and swelling index. The best batch exhibited a high drug entrapment efficiency of 70 % and a swelling index of 1.39; percentage mucoadhesion after one h was 79 %. The drug release was also sustained for more than 12 h. The polymer-to-drug ratio had a more significant effect on the dependent variables. The morphological characteristics of the mucoadhesive microspheres were studied using scanning electron microscopy. *In-vitro* release test showed that amoxicillin released slightly faster in pH 1.0 hydrochloric acid than in pH 7.8 phosphate buffer. *In-vivo H. pylori* clearance tests were also carried out by administering amoxicillin mucoadhesive microspheres and powder, to *H. pylori* infectious Wistar rats under fed conditions at single dose or multiple dose(s) in oral administration. The results showed that amoxicillin mucoadhesive microspheres had a better clearance effect than amoxicillin powder. In conclusion, the prolonged gastrointestinal residence time and enhanced amoxicillin stability resulting from the mucoadhesive microspheres of amoxicillin might make contribution complete eradication of *H. pylori*.

Myung-Kwan Chun et al [33] prepared mucoadhesive microspheres to increase gastric residence time using an interpolymer complexation of poly(acrylic acid) (PAA) with poly(vinyl pyrrolidone) (PVP) and a solvent diffusion method. The

complexation between poly(acrylic acid) and poly(vinyl pyrrolidone) as a result of hydrogen bonding was confirmed by the shift in the carbonyl absorption bands of poly(acrylic acid) using FT-IR. A mixture of ethanol/water was used as the internal phase, corn oil was used as the external phase of emulsion, and span 80 was used as the surfactant. Spherical microspheres were prepared and the inside of the microspheres was completely filled. The optimum solvent ratio of the internal phase (ethanol/water) was 8/2 and 7/3, and the particle size increased as the content of water was increased. The mean particle size increased with the increase in polymer concentration. The adhesive force of microspheres was equivalent to that of Carbopol. The release rate of acetaminophen from the complex microspheres was slower than the PVP microspheres at pH 2.0 and 6.8.

B. Y. Choi et al [34] prepared floating beads from a sodium alginate solution containing $CaCO_3$ or $NaHCO_3$ as gas-forming agents. The solution was dropped to 1% $CaCl_2$ solution containing 10% acetic acid for CO_2 gas and gel formation. The effects of gas-forming agents on bead size and floating properties were investigated. As gas-forming agents increased, the size and floating properties increased. Bead porosity and volume average pore size, as well as the surface and cross-sectional morphology of the beads were examined with Mercury porosimetry and Scanning Electron Microscopy. $NaHCO_3$ significantly increased porosity and pore diameter than $CaCO_3$. Incorporation of $CaCO_3$ into alginate solution resulted in smoother beads than those produced with $NaHCO_3$. Gel strength analysis indicated that bead strength decreased with increasing gas-forming agent from 9 to 4 N. Beads incorporating $CaCO_3$ exhibited significantly increased gel strength over control and $NaHCO_3$-containing samples. Release characteristics of riboflavin as a model drug were studied *in-vitro*. Release rate of riboflavin increased proportionally with addition of $NaHCO_3$. However, increasing weight ratios of $CaCO_3$ did not appreciably accelerate drug release. The results of these studies indicate that $CaCO_3$ is superior to $NaHCO_3$ as a gas forming agent in alginate bead preparations. The enhanced buoyancy and sustained release properties of $CaCO_3$-containing beads make them an excellent candidate for floating drug dosage systems (FDDS).

Colombo et al [35] prepared swellable matrices by compression of a powdered mixture of a hydrophilic polymer and a drug. Their success is linked to the established tabletting technology of manufacturing. Swellable matrix DDS must be differentiated from true swelling-controlled delivery systems. This review focuses on hydrophilic swellable matrix tablets as controlled DDS. Gel-layer behaviour, front movement and release are described to show the dependence of the release kinetics on the swelling behaviour of the system. *In-vivo* behaviour of matrix systems is also considered.

Alvaro et al [36] developed and characterized the delivery properties of swellable drug-polyelectrolyte matrices (SDPM). Solid complexes $(C–D)_X$ of carbomer (C) neutralized with different proportions of model basic drugs (D), in which D is atenolol, lidocaine, and metoclopramide, and X = 25, 50, 75 and 100 mol of D per 100 equivalents of carboxylic groups of C, were prepared and characterized by DSC-TG, IR, and X-ray diffraction studies. Mechanistic studies with hydrophilic and hydrophobic basic drugs were conducted to explore the drug release patterns of SDPM. Besides, release and up-take studies were carried out in water and NaCl solution to examine the influence of ionic effects. The authors concluded that drugs can be loaded in a high proportion on to the polymer and therefore the resulting material could be diluted with other polymers to modulate delivery properties of SDPM. Matrices of atenolol and lidocaine exhibited robust delivery properties with regard to change in proportion of loading D.

J. A. Raval and J. K. Patel [37] investigated the effects of formulation and processing parameters on a floating matrix controlled drug delivery system consisting of a poly (styrene-divinyl benzene) copolymer low density powder, a matrix-forming polymer(s), drug, and diluents (optional). The tablets were prepared by the direct compression technique, using hydrophilic matrix polymers HPMC K4M, HPMC K15M, HPMC K100M, sodium alginate, psyllum, sesbania gum, guar gum, and gum acacia, with or without low density copolymer. Tablets were physically characterized and evaluated for *in-vitro* release characteristics for 8 h in 0.1 mol/l HCl at 37°C. The effect of the addition of low density copolymer and the drug release pattern were also studied. The release rate was

modified by varying the type of matrix-forming polymer, the tablet geometry (radius), and the addition of water-soluble or water-insoluble diluents. At the same time, different concentrations of low-density copolymer were taken to examine any differences in the floating lag-time of the formulation. The *in-vitro* release mechanism was evaluated by kinetic modeling. The similarity factor, floating lag-time, and t_{50} and t_{90} were used as parameters for selection of the best batch. The tablet eroded/swelled upon contact with the release medium, and the relative importance of drug diffusion, polymer swelling and tablet erosion on the resulting release patterns varied significantly with the type of matrix forming polymer. The highly porous copolymer provided a low density and, thus, excellent *in-vitro* floating behavior of the tablets at a concentration of 15% (w/w). It was established that floating behavior of the low-density drug delivery systems could be successfully combined with accurate control and prolongation of the drug release patterns.

Anand Kumar [38] studied preparation and evaluation of floating microspheres with cimetidine as model drug for prolongation of gastric residence time. The microspheres were prepared by the solvent evaporation method using polymers hydroxypropylmethyl cellulose and ethyl cellulose. The shape and surface morphology of prepared microspheres were characterized by optical and scanning electron microscopy, respectively. *In-vitro* drug release studies were performed and drug release kinetics was evaluated using the linear regression method. Effects of the stirring rate during preparation, polymer concentration, solvent composition and dissolution medium on the size of microspheres and drug release were also observed. The prepared microspheres exhibited prolonged drug release (8 hrs) and remained buoyant for > 10 hrs. The mean particle size increased and the drug release rate decreased at higher polymer concentration. No significant effect of the stirring rate during preparation on drug release was observed. *In-vitro* studies demonstrated diffusion-controlled drug release from the microspheres.

Whitehead et al [39] prepared floating dosage forms of Amoxicillin based on alginate to exhibit prolong gastric residence time. A freeze-dried calcium alginate

multiple unit floating dosage form that demonstrated favorable *in-vitro* floating characteristics was developed.

Ganguly S et al [40]developed a novel chitosan-glyceryl monooleate (GMO) in situ gel system for sustained drug delivery & targeting was developed. The delivery system consisted of 3 % (w/v) chitosan & 3 % (w/v) glyceryl monooleate in 0.33M citric acid. In situ gel was formed at a biological pH and *in-vitro* release studies were conducted in Sorensen's phosphate buffer (pH 7.4). Characterization of the gel included the effect of cross-linker, determination of diffusion coefficient and water uptake by thermogravimetric analysis (TGA). Incorporation of a cross-linker (glutaraldehyde) retarded the rate and extent of drug release. Drug release from the gel followed a matrix diffusion controlled mechanism.

Kubo W et al [41] developed oral sustained delivery of paracetamol from in situ-gelling gellan and sodium alginate formulations. The potential for the oral sustained delivery of paracetamol of two formulations with in situ gelling properties was evaluated. *In-vitro* studies demonstrated diffusion-controlled release of paracetamol from the gels over a period of six hrs. The bioavailability of paracetamol from the gels formed in situ in the stomachs of rabbits following oral administration of the liquid formulations was similar to that of a commercially available suspension containing an identical dose of paracetamol.

Dairaku M et al [42] developed insitu gel using pectin.Gels formed in situ following oral administration of dilute aqueous solutions of pectin (1.0 and 1.5 %, w/v) containing calcium ions in complexed form to rats was evaluated as vehicles for the sustained release of the expectorant drug ambroxol hydrochloride. A bioavailability of approximately 64 % of that of a commercially available formulation was achieved from gels containing an identical dose of ambroxol formed in situ in the stomachs of rats, with appreciably lower peak plasma levels, diffusion controlled and sustained release of drug over a period of at least six hrs. The influence of added sorbitol (17 %, w/v) on the rheological and drug release properties of the formulations has been examined.

Fujiwara et al [43] studied in situ gel from *in-vitro* and *in-vivo* release of paracetamol and ambroxol the influence of different polyhydric alcohols like xylitol, mannitol and sorbitol in different concentration of in situ gelling pectin formulations was examined. 2 % (w/v) pectin gels containing 10 % (w/v) sorbitol showed a sustained release of paracetamol and bioavailabilities of approximately 90 % was seen. Sustained release of ambroxol with pectin concentrations of 1.5 and 1 % (w/v) and a sorbitol concentration of 10 % (w/v) was seen.

Peterson et al [44] developed insitu gelling alginates formulations as an alternative to incorporation of various excipients N^4- alkoxy carbonyl cytosine derivatives possessing various physicochemical properties and cytosine regeneration rates was being examined to modify release rate and kinetics from in situ gelling alginate formulations. Release rate constants and square root of solubility showed a linear relationship for suspension. A zero order release of parent cytosine was observed from in situ gelling suspension and diffusion coefficients calculated was observed to be similar for suspension and solution.[10]

Itoh K et al [45]compared the gelation and drug release characteristics of formulations of pectin with high (31%) and low (9%) degrees of methoxylation over a wide pH range (pH 1.2-5.0). Gelation of formulations of pectin with a degree of esterification of 9% (DE9) was observed over the pH range 2.5-5.0 in the presence of 1.6mM Ca (++), but was incomplete in formulations of pectin with a degree of esterification of 31% (DE31). A sustained release of ambroxol was observed following oral administration of pectin DE9 formulations to gastric-acidity controlled rabbits at pH 5.5-5.7 and visual observation of the stomach contents of these rabbits confirmed in situ gelation of these formulations.

Kunihiko Itoh et al [46] studied the influence of a variation of gastric pH & addition of a taste masking agent on gelation of pectin solutions and on *in-vitro*/*in-vivo* release of acetaminophen from gels. Increase of pH above 2.5 and addition of 10% (w/v) D-sorbitol significantly affected ability of 1.5% (w/v) pectin solutions to form coherent gels *in-vitro*. Gelation of sorbitol-free formulations was observed at pH 1.2 and *in-vitro* release of acetaminophen from gel followed diffusion-controlled kinetics; *in-vitro* gelation was incomplete at pH 3.0 resulting in

poor sustained release characteristics. While D-sorbitol inhibited *in-vitro* gelation & noted poor sustained release properties.

Oi H et al [47] developed a thermosensitive in situ gelling and mucoadhesive ophthalmic drug delivery system containing puerarin based on poloxamer analogs (21% (w/v) poloxamer 407/5% (w/v) poloxamer 188) and carbopol (0.1% (w/v) or 0.2% (w/v) carbopol 1342P NF). The combined solutions would convert to firm gels under physiological condition and attach to the ocular mucosal surface for a relative long time. *In-vitro* release studies demonstrated diffusion-controlled release of puerarin from the combined solutions over a period of 8 hrs. *In-vivo* evaluation indicated the combined solutions had better ability to retain drug than poloxamer analogs or carbopol alone.

Kashyap et al [48] developed biodegradable glucose-sensitive in situ gelling system based on chitosan for pulsatile delivery of insulin was developed. The sols/gels were thoroughly characterized for swelling properties, rheology, texture analysis and water content. Insulin load onto the gels was optimized and was found to affect the rheological behavior of these gels, the final preparation used for *in-vitro* contained 1IU/200mul of the sol. These gels released the entrapped insulin in a pulsatile manner in response to the glucose concentration *in-vitro*. The formulations also evaluated for their *in-vivo* efficacy in streptozotocin-induced diabetic rats at a dose of 3IU/kg.

Escobar-Chavez JJ et al [49] developed insitu gel by using Pluronic F-127 PF-127The use of high viscosity hydromiscible vehicles such as hydrophilic gels, is one of various approaches for controlled drug delivery, and represents an important area of pharmaceutical research and development. Of these systems, PF-127 provides the pharmacist with an excellent drug delivery system for a number of routes of administration and is compatible with many different substances. Gels containing penetration enhancers have proven to be especially popular for administering anti-inflammatory medications since they are relatively easy to prepare & very efficacious.

Voorspoels et al [50] studied treatment of bacterial vaginosis with a single application of 100 mg metronidazole in a bioadhesive vaginal tablet was found to

be a valid alternative. Further research in relation to tablet shaping and optimal dose finding might increase the cure rate.

Smart [51] developed *in-vitro* method for the assessment of the adhesive force [i.e. the force required to break an adhesive bond] between a disc of test material and a model mucous membrane. This system showed reasonable reproducibility and produced data in agreement with previous studies. Some factors influencing the adhesive force were assessed and only increasing the rate of application of the tensile force was found to have a significant effect. Some putative mucosa-adhesive formulations were evaluated and some buccal tablets found to have minimal adhesive properties. It was concluded that only a small force is required to retain a dosage form within the buccal cavity. The stability of the adhesive bond was assessed for the two most adhesive materials {poly [acrylic acids] carbopol-934P and EX55} by subjecting to a continuous stress for 8 hrs prior to measuring the adhesive force. The carbopol EX55 [polycarbophil] formed the most stable adhesive bond which remained intact for 8 hrs.

Han-Gon et al [52] studied the release and bioavailability of omeprazole delivered by buccal adhesive tablets composed of sodium alginate, hydroxypropyl methylcellulose [HPMC], magnesium oxide and cross-carmellose sodium. Cross-carmellose sodium enhanced the release of omeprazole from the tablets. The tablet was composed of omeprazole, sodium alginate, HPMC, magnesium-oxide, cross-carmellose sodium [20:24:6:50:10 mg]. It may be attached to the human cheek without collapse and it enhanced the stability of omeprazole in human saliva for at least 4 hrs giving a fast release of omeprazole. Results demonstrate that the omeprazole buccal adhesive tablet would be useful to deliver omeprazole which degrades very rapidly in acidic aqueous medium and undergoes hepatic first-pass metabolism after oral administration.

Codd et al [53] developed two novel antifungal bioadhesive lozenges. Both were two-layered with an upper modified-release drug containing layer and a lower bioadhesive layer composed of drum-dried waxy maize starch and carbopol-980P to facilitate application to the oral mucosa. The first type of lozenge contained miconazole nitrate as a spray-dried form containing acacia and

cremophor-RH40 to increase the dissolution of the poorly soluble azole, plus flavorings. The second type also contained chlorhexidine acetate in the drug layer, as both drugs had been reported to act synergistically. In comparison to a proprietary oral gel formulation, the new bioadhesive lozenges produced much more uniform and effective salivary levels of miconazole nitrate over a prolonged period.

Prudat-Christiaens et al [54] studied the bioadhesive systems are new delivery systems used to reduce bioavailability problems resulting from a too short stay of the pharmaceutical form at the activity or absorption site. Aminophylline bioadhesive tablets were made by wet granulation with different polymers: Carbomers-934P, 974P, EX55, sodium carmellose, hypromellose and hydroxypropyl cellulose [HPC]. Wet granulation is a limiting factor for bioadhesion. The combination of polyacrylic acids with hypromellose or sodium carmellose increases bioadhesion and decreases drug release. Carbomer-974P alone had a lower adhesion than carbomer-934P. Combinations of carbomer-934P/hypromellose-100 gave the best adhesion properties and slow release dissolution.

Hosny et al [55] prepared polycarbophil containing diclofenac sodium tablets using two different size of granules. The granules were obtained by evaporation under reduced pressure of polycarbophil particles loaded with alcoholic solution of the drug. The in-vitro release of these bioadhesive containing tablets was evaluated together with that of Ciba-Geigy commercially available enteric coated tablets 'Voltaren' in simulated gastric fluid for 2 hrs followed by another 2 hrs in simulated intestinal fluid.

Bouckaert et al [56] studied the use of a bioadhesive buccal tablet containing miconazole nitrate has been shown to be effective in the treatment of oral candidosis and the influence of the application site on the buccal levels of miconazole nitrate. The t_{max} the adhesion time and $T^{>MIC}$ were significantly higher [$P < 0.05$] when the gingiva was chosen as the application site in comparison with the cheek. The C_{max}, t_{max} and AUC were not significantly different. The

gingiva is the application site of choice in irradiated patients even with a decreased salivary flow.

Mumtaz et al [57] prepared bioadhesive buccal tablets from different ratios of poly[acrylic acid-2,5-dimethyl-1,5-hexadiene] [PADH] and HPMC with and without triamcinolone acetonide [TAA] has been investigated in the buccal cavities of healthy human volunteers. The results indicate that tablets with a higher ratio of PADH swell faster, causing the disintegration of the tablets and consequently give rise to more rapid release of drug. The inclusion of higher percentages of HPMC provides more prolonged release of drug through its properties of gelling and slow dissolution. However, adhesion of the tablet was reduced in the excessive flow of saliva and there was also a tendency for the tablet to be dislodged from the mucosa. The tablet with a PADH/HPMC ratio of 50:50 seems to provide a suitable compromise for good bioadhesion and prolonged release of drug.

Woolfson et al [58] prepared novel bioadhesive cervical patch drug delivery containing 5-fluorouracil for the treatment of cervical intraepithelial neoplasia [CIN]. The patch was of bilaminar design; with a drug-loaded bioadhesive film cast from a gel containing 2% [w/w] carbopol-981P plasticized with 1% [w/w] glycerin. The casting solvent was ethanol/water 30:70, chosen to give a non-fissuring film with an even particle size distribution. Bioadhesive strength was independent of drug loading in the bioadhesive matrix over the range investigated but was influenced by both the plasticizer concentration in the casting gel and the thickness of the final film. Release of 5-fluorouracil from the bioadhesive layer into an aqueous sink was rapid but was controlled down to an undetectable level through the backing layer.

Miyazaki et al [59] prepared oral mucosal bioadhesive tablets of diltiazem by directly compressing the drug with a mixture of chitosan and sodium alginate. In-vitro adhesion studies indicated adhesion properties comparable to those of a commercial formulation. In-vitro release of diltiazem was rapid and could be modified by changing the mixing ratio of chitosan and sodium alginate; increasing the chitosan content in the tablets and/or the viscosity grade of the alginate

resulted in a decrease in the *in-vitro* release rate. The bioavailability of diltiazem was 69.6% from tablets with a 1:4 chitosan/alginate weight ratio when administered sublingually to rabbits compared with 30.4% by oral administration.

Needleman et al [60] examined the factors important to prolonged adhesion [adhesion time] in organ culture under standardized conditions. A wide variety of bioadhesive were tested in the model and the effect of mucin was also examined. Whilst many gels adhered for 1–5 hrs, others [chitosan and eudispert] showed no retention loss over 4 days. Histologically, chitosan also showed excellent tissue wetting properties. For most materials, however, mucin significantly reduced adhesion times [$P < 0.05$]. In conclusion, the absence of mucin, the control of gel hydration and swelling, and wetting characteristics were identified as key factors for prolonged adhesion.

Wen-Gang et al [61] prepared direct compressed disc systems containing either 10, 15 or 20 mg of propranolol hydrochloride [PL] with a mixture of hydroxypropylcellulose and poly[acrylic acid]. The release data were fitted to the simple power equation and it was found that the release characteristics of PL from these systems were not affected by the amounts of the drug loaded and followed behavior conforming to a non-Fickian mechanism of release. The adhesive bond strength of the systems to the porcine buccal mucosa was evaluated by the tensile strength test and the result showed no significant difference in adhesive bond strength to the porcine buccal mucosa among the three PL-containing discs and drug free discs.

Naffie et al [62] investigated different types of mucoadhesive polymers, intended for buccal tablet formulation, for their comparative mucoadhesive force, swelling behavior, residence time and surface pH. The selected polymers were carbopols [CP-934 and CP-940], polycarbophil [PC], sodiumcarboxymethylcellulose [NaCMC] and pectin representing the anionic type, while chitosan as cationic polymer and hydroxypropylmethyl cellulose [HPMC] as a non-ionic polymer. Results revealed that polyacrylic acid derivatives [PAA] showed the highest bioadhesion force, prolonged residence time and high surface acidity. NaCMC and chitosan ensured promising bioadhesive characteristics, while HPMC and

pectin exhibited weaker bioadhesion. Different polymer combinations as well as formulations were evaluated to improve the mucoadhesive performance of the tablets. Bioadhesive tablet formulations containing either 5% CP934, 65% HPMC and 30% spray-dried lactose or 2% PC, 68% HPMC and 30% mannitol showed optimum mucoadhesion and suitable residence time. NaCMC, when formulated individually, exhibited promising bioadhesion, acceptable swelling, convenient residence time and surface pH. In-vivo trials of these formulations proved non-irritative and prolonged residence of the mucoadhesive tablets on human buccal mucosa for 8 to 13 hrs.

Naffie et al [63] studied from the previous work [Part-I[62]], mucoadhesive formulae containing 5% CP/65% HPMC/30% lactose and 2% PC/68% HPMC/30% mannitol as well as formulae based on sodium carboxymethylcellulose [NaCMC] were selected. Medicated tablets were prepared using diltiazem hydrochloride and metclopramide hydrochloride in two different doses [30 and 60 mg]. The effect of drug and dose on the mucoadhesive properties and in-vitro drug release was evaluated. All formulae produced extended drug release [over 8 to 12 hrs]. Doubling the dose significantly reduced the bioadhesion strength [$p < 0.05$] with a slight improvement in drug release rate. The formulation of bilayer tablets containing drug-free layer and medicated layer enhanced the drug release without affecting the bioadhesive performance. The bilayer tablet formulated with 2% PC/68% HPMC/30% mannitol was selected for studying the in-vivo metoclopramide release in four healthy volunteers. The tablet ensured controlled drug release for 12 hrs, in addition, good correlation [$r = 0.9398$] was observed between in-vitro and in-vivo data. Storage at 40℃ and 75% relative humidity for 6 months didn't influence the mucoadhesive performance, however, an enhanced released rate was observed.

Shan-chul et al [64] developed the new local anesthetic formulations with a suitable bioadhesive property, hydroxypropylmethylcellulose based gel was formulated. The effects of permeation enhancers on the permeation rate of drug through skin were studied using various enhancers, such as the glycols, the nonionic surfactants, and the bile salts. Among the enhancers used,

polyoxyethylene 2-oleyl ether showed the highest enhancing effects on drug permeation through skin. The analgesic activity was examined using a tail-flick analgesimeter.

Chowdary et al [65] prepared mucoadhesive tablets with nifedipine alone and its inclusion complexes with β-cyclodextrin and the mucoadhesive polymers sodium carboxy methyl cellulose and carbopol were investigated with a view to the design of oral controlled release tablets of nifedipine.

Raghuraman et al [66] prepared propranolol hydrochloride buccal films using three different polymers in various proportions and combinations. The physiochemical parameters like weight variation, thickness, folding endurance, drug content, percentage moisture absorption and percentage moisture loss were evaluated.

Perioli et al [67] prepared mucoadhesive tablets using different mixture of cellulose and polyacrylic derivatives in order to obtain new formulations containing metronidazole for periodontal disease treatment.

Patil et al [68] prepared mucoadhesive buccal patches of diclofenac sodium. Patches were fabricated by casting technique with different polymer combinations.

Khurana et al [69] prepared mucoadhesive films of miconazole nitrate for the treatment of oral candidosis. Films were fabricated by casting technique with different polymer combinations and were evaluated for their in-vitro bioadhesive performance and release characteristics.

Nafee et al [70] prepared mucoadhesive patches containing 10 mg miconazole nitrate with ionic polymers, sodiumcarboxymethylcellulose and chitosan or non-ionic polymers polyvinyl alcohol, hydroxyethylcellulose and hydroxypropylmethyl cellulose.

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Chapter 3

Experimental setup

3.1 Materials used in the present investigation

Amoxicillin (powder)	: Zydus Cadila, Ahmedabad.
Chitosan (degree of deacetylation of 85%; intrinsic viscosity, 1390 mL/g in 0.30 M acetic acid and viscometric molecular weight, 4.08×10^5 Da)	: Central Institute of Fisheries Technology, Cochin.
Skirrow's medium	: Himedia Ltd. Mumbai, India.
Petroleum ether 80:20	: S. D. Fine Chemicals Ltd., Mumbai.
Liquid paraffin	: Loba Chemie Pvt. Ltd., Mumbai.
Citric acid	: Merck, Germany.
Hydrochloride acid	: Qualigen Chemicals, India.
Acetic acid	: S. D. Fine Chemicals Ltd., Mumbai.
Glipizide	: USV Ltd., Daman, India.
Sodium bicarbonate	: Merck, Germany.
Famotidine	: Torrent Pharmaceuticals Pvt. Ltd Chhatral, India.
Sodium hydroxide	: Loba Chemie Pvt. Ltd., Mumbai, India.
Alginate	: Sigma Chemicals Ltd, New Delhi, India.
Sodium citrate	: Sigma Chemicals Ltd, New Delhi, India.
Calcium chloride	: Muby Chemicals, Mumbai, India.
Carbopol-934P (CP) (CP, molecular weight [MW] 3×10^6 Da)	: Noveon® Mumbai, India.
Hydroxypropyl methyl cellulose-K4M (HPMC)	: Zydus Research Center, Ahmedabad, India.
Polymethacrylic acid (PMA)	: Zydus Cadila. Ahmedabad, India.

3.2 Instruments used in the present investigation

UV Spectrophotometer	: Shimadzu UV-1601 UV/Vis double beam Spectrophotometer (Kyoto, Japan).
Stirrer	: Propeller Stirrer (Remi, Mumbai, India).
Dissolution test apparatus	: Dissolution test apparatus-TDT-06T (Electrolab, Mumbai, India).
Microscope	: Optical microscope (Labomed CX RIII, Ambala, India).
Scanning electron microscope	: Scanning electron microscope (JSM 5610 LV SEM, JEOL, Datum Ltd, Tokyo, Japan).
pH meter	: Systronic, 335 pH meter.
Sigma plot version 10.0	: Sigma plot software, Jangel Scientific Software, San Rafael, CA.
Tablet compression machine	: Multipunch tablet compression machine (Cadmach Machinery Co. Pvt. Ltd., Ahmedabad, India).
Tablet-disintegrating tester	: USP tablet-disintegrating tester.
Balance	: Modified analytical balance.
Thermogravimetric Analyzer	: TGA-50, Shimadzu, Kyoto, Japan.
Brookfield viscometer	: Model no RVT 81990

3.3 Animals

Six-week-old mixed sexes specific pathogen free Wistar rats (Body weight 200-250 gm) were gifted from Torrent Research Center (Ahmedabad, India) and maintained under standard laboratory conditions (room temperature, 23°±2℃; relative humidity, 55±5%; 12/12 hrs light/dark cycle) with free access to a commercial rodent diet and tap water.

Chapter 4

Formulation and evaluation of stomach specific amoxicillin-loaded carbopol-934P mucoadhesive microspheres for anti-Helicobacter pylori therapy

4.1 Aim of present investigation

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems [1-3]. They have varied applications and are prepared using assorted polymers [4]. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes [5-8]. This can be achieved by coupling mucoadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site [9-12]. Carbopol-934P (acrylic acid homopolymer) is anionic polymer that has been used in mucoadhesive systems by a several researchers [13-17]. Carbopol-934P was selected as a polymer in the preparation of mucoadhesive microspheres because of its good mucoadhesive and biodegradable properties and ethyl cellulose as carrier polymer for microspheres.

In a relatively short time span, Helicobacter pylori (*H. pylori*) have become recognized as a major gastric pathogen with worldwide distribution. *H. pylori* are a spiral-shaped bacterium found in the stomach, which (along with acid secretion) damages stomach and duodenal tissue, causing inflammation and peptic ulcers. *H. pylori*, a prevalent human-specific pathogen, is a causative agent in chronic active gastritis[18], gastric and duodenal ulcers[19], and gastric adenocarcinoma[20], one of the most common forms of cancer in humans. Epidemiological, laboratory, and interventional human studies strongly suggest that *H. pylori* play a pathogenic role in the development of adenocarcinoma of the distal stomach [21]. The mechanisms by which *H. pylori* may cause gastroduodenal

disease and contribute to gastric carcinogenesis are still hypothetical. However, the production of specific virulence factors by the bacterium, the inflammatory response of the host, and the association with environmental contributors may all be responsible [22].

Treatment regimens for *H. pylori* infection have been evolving since the early 1990s, when monotherapy was first recommended. Antimicrobial therapy for this infection is a complex issue, and the following drugs are currently used in combination regimens: proton-pump inhibitors and/or bismuth, metronidazole, clarithromycin, and amoxicillin [23]. Tetracycline is used in the rescue therapy [24]. Although optimal first-line treatment is associated with high cure rates, the rising prevalence of resistance to the antibiotic component of current eradication regimens increasingly threatens to compromise the efficacy of these regimens. Strains resistant to metronidazole [25] and clarithromycin [26] have been well documented, while resistance to amoxicillin [27] and tetracycline was mainly reported in Asia [28]. Therapeutic regimens directed against *H. pylori* infection will continue to evolve. What is required is a simpler and more efficacious strategy for the treatment of *H. pylori* infection. *H. pylori* is susceptible to many antibiotics in-vitro but has proved difficult to eradicate (to root out) in-vivo.

Amoxicillin (α-amino-hydroxybenzylpenicillin) is a semi-synthetic, orally absorbed, board-spectrum antibiotic. It is now widely used in the standard eradication treatment of gastric and duodenal ulcers, which are associated with *H. pylori* infection combined with a second antibiotics and an acid-suppressing agent [29-31]. These tripe therapies are proved to be effective in clinical application. However, some other reports and clinical trials indicate that the therapies cannot bring out compete eradication of *H. pylori* and suggest that the therapeutic effect needs more investigation [32-33]. One reason for the incomplete eradication of *H. pylori* is probably due to short residence time of dosage form in the stomach so that effective antimicrobial concentration cannot be achieved in gastric mucous layer or epithelial cell surfaces where *H. pylori* exist [34-35]. The other may be the degradation of amoxicillin in gastric acid [36-37]. Therefore, some researchers had prepared and reported new amoxicillin formulations such as float tablets,

mucoadhesive tablets, pH-sensitive excipients composition microspheres, etc., which were able to reside in the gastrointestinal tract for an extended period of time for a more effective *H. pylori* eradication [38-39]. In our previous investigation on *H. pylori* clearance effect showed that there was tendency for a more effective *H. pylori* activity of mucoadhesive amoxicillin microspheres prepared using chitosan as mucoadhesive microspheres [40].

In context of the above principles, a strong need was felt to develop a dosage form that delivered amoxicillin in the stomach and would increase the efficiency of the drug, providing sustains action. Thus, an attempt was made in the present investigation to use carbopol-934P as mucoadhesive polymer and ethyl cellulose as carrier polymer and prepare mucoadhesive amoxicillin microspheres. The microspheres were characterized by in-vitro and iv-vivo tests and factorial design was used to optimize the variables.

It is our intention to develop mucoadhesive microspheres that:

1. Adhere to the stomach mucosa for prolonged period of time.
2. Provide an increased gastric residence time resulting in prolonged drug delivery in gastrointestinal tract.
3. Delivers a drug at a controlled rate over a period of time, which is same as or less than the residency period of the delivery system in the gastrointestinal tract.
4. Shows better in-vivo performances than conventional dosage forms.

In context to above intention, following criteria were aimed to achieve:

1. Microspheres should be discrete, spherical and free flowing.
2. Mucoadhesion time of microspheres should be more than 8 hrs.
3. Drug entrapment should be more than 50 %.
4. More than 90 % of drug should be released within 10 hrs.

4.2 Experimental

4.2.1 Preparation of standard calibration curve of amoxicillin

Amoxicillin (10 mg) was dissolved in 0.1 N HCl and volume was made up to 100 mL in 100 mL volumetric flask. This solution (100 mcg/mL) was further diluted with 0.1 N HCl to obtain solution of 5 to 40 mcg/mL. Absorbance of each solution was measured at 228 nm using Shimadzu UV-1601 UV/Vis double beam spectrophotometer and 0.1 N HCl as reference standard. The standard curve was generated for the entire range from 5 to 40 mcg /mL. The results of standard curve preparation are shown in the Table 4.1 and Figure 4.1.

Table 4.1: Standard calibration curve of amoxicillin in 0.1 N HCl						
Sr. No.	**Concentration (µg/mL)**	**Absorbance**			**Average Absorbance**	**Calculated absorbance**
		1	2	3		
1	0	0	0	0	0	-0.0012
2	5	0.110	0.109	0.111	0.110	0.1073
3	10	0.227	0.228	0.226	0.227	0.2158
4	15	0.316	0.314	0.312	0.314	0.3243
5	20	0.419	0.419	0.419	0.419	0.4328
6	25	0.531	0.530	0.529	0.530	0.5413
7	30	0.660	0.659	0.659	0.659	0.6498
8	35	0.770	0.770	0.770	0.770	0.7583
9	40	0.860	0.859	0.861	0.860	0.8668
Correlation Co-efficient : 0.9988 Absorbance= 0.0217x - 0.0012						

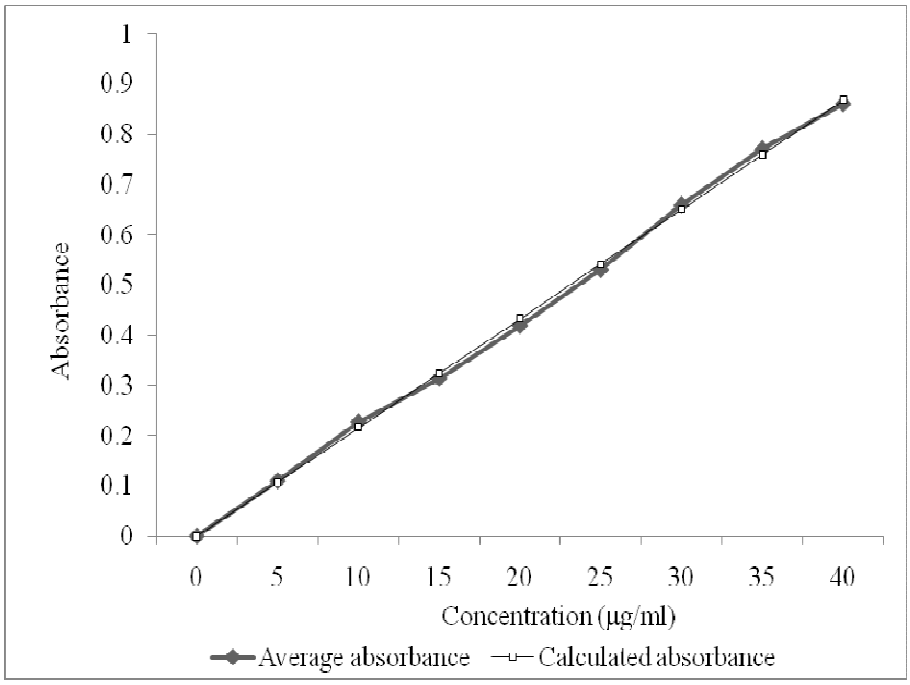

Figure 4.1: ***Standard calibration curve of amoxicillin in 0.1 N HCl***

4.2.2 Preparation of mucoadhesive amoxicillin microspheres

Mucoadhesive microspheres of amoxicillin were prepared containing carbopol-934P as mucoadhesive polymer and ethyl cellulose as carrier polymer by emulsion-solvent evaporation technique. Briefly, ethyl cellulose (1500 mg) was dissolved in 200 mL of ethanol. Each five hundred milligrams of amoxicillin and carbopol-934P were dispersed in the polymer solution of ethyl cellulose under stirring. In preliminary trail batches the drug-to-polymer-to-polymer (amoxicillin-ethyl cellulose-carbopol-934P) ratio was kept constant at 1:3:1. The resultant mixture was extruded through a syringe (gauge No. 20) in 500 mL of liquid paraffin (mixture of heavy and light, 1:1 ratio) containing 2.0 % v/v Span 80 and stirring was carried out using a propeller stirrer (Remi, Mumbai, India) at 1000 rpm. Stirring was continued for three hrs. The amount of emulsifying agent and time for stirring were varied in preliminary trial batches from 1 to 3 % v/v and 1 to 3 hrs respectively. In factorial design batches J1 to J9, 2.0 % v/v Span 80 was used as an emulsifying agent and time for stirring was kept to 3 hrs. The drug-to-polymer-to-polymer ratio and stirring speed were varied in batches J1 to J9 as shown in Table 4.2. All other variables were similar as per in preliminary trial batches. Microspheres thus, obtained were filtered and washed several times with petroleum ether (80:20) to remove traces of oil. The microspheres were then dried at room temperature (25^{o}C and 60 % RH) for 24 hrs. The effect of formulation variables on characteristics of the microspheres of factorial design batches is summarized in Table 4.2.

4.2.3 Optimization of microspheres formulation using 3^2 full factorial design

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \qquad [4.1]$$

Where, Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs, and b_i is the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low

to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity. On the basis of the preliminary trials a 3^2 full factorial design was employed to study the effect of independent variables i.e. drug-to-polymer-to-polymer (X_1) and the stirring speed (X_2) on dependent variables % mucoadhesion, drug entrapment efficiency, particle size and the time required for 80% drug dissolution (t_{80}).

4.2.4 Drug entrapment efficiency

Two hundred milligrams of accurately weighed microspheres were crushed in a glass mortar-pestle and the powdered microspheres were suspended in 10 mL phosphate buffer (pH 7.8). After 24 hrs the solution was filtered and the filtrate was analysed for the drug content. The drug entrapment efficiency was calculated using the following formula: Practical drug content/Theoretical drug content × 100. The drug entrapment efficiency for batches J1 to J9 is reported in Table 4.2.

4.2.5 Particle size of microspheres

The particle size of the microspheres was determined by using optical microscopy method [41]. Approximately 300 microspheres were counted for particle size using a calibrated optical microscope (Labomed CX RIII, Ambala, India). The particle size of microspheres of batches J1 to J9 is reported in Table 4.2.

4.2.6 *In-vitro* wash-off test for microspheres

The mucoadhesive properties of the microspheres were evaluated by *in-vitro* wash-off test as reported by Lehr et al [42]. A 1x1 cm piece of rat stomach mucosa was tied onto a glass slide (-3 inch-by-1inch-) using thread. Microspheres were spread (~50) onto the wet rinsed tissue specimen and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus with continuous oxygen supply. The disintegrating test apparatus was operated whereby the tissue specimen was given regular up and down movements in the beaker of the disintegration apparatus, which contained the gastric fluid (pH 1.2). At the end of 30 min, 1 hr and at hourly intervals upto 12 hrs, the number of microspheres still adhering onto the tissue was counted. The results of *in-vitro*

wash-off test after 1, 5 and 10 hrs of batches J1 to J9 are shown in Table 4.2. Also results of *in-vitro* wash-off test of amoxicillin loaded carbopol-934P mucoadhesive microspheres of batch J4 is shown in Figure 4.2.

4.2.7 Scanning electron microscopy

Scanning electron photomicrographs of drug-loaded carbopol-934P mucoadhesive microspheres were taken. A small amount of microspheres was spread on glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope (JSM 5610 LV SEM, JEOL, Datum Ltd, Tokyo, Japan) chamber. Scanning electron photomicrograph was taken at the acceleration voltage of 20 KV, chamber pressure of 0.6 mm Hg, at different magnification. The photomicrograph of batch J4 is depicted in Figure 4.3.

The photomicrograph of in-vitro wash-off test results after 2 hrs and 8 hrs are depicted in Figure 4.4 and Figure 4.5, respectively.

4.2.8 Drug release study

The drug release study was carried out using USP XXIV basket apparatus (Electrolab, TDT-06T, India) at 37°C±0.5°C and at 100 rpm using 900 mL of phosphate buffer (pH 7.8) as a dissolution medium (n=5) as per USP XXVI dissolution test prescribed for amoxicillin tablets. Microspheres equivalent to 100 mg of amoxicillin were used for the test. Five milliliters of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 µm membrane filter, diluted suitably, and analyzed spectrophotometrically. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage drug dissolved at different time intervals was calculated using the Lambert-Beer's equation. The t_{80} was calculated using the Weibull equation[43]. The average values of t_{80} for batches J1 to J9 are mentioned in Table 4.2. The percentage drug release of batch J4 in pH 1.2 and pH 7.8 is shown in Table 4.3 and Figure 4.6.

4.2.9 Data fitting

An attempt was made to fit the dissolution data into the Hixon-Crowell [43] model represented:

$$m = [(100) * (1/3) - k * t]^3 \qquad [4.2]$$

Where, k is Hixon-Crowell constant [mass/ (time)] $^{1/3}$. In this model the % drug unreleased versus cube root of time is linear.

The data was treated with the Korsmeyer-Peppas model [44] to characterize the mechanism of drug release:

$$Mt / M\infty = Kpt^n \quad [4.3]$$

Mt/M_∞ represents the fraction of drug released at time t and Kp is the kinetic constant characterizing the polymeric system and n stands for the diffusion exponent.

The dissolution data was also analyzed using the Weibull equation[43] to determine the kinetics of drug release from different batches of mucoadhesive microspheres:

$$m = 1- \exp [-(t-ti) b/a] \quad [4.4]$$

Where, 'a' is the scale parameter which defines the time scale of the process, 'ti' is location parameter which represents the lag period before the actual onset of dissolution process (in most cases ti = 0) and 'b' is the shape parameter. In this model the plot of log of time vs- In (1-m) is linear.

The results of *F*-statistics were used for the selection of the most appropriate model. Results of *F*-statistics and summary of results of regression analysis are shown in Tables 4.4 and 4.5, respectively.

The curve fitting, simulation and plotting was performed in Excel (Microsoft Software Inc., USA) and Sigma plot® version 10.0 (Sigma plot soft ware, Jangel Scientific Software, San Rafael, CA). The effects of independent variables on the response parameters were visualized from the contour plots. Numerical optimization using the desirability approach was employed to locate the optimal settings of the formulation variables so as to obtain the desired response [45]. An optimized formulation was developed by setting constraints on the dependent and independent variables. The formulation developed was evaluated for the responses and the experimental values obtained were compared with those

predicted by the mathematical models generated. Counter plot showing the effect of drug-polymer-polymer ratio (X_1) and stirring speed (X_2) on: % mucoadhesion, particle size, drug entrapment efficiency and t_{80} in Figure 4.7.

4.2.10 *In-vivo* clearance of H. pylori

The *H. pylori* infected animal model was established according to Qian's method (China Patent, CN 1304729A). Briefly, 0.3 mL of broth containing 10^9 CFU/mL of *H. pylori,* isolated from patients with gastritis and gastric ulcer, was inoculated into the stomachs of 6-week-old male Wistar rats. Then, the rats were fed for 4 weeks. *H. pylori* infection in rat was detected using the "golden standard" culture, W-S stain and rapid urease test etc. The rapid urease test was carried out by collecting and transferring the bacterial colonies into small tubes containing 0.5 mL of mixture of phosphate buffer, urea (2% w/v) and phenol red (0.03% v/v). If the solution color turned into red in several minutes, the urease test was regarded to be positive, which indicated presence of *H. pylori*. While if the solution color did not turn red in several minutes, the urease test was regarded to be negative, this indicated absence of *H. pylori*.

4.2.10.1 Single-dosage administration

To determine the dose for *H. pylori* clearance, mucoadhesive amoxicillin microspheres and amoxicillin powder were orally administered to the *H. pylori* infected rats at the dosages of 4.0, 7.5 and 15 mg/kg (n=2). Physiological saline was given to rats as control (n=2). One day after administration of drug to the *H. pylori* infectious rats, they were killed, and their stomachs were removed and cut. Then, the gastric tissue was daubed on the modified Skirrow's medium. The plates were incubated for 3 days at 37 ^oC under microaerobic conditions. *H. pylori* clearance effect was judged by both bacterial colony counts and rapid urease test. The rapid urease test was carried out by collecting and transferring the bacterial colonies into small tubes containing 0.5 mL of mixture of phosphate buffer, urea (2% w/v) and phenol red (0.03% v/v). If the solution color turned into red in several minutes, the urease test was regarded to be positive, which indicated for *H. pylori* detection. *H .pylori* clearance effect of amoxicillin at different doses in different formulations (n=2) are shown in Tables 4.6 and 4.7.

4.2.10.2 Multidose administration

To determine whether the mucoadhesive amoxicillin microspheres could completely eradicate *H. pylori*, a multidose administration therapy was carried out. Briefly, amoxicillin was orally administrated twice a day for three days at a dose of 3.5 mg/kg in the form of either amoxicillin mucoadhesive microspheres or powder (n=2). Physiological saline was given to rat as control (n=2). One day after administration the *H. pylori* infectious rats were killed, and their stomachs were removed and cut. Then, the gastric tissue was daubed on the modified Skirrow's medium. *H. pylori* clearance effect was studied using the same method as described for single doge administration. *H .pylori* clearance effect of amoxicillin at different doses in different formulations is shown in Figure 4.8.

Table 4.2: Amoxicillin mucoadhesive microspheres batches using 3^2 full factorial design layout

Batch code	Variable levels in coded from		*In-vitro* wash-off test (% mucoadhesion after)			Drug entrapment efficiency (%)	Particle size (μm)	t_{80} (min)
	X_1	X_2	1 h	5 h	10 h			
J1	-1	-1	57	50	45	26	99	592
J2	-1	0	55	49	44	21	93	645
J3	-1	1	40	35	29	20	86	727
J4	0	-1	80	72	60	56	109	502
J5	0	0	77	70	55	52	101	542
J6	0	1	71	65	50	41	94	582
J7	1	-1	90	80	72	47	112	299
J8	1	0	81	75	64	41	110	308
J9	1	1	73	64	50	34	99	340

Translation of coded levels in actual units

Variables level	Low (-1)	Medium (0)	High (+1)
Drug-to-polymer-to polymer ratio (X_1) (amoxicillin-ethyl cellulose-carbopol-934P)	1:3:0.5	1:3:1	1:3:1.5
Stirring speed (X_2) rpm	800	1000	1200

All the batches were prepared using 2 % v/v Span 80 and stirring time of 3 hrs

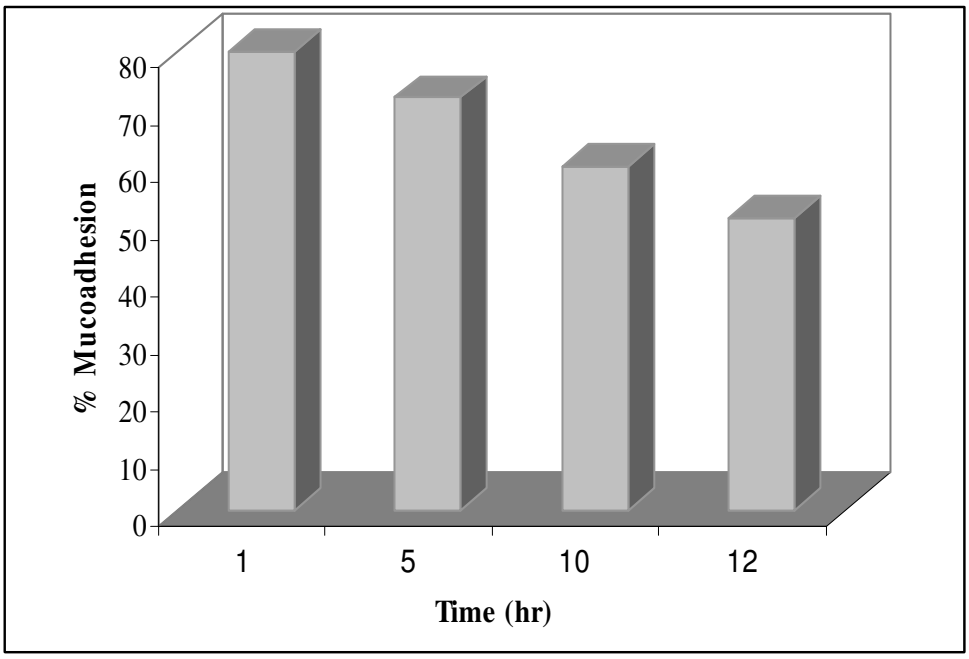

Figure 4.2: ***% mucoadhesion of amoxicillin loaded carbopol-934P mucoadhesive microspheres (batch J4) after 1, 5, 10 and 12 hrs***

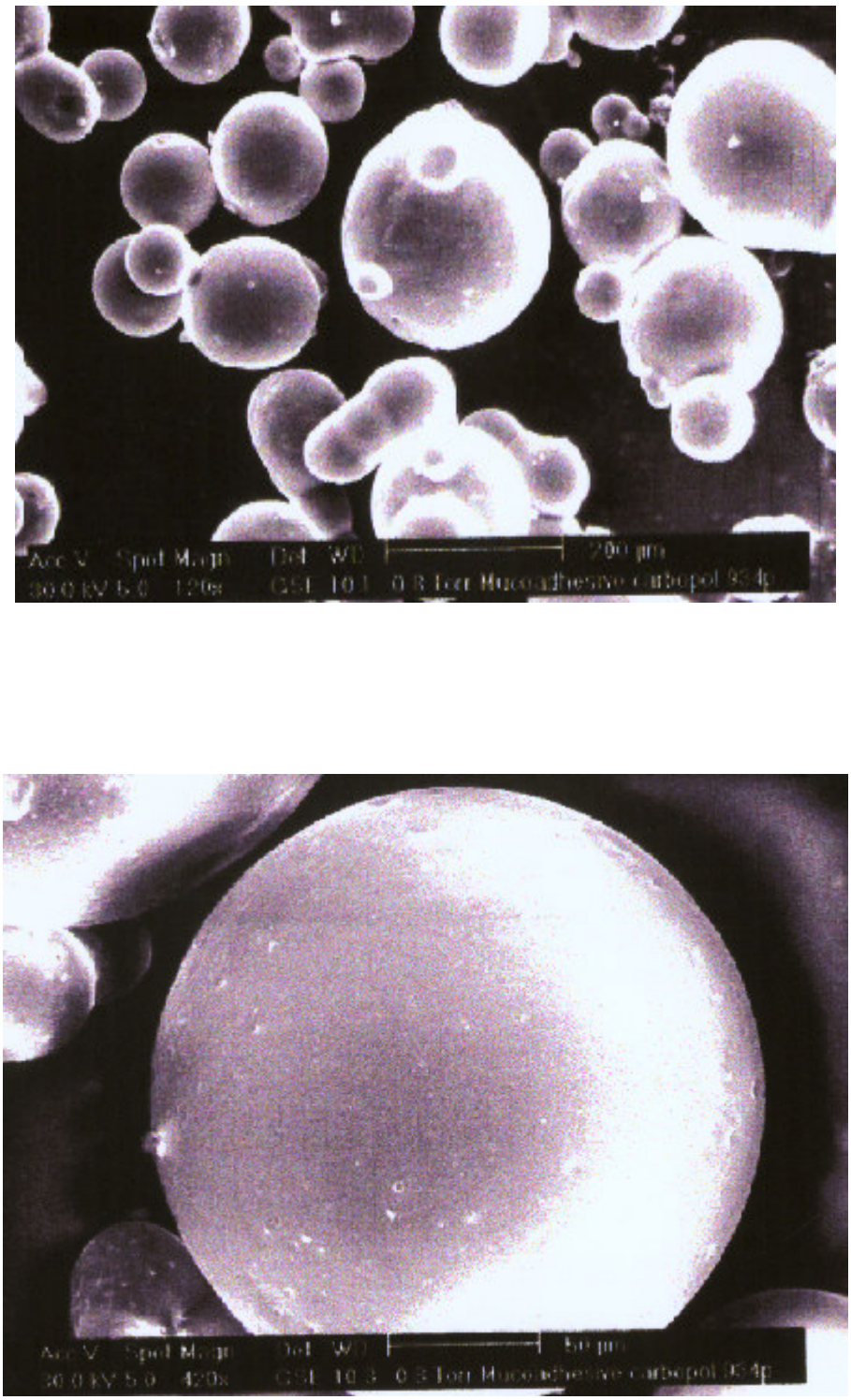

Figure 4.3: ***Scanning electron photomicrograph of amoxicillin loaded carbopol-934P mucoadhesive microspheres (batch J4)***

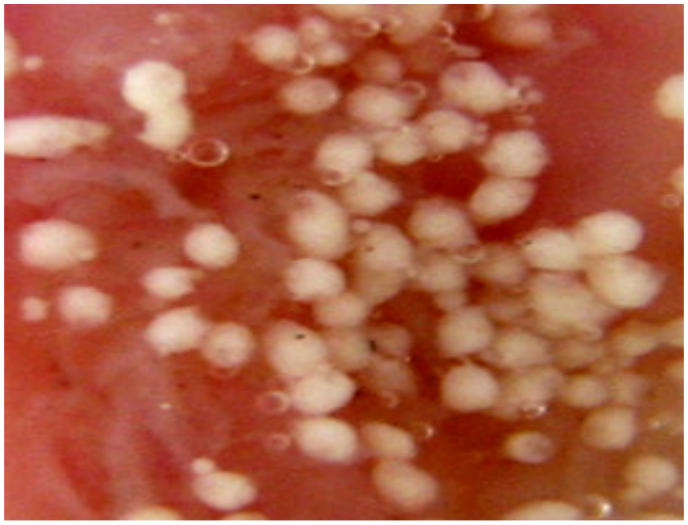

Figure 4.4: ***In-vitro wash-off test of amoxicillin loaded carbopol-934P mucoadhesive microspheres (batch J4) on rat stomach after 2 hrs***

Figure 4.5: ***In-vitro wash-off test of amoxicillin loaded carbopol-934P mucoadhesive microspheres (batch J4) on rat stomach after 8 hrs***

Table 4.3: In-vitro dissolution of amoxicillin from mucoadhesive microspheres of batch J4		
Time(min)	**pH=1.2**	**pH=7.8**
0	0	0
30	17±4	11±3
60	27±3	23±4
120	41±3	35±3
180	51±4	42±4
240	56±3	50±3
300	65±3	59±3
360	70±4	66±4
420	75±3	69±2
480	78±2	72±5
540	90±2	80±3
600	94±5	90±4

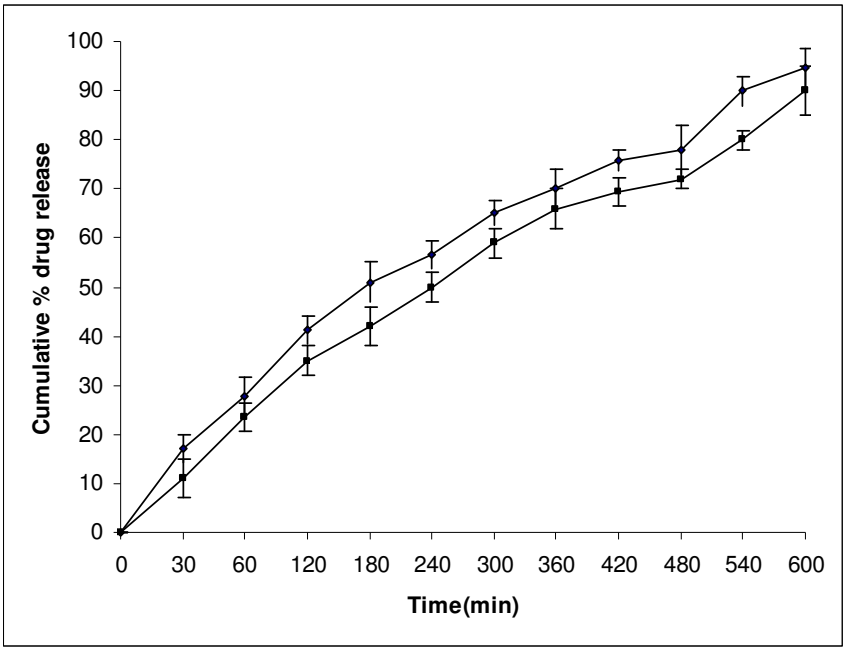

Figure 4.6: ***In-vitro dissolution of amoxicillin from mucoadhesive microspheres of batch J4 (-♦- pH=1.2 and -■- pH=7.8)***

Table 4.4: Results of models fitting of batch J4							
Time (min)	**Cumulative percent release**	**Hixon-Crowell**		**Korsemeyer and Peppas**		**Weibull**	
		Calculated cumulative percent release	**Residual square**	**Calculated cumulative percent release**	**Residual square**	**Calculated cumulative percent release**	**Residual square**
0	0	-6.86	47.00	-	-	-	-
60	11.0	10.57	4.51	14.01	1.71	11.69	1.01
120	35.0	26.00	0.48	25.45	0.02	25.98	0.45
180	42.1	39.54	1.29	36.09	5.35	39.61	1.45
240	50.2	51.32	0.77	46.23	35.64	51.70	0.25
300	60.2	61.46	0.43	56.02	22.80	61.98	1.38
360	66.1	70.08	8.30	65.55	2.72	70.48	10.73
420	69.9	77.31	10.32	74.85	0.56	77.34	10.53
480	72.3	83.27	5.63	83.97	9.45	82.80	3.60
540	80.4	88.09	3.19	92.94	44.07	87.07	0.58
600	90.2	91.87	12.43	101.77	40.51	90.36	25.39
SSR		94.41		162.88		55.43	
F-value		10.49		20.36		**6.92**	
Correlation Coefficient		0.9889		0.9935		0.9931	
R-Square		0.9780		0.9871		0.9863	
Slope		0.0046		0.8611		1.2745	
Intercept		-0.1038		-2.38		-3.17	

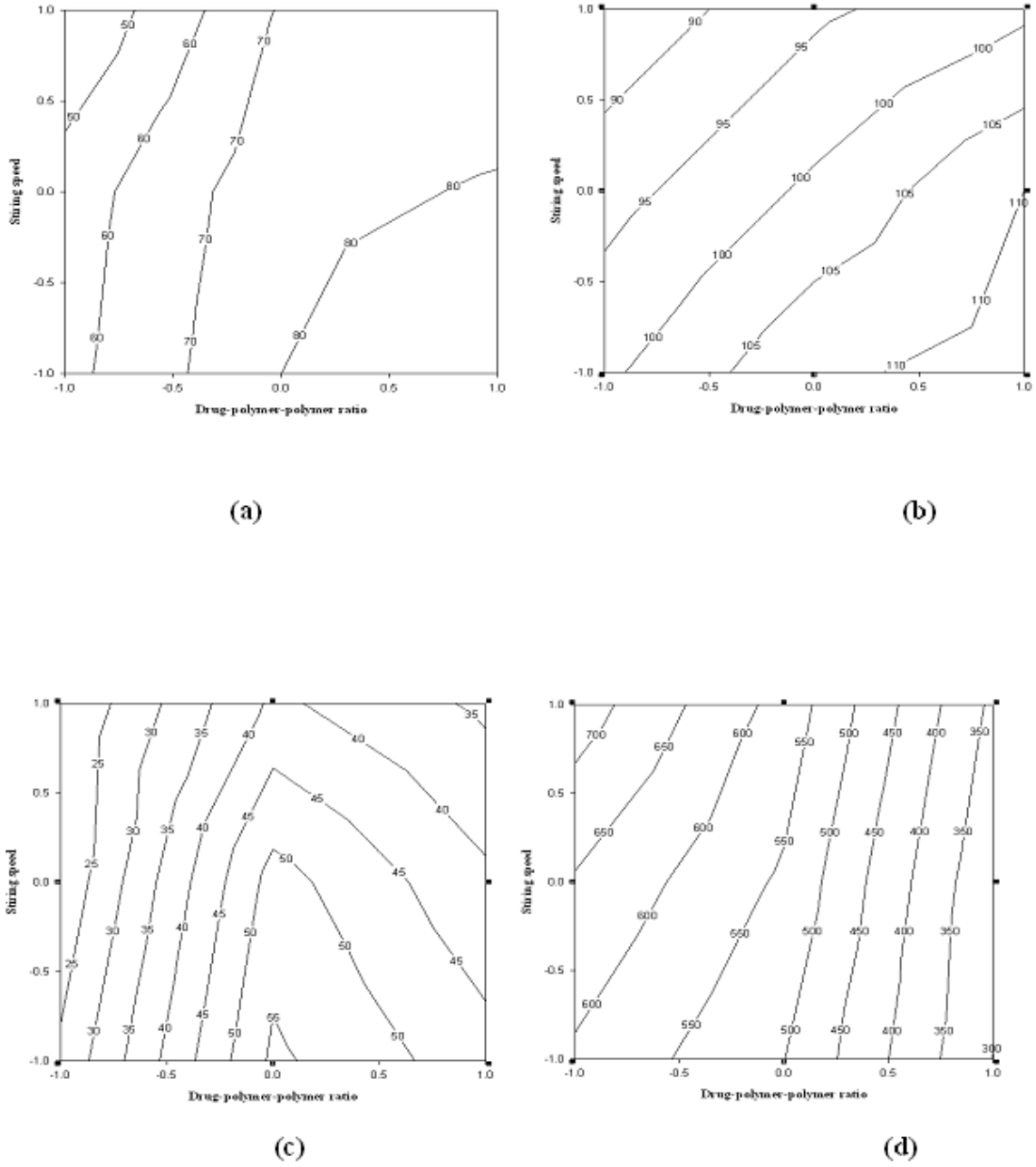

Figure 4.7: ***Counter plot showing the effect of drug-polymer-polymer ratio(X_1) and stirring speed (X_2) on: % mucoadhesion (a), particle size (b), drug entrapment efficiency (c) and t_{80} (d)***

Table 4.5: Summary of results of regression analysis

Coefficient	b_0	b_1	b_2	b_{11}	b_{22}	b_{12}	R^2
% Mucoadhesion	77.66	15.33	-7.16	0	-10.0	-2.5	0.9803
Drug entrapment efficiency	50.11	9.16	-5.66	-1.75	-18.16	-0.66	0.9954
Particle size	102.33	7.16	-6.83	0	-1.5	-1.5	0.9824
t_{80}	536.22	-169.5	42.66	-23.5	-56.83	8.66	0.9994

Table 4.6: *H .pylori* clearance effect of amoxicillin at different doses in different formulations (n=2)

Colony counts	Doses (mg/kg)					
	4		7.5		15	
Amoxicillin mucoadhesive microspheres	20	26	8	6	2	2
	(23±4.24)*		(7.0±1.41)*		(2±0)*	
Amoxicillin powder	72	84	25	33	5	30
	(78±8.48)*		(29±5.65)*		(18.0±16.97)*	
Physiological saline	98	90	99	85	105	80
	(94±5.65)*		(92±9.89)*		(92.5±17.67)*	

* Figure showed mean ± S.D.

Table 4.7: *In-vivo* clearance of *H .pylori* after the administration of amoxicillin powder, mucoadhesive amoxicillin microspheres and physiological saline (n=2)

***H .pylori* Condition**	**Mucoadhesive amoxicillin microspheres**		**Amoxicillin powder**		**Physiological saline**	
	1	2	1	2	1	2
	-/-	-/-	+/+	+/+	+/+	+/+

Negative (-) means neither bacterial colony was found nor rapid urease test was positive (+) means either bacterial colony was found or rapid urease test was positive

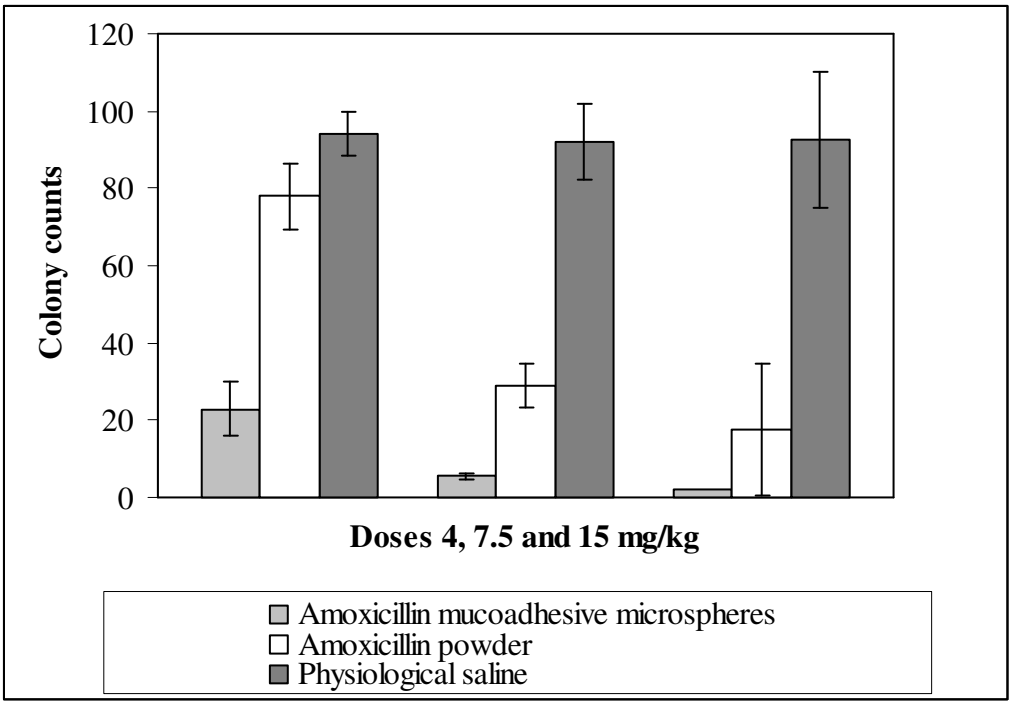

Figure 4.8: *H .pylori clearance effect of amoxicillin at different doses in different formulations*

4.3 Results and discussion

4.3.1 Preliminary trials

The mucoadhesive microspheres of amoxicillin using carbopol-934P and ethyl cellulose were prepared by emulsion-solvent evaporation technique. Carbopol-934P was selected as a polymer for the preparation of mucoadhesive microspheres owing to its biodegradable and mucoadhesive properties. Ethyl cellulose was used as carrier polymer. Different concentrations of span 80 from 1% v/v to 3% v/v were used as emulsifying agent. Significant effect of concentration of span 80 was observed on percentage mucoadhesion, particles size and drug entrapment efficiency. Results showed that increase in the concentration of span 80 increase the particles size and percentage mucoadhesion but decrease the drug entrapment efficiency. At 1% v/v, percentage mucoadhesion, particles size and drug entrapment efficiency were 66%, 80 µm and 65 %, respectively but irregular shape of microspheres was observed. While at 3% v/v, percentage mucoadhesion, particles size and drug entrapment efficiency were 82%, 210 µm and 38% respectively; spherical shape of microspheres was observed but particles were coalesced. Therefore, 2 % v/v of concentrations of span 80 was used for further study.

One of the important factors related to microspheres as reported by Lee et al [46] is the viscosity of the polymer solution. Polymers concentrations of 0.5%, 1%, and 2% w/v were selected for preliminary trials. Flake formation was observed when ethyl cellulose and carbopol-934P concentration was used at a level of 0.5% w/v, whereas maximum sphericity was observed at the 1% w/v level. Non spherical microspheres were found when polymer concentration was used at the 2% w/v level. Therefore, 1% w/v of ethyl cellulose and carbopol-934P each, in ethanol was found to be the optimum concentration for the polymer solution. A 1:1 mixture of heavy and light liquid paraffin was found to be suitable as a dispersion medium.

Preliminary trial batches were prepared to study the effect of the time for stirring and stirring speed on the percentage mucoadhesion, drug entrapment efficiency, and characteristics of the microspheres. Increase in the time for stirring from 1 to

3 hrs, showed increase in percentages of mucoadhesion but decrease in drug entrapment efficiency and particles size. Thus, 3 hrs of stirring time was selected for further study. Since stirring speed has significant effect on percentage mucoadhesion, drug entrapment efficiency and particles size, it was selected as one an important factor for further study.

On the basis of the preliminary trials a 3^2 full factorial design was employed to study the effect of independent variables (i.e, drug-to-polymer-to-polymer ratio [X_1] and the stirring speed [X_2]) on dependent variables percentage mucoadhesion, drug entrapment efficiency, particle size and t_{80}. The results depicted in Table 4.2 clearly indicate that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the 9 batches (J1 to J9). The fitted equations (full models) relating the responses (ie, percentage mucoadhesion, drug entrapment efficiency, particle size and t_{80}) to the transformed factor are shown in Table 4.5. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (ie, positive or negative). The high values of correlation coefficient (Table 4.5) for the dependent variables indicate a good fit. The equations may be used to obtain estimates of the response since small error of variance was noticed in the replicates.

4.3.2 Factorial equation for % mucoadhesion

The *in-vitro* mucoadhesiveness test showed that the percentage of mucoadhesive microspheres remaining on the stomach mucosa (Table 4.2). Figure 4.2 showed that even after 12 hrs, 52 % microspheres were adhered to gastric mucous layer. The mucoadhesive microspheres of all the batches of the factorial design were spherical (Figure 4.3, batch J4) and free flowing.

The linear model generated for % mucoadhesion was found to be significant with an *F*-value of 29.96 ($p<0.0001$) and R^2 value of 0.9803:

$$\% \text{ mucoadhesion} = 77.66 + 15.33X_1 - 7.16X_2 - 2.5X_1X_2 - 10\,X_2^2 \qquad [4.5]$$

The counter plot (Figure 4.7a) shows that the *in-vitro* wash-off test for % mucoadhesion of microspheres increased from 40 to 57 and 73 to 90 at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as stirring speed decreased. Results of equation indicate that the effect of X_1 (drug-to-polymer-to-polymer) is more significant than X_2 (stirring speed). Moreover, stirring speed had a negative effect on the percentage mucoadhesion (ie, as the stirring speed increased, the percentage mucoadhesion decreased). This finding may be attributed to the change in particle size that affects mucoadhesion. As the drug -to-polymer-to-polymer ratio increases, the % mucoadhesion also increases; because more amounts of polymer results in higher amount of free –COOH groups [17], which are responsible for binding with sialic acid groups in mucus membrane and thus results in increase in mucoadhesive properties of microspheres. *In-vitro* mucoadhesive test showed that amoxicillin mucoadhesive microspheres a-dhered more strongly to gastric mucous layer and could retain in gastrointestinal tract for an extended period of time (Figures 4.4 and 4.5). Figure 4.5 showed that even after 8 hrs, some of microspheres were adhered to gastric mucous layer. All factorial batches showed more than 50 % mucoadhesion even after 10 hrs.

4.3.3 Factorial equation for particle size

The linear model generated for particle size was found to be significant with an *F*-value of 33.6 ($p<0.0001$) and R^2 value of 0.9824:

$$\text{Particle size} = 102.33 + 7.16X_1 - 6.83X_2 - 1.5X_1X_2 - 1.5X_2^2 \quad [4.6]$$

The counter plot (Figure 4.7b) shows that the particle size of microspheres increased from 86.0 to 99.0 µm and 99.0 to 112 µm at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as stirring speed decreased. Results indicate that the effect of X_1 (drug-to-polymer-to-polymer) is more significant than X_2 (stirring speed). Means, as the stirring speed increased, the particle sizes decreased that directly affect the percentage mucoadhesion.

Thus, we can conclude that the amount of polymer (carbopol-934P) and stirring speed directly affects the percentage mucoadhesion and particles size.

4.3.4 Factorial equation for drug entrapment efficiency

The drug entrapment efficiency and t_{80} are important variables for assessing the drug loading capacity of microspheres and their drug release profile, thus suggesting the amount of drug availability at site. These parameters are dependent on the process of preparation, physicochemical properties of drug, and formulation variables. The linear model generated for drug entrapment efficiency was found to be significant with an *F*-value of 40.70 ($p<0.0001$) and R^2 value of 0.9954:

$$\text{Drug entrapment efficiency} = 50.11+9.16X_1 -5.66X_2 -0.66X_1X_2 -1.75X_1^2-18.16X_2^2 \quad [4.7]$$

The counter plot (Figure 4.7c) shows that the % drug entrapment efficiency of microspheres increased from 20.0 to 26.0 and 34.0 to 47.0 at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as stirring speed decreased. But at medium level of drug-to-polymer-to-polymer ratio, as stirring speed decreased, % drug entrapment efficiency of microspheres showed increase from 47.0 to 52.0. Results of equation indicate that the effect of X_1 (drug -to-polymer-to-polymer) is more significant than X_2 (stirring speed). Moreover, the stirring speed had a negative effect on the percentage drug entrapment efficiency (ie, as the stirring speed increased, the particle size decreased, and thus drug entrapment efficiency decreased).

4.3.5 Factorial equation for t_{80}

The linear model generated for t_{80} was found to be significant with an *F*-value of 115.91 ($p<0.0001$) and R^2 value of 0.9994:

$$t_{80} = 536.22 - 169.5X_1+ 42.66X_2 -23.5X_1X_2 -56.83X_1^2 -8.66\ X_2^2 \quad [4.8]$$

The counter plot (Figure 4.7d) shows that the % drug released *in-vitro* from microspheres decreased from 727 to 592 min and 340 to 299 min at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as stirring speed decreased. Results depicted in Table 4.5 indicate that the % drug released *in-vitro* is highly dependent on the drug-to-polymer-to-polymer and stirring speed. The drug-to-polymer-to-polymer ratio has a negative effect on t_{80}, while stirring speed has a positive effect on t_{80}, because as the particle size decreases the drug release decreases.

A numerical optimization technique using the desirability approach was employed to develop a new formulation with the desired responses. Constraints like maximizing % mucoadhesion, drug entrapment efficiency, particle size and release at the end of 12 hrs in addition to minimizing the t_{80} were set as goals to locate the optimum settings of independent variables in the new formulation. The optimized microsphere formulation (J10) was developed using 1:3:1.25 drug-to-polymer-to-polymer ratio and 950 rpm stirring speed. The optimized formulation was evaluated for % mucoadhesion, drug entrapment efficiency, particle size and drug release. The results of experimentally observed responses and those predicted by mathematical models along with the percentage prediction errors were compared. The prediction error for the response parameters ranged between 0.50 and 2.15% with the value of absolute error of 1.36 ± 0.70%. The low values of error indicate the high prognostic ability of factorial equation and counter plot methodology. The drug release from the optimized formulation was found to low t_{80} (410 min) thus, we selected batch J4 for further study, which exhibited a high t_{80} of 502 min and seems to be a promising candidate for achieving drug release upto 12 hrs. The drug release profile of batch J4 is shown in Figure 4.6. The figure reveals that drug release rate slowed after 4 hrs. The study focus was the preparation of mucoadhesive microspheres, thus the microspheres of batch J4 were also evaluated in simulated gastric fluid USP (pH 1.2). *In-vitro* release test showed that amoxicillin released faster in pH 1.2 hydrochloric acid than in pH 7.8 phosphate buffer but the results indicated that no

significant difference was observed between dissolution profiles at pH 7.8 and pH 1.2 as the f_2 (similarity factor) value was 63.48.

The results of curve fitting of best batch into different mathematical models are given in Table 4.4. The mechanism of drug release from the microspheres was found to be diffusion controlled because plots of percent cumulative drug release vs. square root of time were found to be linear with the regression coefficient (R^2) values ranging from 0.9780 to 0.9871 for the best batch. Results of *F*-statistics are shown in Table 4.4. The release profile fitted to Weibull equation *F* value was found 6.92. The value of correlation coefficient was found to be 0.9931. The values of slope and intercept were found to be 1.27 and –3.17 respectively. While release profile fitted to Korsmeyer-Peppas equation, *F* value was found 20.36. The value of correlation coefficient was found to be 0.9935. The values of slope and intercept were found to be 0.8611 and –2.38 respectively; and release profile fitted to Hixon-Crowell equation, *F* value was found 10.49. The value of correlation coefficient was found to be 0.9889. The values of slope and intercept were found to be 0.0046 and –0.1038 respectively. The results of *F*-statistics were used for the selection of the most appropriate model, thus we concluded that the release profile fitted best to Weibull equation (*F* =6.92).

4.3.6 *In-vivo* study

At present, most studies of mucoadhesive formulations loading amoxicillin for anti- *H. pylori* focused on prolonging the gastric retarding time. The stability of amoxicillin in acidic medium was neglected. In fact, lots of antibiotics, such as erythromycin, clarithromycin, were reported with strong *in-vitro H. pylori* clearance effect but with poor *in-vivo* results. Ogwal and Xide [47] suggested that one of the reasons was due to their instability in acidic medium. Amoxicillin was also reported to be unstable in mediums with pH below 2 [48-50]. Amoxicillin can be quickly absorbed after its conventional dosage forms are orally administered. Therefore, its residence time in the stomach is expected to be short [34], which might cover up its shortcoming of being unstable in acidic medium. But for the mucoadhesive microspheres, which would stay in the stomach for a much longer time, the stability of amoxicillin should be seriously considered. In this study, we

found that, amoxicillin microspheres were more stable in pH 1.2 HCl than amoxicillin powder.

From the result of the *in-vivo H. pylori* clearance test, we observed that, with the increase of amoxicillin's doses, the *H. pylori* clearance effect was enhanced in mucoadhesive amoxicillin microspheres formulation. In the single dosage administration test, it was found that the total colony counts decreased markedly with the increase of the amoxicillin dose in both groups. Means, 4 mg/kg dose of amoxicillin mucoadhesive microspheres administrated colony counts were 23±4.24, and on increase in the doses to 7.5 and 15 mg/kg colony counts were 7.0±1.41 and 2±0, respectively. On administrating Amoxicillin powder 4 mg/kg dose colony counts were 78±8.48, and on increase in the doses to 7.5 and 15 mg/kg colony counts were 29±5.65 and 18.0±16.97, respectively. While in case of Physiological saline 4 mg/kg dose administrated colony counts were 94±5.65, and than increases the doses 7.5 and 15 mg/kg colony counts were 92±9.89 and 92.5±17.67, respectively. Physiological saline did not show any decrease in colony count. But the ratio of colony counts between amoxicillin powder and mucoadhesive microspheres increased rapidly from 3.39 at 4 mg/kg to 9.0 at 15 mg/kg. (Table 4.6, Figure 4.8). This phenomenon indicated that with the increase in dose, mucoadhesive amoxicillin microspheres showed more effective clearance of *H. pylori* than that in case of amoxicillin powder. We inferred that this might be due to the lack of repetition of drug administration. Therefore, another multidose administration regimen was tried. The results showed that, at the dose of 3.5 mg/kg, when amoxicillin microspheres, powder or physiological saline was administrated, respectively, to the *H. pylori* infectious rat twice a day for three consecutive days (Table 4.7). Neither *H. pylori* colony was found nor was urease test positive in rats whom mucoadhesive amoxicillin microspheres were administrated. We conclude that the mucoadhesive amoxicillin microspheres showed a more complete *H. pylori* clearance effect.

4.4 Conclusion

Amoxicillin mucoadhesive microspheres containing carbopol-934P as mucoadhesive polymer and ethyl cellulose as carrier polymer, were prepared by emulsion-solvent evaporation technique. The results of a 3^2 full factorial design revealed that the drug-to-polymer-to-polymer (amoxicillin-ethyl cellulose-carbopol-934P) and stirring speed significantly affected the dependent variables % mucoadhesion, drug entrapment efficiency, particle size and $t_{80.}$The microspheres of the best batch (J4) exhibited a high % mucoadhesion of 80% after 1 hr, 56 % drug entrapment efficiency and mean particles size of 109 µm. The t_{80} of 502 min indicates that the mucoadhesive microspheres of amoxicillin could sustain the release of the drug for more than 10 hrs. The investigation on *H. pylori* clearance effect showed that there was a tendency for a more effective *H. pylori* activity of mucoadhesive amoxicillin microspheres compare to amoxicillin powder and physiological saline, which might indicate a potential use of mucoadhesive amoxicillin microspheres in treating *H. pylori* infection.

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Chapter 5

Floating in situ gel based on alginate as carrier for stomach-specific drug delivery of famotidine

5.1 Aim of present investigation

Famotidine is a histamine H2-receptor antagonist. It is widely prescribed in gastric ulcers, duodenal ulcers, Zollinger-Ellison syndrome and gastroesophageal reflux disease. In the management of benign gastric and duodenal ulceration the dose is 40 mg daily by mouth at bedtime, for 4 to 8 weeks. In gastroesophageal reflux disease the recommended dose is 20 mg by mouth twice daily for 6 to 12 weeks; where gastroesophageal reflux disease is associated with esophageal ulceration, the recommended dosage is 40 mg twice daily for a similar period. For the short term symptomatic relief of heartburn or non-ulcer dyspepsia a dose of 10 mg up to twice daily is suggested. In the Zollinger-Ellision syndrome the initial dose by mouth is 20 mg every 6 hrs, increased as necessary; dose up to 80 mg daily have been employed [1]. Its low bioavailability (40-45%) and short biological half-life (2.5-4.0 hrs) following oral administration favors development of a sustained release formulation.

The gastroretentive drug delivery systems can be retained in the stomach and contribute in improving the oral sustained delivery of drugs that have an absorption window in a particular region of the gastrointestinal tract. These systems help in continuously releasing the drug before it reaches the absorption window, thus ensuring optimal bioavailability [2]. It has been reported that the oral treatment of gastric disorders with an H2 receptor antagonist like famotidine or ranitidine used in combination with antacids promotes local delivery of these drugs to the receptor of parietal cell wall. Local delivery also increases the stomach wall receptor site bioavailability and increases efficacy of drugs to reduce acid secretion. Hence this principle may be applied for improving systemic as well as local delivery of famotidine, which would efficiently reduce gastric acid secretion [3].

There are a number of approaches that can be used to prolong gastric retention time, like these include floating drug delivery systems, also known as hydrodynamically balanced systems, swelling and expanding systems, polymeric bioadhesive systems, modified-shape systems, high-density systems, and other delayed gastric emptying devices [4-10]. A floating drug delivery system, being less

dense than gastric juice due to the incorporation of at least one porous structural element was described [11]. Recently, research has been done using famotidine as an effervescent type drug delivery system [12]. Also, a new type of multiparticulate floating drug delivery system consisting of a highly porous carrier material (foam powder), drug and polymer: low density microparticles have been proposed [13-14].

In situ gel, or *in-vivo* gel, environment sensitive gel is a new dosage form which has been used in stomach-specific drug delivery recently. Oral administration of in situ gels as low viscosity solution and upon contact with the simulated gastric fluid, the polymer changes conformation producing a gel, so it cannot only prolong the contact time between the drug and the absorptive sites at the stomach, but also release drug slowly and continuously[15-16]. The alginate based in situ gelling liquid formulation containing calcium ion in complexed form gets converted into gel when reaches to acidic environment of stomach and make the formulation to float for prolong period of time. The optimum quantity of sodium citrate was added to the above formulation to maintain its fluidity at room temperature before administration. The proposed alginate based floating in situ gelling systems of famotidine (FIGF), would have the advantage of ease of administration, as being a liquid, and is more patient compliance.

It is our intention to develop floating in situ gelling systems of famotidine that:

1. Formation of gel in gastric fluid and float to the stomach for prolonged period of time.
2. Provide an increased gastric residence time resulting in prolonged drug delivery in gastrointestinal tract.
3. Delivers a drug at a controlled rate over a period of time, which is same as or less than the residency period of the delivery system in the gastrointestinal tract.
4. Shows better *in-vivo* performances than conventional dosage forms.

In context to above intention, following criteria were aimed to achieve:

1. The proposed alginate based floating in situ gelling systems of famotidine (FIGF), would have the advantage of ease of administration, as being a liquid, and is more patient compliance.
2. Floating time of in situ gel should be more than 8 hrs.
3. Drug entrapment should be more than 50 %.
4. More than 90 % of drug should be released within 10 hrs.

5.2 Experimental

5.2.1 Estimation of famotidine

A solution of Famotidine was prepared in 0.1 N HCl and Phosphate buffer pH 4.5 and UV spectrum was taken using Shimadzu UV-1601 UV/Vis double beam Spectrophotometer (Kyoto, Japan), The UV maxima of Famotidine was found to be 265 nm in 0.1 N HCl and also same in Phosphate buffer pH 4.5

5.2.1.1 Preparation of standard curve of famotidine in 0.1 N HCl

Famotidine (10 mg) was dissolve in 0.1 N HCl and volume is made up to 100 mL in volumetric flask.1 mL of stock solution (100 μg/mL) was further diluted with 0.1 N HCl to obtained solution of 10 μg/mL to 25 μg/mL. Absorbance of each solution was measured at 265 nm using Shimadzu UV-1601 UV/Vis double beam Spectrophotometer (Kyoto, Japan) and 0.1 N HCl as a reference standard. The standard curve was generated for entire range from 10 to 25 μg/mL. The experiment was performed in triplicate and based on average absorbance; the equation for the best line was generated. The results of standard curve preparation are shown in Figure 5.1.

Table 5.1: Standard curve of famotidine in 0.1 N HCl						
Sr. No.	**Concentration (μg/mL)**	**Absorbance**			**Average Absorbance**	**Calculated Absorbance**
		1	**2**	**3**		
1	5	0.180	0.182	0.181	0.181	0.184
2	10	0.356	0.360	0.358	0.358	0.354
3	15	0.523	0.523	0.521	0.521	0.531
4	20	0.702	0.710	0.706	0.706	0.714
5	25	0.884	0.892	0.889	0.889	0.890
Correlation Coefficient = 0.9995 Absorption = 0.0353x + 0.0018						

Figure 5.1: ***Standard curve of famotidine in 0.1 N HCl***

5.2.1.2 Preparation of standard curve of famotidine in phosphate buffer (pH 4.5)

Famotidine (10 mg) was dissolve in phosphate buffer (pH 4.5) and volume is made up to 100 mL in volumetric flask. 1 mL of stock solution (100 µg/mL) was further diluted with phosphate buffer (pH 4.5) to obtained solution of 10 µg/mL to 25 µg/mL. Absorbance of each solution was measured at 265 nm using Shimadzu UV-1601 UV/Vis double beam Spectrophotometer (Kyoto, Japan) and phosphate buffer (pH 4.5) as a reference standard. The standard curve was generated for entire range from 10 to 25 µg/mL. The experiment was performed in triplicate and based on average absorbance; the equation for the best line was generated .The results of standard curve preparation are shown in Figure 5.2.

Table 5.2: Standard curve of famotidine in phosphate buffer (pH 4.5)						
Sr. No.	**Concentration (μg/mL)**	**Absorbance**			**Average Absorbance**	**Calculated Absorbance**
		1	**2**	**3**		
1	5	0.188	0.191	0.190	0.190	0.186
2	10	0.331	0.339	0.335	0.335	0.341
3	15	0.499	0.504	0.504	0.502	0.497
4	20	0.654	0.646	0.650	0.650	0.651
5	25	0.803	0.813	0.808	0.808	0.806
Correlation Coefficient = 0.9996 Absorption = 0.031x + 0.0317						

Figure 5.2: ***Preparation of standard curve of famotidine in phosphate buffer (pH 4.5)***

5.2.2 Preparation of in situ gelling solution

Sodium alginate solutions of concentrations 0.25, 0.5, 1.0 and 1.5 % (w/v) were prepared by adding the alginate to ultrapure water containing 0.25% (w/v) sodium citrate and 0.075% (w/v) calcium chloride and heating to 60 ℃ while stirring. Famotidine (40 mg) was then dissolved in 10 mL of 0.1N hydrochloride acid solution (pH 1.2) and added in the resulting solution after cooling to below 40 ℃. The solution was neutralized by 0.1N sodium hydroxide. A 1% (w/v) control solution (for use in the in vitro release experiments) was prepared by dissolving famotidine in a 0.6% (w/v) aqueous solution of sodium alginate. A 1% (w/v) solution of famotidine was prepared in ultrapure water. The resulting alginate in situ gel solution containing famotidine was checked for viscosity and gelling property (Figure 5.3) and finally stored in amber color narrow mouth bottles until further use. In the preliminary batches J1 to J12 the concentration of calcium chloride and sodium citrate were kept constant at 0.075 and 0.25 % w/v, respectively. The concentration of the alginate was varied in batches J1 to J12 from 0.25 to1.5 % w/v. The effect of formulation variables on characteristics of the sodium alginate based in situ gel of famotidine are summarized in Tables 5.2.3 and 5.2.4. In factorial design batches F1 to F9, the concentration sodium alginate (X_1) and the concentration of calcium chloride (X_2) were varied from 0.5 to 1.5 % w/v and 0.05 to 0.1 % w/v respectively, as shown in Table 5.4.

5.2.3 Optimization by using 3^2 full factorial design

On the basis of the preliminary trials in the present study a 3^2 full factorial design was employed to study the effect of independent variables, i.e. concentration of sodium alginate (X_1) and the concentration of calcium chloride (X_2) on dependent variables; drug content, viscosity, % drug released at 4 hrs (Q_{50}) and 8 hrs (Q_{80}). A statistical model (see equation) incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y = b0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_{12} + b_{22}X_{22} \quad [5.1]$$

Where, Y is the dependent variables, b0 is the arithmetic mean response of the nine runs, and b1 is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_{12} and X_{22}) are included to investigate non-linearity. The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The high values of correlation coefficient for the dependent variables indicate a good fit. The equation may be used to obtain estimate of the response because small error of variance was noticed in the replicates.

5.2.4 Physical appearance and pH

All the prepared alginate based in situ solutions of famotidine were checked for their clarity and the time required for gel formation. The pH was measured of in situ solutions of famotidine using a calibrated digital pH meter at 25°C. All measurements of pH were made in triplicate and the results are given in Table 5.3.

5.2.5 In vitro gelation study and viscosity measurement of in situ gels

Famotidine in situ solution (5 mL) and artificial simulated gastric fluid (100 mL) were mixed (1:20, v/v) and gelation was observed by visual examination. The viscosity of the sodium alginate solution either in solution or in gel made with artificial simulated gastric fluid were determined with a Brookfield viscometer (Model no RVT 81990) using a 20 mL aliquot of the sample. Measurements were performed using suitable spindle number at 6, 12, 30, 60 rpm, and the temperature was maintained at 25±1°C. The viscosity was read directly from the viscometer display. Gelation was also checked in collected gastric juice from the rats. All measurements were made in triplicate and the results are given in Tables 5.3 and 5.4.

5.2.6 Determination of drug content

The amount of famotidine in each sample was determined by spectrophotometer (UV-1601, Shimadzu Co Ltd., Kyoto, Japan). The UV absorbance of the sample

was determined at a wavelength of 265 nm. The drug content for batches J1 to J12 and F1 to F9 are depicted in Tables 5.3, 5.4 and Figure 5.4.

5.2.7 Measurement of in vitro drug release

The release of famotidine from floating in situ gel were determined as described by Zatz and Woodford [17] with some modification using USP dissolution test apparatus (USP 24) with a paddle stirrer at 50 rpm. This speed was slow enough to avoid the breaking of gelled formulation and was maintaining with the mild agitation conditions believed to exist *in-vivo*. The dissolution medium used was 500 mL of 0.1N HCL (pH 1.2), and temperature was maintained at 37 ± 0.2 ℃. Ten mL of formulation was drawn up using disposable syringe, the needle was wiped clean and excess formulation was removed from the needle end. The syringe end was then placed into the Petri dish (4.5 mm internal diameter) and the syringe plunger depressed slowly to extrude 10 mL and finally Petri dish containing formulation was kept in the dissolution vessel containing dissolution medium without much disturbance. At each time interval, a precisely measured sample of the dissolution medium was removed and replenished with pre warmed (37℃) fresh medium. Samples were withdrawn at predetermined time intervals, filtered through a 0.45 µm membrane filter, dilute suitably and analyzed spectrophotometrically. The experiments were conducted in triplicate. The amount of drug released at 4 hrs (Q_{50}) and 8 hrs (Q_{80}) were calculated [18-19]. The average value of Q_{50} and Q_{80} for batches F1 to F9 is mentioned in Tables 5.4 and Figure 5.5.

5.2.8 Measurement of water uptake by the gel

The water uptake by the gel was determined using a Thermogravimetric Analyzer (TGA-50, Shimadzu, Kyoto, Japan). The in situ gels formed in 40 mL of Sorensen's phosphate buffer were used for this study. At periodic time intervals a portion of the gel was carefully removed. The sample was immediately loaded onto a TGA pan after removal of surface water by an absorbing tissue. The sample was subjected to a controlled temperature program (10 ℃/min). The weight loss (% (w/w)) on heating was measured over 30–200 ℃. Water uptake

of in situ gels containing various cross-linker concentrations and different reaction times was examined over 6 hrs. All studies were carried out in triplicate.

Table 5.3: Results of preliminary trial batches*

Batch No.	Concentration of Sodium alginate (%)	pH	Viscosity (cp)	Drug content (%)	Characteristic of In situ gels
J1	0.25	7.4	90	83.25	Gel is not form properly
J2	0.25	7.4	92	86.22	
J3	0.25	7.3	91	84.55	
J4	0.5	7.1	150	91.92	Gel formation
J5	0.5	7.1	153	93.80	
J6	0.5	7.2	155	92.35	
J7	1	7.0	236	97.87	Gel formation
J8	1	6.9	238	98.98	
J9	1	7.0	235	98.25	
J10	1.5	6.8	331	96.56	Gel formation
J11	1.5	6.7	299	98.11	
J12	1.5	6.8	332	97.12	

*All the batches were prepared using 0.075% (w/v) calcium chloride and 0.25% (w/v) sodium citrate

Table 5.4: 3^2 full factorial design layout*

Batch No.	Variables levels in coded form		Viscosity (cp)	Drug content (%)	% Drug release (Q_{50})	% Drug release (Q_{80})
	X_1	X_2				
F1	-1	-1	98	92.12	98.18	98.18
F2	-1	0	134	93.65	97.66	97.66
F3	-1	+1	155	94.78	91.39	99.23
F4	0	-1	192	95.92	75.76	98.22
F5	0	0	236	98.72	54.81	92.40
F6	0	+1	266	95.54	50.29	81.26
F7	+1	-1	296	96.22	46.17	78.66
F8	+1	0	335	97.95	43.10	75.43
F9	+1	+1	365	95.75	37.71	71.21

Translation of coded levels in actual units

Variables level	Low (-1)	Medium (0)	High (+1)
Concentration of sodium alginate (X_1)	0.5%	1%	1.5%
Concentration of Calcium chloride (X_2)	0.05%	0.075%	0.1%

*All the batches contain 40 mg famotidine, viscosity measured at 150 rpm.

Figure 5.3: ***Gel formation of alginate based in situ gel in simulated gastric fluid (batch F5)***

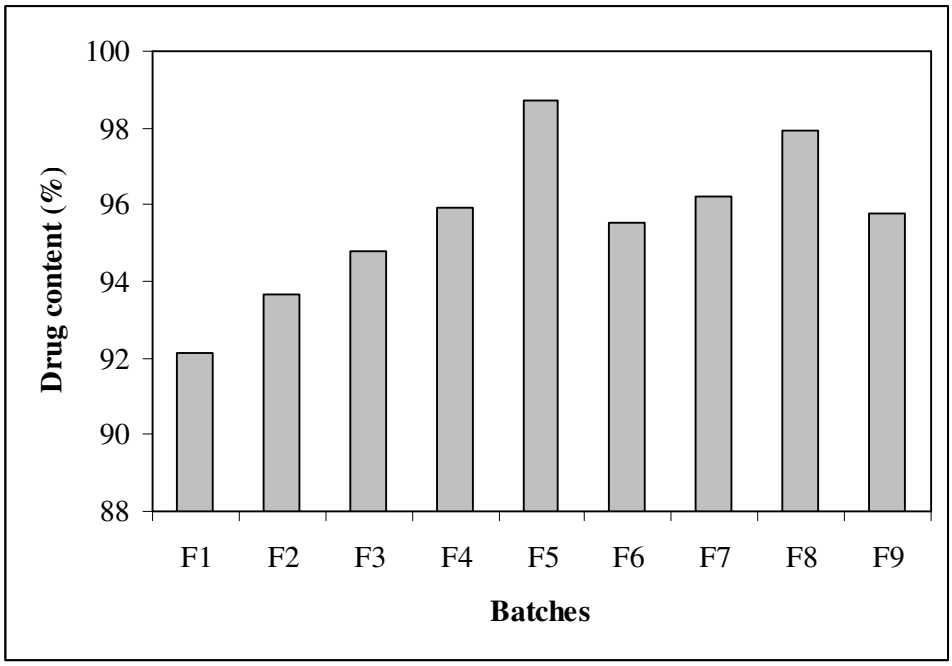

Figure 5.4: ***Drug content of alginate based in situ gel batches F1-F9***

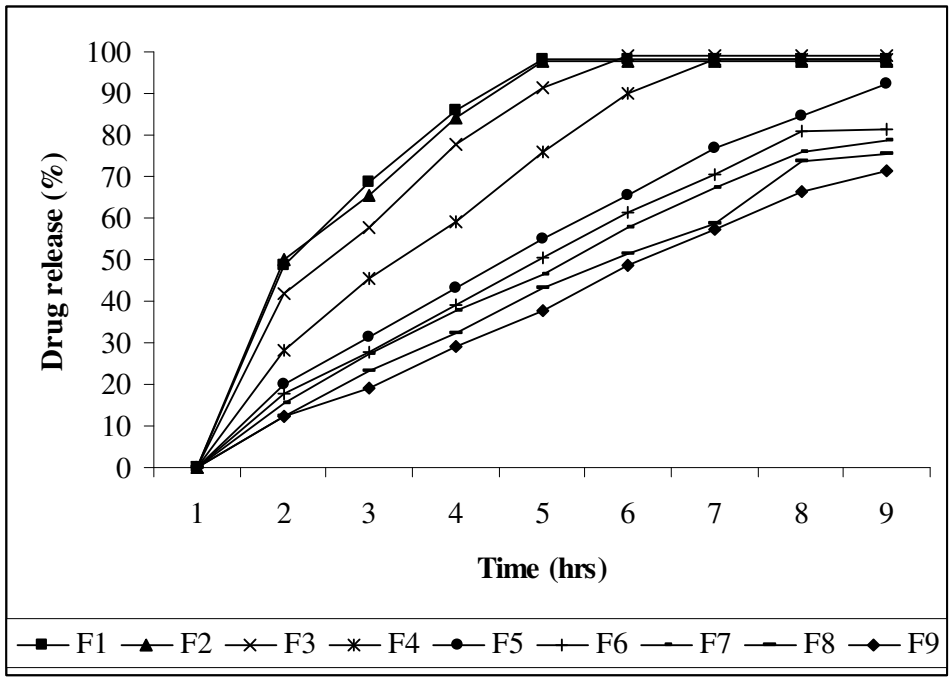

Figure 5.5: ***Percent drug release from in situ gel batches F1-F9***

Table 5.5: Summary of results of regression analysis

Coefficient	B0	B1	B2	B11	B22	B12	R^2
Viscosity	236.381	102.73	34.57	1.14	-2.07	-7.57	0.9996
Drug content (%)	98.02	1.78	0.52	-1.11	-1.87	-1.94	0.8361
Q_{50} (%)	56.84	-30.47	-10.5	-5.23	12.5	5.16	0.9853
Q_{80} (%)	90.11	-13.09	-5.35	0.07	-2.43	0.76	0.9416

5.2.9 Stability studies

Stability studies were carried out on gel formulation according to ICH (International Conference on Harmonization) guidelines. A sufficient quantity of in situ gel in glass bottles was stored in desiccator containing saturated solution of sodium chloride, which gave a relative humidity of 75±5%. The desiccator was placed in a hot air oven maintained at 40±2 ^{o}C and samples were withdrawn at 0, 30, 60, and 90 days. The physical stability of gel was observed periodically the occurrence of turbidity or gelation. The drug content remaining and the viscosity of formulation were measured at predetermined time interval. The results of the stability study for the selected batch of alginate based in situ formulation is given in Figure 5.6.

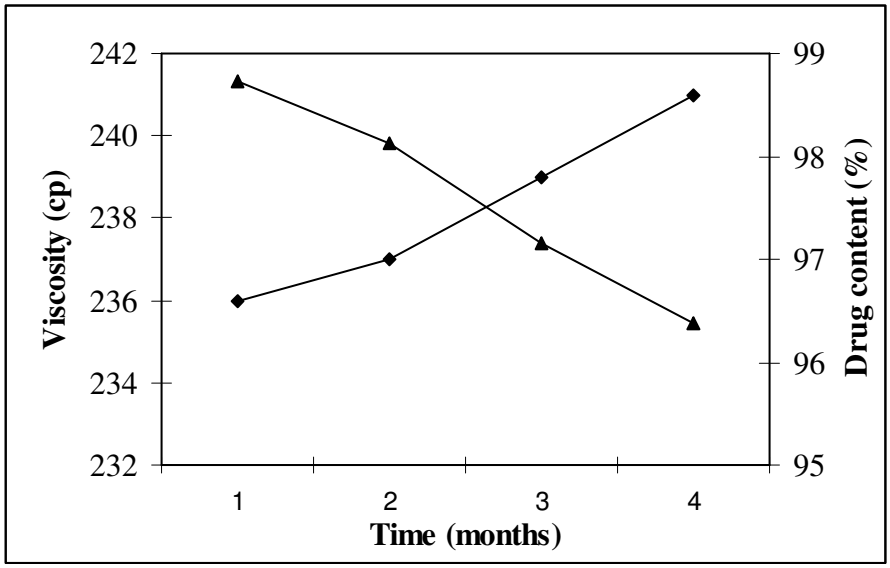

Figure 5.6: ***Stability study of famotidine in situ gel***

5.2.10 Data fitting

An attempt was made to fit the dissolution data in to Zero order[19] release kinetics represented:

$$m = k * t \qquad [5.2]$$

Where, k is zero-order constant, m is the % drug unreleased and t is the time. The plot of % drug unreleased (released) versus time is the linear.

The data was treated with the First order[20] release kinetics to characterize the mechanism of drug release:

$$m = ea * e\text{-}bt \qquad [5.3]$$

Where, a is the intercept and b is the slope. It assumes that the drug molecules, diffuses out through a gel like layer formed around the drug during the dissolution process. A plot of log % drug release versus time is the linear.

The data was treated with the Higuchi [21] model to characterize the mechanism of drug release:

$$m = 100 - q * \text{square root of time} \quad [5.4]$$

Where q is the Higuchi constant (% per square root of time). In this model, a plot of % drug unreleased (released) versus square root of time is linear.

The dissolution data was also analyzed using the Krosmeyer-Peppas model [22] to determine the kinetic of drug release from different batches of in situ gel:

$$M_t/M_\infty = Kpt^n \quad [5.5]$$

Where, M_t/M_∞ represent the fraction of drug released at time t and Kp is the kinetic constant characterizing the polymeric system and n stands for the diffusion exponent.

The results of *F*-statistics were used for the selection of the most appropriate model. Results of summary of results of regression analysis and data fitting are shown in Tables 5.5 and 5.6, respectively. The curve fitting, simulation and plotting was performed in Excel (Microsoft Software Inc., USA) and Sigma plot version 10.0 (Sigma plot software, Jangel Scientific Software, San Rafael, CA). The effects of independent variables on the response parameters were visualized from the contour plots. Numerical optimization using the desirability approach was employed to locate the optimal settings of the formulation variables so as to obtain the desired response [23]. An optimized formulation was developed by setting constraints on the dependent and independent variables. The formulation developed was evaluated for the responses and the experimental values obtained were compared with those predicted by the mathematical models generated. Counter plots showing the effect of the concentration of sodium alginate (X_1) and the concentration of calcium chloride (X_2) on drug content, viscosity, % drug released at 4 hrs (Q_{50}) and 8 hrs (Q_{80}) appear in Figure 5.7.

Table 5.6: Results of models fitting of batches F1-F9				
Batch no.	**Regression**			
	Zero order kinetic	**First order kinetic**	**Higuchi kinetic**	**Krosmeyer peppas**
F1	0.7068	0.4951	0.8299	0.9124
F2	0.7491	0.5110	0.8637	0.9314
F3	0.8128	0.5550	0.9048	0.9505
F4	0.9149	0.9300	0.9651	0.9848
F5	0.9959	0.8790	0.9858	0.9986
F6	0.9841	0.9679	0.9804	0.9929
F7	0.9872	0.9731	0.9879	0.9972
F8	0.9953	0.9680	0.9773	0.9927
F9	0.9953	0.9797	0.9651	0.9812

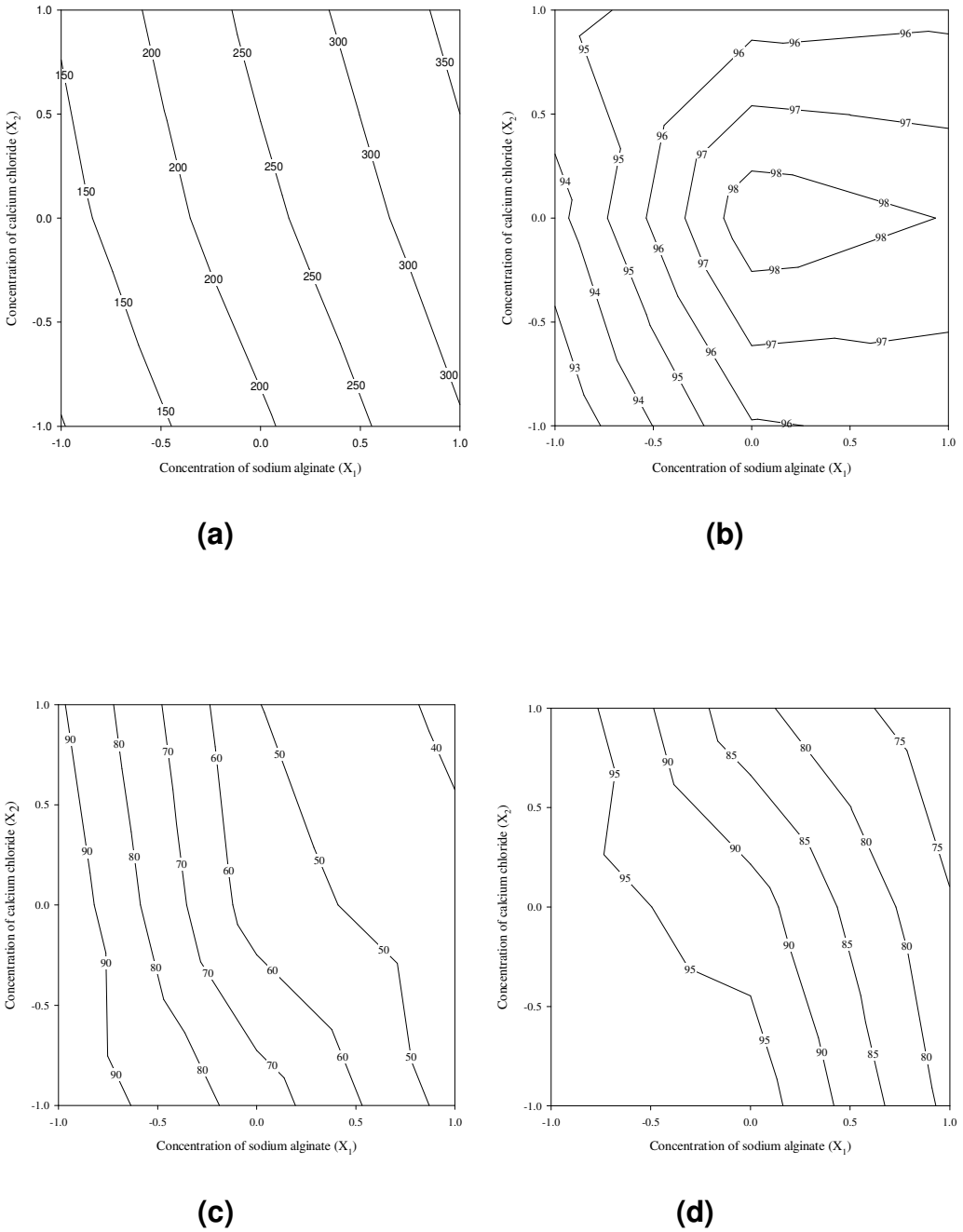

Figure 5.7: ***Counter plots showing the effect of the concentration of sodium alginate (X_1) and the concentration of calcium chloride (X_2) on viscosity (a), drug content (b), Q_{50} (c) and Q_{80} (d)***

5.11 *In-vivo* study

In-vivo evaluation studies for optimized formulation were performed on normal healthy Wistar rats weighing 200-250 gm each as per pyrolus ligation method [24]. The approval of the Institutional Animal Ethics Committee was obtained before starting the study. The study was conducted in accordance with standard intuitional guidelines. Three groups of Wistar rats (5 in each group) that were fasted (with water) at least 24 hrs before experiments were used for the study and divided first group as control, second as control plus immediate treatment and third as treated (in situ famotidine gel). Wistar rats were anaesthetized with ether and a portion of the abdomen was opened by a small midline incision below the xiphoid process. The pylorus portion of the stomach was lifted and ligated. During this process, care was taken to avoid the traction to the pylorus or damage to its blood supply the stomach was closed by interrupted sutures. In first group after 5 hrs the animals were sacrificed and the stomachs were removed, cut along the greater curvature and subjected to measurement of ulcer index. In second group alginate based in situ gel of famotidine were administered orally after 5 hrs of ligation, after 20 min the animals were sacrificed and the stomachs were removed, cut along the greater curvature observed whether gel is form or not and subjected to measurement of ulcer index. And in third group the alginate based in situ gel of famotidine were administered orally 30 min before starting the experiment in 24 hrs fasted rats and after 8 hrs of animals were sacrificed and observed for the effect of drug by counting the ulcer index. The ulcer index was determined using the formula: [24-26] Ulcer index = 10/X, Where X = Total mucosal area/Total ulcerated area. The results of *in-vivo* study are depicted in Figures 5.8 to 5.11.

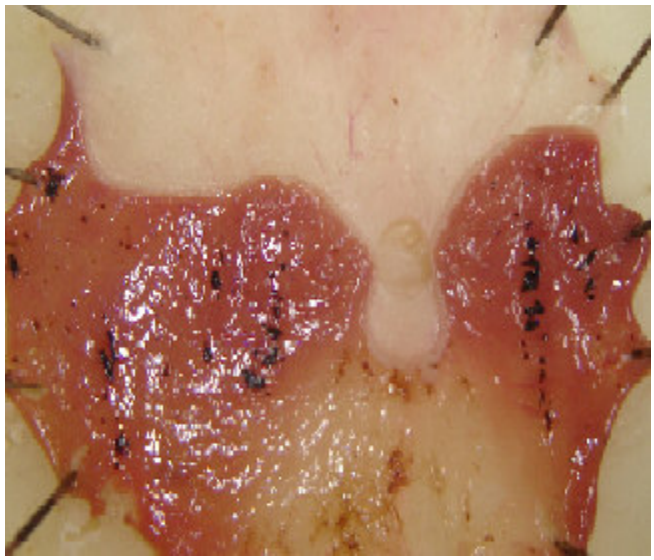

Figure 5.8: ***Group 1 as a control***

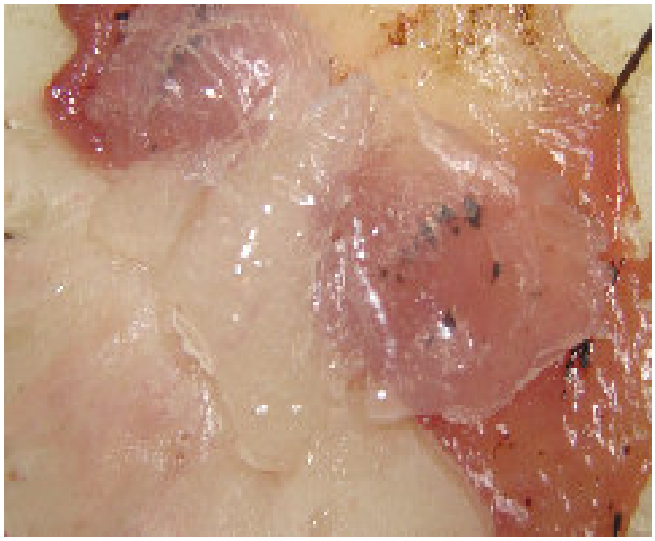

Figure 5.9: ***Group 2 as control + immediate treatment by alginate based in situ gel***

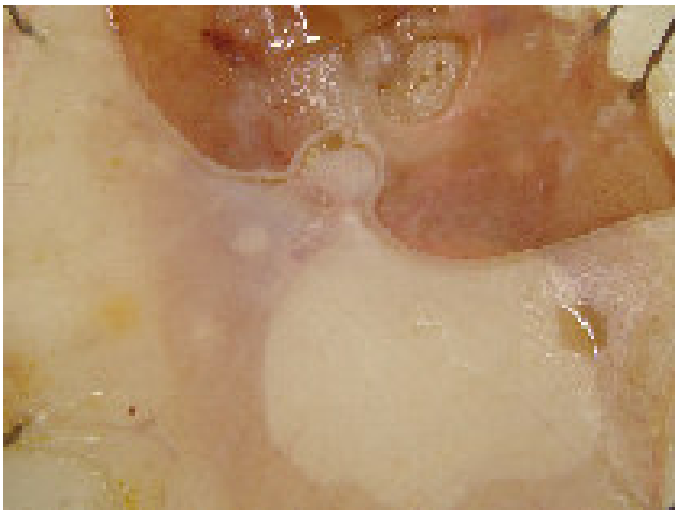

Figure 5.10: ***Group 3 as treated + alginate based in situ gel***

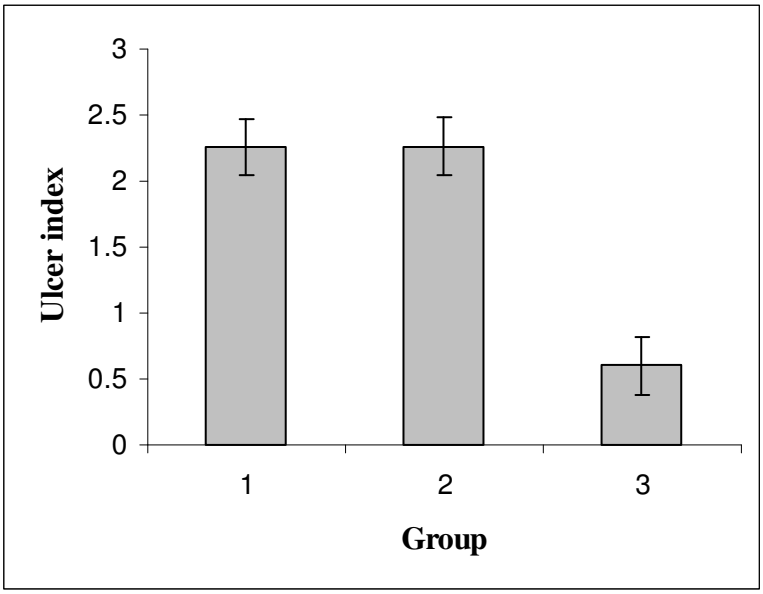

Figure 5.11: ***Ulcer index of alginate based in situ of famotidine***

5.3 Results and Discussion

5.3.1 Preliminary trials

The floating in situ gels of famotidine were prepared by ion activation technique, dissolving varying concentrations of alginate in deionized water containing sodium citrate, to which varying concentrations of drug and calcium chloride was added. In preliminary trial batches of J1 to J12 (Table 5.3) were prepared using different concentration of sodium alginate to see the effect on the viscosity of the solution, drug content, pH and the physical properties of the gel in simulated gastric fluid (pH 1.2). The concentration of sodium alginate was varied from 0.25 to 1.5 % w/v. In the batches J1 to J3 (0.25 % w/v) improper gelation was observed which leads the rapid flow of the formulation. Also the time required for gelation and drug content was very low. Batches J4 to J6 prepared using 0.5 % w/v of sodium alginate the gelation, time required for gelation and drug content were slightly better than batches J1 to J3. While in the batches J7 to J12 all the characteristics of the gels were good but, in the batches of J10 to J12 the viscosity of the solutions were very high because of the higher concentration of sodium alginate which was difficult to pour while it was not observed in batches J7 to J9. Thus, we can conclude that 1 % w/v sodium alginate was the optimum concentration. The concentration of sodium citrate was constant in all the batches (0.25 % w/v) and observed no significant effect.

On the basis of the preliminary trials in the present study a 3^2 full factorial design was employed to study the effect of independent variables, i.e. concentration of sodium alginate (X_1) and concentration of calcium chloride (X_2) on dependent variables viscosity, drug content, drug released at 4 hrs (Q_{50}) and 8 hrs (Q_{80}). The results summarized in Table 5.4 clearly indicate that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the nine batches (F1 to F9). Fitted equations (full models) relating the responses i.e. viscosity, drug content, Q_{50} and Q_{80} to the transformed factor are shown in Table 5.5. The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The high values of

correlation coefficient (Table 5.5) for the dependent variables indicate a good fit. The equation may be used to obtain estimate of the response because small error of variance was noticed in the replicates.

5.3.2 Factorial equation for viscosity

The viscosity is an important variable because it affects the gelation of the solutions, the flow of the formulation and time required for the gelation. The viscosity is dependent on the concentrations of the polymer and calcium chloride. The linear model generated for viscosity was found to be significant with an *F*-value of 590.35 ($p<0.0001$) and R^2 value of 0.9993:

$$\text{Viscosity (cp)} = 236.381+102.73X_1+34.57X_2+1.14X_1X_2 - 2.07X_1^2 -7.57X_2^2 \quad [5.6]$$

The counter plot (Figure 5.7a) shows that the viscosity of solution increased from 98 to 155 cp and 296 to 365 cp at lower and higher levels of concentration of sodium alginate, respectively, as concentration of calcium chloride increased. The results of the equation indicate that the effect of X_1 (concentration of sodium alginate) is more significant than X_2 (concentration of calcium chloride). Moreover, amount of calcium chloride had a positive effect on the viscosity, i.e. as the volume of cross-linking agent increase, the viscosity increases.

5.3.3 Factorial equation for drug content

The linear model generated for drug content was found to be significant with an *F*-value of 2.041 ($p<0.0001$) and R^2 value of 0.8361:

$$\text{Drug content} =98.02+1.78X_1+0.52X_2-1.11X_1X_2 - 1.87X_1^2 -1.94X_2^2 \quad [5.7]$$

The counter plot (Figure 5.7b) shows that the drug content increased from 92.12 to 94.78 % at lower levels of concentration of sodium alginate and decreased from 97.75 to 96.22 % at higher levels of concentration of sodium alginate as concentration of calcium chloride increased. The results of the equation indicated that both the concentration of the X_1 and X_2 were responsible for the drug content of the in situ formulations but the effect of X_1 (concentration of sodium alginate) is

more significant than X_2 (concentration of calcium chloride), the effect of the X_2 was very less so, it was considered non significant compared to the concentration of sodium alginate.

5.3.4 Factorial equation for Q_{50}

The amount of drug released in an important parameter for sustained release action of the in situ gel of famotidine. The linear model generated for drug released at 4 hrs was found to be significant with an *F*-value of 26.97 ($p<0.005$) and R^2 value of 0.9853:

$$Q_{50} = 56.84 - 30.47X_1 - 10.55X_2 + 5.23X_1X_2 + 12.517X_1^2 + 5.16X_2^2 \quad [5.8]$$

The counter plot (Figure 5.7c) shows that the drug release at 4 hrs (Q_{50}) decreased from 98.18 to 91.39 at lower and 46.17 to 37.71 at higher levels of concentration of sodium alginate, respectively, as concentration of calcium chloride increased. The results depicted in Table 5.4 indicate that the percentage drug released in vitro is highly depended on the concentration of sodium alginate (X_1) and the concentration of calcium chloride (X_2). The concentration of calcium chloride (X_2) has a negative effect on $Q_{50,}$ while the concentration of sodium alginate had lower effect on $Q_{50.}$

5.3.5 Factorial equation for Q_{80}

The linear model generated for drug released at 8 hrs found to be significant with an *F*-value of 6.45 ($p<0.005$) and R^2 value of 0.9416:

$$Q_{80} = 90.11 - 13.09X_1 - 5.35X_2 + 0.07X_1X_2 - 2.43X_1^2 + 0.76X_2^2 \quad [5.9]$$

The counter plot (Figure 5.7d) shows that the drug release at 8 hrs (Q_{80}) increased from 98.18 to 99.23 at lower and decreased from 78.66 to 71.21 at higher levels of concentration of sodium alginate, respectively, as concentration of calcium chloride increased. The results depicted in Table 5.5 indicate that the percentage drug released in vitro is highly depended on the concentration of sodium alginate (X_1) and the concentration of calcium chloride (X_2). The

concentration of calcium chloride (X_2) has a negative effect on $Q_{80,}$ while the concentration of sodium alginate had lower effect on $Q_{80.}$

5.3.6 Release mechanism

Release of the drug from a polymeric matrix depends on the amount of water associated with the system. The release of the drug may involve the penetration of water into the matrix and simultaneous release of the drug via diffusion or dissolution as governed by Ficks law [27-28]. The results of curve fitting of factorial batches into different mathematical models are given in Table 5.6. The mechanism of drug release from the in situ gel was found to be diffusion controlled because plots of percentage cumulative drug release vs square root of time were found to be linear with the regression coefficient (R^2) values ranging from 0.9124–0.9986 for the factorial batches. The release profile of batch F5 fitted to Korsmeyer-Peppas equation, *F*-value was found to be 10.16. The value of correlation coefficient was found to be 0.9986. The values of slope and intercept were found to be 0.8621 and -1.42, respectively. The results of *F*-statistics were used for the selection of the most appropriate model, thus it was concluded that the release profile fitted best to Korsmeyer-Peppas equation (*F*=10.16).

5.3.7 Optimized batch

A numerical optimization technique using the desirability approach was employed to develop a new formulation with the desired responses. Constraints like maximizing drug content, minimizing the viscosity and release at the end of 10 hrs in addition to manimizing the Q_{50} and Q_{80} were to set as goals to locate the optimum setting of independent variables in the new formulation. The optimized in situ gel formulation (J10) was developed using a 0.75 % w/v of sodium alginate and 0.0625 % w/v of calcium chloride. The optimized formulation was evaluated for percentage viscosity, drug content, Q_{50} and Q_{80}. The results of experimentally observed responses and those predicted errors for the response parameters ranged between 0.46-1.95 percent, with the value of absolute error of 1.25±0.56 %. The low value of error indicates the high prognostic ability of factorial equation and counter plot methodology. The drug content from the

optimized formulation was found to be low 96.5% and viscosity of 208 cp, thus batch F5 was selected for further study, which exhibited a high drug content of 98.72 and the viscosity of 236 cp which is easy for swallowing and good ability for gelation immediately after oral administration.

The water associated with the formulation at any point in time in the release medium was studied by TGA. The percentage of weight loss was thought to be due to water loss during heating. TGA was also used to study the effect of cross-linking on water uptake by the gels. The result of the water uptake by the sodium alginate based in situ gel of famotidine at 8 hrs was 71.72 % and the good correlation coefficient (0.9983). There was a sudden increase in water uptake followed by a decrease. This decrease is particularly prominent for gels without cross-linker and has been observed in lower concentrations of cross-linker. This decrease in water uptake can be explained by the collapsing of gels with time. There was also a decrease in water uptake by the gels with cross-linker. The formation of cross-linked networks provided an additional barrier to water penetration. As the concentration of the cross-linker in the delivery system increased, the time taken to reach maximum water uptake increased. At a higher cross-linker concentration the collapsing of the gel was negligible compared to gels without a cross-linker.

Based on visual identification, the in situ gel has remained as liquid for a period of 3 months without the occurrence of turbidity or gelation at 40±2 ^{o}C. As illustrated in Figure 4, the viscosity of the gel slightly changed from 236 cp at 0 month to 241 cp at the 3rd month. The samples also were analyzed for famotidine content by spectrophotometer. The results showed that about 2.32 % content decrease was found when the in situ gel was kept at 40±2 ^{o}C for 3 months. Since the overall degradation is <5%, a tentative shelf life of 2 years may be assigned to the formulation.

5.3.8 Results of *in-vivo* study

The *in-vivo* study was carried out by pylorus ligation method in rats to see whether the gel was formed or not in the stomach of the rats and also checked the effect of the drug by counting the ulcer index. Results of group 1 showed

ulcer (Figure 5.8) while results of group 2 showed gel was formed but the ulcers was also identified (Figure 5.9). Results of group 3 showed gel was after 5 hrs of the treatment and an ulcer was reduced (Figure 5.10). The gel formation was checked in collected gastric juice of the rats and results showed immediately formation of gel in gastric juice of the rats. The ulcer index of group 1, 2 and 3 were 2.25, 2.26 and 0.5995, respectively (Figure 5.11). Thus we concluded that the gel formation in the stomach of the rats and significant reduction of ulcers were also observed.

5.4 Conclusion

This study reports that oral administration of aqueous solutions of famotidine containing sodium alginate results in formation of in situ gel at the stomach site. The results of a 3^2 full factorial design revealed that the concentration of sodium alginate and concentration of calcium chloride significantly affected on the dependent variables like viscosity, drug content, Q_{50} and Q_{80}. The *in-vivo* study demonstrated the excellent gel formation in the stomach of the rat and significant anti-ulcer effect of alginate based in situ gel of famotidine over longer period of time.

5.5 References

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Chapter 6

Formulation and evaluation of glipizide floating-bioadhesive tablets

6.1 Aim of present investigation

The earliest studies in the field of modified drug delivery date back to the 1950s. Since then, a large number of drug products, mainly in the form of tablet and capsule with controlled release characteristics, have been introduced. Das and Das predicted a minimum growth of 9% per year for this market through 2008[1]. This incredible growth can be attributed to several advantages that these products offer, including improved patient compliance, better therapeutic efficiency, potential for cost saving and patentability, and opportunity for extending product life-cycle.

Oral sustained-release technology provides oral delivery for 24 hr; however, in substances that cannot be well absorbed throughout the whole gastrointestinal tract, it may be disadvantageous[2]. Extended-release dosage forms with prolonged residence times in the stomach are highly desirable for drugs with narrow absorption windows, stability problems in the intestinal or colonic environments, locally acting in the stomach, and poor solubility in the intestine[3]. Recent approaches to increase the gastric residence time of drug delivery systems include bioadhesive devices[4-6], swelling devices that increase their size[7,8], low density devices[3,8], floating systems[9,10], high density systems[11,12], magnetic systems, unfoldable and expandable systems, magnetic systems, superporous, biodegradable hydrogel systems[13] and microparticulate systems[6].

The otherwise-excellent concept of floating system suffers from a disadvantage that it is effective only when the fluid level in the stomach is sufficient high; however, as the stomach empties and the tablet is at the pylorus, the buoyancy of the dosage form may be impeded[14]. This serious limitation can be achieved by coupling bioadhesion characteristics to dosage form and developing bioadhesive tablets. Floating and bioadhesive drug delivery systems have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site[15,16]. The various buoyant preparations include microballoons, microspheres, granules, powders, gel, capsules, tablets, and laminated films[13]. Based on the mechanism of buoyancy,

two distinctly different technologies, i.e., noneffervescent and effervescent systems have been utilized in the development of floating systems:

1. Noneffervescent systems that use commonly gel-forming or highly swellable cellulose-type hydrocolloids, polysaccharides, and matrix forming polymers such as polycarbonate, polyacrylate, polymethacrylate, and polystyrene[13].

2. Effervescent systems that utilize matrices prepared with swellable polymers such as HPMC or chitosan and effervescent compounds, e.g., sodium bicarbonate and citric or tartaric acid[17] or matrices containing chambers of liquid that gasify at body temperature[18].

Matrix tablets based on hydroxypropyl methylcellulose (HPMC K4M) have been developed by Li et al[19,20]. Natural gums in combination with HPMC also have been evaluated for gel-forming properties[10]. Microparticulate systems using natural polymers have been evaluated for stomach specific drug delivery of glipizide[21]. Different mass transport processes may occur during drug release from polymer-based matrix tablets, including water imbibition into the system, polymer swelling, drug dissolution, drug diffusion out of tablet, and polymer dissolution[22].

Glipizide is a second-generation sulfonylurea that can acutely lower the blood glucose level in humans by stimulating the release of insulin from the pancreas and is typically prescribed to treat type II diabetes (non-insulin-dependent diabetes mellitus). Its short biological half-life (3.4 ± 0.7 hr) necessitates that it be administered in 2 or 3 doses of 2.5 to 10 mg per day[23]. Glipizide is available in a Gastrointestinal Therapeutic System (GITS) extended-release formulation. Glipizide GITS provides more stable plasma drug concentrations than the immediate-release formulation and the once-daily regimen may optimize patient compliance. In patients with type II diabetes mellitus, glipizide GITS is at least as effective as the immediate-release formulation of glipizide in providing glycaemic control, and may have a greater effect on fasting plasma glucose levels. Any therapeutic advantage over other antidiabetic agents remains to be established, but in a preliminary report (n = 40) glipizide GITS provided better glycaemic control and produced less fasting insulinaemia than glibenclamide (glyburide) [23].

Menger et al[24] compared the pharmacokinetic and short-term pharmacodynamic profile of extended-release glipizide (GITS) given with that of immediate-release glipizide in patients with type II diabetes mellitus. At steady state, the mean C_{max} after immediate-release glipizide was significantly greater than after glipizide GITS, and the t_{max} was considerably shorter. Although the mean C_{min} with glipizide GITS was about 80% higher than with immediate-release glipizide, the mean AUC 0-24 was significantly lower. Despite the lower plasma concentrations with glipizide GITS in this short-term study, the two formulations had similar effects on serum concentrations of glucose, insulin, and C-peptide. The absence of a pronounced peak plasma concentration with the GITS formulation might confer advantages in terms of maintaining clinical effectiveness and reducing the potential to cause adverse effects. Thus, the development of controlled/extended release dosage forms of glipizide would clearly be advantageous. Researchers have formulated oral controlled-release products of glipizide by various techniques[21,25,26] .

The hypothesis for this research work is that if glipizide can be delivered in a controlled manner to the duodenum at a rate that does not exceed the maximum rate of its absorption, then the oral bioavailability of glipizide could be improved. Based on this hypothesis, the gastric floating and bioadhesive tablets were designed in such a way that they should be retained in the stomach for a prolonged period of time, thus maximizing the exposure of this drug to its absorption site.

6.2 Experimental

6.2.1 Preparation of standard curve of glipizide

Glipizide (50 mg) was dissolved in 1 mL phosphate buffer (pH 7.4) and volume was made upto 50 mL volumetric flask using phosphate buffer (pH 7.4). Five microliters of stock solution (1 mg/mL) was further diluted with phosphate buffer (pH 7.4) to 50 mL. This solution (100 µg/mL) was further diluted to phosphate buffer (pH 7.4) to obtain solutions of 5 to 50 µg/mL. Absorption of each solution was measured at 276 nm using Shimadzu UV-1601 UV/Vis double beam spectrophotometer and phosphate buffer (pH 7.4) as a reference standard. The method was validated for linearity, accuracy and precision. The method was validated for linearity, accuracy and precision. The method obeys Beer-Lambert's Law in the concentration range of 5-50 µg/mL. When a standard drug solution was analysed repeatedly (n=5) the mean error (accuracy) and relative standard deviation (precision) were found to be 0.8 % and 1.3 % respectively. The results of standard curve preparation are shown in Table 6.1 and Figure 6.1.

Table 6.1: Standard curve of glipizide in phosphate buffer pH (7.4) at 276 nm					
Sr. No.	**Concentration (µ/mL)**	**Absorbance**			**Average Absorbance**
		1	**2**	**3**	
1	0	0	0	0	0
2	5	0.120	0.122	0.121	0.121
3	10	0.222	0.220	0.221	0.221
4	15	0.350	0.346	0.348	0.348
5	20	0.467	0.465	0.463	0.465
5	25	0.544	0.544	0.543	0.544
6	30	0.699	0.697	0.695	0.697
7	35	0.760	0.762	0.761	0.761
8	40	0.890	0.891	0.891	0.891
Correlation Coefficient = 0.9979 Average Absorption = 0.0218 x Concentration + 0.0147					

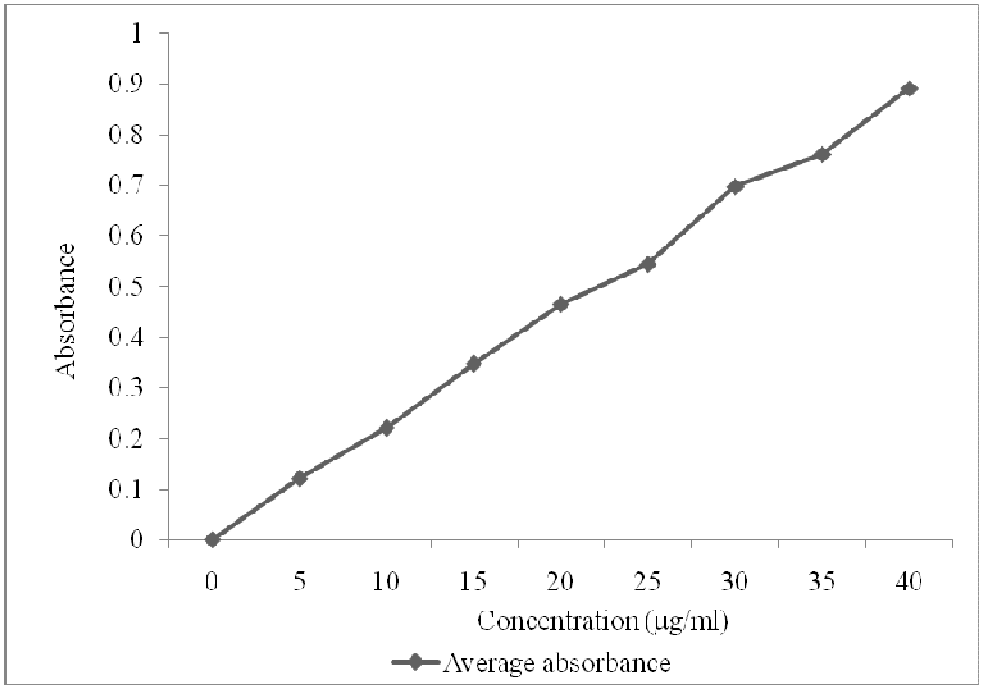


Figure 6.1: ***Standard curve of glipizide in phosphate buffer (pH 7.4)***

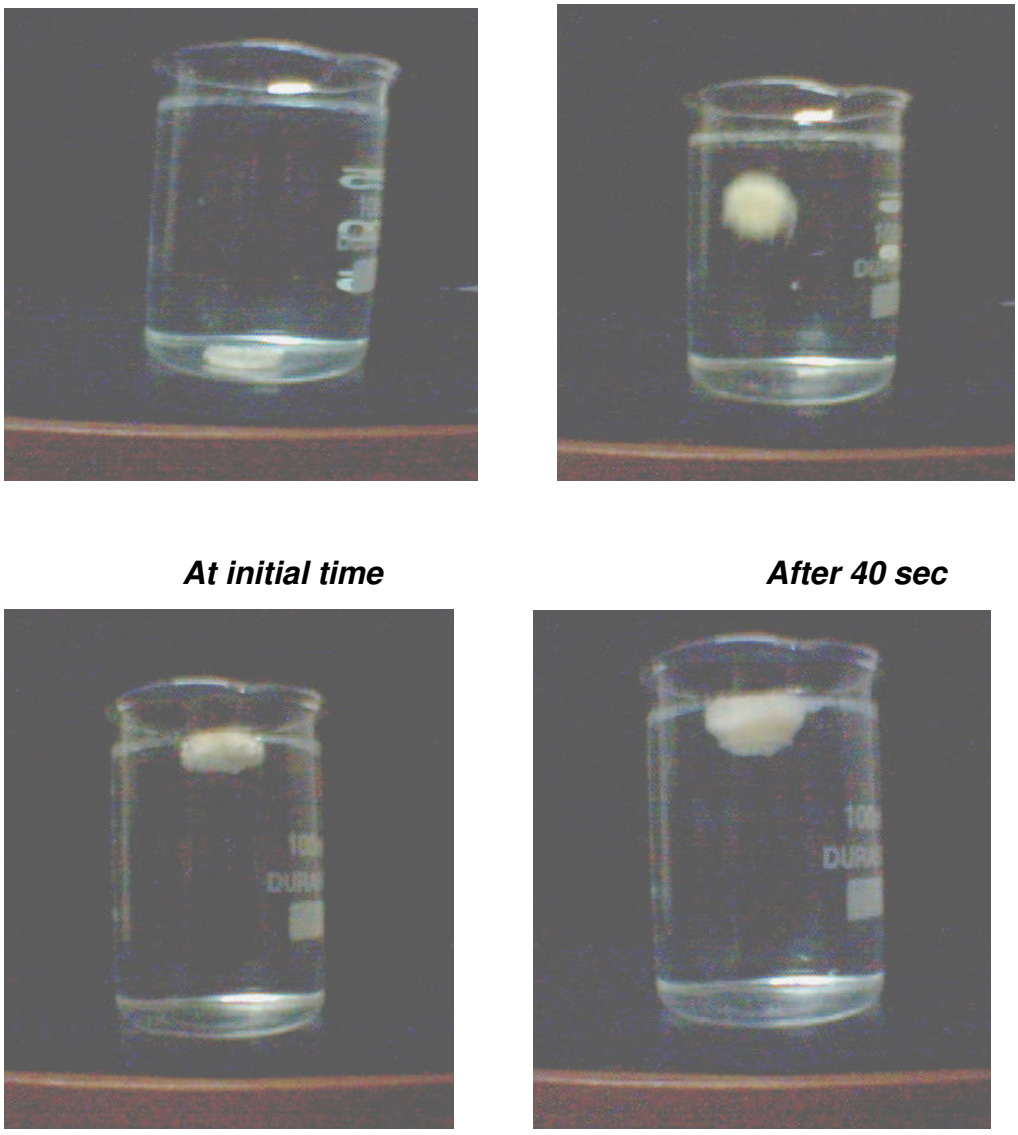
6.2.2 Preparation of bioadhesive and floating tablets

In the tablet formulation, HPMC/CH or CP934/PMA was used as bioadhesive agents. These polymers produce gel-forming matrices and, in contact with gastric fluid, possess sufficient structure to form a gel layer and achieve an overall specific gravity lower than that of gastric fluid. Citric acid and sodium bicarbonate were used as effervescent base to generate carbon dioxide and to enhance the buoyancy of the tablets. All powders except magnesium stearate were sieved through sieve of mesh size 20. The components of the formulation were mixed for 20 min in a cubic mixer. Magnesium stearate (60-mesh sieved) was added into powder blend as a lubricant and mixed for an additional 3 min before compaction process. Then 200 mg tablets containing 10 mg glipizide prepared via a lab press (Cadmach, India) under a pressure of 50 kg/cm^2 using two flat face punches with a 7.9-mm diameter. The tablet formulations are shown in Table 6.2.

6.2.3 Floating behavior of tablets

The in-vitro floating behavior of the tablets was studied in 500 mL preheated 0.1NHCl (pH 1.2, 37^{o}C, no enzyme) and stirred at 50 rpm with a paddle (USP paddle method). The floating lag times (time period between placing the tablet in the medium and tablet floating) and floating durations of the tablets were determined by visual observation. The results of the in-vitro buoyancy study of batch JE10H20CH80 are shown in Figure 6.2.

Table 6.2: Ingredients in mg of floating-bioadhesive tablets of glipizide						
Formulation code	**Sodium bicarbonate**	**Citric acid**	**Hydroxypropyl methylcellulose**	**Chitosan**	**Polymethacrylic acid**	**Carbopol P934**
JE5H100	9.5	9.5	169	0	-	-
JE5H80CH20	9.5	9.5	132	37	-	-
JE5H60CH40	9.5	9.5	113	56	-	-
JE5H40CH60	9.5	9.5	56	113	-	-
JE5H20CH80	9.5	9.5	37	132	-	-
JE5CH100	9.5	9.5	0	169	-	-
JE10H100	18.5	18.5	151	0	-	-
JE10H80CH20	18.5	18.5	121	30	-	-
JE10H60CH40	18.5	18.5	90	61	-	-
JE10H40CH60	18.5	18.5	61	90	-	-
JE10H20CH80	18.5	18.5	30	121	-	-
JE10CH100	18.5	18.5	0	151	-	-
JE10P100	18.5	18.5	-	-	151	0
JE10P80CP20	18.5	18.5	-	-	121	30
JE10P60CP40	18.5	18.5	-	-	90	61
JE10P40CP60	18.5	18.5	-	-	61	90
JE10P20CP80	18.5	18.5	-	-	30	121
JE10CP100	18.5	18.5	-	-	0	151
All tablets contain 10 mg glipizide and 2 mg magnesium stearate as lubricant						



At initial time ***After 40 sec***

After 45 sec ***After 15 hr***

Figure 6.2: ***In-vitro buoyancy studies of batch JE10H20CH80***

6.2.4 Measurement of bioadhesive strength of the tablets

Bioadhesive strength of the prepared tablets was measured on a modified physical balance[27,28]. Albino rat stomach was used as the membrane and isotonic phosphate buffer (IPB) pH 6.6 was used as the moistening fluid. The stomach membrane was excised by removing the underlying tissues. It was washed thoroughly with isotonic phosphate buffer (IPB) pH6.6 and then tied over the protrusion in the Teflon block using a thread. The block was lowered into the glass container filled with IPB pH 6.6 at 37±2℃ such that the buffer just touched the sides of the stomach membrane. The two sides of the balance were made equal, before the study, by keeping 5.0 gm weight on the right pan. The glass container was kept below the left hand side of the balance. The tablet was stuck onto the lower side of the hanging Teflon cylinder using either a little moisture or a double sided tape. The surface of the stomach membrane was blotted with a whattman filter paper and 25 µL of IPB pH 6.6 was added to the stomach surface. This was done in order to obtain reproducible results. The 5.0 gm weight from the right pan was removed. This lowered the Teflon cylinder along the patch over the stomach membrane with a weight of 5.0 gm. This was kept undisturbed for 2.0 min. Then the weights on the right hand side were slowly added in increments of 0.5 gm till the tablet just separated from the stomach membrane surface. The excess weight on the right pan, that is, total weight minus 5.0 gm was taken as a measure of the bioadhesive strength. The equipment was located in an air-conditioned room at 22^{o}C and 60% relative humidity.

6.2.5 Density measurements

The apparent densities of the tablets were calculated from their volumes and masses in triplicate. The volumes V of the cylindrical tablets were calculated from their heights h and radius r (both determined with a micrometer gauge) using the mathematical equation for a cylinder ($V = \pi \times r^2 \times h$). The tablets with ~1 g/cm^3 density or less were chosen for further studies[14].

6.2.6 Drug release study

The drug-release study was carried out using a USP XXIV basket apparatus (Electrolab, TDT-06T, India) at 37°C ± 0.5°C and at 50 rpm using 250 mL of phosphate buffer (pH 7.4) as a dissolution medium (n = 5) as per the USP XXVI dissolution test prescribed for glipizide extended-release tablets (USP, 2003). Floating-bioadhesive tablets of glipizide (10 mg) were used for the test. A 5-mL sample solution was withdrawn at predetermined time intervals, filtered through a 0.45-micrometer membrane filter, diluted suitably, and analyzed spectrophotometrically. An equal amount of fresh dissolution medium was replaced immediately following withdrawal of the test sample. The percentage of drug dissolved at different time intervals was calculated using the Lambert-Beer's equation (Average Absorption = 0.0218 x Concentration + 0.0147, R^2 = 0.9979).

6.2.7 Data fitting

Dissolution efficiency (DE)[29] after 8 hr of release test was used to compare the results of dissolution tests of different formulations:

$$DE_8\% = \frac{\int_0^t y\,dt}{y_{100}t} \times 100 \qquad [6.1]$$

The other dissolution parameter used for comparing the different formulations was mean dissolution time or MDT that is calculated from the amount of drug released to the total cumulative drug. MDT is a measure of the rate of the dissolution process: the higher the MDT, the slower the release rate. The following equation was used to calculate the MDT from the mean dissolution data:

$$MDT = \frac{\sum_{i=1}^{i=n} t_{mid} \times \Delta M}{\sum_{i=1}^{i=n} \Delta M} \qquad [6.2]$$

Where i is the dissolution sample number, n is the number of dissolution sample time, t_{mid} is the time at the midpoint between i and i – 1, and ΔM is the additional amount of drug dissolved between i and i – 1 [30].

6.3 Results

Table 6.3 shows the results of floating time and density of tablets. As this table shows increasing the effervescent base of tablets from 5% to 10% significantly lowers the lag time of floating from about 105 sec to 45 sec. All the batches showed good in-vitro buoyancy. The results of the in-vitro buoyancy study of batch JE10H20CH80 are shown in Figure 6.2. The figure clearly indicates the floating lag time (45 sec) of the glipizide tablets and the floating and swelling tendency of the formulation. The tablet swelled radially and axially. The figure also indicates that the tablet remained buoyant for 15 hr, but the tablet actually floated throughout the entire study. The in-vitro buoyancy study was also conducted at an elevated pH condition (~4.5). The floating tendency remained unaltered at higher pH. In all studied formulations the density was ~1 or less than 1 g/cm^3.

Table 6.3: Physical properties of floating-bioadhesive tablets of glipizide (n=3)

Formulation code	Density (g/cm^3)	Floating lag-time (sec)	Floating duration (hr)
JE5H100	0.982 ± 0.016	105.0 ± 5.4	23.5 ± 1.0
JE5H80CH20	1.078 ± 0.023	105.0 ± 8.2	24.0 ± 1.5
JE5H60CH40	1.011 ± 0.043	75.4 ± 3.7	23.5 ± 1.5
JE5H40CH60	1.103 ± 0.076	87.2 ± 11.4	24.0 ± 1.5
JE5H20CH80	0.992 ± 0.026	84.4 ± 12.4	23.5 ± 2.0
JE5CH100	1.102 ± 0.065	89.5 ± 4.6	24.5 ± 2.5
JE10H100	0.970 ± 0.058	47.8 ± 5.4	24.0 ± 2.0
JE10H80CH20	1.067 ± 0.098	49.2 ± 10.0	23.5 ± 2.5
JE10H60CH40	1.072 ± 0.097	54.0 ± 11.1	23.5 ± 2.0
JE10H40CH60	1.042 ± 0.145	53.1 ± 9.6	24.0 ± 2.5
JE10H20CH80	1.045 ± 0.078	45.9 ± 3.2	23.0 ± 1.0
JE10CH100	1.045 ± 0.098	52.5 ± 4.4	23.0 ± 1.0
JE10P100	1.013 ± 0.058	51.7± 9.0	23.0 ± 3.0
JE10P80CP20	1.065 ± 0.065	51.0 ± 8.9	24.5 ± 2.5
JE10P60CP40	1.045 ± 0.043	53.2 ± 11.2	23.5 ± 2.5
JE10P40CP60	0.995 ± 0.057	54.0 ± 8.6	23.0 ± 2.0
JE10P20CP80	0.976 ± 0.104	45.8 ± 4.6	23.5 ± 3.0
JE10CP100	1.042 ± 0.097	52.0 ± 10.3	24.0 ± 1.0

The results of bioadhesion studies are shown in Figure 6.3. Tablets with 5% or 10% effervescent base in a matrix of HPMC/CH and 10% effervescent base in matrix of CP/PMA are compared for the bioadhesion in this figure.

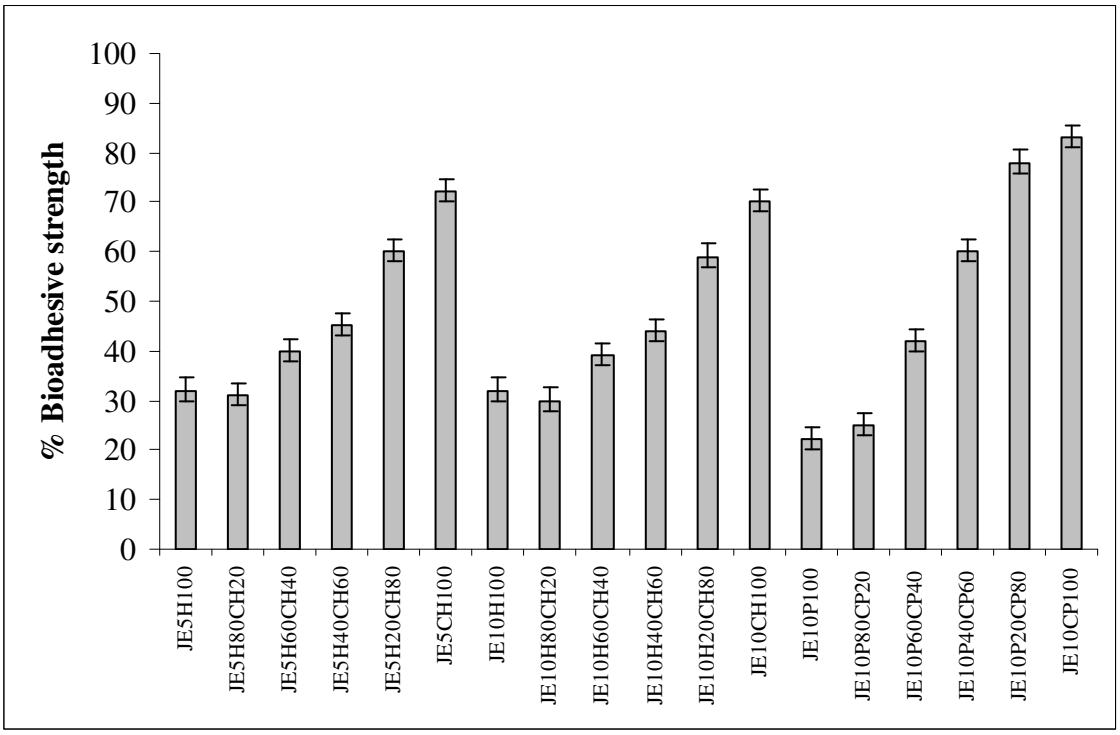


Figure 6.3: ***Bioadhesive strength of different floating-bioadhesive tablets of glipizide***

Figures 6.4 and 6.5 show the effect of different ratios of HPMC and CH in tablets with two different percentages of effervescent base on drug release profiles.

Table 6.4: Glipizide release profiles in phosphate buffer solution (pH 7.4) from floating-bioadhesive tablets containing 5 % effervescent base and HPMC/CH bland

Batch Time (hr)	JE5H100	JE5H20CH80	JE5H80CH20	JE5H60CH40	JE5H40CH60	JE5CH100
0	0	0	0	0	0	0
1	14	12	5	8	10	11
2	32	32	15	17	20	25
3	38	35	20	24	26	30
4	51	45	25	32	37	36
5	59	50	30	41	45	41
6	64	55	35	42	46	46
7	68	61	39	45	51	54
8	70	65	43	51	57	58
9	72	67	47	55	58	64
10	74	69	52	61	65	68

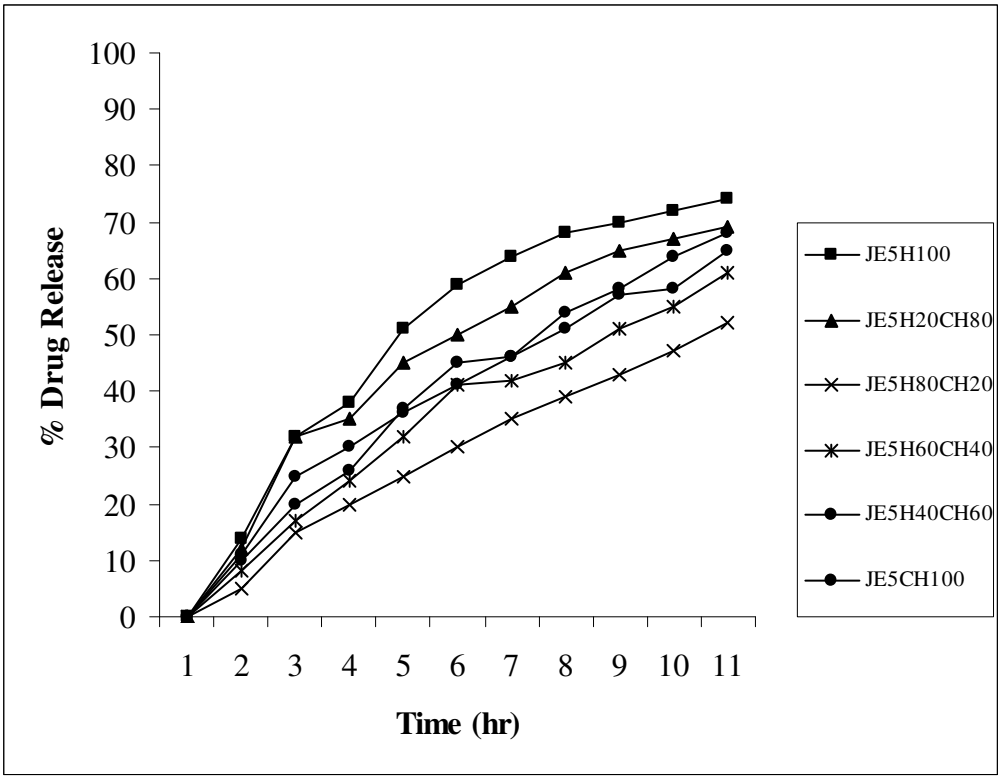


Figure 6.4: ***Glipizide release profiles in phosphate buffer solution (pH 7.4) from floating-bioadhesive tablets containing 5 % effervescent base and HPMC/CH bland***

Table 6.5: Glipizide release profiles in phosphate buffer solution (pH 7.4) from floating-bioadhesive tablets containing 10 % effervescent base and HPMC/CH bland

Batch Time (hr)	JE10H100	JE10H20 CH80	JE10H80 CH20	JE10H60 CH40	JE10H40 CH60	JE10 CH100
0	0	0	0	0	0	0
1	21	18	10	14	14	15
2	41	35	20	23	25	28
3	45	40	25	26	31	34
4	53	48	32	38	39	39
5	60	52	34	45	49	45
6	65	57	36	48	51	49
7	69	61	41	49	54	55
8	71	65	43	52	58	59
9	72	68	52	57	59	65
10	75	69	55	62	66	70

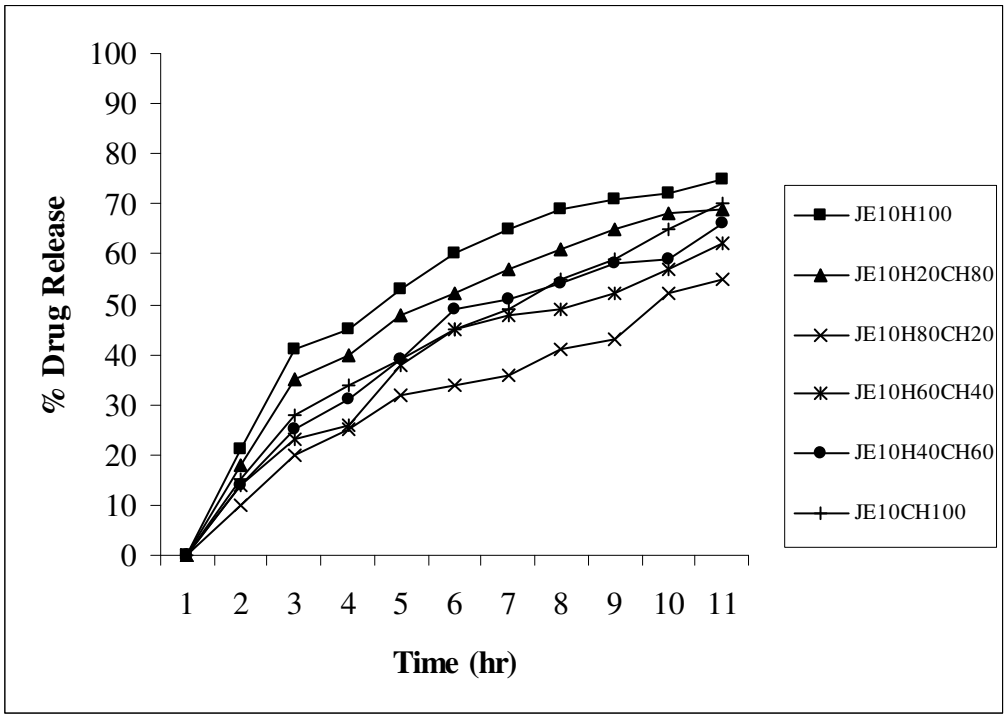


Figure 6.5: ***Glipizide release profiles in phosphate buffer solution (pH 7.4) from floating-bioadhesive tablets containing 10 % effervescent base and HPMC/CH bland***

Figure 6.6 compares the effect of gas generating agent concentration on drug release rate of HPMC/CH tablets. As this figure shows tablets with higher gas-forming agent facilitates drug release.

Table 6.6: Comparison between glipizide release profiles in phosphate buffer solution (pH 7.4) from floating-bioadhesive tablets containing 5 % or 10 % effervescent base and HPMC/CH bland

Batch Time (hr)	JE5H60CH40	JE10H60CH40	JE5CH100	JE10CH100
0	0	0	0	0
1	8	14	11	15
2	17	23	25	28
3	24	26	30	34
4	32	38	36	39
5	41	45	41	45
6	42	48	46	49
7	45	49	54	55
8	51	52	58	59

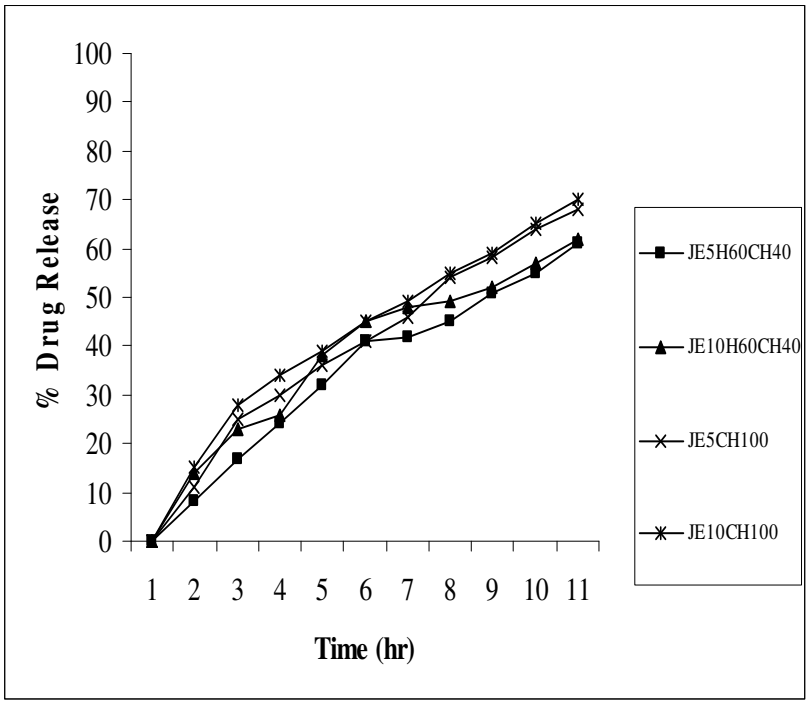


Figure 6.6: ***Comparison between glipizide release profiles in phosphate buffer solution (pH 7.4) from floating-bioadhesive tablets containing 5 % or 10 % effervescent base and HPMC/CH bland***

Figure 6.7 compares the different ratios of CP/PMA from a release characteristic point of view.

Table 6.7: Glipizide release profiles in phosphate buffer solution (pH 7.4) from floating-bioadhesive tablets containing 10 % effervescent base and CP/PMA bland

Batch Time (hr)	JE10H100	JE10H20CH80	JE10H80CH20	JE10H60CH40	JE10H40CH60	JE10CH100
0	0	0	0	0	0	0
1	22	18	15	8	7	4
2	35	31	24	16	15	12
3	40	36	33	25	22	18
4	50	45	41	32	30	22
5	60	54	50	39	37	32
6	70	62	57	45	42	36
7	72	65	60	50	45	40
8	73	71	65	53	48	44
9	77	72	67	58	51	46
10	80	75	70	60	53	48

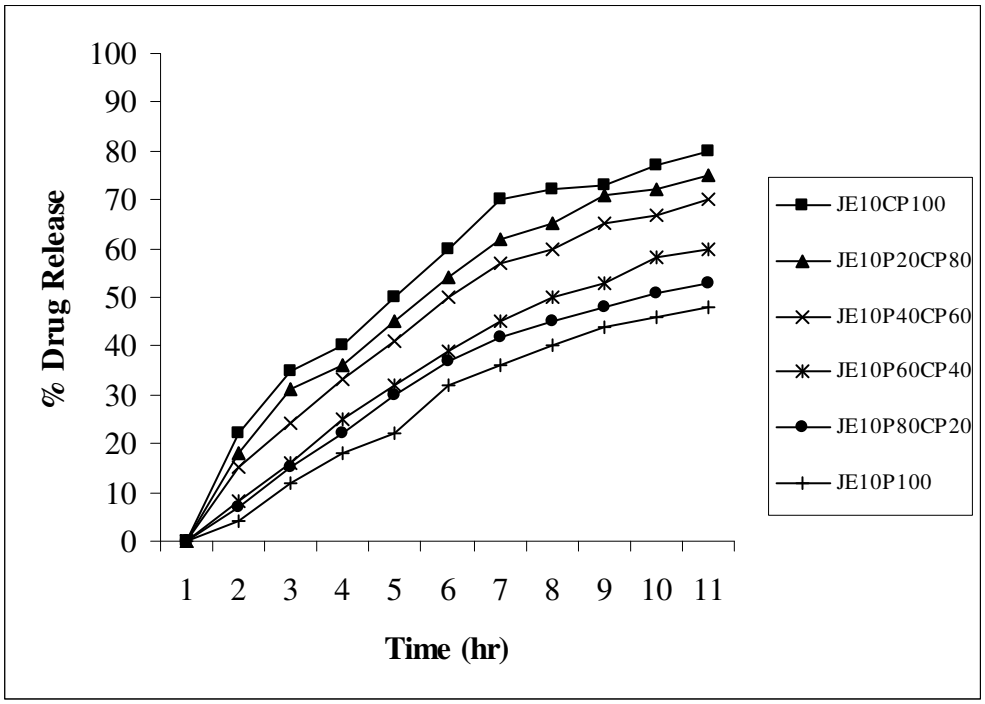


Figure 6.7: ***Glipizide release profiles in phosphate buffer solution (pH 7.4) from floating-bioadhesive tablets containing 10 % effervescent base and CP/PMA bland***

In all formulations curve-fitting method was used to determine the drug release kinetics (Tables 6.8 and 6.9).

Dissolution results were analyzed using the semiempirical equation:

$$M_t/M_\infty = Kt^n \qquad [6.3]$$

where Mt/M_∞ represents the fraction of drug released at time *t, K* is the diffusional constant characteristic of the drug/polymer system, *t* is the release time, and *n* is an exponent characterizing the mechanism of release of the drugs[31].

Table 6.8: Results of mean dissolution time (MDT), dissolution efficiency after 8 hr ($DE_{8\%}$), time required for release 50% of drug ($T_{50\%}$), and diffusion exponent (n)

Formulation code	N	MDT (hr)	$DE_{8\%}$	$T_{50\%}$ (hr)
JE5H100	0.77	3.52	59.24	8.54
JE5H80CH20	0.87	3.41	55.23	8.65
JE5H60CH40	0.71	3.22	62.65	6.54
JE5H40CH60	0.50	3.21	67.75	5.78
JE5H20CH80	0.51	3.22	68.93	4.76
JE5CH100	0.53	2.81	75.34	3.67
JE10H100	0.58	3.08	67.23	7.98
JE10H80CH20	0.47	2.52	76.23	8.14
JE10H60CH40	0.46	3.08	72.31	6.76
JE10H40CH60	0.50	2.86	78.97	6.47
JE10H20CH80	0.52	3.42	82.12	7.83
JE10CH100	0.52	2.52	84.22	5.84
JE10P100	0.69	3.57	65.74	26.54
JE10P80CP20	0.70	2.12	98.25	12.56
JE10P60CP40	0.68	2.25	96.57	5.09
JE10P40CP60	0.67	2.32	94.75	2.37
JE10P20CP80	0.57	2.34	86.98	2.45
JE10CP100	0.47	2.52	86.40	3.12

A table 6.8 and 6.9 summarizes the range of values of the diffusional exponent *n* and the corresponding release mechanism. The *n* values are in the range of 0.45–0.85 representing a non-Fickian or anomalous transport. This table also represents the release parameters i.e., MDT, $DE_{8\%}$, and $t_{50\%}$.

Table 6.9: Correlation coefficient of release data of floating-bioadhesive tablets of glipizide

Formulation code	r^2		
	Zero-order	First-order	Higuchi model
JE5H100	0.9862	0.9863	0.9902
JE5H80CH20	0.9889	0.9891	0.9972
JE5H60CH40	0.9575	0.9765	0.9854
JE5H40CH60	0.9467	0.9790	0.9989
JE5H20CH80	0.9365	0.9786	0.9844
JE5CH100	0.9116	0.9702	0.9876
JE10H100	0.9566	0.9732	0.9878
JE10H80CH20	0.9109	0.9542	0.9809
JE10H60CH40	0.9045	0.9598	0.9877
JE10H40CH60	0.8154	0.9034	0.9531
JE10H20CH80	0.6589	0.8456	0.8794
JE10CH100	0.6439	0.8325	0.8567
JE10P100	0.9453	0.9532	0.9822
JE10P80CP20	0.9452	0.9598	0.9834
JE10P60CP40	0.8764	0.9412	0.9642
JE10P40CP60	0.8498	0.9501	0.9608
JE10P20CP80	0.8432	0.9523	0.9678
JE10CP100	0.6098	0.8145	0.8324

6.4 Discussion

Studies show that some polymers like carbopolP934, polymethacrylic acid, chitosan, and hydroxypropyl methylcellulose are among the floating polymers that show bioadhesive properties more than other polymers and have been used in production of bioadhesive tablets. As these polymers are well hydrated and can adhere to the mucosal membranes, especially if a combination of them is used, their properties are improved[32]. Bioadhesive 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[33]. The approach involves the use of bioadhesive polymers that can adhere to the epithelial surface of the gastrointestinal tract. The proposed mechanism of bioadhesion is the formation of hydrogen and electrostatic bonding at the mucus polymer boundary[34].

Floating dosage forms are meant to remain buoyant on the gastric fluid when the stomach is full after a meal; however, as the stomach empties and the tablet is at the pylorus the buoyancy of the dosage form may be impeded[35]. It then becomes increasingly likely that the dosage form will pass through the pylorus into the small intestine. Thus, the buoyant ability of a floating drug delivery system in the stomach could be limited to only 3–4 hrs. In a bioadhesive drug delivery system, it is quite likely that the system becomes dislodged from the stomach mucosa wall when the stomach is full and semi liquid contents are churning around under the influence of peristaltic movement[14].

A synergism between a bioadhesive system and a floating system also has been explored. Chitnis et al[36] synthesized a series of bioadhesive polymers that were cross-linked polymers of PMA and CP. Floating tablets of isosorbide mononitrate were prepared and then coated with these polymers. The results showed good bioadhesion and low densities, indicating that the coat might confer buoyancy to these tablets. Patel et al[21] prepared chitosan microspheres using cross-linked method. The results showed longer glycemic effect indicating good bioadhesion and low densities of these microspheres. The results of bioadhesion test (Figure 6.3) shows that the bioadhesion was significantly higher in JE10CP100 tablets

than other formulations ($p < 0.05$) and the following order is seen: JE10P100 <JE10H100 < JE10CH100 < JE10CP100.

Statistical analysis of bioadhesion between two groups of tablets containing HPMC/CH or CP/PMA shows that tablets with 80–100% and 60% of CP have higher bioadhesion than tablets containing the comparable amounts of CH (p <0.05). However, tablets with 20–40% CH, or those with pure HPMC, have high bioadhesion compared with tablets with similar amounts of CP or without CP ($p <$ 0.05). Increasing the content of CP in a series prepared with CP/PMA increased the bioadhesion ($p < 0.05$). Chng et al[37] also reported that CP polymer adheres to the surface mucin of the epithelial cells and this cause a longer gastrointestinal transit time compared with PMA polymer. This is related to the charge of CP and neutral nature of PMA[37].

In the design of floating-bioadhesive glipizide tablets, the floatation was accomplished by incorporating gas-generating salts such as sodium bicarbonate and citric acid into a swellable matrix. The overall make-up of this particular matrix is of swellable polymers. As the dissolution medium was imbibed into the matrix, the interaction of fluid with effervescent base resulted in the formation and entrapment of carbon dioxide gas within the swollen gel, thus causing floatation as the matrix volume expanded and its density decreased. We observed that the amount of gas-generating effervescent base had a significant effect on the lag time of the system buoyancy (Table 6.3). However, statistical analysis of duration of floating time in HPMC/CH and CP/PMA tablets with 10% effervescent base showed no change ($p < 0.05$) in duration of system buoyancy by changing the percentage or the type of polymer mixtures (Table 6.3). In other words, the amount of gas-generating agent is just effective on the buoyancy lag time, but as the gas is generated at the early times of contact of fluid medium with effervescent base, the swelling of polymers is controlling the duration of system buoyancy.

Yang et al[38] used a mixture of sodium bicarbonate and calcium carbonate to induce gas formation in intragastric floating tablets of tetracycline/metronidazole tablets. Li et al [19,20] used citric acid as gas-generating agent in floating capsules

of calcium carbonate. A 1:1 mixture of potassium bicarbonate: monobasic potassium citrate as effervescent base of verapamil floating capsules has been reported by Gan-Lin and Wei-Hua[39]. Dave et al[10] used sodium bicarbonate and citric acid as gas-generating agent in floating tablets of ranitidine hydrochloride.

Drug release studies were made to determine whether the release of the drug is slow enough, i.e., which polymer percentage is enough to sustain the release of the drug for at least 8 hr. As Figures 6.4 and 6.5 shows, increasing the CH content of tablets significantly increases the percentage of drug released at comparable times ($p < 0.05$). This is because of rapid swelling and erosion of CH in contact with gastric fluid. Comparison of tablets with the same formulations but different effervescent base concentrations (Figure 6.7) shows faster release rate of drug and $DE_{8\%}$ (Table 6.8) in tablets with 10% of gas-generating agent than 5%. This is because of greater expansion of polymer matrix, better penetration of liquid medium into the tablet, and faster diffusion of drug. Table 6.8 shows that increasing CH content of tablets reduces MDT and $T_{50\%}$ while increasing the $DE_{8\%}$ ($p < 0.05$). Comparison of $T_{50\%}$ and MDT of tablets with the same ratio of HPMC/CH but different effervescent bases shows a decrease in these parameters in tablets with 10% of gas-generating agent than 5% ($p < 0.05$) (Table 6.8).

In spite of more suitable sustained-release effect of tablets with 5% effervescent base (Table 6.8), but as their long lag-time of buoyancy (Table 6.3), tablets of JE10H60CH40 and JE10H40CH60 were chosen as optimum formulations (Figure 6.5 and Table 6.8). However, as there were some difficulties in flow rate of powder in preparation of these tablets, formulation JE10H20CH80 also seems optimum from floating lag time, bioadhesion, and sustained-release point of view.

Tablets composed of a polymeric matrix build a gel layer around the tablet core on contact with gastric fluid, which controls the drug release. Drug release from HPMC matrices is controlled by diffusion through the gel layer for water-soluble drugs or by erosion of the outer polymer chains for poorly soluble drugs[40]. The drug characteristics are as important as those of the gel. The size, shape, and ionization of the drug affect its diffusion through the gel layer[41].

In tablets prepared from acrylate series, increasing the CP content, decreases the MDT and $T_{50\%}$ but increases the $DE_{8\%}$ significantly (p <0.05) (Table 6.8). Considering MDT and $DE_{8\%}$, tablets of JE10CP80P20, JE10CP60P40, and JE10CP40P60 seem suitable for sustained–release of drug in the stomach (Table 6.8).

Curve fitting method according to zero-order, first-order, or Higuchi model for analysis of drug release kinetics are shown in Table 6.9. In all cases the Higuchi model is dominant and shows that the passage of glipizide, the water insoluble drug through the hydrated gel layer around the matrix tablet, is approximately dependent on the square root of time and can be described in the following form[42]:

$$Q_t = Kt^{1/2} \qquad [6.4]$$

where *Qt* is the amount of the released drug in time *t, k* is the kinetic constant, and *t* is time. To predict the mechanism of diffusional release, the following semiempirical equation of

$$M_t/M_\infty = Kt^n \qquad [6.5]$$

was used to analyze data of controlled–release of this water soluble drug from the studied polymer matrices[43]. In this equation *Mt* is amount of the released drug at time *t, M*∞ is the overall amount of the drug (whole dose), *k* is the constant incorporating structural and geometric characteristics of the controlled–release device, and *n* is the release exponent indicative of the drug release mechanism. For tablets of a known geometry (in this case a slab) $n = 0.5$ means Fickian diffusion, $0.5 < n < 1.0$ non-Fickian diffusion, and $n = 1.0$ Case II diffusion[43]. Considering the *n* values calculated for the studied tablets (Table 6.8), almost in most cases a non-Fickian mechanism is dominant. The drug diffusion through most types of polymeric systems is often best described by Fickian diffusion, but other processes in addition to diffusion are important. In this

case the non- Fickian or anomalous diffusion shows also a relaxation of the polymeric chains, and influences the drug release. Release from initially dry, hydrophilic glassy polymers that swell in contact of water and become rubbery show anomalous diffusion as a result of the rearrangement of macromolecular chains[44]. The thermodynamic state of the polymer and the penetrate concentration are responsible for the different types of the diffusion. A third class of diffusion is case II diffusion, which is a special case of non-Fickian diffusion[40,43]. The results of the calculated *n* (Table 6.8) reveal a non-Fickian type of drug diffusion, which means that the process of diffusion and relaxation run at comparable rates.

6.5 Conclusion

In the current work, a matrix floating-bioadhesive tablet incorporating an insoluble active substance is described. The most successful tablets with the least lag time of buoyancy were those prepared with 10% of effervescent base but changing the polymer type of mixture ratio did not change the duration of buoyancy. Tablets containing 20% of HPMC and 80% CH or 80% of CP and 20% of PMA were optimum from both the bioadhesion and prolonged drug release rate point of view.

6.6 References

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Chapter 7

Research papers presented and published

7 Research papers presented and published

7.1 Publications

1. Jayvadan K. Patel and Jayant R. Chavda. Formulation and evaluation of stomach-specific amoxicillin-loaded carbopol-934P mucoadhesive microspheres for anti-Helicobacter pylori therapy. *Journal of Microencapsulation*. 2009; 26(4): 365–376.
2. Jayvadan K. Patel and Jayant R. Chavda. Floating in situ gel based on alginate as carrier for stomach-specific delivery of famotidine. *International Journal of Pharmaceutical Sciences and Nanotechnology*. (Accepted).
3. J.K. Patel and J. R. Chavda. Formulation and evaluation of glipizide floating-bioadhesive tablets. *Brazilian Archives of Biology and Technology*. (Accepted).

7.2 Presentation

1. Floating in situ gel based on alginate as carrier for stomach-specific delivery of famotidine at 2009 AAPS National Biotechnological Conference, Washington, USA, June 21-24, 2009.

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Formulation and evaluation of stomach-specific amoxicillin-loaded carbopol-934P mucoadhesive microspheres for anti-*Helicobacter pylori* therapy

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Abstract

The purpose of this research was to formulate and systemically evaluate *in vitro* and *in vivo* performances of mucoadhesive amoxicillin microspheres for the potential use in the treatment of gastric and duodenal ulcers, which were associated with *Helicobacter pylori*. Amoxicillin mucoadhesive microspheres containing carbopol-934P as mucoadhesive polymer and ethyl cellulose as carrier polymer were prepared by an emulsion-solvent evaporation technique. Results of preliminary trials indicate that quantity of emulsifying agent, time for stirring, drug-to-polymers ratio and speed of rotation affected the characteristics of microspheres. Microspheres were discrete, spherical, free flowing and showed a good percentage of drug entrapment efficiency. An *in vitro* mucoadhesive test showed that amoxicillin mucoadhesive microspheres adhered more strongly to the gastric mucous layer and could retain in the gastrointestinal tract for an extended period of time. A 3^2 full factorial design was employed to study the effect of independent variables, drug-to-polymer-to-polymer ratio (amoxicillin-ethyl cellulose-carbopol-934P) (X_1) and stirring speed (X_2) on dependent variables, i.e. percentage mucoadhesion, drug entrapment efficiency, particle size and t_{80}. The best batch exhibited a high drug entrapment efficiency of 56%; mucoadhesion percentage after 1 h was 80% and the particle size was 109 μm. A sustained drug release was obtained for more than 12 h. The drug-to-polymer-to-polymer ratio had a more significant effect on the dependent variables. The morphological characteristics of the mucoadhesive microspheres were studied under a scanning electron microscope. *In vitro* release test showed that amoxicillin released slightly faster in pH 1.2 hydrochloric acid than in pH 7.8 phosphate buffer. *In vivo H. pylori* clearance tests were also carried out by administering amoxicillin powder and mucoadhesive microspheres to *H. pylori* infectious Wistar rats under fed conditions at single dose or multiple dose(s) in oral administration. The results showed that amoxicillin mucoadhesive microspheres had a better clearance effect than amoxicillin powder. In conclusion, the prolonged gastrointestinal residence time and enhanced amoxicillin stability resulting from the mucoadhesive microspheres of amoxicillin might make a contribution to *H. pylori* complete eradication.

Key words: *Mucoadhesive; amoxicillin; microspheres; factorial design;* H. pylori

Introduction

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems[1–3]. They have varied applications and are prepared using assorted polymers[4]. However, the success of these microspheres is limited, owing to their short residence time at the site of absorption. It would, therefore, be advantageous

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to have means for providing an intimate contact of the drug delivery system with the absorbing membranes[5–8]. This can be achieved by coupling mucoadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site[9–12]. Carbopol-934P (acrylic acid homopolymer) is an anionic polymer that has been used in mucoadhesive systems by several researchers[13–17]. Carbopol-934P was selected as a polymer in the preparation of mucoadhesive microspheres because of its good mucoadhesive and biodegradable properties and ethyl cellulose as carrier polymer for microspheres.

In a relatively short time span, *Helicobacter pylori* (*H. pylori*) have become recognized as a major gastric pathogen with worldwide distribution. *H. pylori* are a spiral-shaped bacterium found in the stomach, which (along with acid secretion) damages stomach and duodenal tissue, causing inflammation and peptic ulcers. *H. pylori*, a prevalent human-specific pathogen, is a causative agent in chronic active gastritis[18], gastric and duodenal ulcers[19] and gastric adenocarcinoma[20], one of the most common forms of cancer in humans. Epidemiological, laboratory and interventional human studies strongly suggest that *H. pylori* play a pathogenic role in the development of adenocarcinoma of the distal stomach[21]. The mechanisms by which *H. pylori* may cause gastroduodenal disease and contribute to gastric carcinogenesis are still hypothetical. However, the production of specific virulence factors by the bacterium, the inflammatory response of the host and the association with environmental contributors may all be responsible[22].

Treatment regimens for *H. pylori* infection have been evolving since the early 1990s, when monotherapy was first recommended. Antimicrobial therapy for this infection is a complex issue and the following drugs are currently used in combination regimens: proton-pump inhibitors and/or bismuth, metronidazole, clarithromycin and amoxicillin[23]. Tetracycline is used in the rescue therapy[24]. Although optimal first-line treatment is associated with high cure rates, the rising prevalence of resistance to the antibiotic component of current eradication regimens increasingly threatens to compromise the efficacy of these regimens. Strains resistant to metronidazole[25] and clarithromycin[26] have been well documented, while resistance to amoxicillin[27] and tetracycline was mainly reported in Asia[28]. Therapeutic regimens directed against *H. pylori* infection will continue to evolve. What is required is a simpler and more efficacious strategy for the treatment of *H. pylori* infection. *H. pylori* is susceptible to many antibiotics *in-vitro*, but has proved difficult to eradicate (to root out) *in-vivo*.

Amoxicillin (α-amino-hydroxybenzylpenicillin) is a semi-synthetic, orally absorbed, broad-spectrum antibiotic. It is now widely used in the standard eradication treatment of gastric and duodenal ulcers, which are associated with *H. pylori* infection combined with a second antibiotic and an acid-suppressing agent[29–31]. These tripe therapies have proved to be effective in clinical application. However, some other reports and clinical trials indicate that the therapies cannot bring out compete eradication of *H. pylori* and suggest that the therapeutic effect needs more investigation[32–33]. One reason for the incomplete eradication of *H. pylori* is probably due to the short residence time of dosage form in the stomach so that effective antimicrobial concentration cannot be achieved in gastric mucous layer or epithelial cell surfaces where *H. pylori* exist[34–35]. The other may be the degradation of amoxicillin in gastric acid[36–37]. Therefore, some researchers had prepared and reported new amoxicillin formulations such as float tablets, mucoadhesive tablets, pH-sensitive excipients composition microspheres, etc., which were able to reside in the gastrointestinal tract for an extended period of time for a more effective *H. pylori* eradication[38–39]. A previous investigation on *H. pylori* clearance effect showed that there was a tendency for a more effective *H. pylori* activity of mucoadhesive amoxicillin microspheres prepared using chitosan as mucoadhesive microspheres[40].

In context of the above principles, a strong need was felt to develop a dosage form that delivered amoxicillin in the stomach and would increase the efficiency of the drug, providing sustained action. Thus, an attempt was made in the present investigation to use carbopol-934P as a mucoadhesive polymer and ethyl cellulose as a carrier polymer and prepare mucoadhesive amoxicillin microspheres. The microspheres were characterized by *in-vitro* and *iv-vivo* tests and factorial design was used to optimize the variables.

Materials

Amoxicillin (powder) was obtained as a gift sample from Zydus Cadila (Ahmedabad, India). Carbopol-934P (CP, molecular weight [MW] 3×10^6 Da) was obtained as a gift sample from Noveon® (Mumbai, India). Ethyl cellulose and petroleum ether 80 : 20 were procured from Willson Lab (Mumbai, India) and S. D. Fine Chemicals Ltd (Mumbai, India), respectively. Liquid paraffin and span 80 were purchased from Loba Chemie Pvt Ltd (Mumbai, India). Wistar rats (300 ± 50 g) were obtained as a gift sample from Zydus Cadila (Ahmedabad, India). Skirrow's medium was purchased from Himedia Ltd.

(Mumbai, India). All other ingredients were of analytical grade.

Methods

Preparation of mucoadhesive amoxicillin microspheres

Mucoadhesive microspheres of amoxicillin were prepared containing carbopol-934P as a mucoadhesive polymer and ethyl cellulose as a carrier polymer by emulsion-solvent evaporation technique. Briefly, ethyl cellulose (1500 mg) was dissolved in 200 mL of ethanol. Each 500 mg of amoxicillin and carbopol-934P were dispersed in the polymer solution of ethyl cellulose under stirring. In preliminary trail batches the drug-to-polymer-to-polymer (amoxicillin-ethyl cellulose-carbopol-934P) ratio was kept constant at 1 : 3 : 1. The resultant mixture was extruded through a syringe (gauge No. 20) in 500 mL of liquid paraffin (mixture of heavy and light, 1 : 1 ratio) containing 2.0% v/v Span 80 and stirring was carried out using a propeller stirrer (Remi, Mumbai, India) at 1000 rpm. Stirring was continued for 3 h. The amount of emulsifying agent and time for stirring were varied in preliminary trial batches from 1–3% v/v and 1–3 h, respectively. In factorial design batches J1–J9, 2.0% v/v Span 80 was used as an emulsifying agent and time for stirring was kept to 3 h. The drug-to-polymer-to-polymer ratio and stirring speed were varied in batches J1–J9, as shown in Table 1. All other variables were similar as in preliminary trial batches. Microspheres thus obtained were filtered and washed several times with petroleum ether (80 : 20) to remove traces of oil. The microspheres were then dried at room temperature (25°C and 60% RH) for 24 h. The effect of formulation variables on characteristics of the microspheres of factorial design batches is summarized in Table 1.

Optimization of microspheres formulation using 3^2 full factorial design

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (1)$$

where Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs and b_i is the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity. On the basis of the preliminary trials a 3^2 full factorial design was employed to study the effect of independent variables, i.e. drug-to-polymer-to-polymer (X_1) and the stirring speed (X_2) on dependent variables percentage mucoadhesion, drug entrapment efficiency, particle size and the time required for 80% drug dissolution (t_{80}).

Determination of amoxicillin

Amoxicillin was estimated by a UV/Vis spectrophotometric method (Shimadzu UV-1700 UV/Vis double beam spectrophotometer, Kyoto, Japan). Aqueous solutions of amoxicillin were prepared in phosphate buffer (pH 7.8) and absorbance was measured on a Shimadzu UV/Vis

Table 1. Amoxicillin mucoadhesive microspheres batches using 3^2 full factorial design layout.

	Variable levels in coded form		*In-vitro* wash-off test (% mucoadhesion after)					
Batch code	X_1	X_2	1 h	5 h	10 h	Drug entrapment efficiency (%)	Particle size (μm)	t_{80} (min)
J1	−1	−1	57	50	45	26	99	592
J2	−1	0	55	49	44	21	93	645
J3	−1	1	40	35	29	20	86	727
J4	0	−1	80	72	60	56	109	502
J5	0	0	77	70	55	52	101	542
J6	0	1	71	65	50	41	94	582
J7	1	−1	90	80	72	47	112	299
J8	1	0	81	75	64	41	110	308
J9	1	1	73	64	50	34	99	340

Translation of coded levels in actual units.
Variables level: drug-to-polymer-to polymer ratio (X_1) (amoxicillin-ethyl cellulose-carbopol-934P), Low (−1) 1 : 3 : 0.5, Medium (0) 1 : 3 : 1, High (+1) 1:3:1.5; Stirring speed (X_2), rpm, Low 800, Medium 1000, High 1200.
All the batches were prepared using 2% v/v Span 80 and stirring time of 3 h.

spectrophotometer at 272 nm. The method was validated for linearity, accuracy and precision.

Drug entrapment efficiency

Two-hundred milligrams of accurately weighed microspheres were crushed in a glass mortar-pestle and the powdered microspheres were suspended in 10 mL phosphate buffer (pH 7.8). After 24 hs the solution was filtered and the filtrate was analysed for the drug content. The drug entrapment efficiency was calculated using the following formula: Practical drug content/Theoretical drug content $\times$ 100. The drug entrapment efficiency for batches J1–J9 is reported in Table 1.

Particle size of microspheres

The particle size of the microspheres was determined by using an optical microscopy method[41]. Approximately 300 microspheres were counted for particle size using a calibrated optical microscope (Labomed CX RIII, Ambala, India). The particle size of microspheres of batches J1–J9 is reported in Table 1.

In vitro *wash-off test for microspheres*

The mucoadhesive properties of the microspheres were evaluated by *in vitro* wash-off test, as reported by Lehr et al.[42]. A 1 $\times$ 1 cm piece of rat stomach mucosa was tied onto a glass slide (3 inch-by-1 inch) using thread. Microspheres were spread ($\sim$50) onto the wet rinsed tissue specimen and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus with continuous oxygen supply. The disintegrating test apparatus was operated whereby the tissue specimen was given regular up and down movements in the beaker of the disintegration apparatus, which contained the gastric fluid (pH 1.2). At the end of 30 min, 1 h and at hourly intervals up to 12 h, the number of microspheres still adhering onto the tissue was counted. The results of *in vitro* wash-off test after 1, 5 and 10 h of batches J1–J9 are shown in Table 1. Also, results of *in vitro* wash-off test of amoxicillin-loaded carbopol-934P mucoadhesive microspheres of batch J4 is shown in Figure 1.

Scanning electron microscopy

Scanning electron photomicrographs of drug-loaded carbopol-934P mucoadhesive microspheres were taken. A small amount of microspheres was spread on a glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope (JSM 5610 LV SEM, JEOL, Datum Ltd, Tokyo, Japan) chamber. A scanning electron photomicrograph was taken at the acceleration voltage of 20 KV, chamber pressure of 0.6 mm Hg, at different magnification. The photomicrograph of batch J4 is depicted in Figure 2.

The photomicrographs of *in-vitro* wash-off test results after 2 h and 8 h are depicted in Figures 3 and 4, respectively.

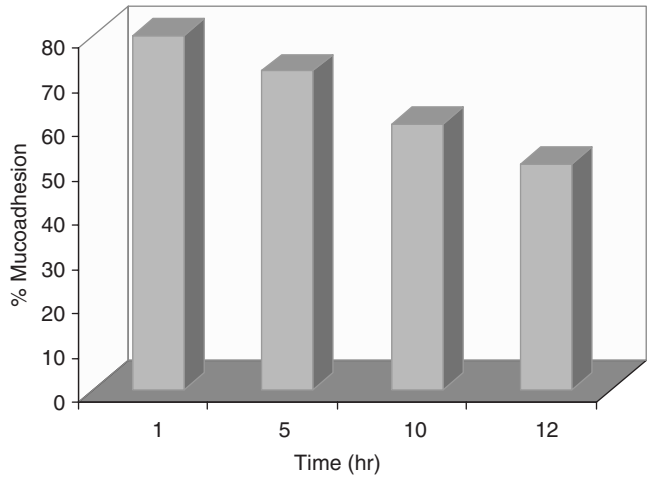

Figure 1. Percentage mucoadhesion of amoxicillin-loaded carbopol-934P mucoadhesive microspheres (batch J4) after 1, 5, 10 and 12 h.

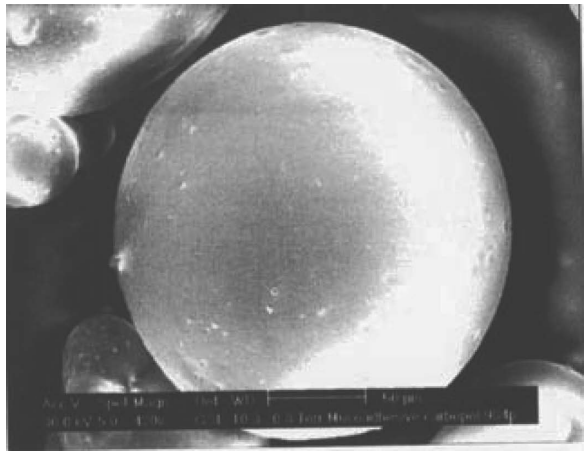

Figure 2. Scanning electron photomicrograph of amoxicillin-loaded carbopol-934P mucoadhesive microspheres (batch J4).

Drug release study

The drug release study was carried out using USP XXIV basket apparatus (Electrolab, TDT-06T, India) at 37°C $\pm$ 0.5°C and at 100 rpm using 900 mL of phosphate

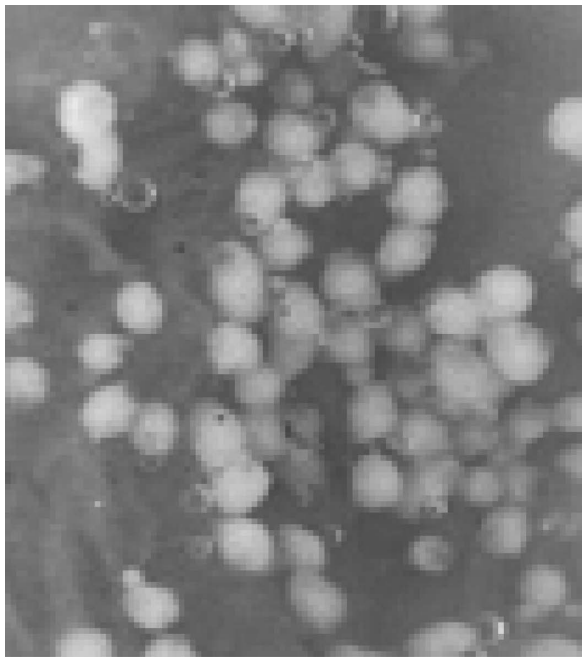

Figure 3. *In-vitro* wash-off test of amoxicillin-loaded carbopol-934P mucoadhesive microspheres (batch J4) on rat stomach after 2 h.

Figure 4. *In vitro* wash-off test of amoxicillin-loaded carbopol-934P mucoadhesive microspheres (batch J4) on rat stomach after 8 h.

buffer (pH 7.8) as a dissolution medium ($n = 5$) as per USP XXVI dissolution test prescribed for amoxicillin tablets. Microspheres equivalent to 100 mg of amoxicillin were used for the test. Five millilitres of sample solution was withdrawn at pre-determined time intervals, filtered through a 0.45 μm membrane filter, diluted suitably and analysed spectrophotometrically. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage drug dissolved at different time intervals was calculated using the Lambert-Beer's equation. The t_{80} was calculated using the Weibull equation[43]. The average values of t_{80} for batches J1–J9 are mentioned in Table 1. The percentage drug release of batch J4 in pH 1.2 and pH 7.8 is shown in Figure 5.

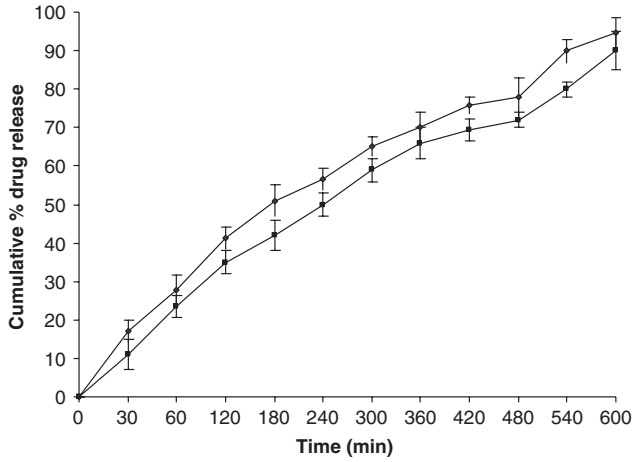

Figure 5. *In vitro* dissolution of amoxicillin from mucoadhesive microspheres of batch J4 (-◆- pH = 1.2 and -■- pH = 7.8).

Data fitting

An attempt was made to fit the dissolution data into the Hixon-Crowell[43] model represented:

$$m = \left[(100) * \left(\frac{1}{3}\right) - k * t\right]^3 \tag{2}$$

where k is Hixon-Crowell constant $[\text{mass}/(\text{time})]^{1/3}$. In this model the percentage drug unreleased vs. cube root of time is linear.

The data was treated with the Korsmeyer-Peppas model[44] to characterize the mechanism of drug release:

$$\frac{M_t}{M_\infty} = Kptn \tag{3}$$

where M_t/M_∞ represents the fraction of drug released at time t and Kp is the kinetic constant characterizing the polymeric system and n stands for the diffusion exponent.

The dissolution data was also analysed using the Weibull equation[43] to determine the kinetics of drug release from different batches of mucoadhesive microspheres:

$$m = 1 - \exp\left[-(t - ti)\frac{b}{a}\right] \tag{4}$$

Table 2. Results of models fitting of batch J4.

Time (min)	Cumulative percentage release	Hixon-Crowell		Korsemeyer and Peppas		Weibull	
		Calculated cumulative percentage release	Residual square	Calculated cumulative percentage release	Residual square	Calculated cumulative percentage release	Residual square
0	0	−6.86	47.00	—	—	—	—
60	11.0	10.57	4.51	14.01	1.71	11.69	1.01
120	35.0	26.00	0.48	25.45	0.02	25.98	0.45
180	42.1	39.54	1.29	36.09	5.35	39.61	1.45
240	50.2	51.32	0.77	46.23	35.64	51.70	0.25
300	60.2	61.46	0.43	56.02	22.80	61.98	1.38
360	66.1	70.08	8.30	65.55	2.72	70.48	10.73
420	69.9	77.31	10.32	74.85	0.56	77.34	10.53
480	72.3	83.27	5.63	83.97	9.45	82.80	3.60
540	80.4	88.09	3.19	92.94	44.07	87.07	0.58
600	90.2	91.87	12.43	101.77	40.51	90.36	25.39
SSR		94.41		162.88		55.43	
F-value		10.49		20.36		6.92	
Correlation coefficient		0.9889		0.9935		0.9931	
R^2		0.9780		0.9871		0.9863	
Slope		0.0046		0.8611		1.2745	
Intercept		−0.1038		−2.38		−3.17	

Table 3. Summary of results of regression analysis.

Coefficient	b_0	b_1	b_2	b_{11}	b_{22}	b_{12}	R^2
% Mucoadhesion	77.66	15.33	−7.16	0	−10.0	−2.5	0.9803
Drug entrapment efficiency	50.11	9.16	−5.66	−1.75	−18.16	−0.66	0.9954
Particle size	102.33	7.16	−6.83	0	−1.5	−1.5	0.9824
t_{80}	536.22	−169.5	42.66	−23.5	−56.83	8.66	0.9994

where *a* is the scale parameter which defines the time scale of the process, *ti* is the location parameter which represents the lag period before the actual onset of dissolution process (in most cases $ti = 0$) and *b* is the shape parameter. In this model the plot of log of time vs. ln $(1 - m)$ is linear.

The results of *F*-statistics were used for the selection of the most appropriate model. Results of *F*-statistics and summary of results of regression analysis are shown in Tables 2 and 3, respectively.

The curve fitting, simulation and plotting was performed in Excel (Microsoft Software Inc., USA) and Sigma plot® version 10.0 (Sigma plot software, Jangel Scientific Software, San Rafael, CA). The effects of independent variables on the response parameters were visualized from the contour plots. Numerical optimization using the desirability approach was employed to locate the optimal settings of the formulation variables so as to obtain the desired response[45]. An optimized formulation was developed by setting constraints on the dependent and independent variables. The formulation developed was evaluated for the responses and the experimental values obtained were compared with those predicted by the mathematical models generated. Counter plots showing the effect of drug-polymer-polymer ratio (X_1) and stirring speed (X_2) on percentage mucoadhesion, particle size, drug entrapment efficiency and t_{80} appear in Figure 6.

In vivo *clearance of H. pylori*

The *H. pylori* infected animal model was established according to Qian's method (China Patent, CN 1304729A). Briefly, 0.3 mL of broth containing 10^9 CFU ml^{-1} of *H. pylori*, isolated from patients with gastritis and gastric ulcer, was inoculated into the stomachs of 6-week-old male Wistar rats. Then, the rats were fed for 4 weeks. *H. pylori* infection in rat was detected using the 'golden standard' culture, W-S stain and rapid urease test, etc. The rapid urease test was carried out by collecting and transferring the bacterial colonies into small tubes containing 0.5 mL of mixture of phosphate buffer, urea

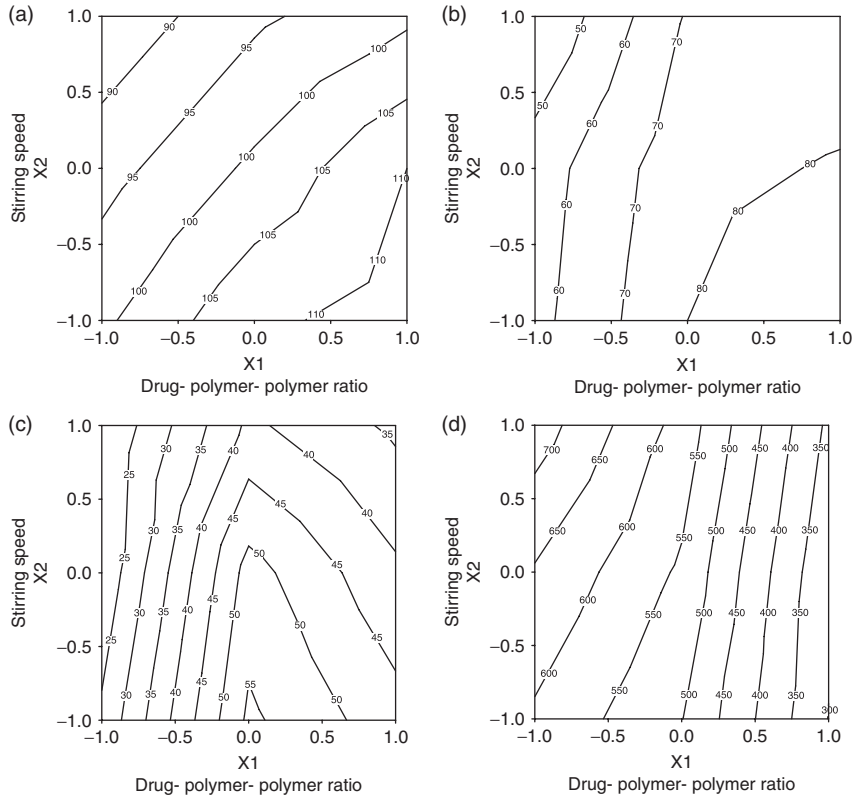

Figure 6. Counter plot showing the effect of drug-polymer-polymer ratio (X_1) and stirring speed (X_2) on percentage mucoadhesion (a), particle size (b), drug entrapment efficiency (c) and t_{80} (d).

(2% w/v) and phenol red (0.03% v/v). If the solution colour turned into red in several minutes, the urease test was regarded to be positive, which indicated the presence of *H. pylori*. While if the solution colour did not turn red in several minutes, the urease test was regarded to be negative, this indicated the absence of *H. pylori*.

Single-dosage administration

To determine the dose for *H. pylori* clearance, mucoadhesive amoxicillin microspheres and amoxicillin powder were orally administered to the *H. pylori* infected rats at the dosages of 4.0, 7.5 and 15 mg kg^{-1} ($n = 2$). Physiological saline was given to rats as control ($n = 2$). One day after administration of drug to the *H. pylori* infected rats, they were killed and their stomachs were removed and cut. Then, the gastric tissue was daubed on the modified Skirrow's medium. The plates were incubated for 3 days at 37°C under microaerobic conditions. *H. pylori* clearance effect was judged by both bacterial colony counts and rapid urease test. The rapid urease test was carried out by collecting and transferring the bacterial colonies into small tubes containing 0.5 mL of mixture of phosphate buffer, urea (2% w/v) and phenol red (0.03% v/v). If the solution colour turned into red in several minutes, the urease test was regarded to be positive, which indicated *H. pylori* detection. *H. pylori* clearance effects of amoxicillin at different doses in different formulations ($n = 2$) are shown in Tables 4 and 5.

Multidose administration

To determine whether the mucoadhesive amoxicillin microspheres could completely eradicate *H. pylori*, a multidose administration therapy was carried out. Briefly, amoxicillin was orally administrated twice a day for 3 days at a dose of 3.5 mg kg^{-1} in the form of either amoxicillin mucoadhesive microspheres or powder ($n = 2$). Physiological saline was given to rat as control ($n = 2$). One day after administration the *H. pylori* infectious rats were killed and their stomachs were removed and cut. Then, the gastric tissue was daubed on the modified Skirrow's medium. The *H. pylori* clearance effect was studied using the same method as described for single doge administration. *H. pylori* clearance effect of amoxicillin at different doses in different formulations is shown in Figure 7.

Table 4. *H. pylori* clearance effect of amoxicillin at different doses in different formulations ($n = 2$).

	Doses (mg kg^{-1})					
Colony counts	4		7.5		15	
Amoxicillin mucoadhesive microspheres	20 (23 ± 4.24)*	26	8 (7.0 ± 1.41)*	6	2 (2 ± 0)*	2
Amoxicillin powder	72 (78 ± 8.48)*	84	25 (29 ± 5.65)*	33	5 (18.0 ± 16.97)*	30
Physiological saline	98 (94 ± 5.65)*	90	99 (92 ± 9.89)*	85	105 (92.5 ± 17.67)*	80

*Figure showed mean ± SD.

Table 5. *In vivo* clearance of *H. pylori* after the administration of amoxicillin powder, mucoadhesive amoxicillin microspheres and physiological saline ($n = 2$).

	Mucoadhesive amoxicillin microspheres		Amoxicillin powder		Physiological saline	
H. pylori	1	2	1	2	1	2
Condition	−/−	−/−	+/+	+/+	+/+	+/+

Negative (−) means neither bacterial colony was found nor rapid urease test was positive; (+) means either bacterial colony was found or rapid urease test was positive.

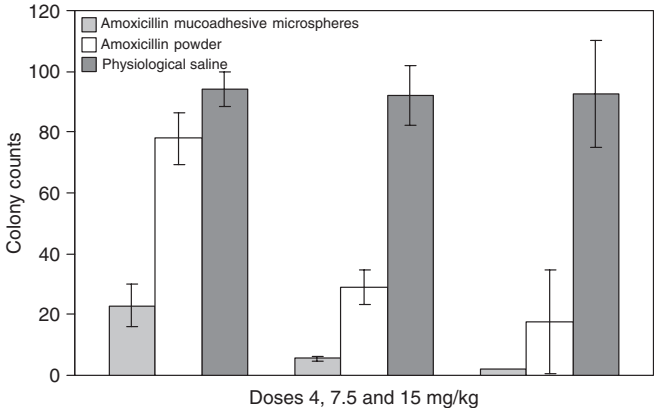

Figure 7. *H. pylori* clearance effect of amoxicillin at different doses in different formulations.

Results and discussion

Preliminary trials

The mucoadhesive microspheres of amoxicillin using carbopol-934P and ethyl cellulose were prepared by emulsion-solvent evaporation technique. Carbopol-934P was selected as a polymer for the preparation of mucoadhesive microspheres owing to its biodegradable and mucoadhesive properties. Ethyl cellulose was used as carrier polymer. Different concentrations of span 80 from 1–3% v/v were used as emulsifying agent. Significant effect of concentration of span 80 was observed on percentage mucoadhesion, particles size and drug entrapment efficiency. Results showed that increase in the concentration of span 80 increase the particles size and percentage mucoadhesion but decrease the drug entrapment efficiency. At 1% v/v, percentage mucoadhesion, particles size and drug entrapment efficiency were 66%, 80 μm and 65%, respectively, but irregular shape of microspheres was observed. While at 3% v/v, percentage mucoadhesion, particles size and drug entrapment efficiency were 82%, 210 μm and 38%, respectively; spherical shape of microspheres was observed but particles were coalesced. Therefore, 2% v/v of concentrations of span 80 was used for further study.

One of the important factors related to microspheres as reported by Lee et al.[46] is the viscosity of the polymer solution. Polymer concentrations of 0.5%, 1% and 2% w/v were selected for preliminary trials. Flake formation was observed when ethyl cellulose and carbopol-934P concentration was used at a level of 0.5% w/v, whereas maximum sphericity was observed at the 1% w/v level. Non-spherical microspheres were found when polymer concentration was used at the 2% w/v level. Therefore, 1% w/v of ethyl cellulose and carbopol-934P each in ethanol was found to be the optimum concentration for the polymer solution. A 1 : 1 mixture of heavy and light liquid paraffin was found to be suitable as a dispersion medium.

Preliminary trials batches were prepared to study the effect of the time for stirring and stirring speed on the

percentage mucoadhesion, drug entrapment efficiency and characteristics of the microspheres. Increase in the time for stirring from 1 to 3 h showed an increase in percentages of mucoadhesion, but a decrease in drug entrapment efficiency and particles size. Thus, 3 h of stirring time was selected for further study. Since stirring speed has a significant effect on percentage mucoadhesion, drug entrapment efficiency and particles size, it was selected as an important factor for further study.

On the basis of the preliminary trials a 3^2 full factorial design was employed to study the effect of independent variables (i.e. drug-to-polymer-to-polymer ratio [X_1] and the stirring speed [X_2]) on dependent variables percentage mucoadhesion, drug entrapment efficiency, particle size and t_{80}. The results depicted in Table 1 clearly indicate that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the nine batches (J1–J9). The fitted equations (full models) relating the responses (i.e. percentage mucoadhesion, drug entrapment efficiency, particle size and t_{80}) to the transformed factor are shown in Table 3. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e. positive or negative). The high values of correlation coefficient (Table 3) for the dependent variables indicate a good fit. The equations may be used to obtain estimates of the response since small error of variance was noticed in the replicates.

Factorial equation for percentage mucoadhesion

The *in vitro* mucoadhesiveness test showed that the percentage of mucoadhesive microspheres remaining on the stomach mucosa (Table 1). Figure 1 showed that even after 12 h, 52% microspheres were adhered to the gastric mucous layer. The mucoadhesive microspheres of all the batches of the factorial design were spherical (Figure 2, batch J4) and free flowing.

The linear model generated for percentage mucoadhesion was found to be significant with an F-value of 29.96 ($p < 0.0001$) and R^2 value of 0.9803:

$$\%\ \text{Mucoadhesion} = 77.66 + 15.33X_1 - 7.16X_2 - 2.5X_1X_2 - 10X_2^2 \quad (5)$$

The counter plot (Figure 6(a)) shows that the *in vitro* wash-off test for percentage mucoadhesion of microspheres increased from 40 to 57 and 73 to 90 at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as stirring speed decreased. Results of equation indicate that the effect of X_1 (drug-to-polymer-to-polymer) is more significant than X_2 (stirring speed). Moreover, stirring speed had a negative effect on the percentage mucoadhesion (i.e. as the stirring speed increased, the percentage mucoadhesion decreased). This finding may be attributed to the change in particle size that affects mucoadhesion. As the drug-to-polymer-to-polymer ratio increases, the percentage mucoadhesion also increases; because more amounts of polymer results in higher amount of free –COOH groups[13], which are responsible for binding with sialic acid groups in mucus membrane and thus results in increase in mucoadhesive properties of microspheres. *In vitro* mucoadhesive test showed that amoxicillin mucoadhesive microspheres adhered more strongly to gastric mucous layer and could retain in gastrointestinal tract for an extended period of time (Figures 3 and 4.). Figure 4 showed that even after 8 h, some of the microspheres were adhered to the gastric mucous layer. All factorial batches showed more than 50 mucoadhesion even after 10 h.

Factorial equation for particle size

The linear model generated for particle size was found to be significant with an F-value of 33.6 ($p < 0.0001$) and R^2 value of 0.9824:

$$\text{Particle size} = 102.33 + 7.16X_1 - 6.83X_2 - 1.5X_1X_2 - 1.5X_2^2 \quad (6)$$

The counter plot (Figure 6(b)) shows that the particle size of microspheres increased from 86.0 to 99.0 μm and 99.0 to 112 μm at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as stirring speed decreased. Results indicate that the effect of X_1 (drug-to-polymer-to-polymer) is more significant than X_2 (stirring speed). This means, as the stirring speed increased, the particle sizes decreased, which directly affected the percentage mucoadhesion.

Thus, one can conclude that the amount of polymer (carbopol-934P) and stirring speed directly affects the percentage mucoadhesion and particles size.

Factorial equation for drug entrapment efficiency

The drug entrapment efficiency and t_{80} are important variables for assessing the drug loading capacity of microspheres and their drug release profile, thus suggesting the amount of drug availability at site. These parameters are dependent on the process of preparation, physicochemical properties of drug and formulation variables. The linear model generated for drug entrapment efficiency

was found to be significant with an *F*-value of 40.70 ($p < 0.0001$) and R^2 value of 0.9954:

$$\text{Drug entrapment efficiency} = 50.11 + 9.16X_1 - 5.66X_2 - 0.66X_1X_2 - 1.75X_1^2 - 18.16X_2^2 \quad (7)$$

The counter plot (Figure 6(c)) shows that the percentage drug entrapment efficiency of microspheres increased from 20.0 to 26.0 and 34.0 to 47.0 at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as stirring speed decreased. However, at medium level of drug-to-polymer-to-polymer ratio, as stirring speed decreased, percentage drug entrapment efficiency of microspheres showed an increase from 41.0 to 56.0. The results of this equation indicate that the effect of X_1 (drug-to-polymer-to-polymer) is more significant than X_2 (stirring speed). Moreover, the stirring speed had a negative effect on the percentage drug entrapment efficiency (i.e. as the stirring speed increased, the particle size decreased and thus drug entrapment efficiency decreased).

Factorial equation for t_{80}

The linear model generated for t_{80} was found to be significant with an *F*-value of 115.91 ($p < 0.0001$) and R^2 value of 0.9994:

$$t_{80} = 536.22 - 169.5X_1 + 42.66X_2 - 23.5X_1X_2 - 56.83X_1^2 - 8.66X_2^2 \quad (8)$$

The counter plot (Figure 6(d)) shows that the percentage drug released *in vitro* from microspheres decreased from 727 to 592 min and 340 to 299 min at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as stirring speed decreased. The results depicted in Table 3 indicate that the percentage drug released *in vitro* is highly dependent on the drug-to-polymer-to-polymer ratio and stirring speed. The drug-to-polymer-to-polymer ratio has a negative effect on t_{80}, while stirring speed has a positive effect on t_{80}, because as the particle size decreases the drug release decreases.

A numerical optimization technique using the desirability approach was employed to develop a new formulation with the desired responses. Constraints like maximizing percentage mucoadhesion, drug entrapment efficiency, particle size and release at the end of 12 h in addition to minimizing the t_{80} were set as goals to locate the optimum settings of independent variables in the new formulation. The optimized microsphere formulation (J10) was developed using a 1 : 3 : 1.25 drug-to-polymer-to-polymer ratio and 950 rpm stirring speed. The optimized formulation was evaluated for percentage mucoadhesion, drug entrapment efficiency, particle size and drug release. The results of experimentally observed responses and those predicted by mathematical models along with the percentage prediction errors were compared. The prediction error for the response parameters ranged between 0.50–2.15%, with the value of absolute error of $1.36 \pm 0.70\%$. The low values of error indicate the high prognostic ability of factorial equation and counter plot methodology. The drug release from the optimized formulation was found to be low t_{80} (410 min), thus, batch J4 was selected for further study, which exhibited a high t_{80} of 502 min and seemed to be a promising candidate for achieving drug release of up to 12 h. The drug release profile of batch J4 is shown in Figure 5. The figure reveals that drug release rate slowed after 4 h. The study focus was the preparation of mucoadhesive microspheres, thus the microspheres of batch J4 were also evaluated in simulated gastric fluid USP (pH 1.2). *In vitro* release test showed that amoxicillin released faster in pH 1.2 hydrochloric acid than in pH 7.8 phosphate buffer, but the results indicated that no significant difference was observed between dissolution profiles at pH 7.8 and pH 1.2 as the f_2 (similarity factor) value was 63.48.

The results of curve fitting of best batch into different mathematical models are given in Table 2. The mechanism of drug release from the microspheres was found to be diffusion controlled because plots of percentage cumulative drug release vs square root of time were found to be linear with the regression coefficient (R^2) values ranging from 0.9780–0.9871 for the best batch. Results of *F*-statistics are shown in Table 2. The release profile fitted to Weibull equation *F*-value was found to be 6.92. The value of correlation coefficient was found to be 0.9931. The values of slope and intercept were found to be 1.27 and −3.17, respectively. The release profile fitted to Korsmeyer-Peppas equation, *F*-value was found to be 20.36. The value of correlation coefficient was found to be 0.9935. The values of slope and intercept were found to be 0.8611 and −2.38, respectively; and release profile fitted to Hixon-Crowell equation, *F*-value was found to be 10.49. The value of correlation coefficient was found to be 0.9889. The values of slope and intercept were found to be 0.0046 and −0.1038, respectively. The results of *F*-statistics were used for the selection of the most appropriate model, thus it was concluded that the release profile fitted best to Weibull equation ($F = 6.92$).

In vivo *study*

At present, most studies of mucoadhesive formulations loading amoxicillin for anti-*H. pylori* focused on prolonging the gastric retarding time. The stability of amoxicillin in acidic medium was neglected. In fact, lots of antibiotics,

such as erythromycin, clarithromycin, were reported with strong *in vitro H. pylori* clearance effect but with poor *in vivo* results. Ogwal and Xide[47] suggested that one of the reasons was due to their instability in acidic medium. Amoxicillin was also reported to be unstable in mediums with pH below 2[48–50]. Amoxicillin can be quickly absorbed after its conventional dosage forms are orally administered. Therefore, its residence time in the stomach is expected to be short[34], which might cover up its shortcoming of being unstable in acidic medium. However, for the mucoadhesive microspheres, which would stay in the stomach for a much longer time, the stability of amoxicillin should be seriously considered. This study found that amoxicillin microspheres were more stable in pH 1.2 HCL than amoxicillin powder.

From the result of the *in vivo H. pylori* clearance test, it was observed that, with the increase of amoxicillin's doses, the *H. pylori* clearance effect was enhanced in mucoadhesive amoxicillin microspheres formulation. In the single dosage administration test, it was found that the total colony counts decreased markedly with the increase of the amoxicillin dose in both groups. Means, $4\,mg\,kg^{-1}$ dose of amoxicillin mucoadhesive microspheres administrated colony counts were 23 ± 4.24 and on increase in the doses to 7.5 and $15\,mg\,kg^{-1}$ colony counts were 7.0 ± 1.41 and 2 ± 0, respectively. On administrating Amoxicillin powder $4\,mg\,kg^{-1}$ dose colony counts were 78 ± 8.48 and on increase in the doses to 7.5 and $15\,mg\,kg^{-1}$ colony counts were 29 ± 5.65 and 18.0 ± 16.97, respectively. While in the case of physiological saline $4\,mg\,kg^{-1}$ dose administrated colony counts were 94 ± 5.65 and then increases in the doses to 7.5 and $15\,mg\,kg^{-1}$ colony counts were 92 ± 9.89 and 92.5 ± 17.67, respectively. Physiological saline did not show any decrease in colony count. However, the ratio of colony counts between amoxicillin powder and mucoadhesive microspheres increased rapidly from 3.39 at $4\,mg\,kg^{-1}$ to 9.0 at $15\,mg\,kg^{-1}$ (Table 4, Figure 7). This phenomenon indicated that, with the increase in dose, mucoadhesive amoxicillin microspheres showed more effective clearance of *H. pylori* than that in the case of amoxicillin powder. It is inferred that this might be due to the lack of repetition of drug administration. Therefore, another multidose administration regimen was tried. The results showed that, at the dose of $3.5\,mg\,kg^{-1}$, when amoxicillin microspheres, powder or physiological saline was administrated, respectively, to the *H. pylori* infectious rat twice a day for three consecutive days (Table 5). Neither *H. pylori* colony was found nor was urease test positive in rats whom mucoadhesive amoxicillin microspheres were administrated. It is concluded that the mucoadhesive amoxicillin microspheres showed a more complete *H. pylori* clearance effect.

Conclusion

The Amoxicillin mucoadhesive microspheres developed employing a 3^2 full factorial design showed high percentage mucoadhesion, drug entrapment efficiency and exhibited a sustained release property for peroral use in the form of capsules. Drug-to-polymer-to-polymer (amoxicillin-ethyl cellulose-carbopol-934P) and stirring speed significant influence on percentage mucoadhesion, drug entrapment efficiency, particle size and t_{80}. The optimized formulation, developed using the desirability approach, showed more effective *H. pylori* activity of mucoadhesive amoxicillin microspheres compared to amoxicillin powder and physiological saline, which might indicate a potential use of mucoadhesive amoxicillin microspheres in treating *H. pylori* infection.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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List of Abbreviation

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DDS	: Drug delivery system
GRDDS	: Gastroretentive drug delivery system
GIT	: Gastro intestinal tract
GI	: Gastro intestinal
BA	: Bioavailability
MMC	: Migrating motor complex
CRDDS	: Controlled release drug delivery system
GRT	: Gastric residence time
GET	: Gastric emptying time
HPMC	: Hydroxyl propyl methyl cellulose
NaCMC	: Sodium carboxyl methyl cellulose
PVA	: Polyvinyl alcohol
NaAl	: Sodium alginate
PEG	: Polyethylene glycol
KSI	: Kilopond per square inch
FDDS	: Floating drug delivery systems
HSPM	: Hot stage polarizing microscopy
SEM	: Scanning electron microscopy
DSC	: Differential scanning calorimetry
FDF	: Floating type dosage form
PEO	: Polyethylene oxide
TFTGR	: Two-layer floating tablet for gastric retention
H. pylori	: Helicobacter pylori
GITS	: Gastrointestinal therapeutic system
PMA	: Polymethacrylic acid
CH	: Chitosan
CP	: CarbopolP934
MDT	: Mean dissolution time

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