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College of Pharmacy

Formulation of Furosemide as a Gastroretentive Floating In-Situ Gelling System for an Oral Controlled Release Dosage Form

A Thesis

Submitted to the Department of Pharmaceutics and the Committee of Graduate Studies of the College of Pharmacy/University of Al-Mustansiriya in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacy "Pharmaceutics"

By

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(B.Sc. pharmacy 2007)

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قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا ۖ إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ٣٢
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Dedication

To my parents whose words of encouragement and push for tenacity ring in my ears. I thank you for your continuing long distance prayers and moral support.

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I dedicate my thesis with love

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Abbreviations

3D	Three dimensional
BCS	Biopharmaceutical Classification System
CHF	Congestive Heart Failure
$CaCl_2$	Calcium Chloride
cp	Centipoise
CR	Controlled Release Dosage Form
ER	Extended Release Dosage Form
FR	Furosemide
FT-IR	Fourier transform infrared spectroscopy
GG	Gellan Gum
GI	Gastrointestinal
GIT	Gastrointestinal tract
GR	Gastroretentive Release Dosage Form
GRDDS	Gastroretentive drug delivery system
GRDF	Gastroretentive Dosage Form
GRT	Gastric Retention Time
HPMC	Hydroxypropylmethylcellulose
IMMC	Interdigestive Myoelectric Motor Complex
IR	Immediate release
IV	Intravenous
log	logarithm
MMC	Migrating Myloelectric Cycle
Mmol/l	Micromole/liter
N	Newton
NaAg	Sodium Alginate
nm	Nanometer
PEG	Polyethylene glycol
pH	Negative Logarithm of Hydrogen Ion Concentration
Q.S.	Sufficient quantity
RPM	Round per minute
GF	Gastric Fluid
SR	Sustained Release
USP	United states pharmacopeia
UV	Ultraviolet
w/v	weight/volume
λ max	Wave length with maximum absorbance

Abstract

Floating in-situ gel is one of the gastroretentive drug delivery systems represents a revolution in the oral controlled release dosage forms in comparative with conventional oral liquids. They prolong the residence time of the drug that has a narrow absorption window in the absorptive sites like stomach or upper gastrointestinal (GI) tract; since they have a bulk density lower than gastric fluids. Thus they remain buoyant in the stomach without affecting the gastric emptying rate until all the drug release continuously at a slow rate.

Furosemide is a high ceiling loop diuretic widely used for patients with congestive heart failure (CHF) to get rid of excess body water, reducing blood pressure, and mobilizing edemas.

This study involved formulation of furosemide oral solution which undergoes gelation upon direct contact with gastric fluid and floated using either primary polymers as sodium alginate and gellan gum or in combination with secondary polymers as iota carrageenan and HPMC (K100M and K4M).

Different evaluations were performed on all 35 in-situ gel formulas to measure the gel strength, gelation time, swelling index, content uniformity, floating lag time, floating duration, pH and density. In addition different variables that affect drug release like types and concentrations of polymer, combination of polymers, gas generating agent, cross linking agent, drug concentrations and taste making agents (sweetening agent) were studied for optimization and selection of the best formula.

Increasing the concentration of the primary polymer (sodium alginate) led to increase swelling index, gel strength, viscosity and consequently reduction in drug release rate. While increasing iota

carrageenan concentration as secondary polymer in the presence of sodium bicarbonate ($NaHCO_3$) led to reduction in gelation time, floating lag time, density and further retardation in the drug release. Increasing $NaHCO_3$ concentration led to increase in drug release while increasing drug and taste masking agent concentrations led to reduction in drug release.

The results revealed that formula (F21) containing (1% w/v) sodium alginate and (0.25% w/v) iota carrageenan was the best formula regarding gel strength (10.96 N/m^2), gelation time (2 seconds), floating lag time (35 seconds), floating duration (24 hr), pH (7.5), density (0.8 g/cm^3) and swelling index (19.7%) with drug release (94.9%) after 5 hrs.

In-vivo test of the selected formula (F21) in comparison to the conventional oral solution of furosemide (Fudesix®) was performed using male albino Wister rats. The results revealed that there was reduction in the excretion rate (urine volume and electrolyte concentrations) during the first hour. While it was increased after 5 and 24 hr unlike that obtained from the conventional solution; indicating that the diuretic profile of furosemide had been affected by the gastroretentive property of the selected formula that gave floating in-situ gel upon contact with stomach content. The results of in-vivo test were agreed with the in-vitro release study and the proposed kinetic mathematical modeling.

The expiration date of furosemide in the selected formula was estimated using accelerated stability study and found to be 2.9 years.

It was concluded that the formulation of furosemide as gastroretentive floating in-situ gel, controls the release leading to improvement in drug absorption and bioavailability.

1. Introduction

In our contemporary epoch innumerable technologies have been made to develop different routes of administration, through which the drug is administered into the body for treatment of various diseases and disorders.

Various routes of administration are classified into the following categories:

1. Systemic routes; enteral route (oral, sublingual, rectal, vaginal) and parenteral route (intravascular, intramuscular, subcutaneous)
2. Local routes; mucosal membranes (nasal, ocular) and topical/skin (dermal, transdermal).
3. Other routes; inhalation (orally, nasally) and intrathecal/ intraventricular [(1)].

1.1 Oral Dosage Form

Among all the routes of administration, the oral route is considered as the most favored, popular and practiced way of drug administration, because of its ease of administration, flexibility in designing, ease of production and the low cost [(2)].

1.1.1 Types of Oral Dosage Forms

Oral route differentiates into two categories:

1- Liquids (i.e. solutions, suspensions)

2- Solids (i.e. tablets, capsules, powders, granules, lozenges, pills) [(3)].

1.1.2. Oral Liquid Dosage Form

Oral liquid dosage forms are homogenous preparations containing one or more active ingredients in a suitable vehicle intended to be swallowed either diluted or after dilution of concentrated liquid preparations, or from powders or granules. They may contain suitable preservatives, antioxidants and other excipients such as dispersing,

suspending, thickening, emulsifying, buffering, wetting, solubilizing, stabilizing, flavors, sweetening and coloring agents [4].

1.1.3. Needs for Oral Liquid Dosage Form

Although solid dosage forms like tablets and capsules are widely used but the selection of oral liquid preparations is due to:

- The dose is adjusted easily by dilution, makes it easier to swallow than solids and is therefore acceptable for pediatric and geriatric use.
- The drug is immediately available for absorption, therefore, the therapeutic response is faster than solid dosage form, which must first disintegrate in order for the drug to be dissolved in the gastrointestinal fluid before absorption begin [5,6].
- It is a homogenous system and therefore the drug will be uniformly distributed throughout the preparation.
- Reduce irritation, because of the immediate dilution by the gastric contents. For example aspirin when administered as solid dosage form cause irritation and damage to the gastric mucosa, since it is localized in one area after the ingestion [6].

1.1.4 Classification of Oral Liquid Dosage Forms

Oral liquid dosage forms can be classified as:

- *Conventional oral liquid dosage form:*

  This drug delivery system results in suboptimal therapy and/or systemic side effects [7]. Several preparations are distinguished including: oral solutions, emulsions, suspensions, elixirs, oral drops, spirits and syrups [8].

❖ *Non-conventional oral liquid dosage form including:*

☞ Extended /Sustained release dosage form (ER/SR): The attractiveness of ER dosage form is the success to ensure safety, improve the efficiency of drug, reduce the dose frequency and thus reduction in the side effects and improvement of bioavailability could be expected. As a result more patient compliance especially for pediatric and geriatric patients or patients that are unable to tolerate solid dosage forms [9].

☞ Controlled/Gastroretentive release dosage form (CR/GR): It offers an alternative and novel strategy for achieving extended release profile, where the formulation will remain in the stomach for a prolonged period, releasing the drug in-situ, which will then dissolve in the liquid contents and slowly pass into the small intestine [10]. Figure 1.1 shows the absorption of drug from both classes.

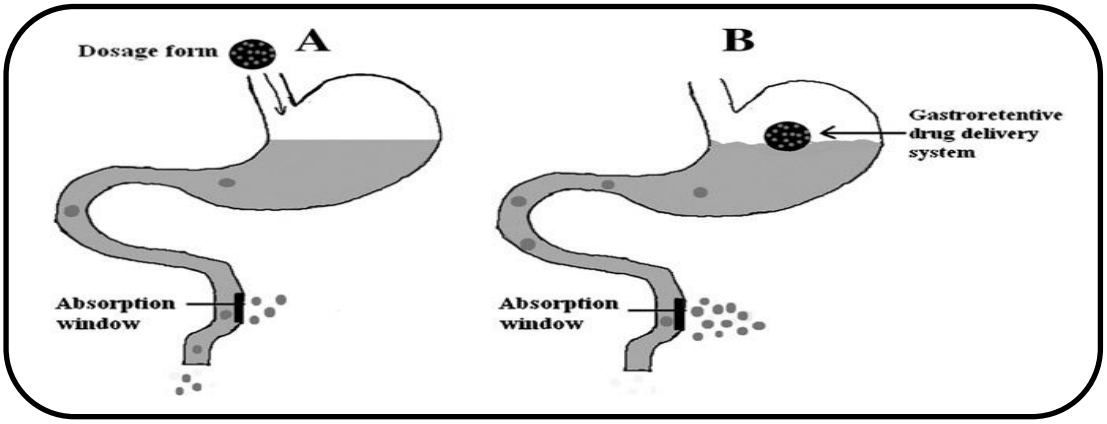


Figure 1.1: Drug Absorption in: (A) Conventional Dosage Form (B) Floating Gastroretentive Drug Delivery System [12]

1.1.5. Advantages of Oral Gastroretentive Release Dosage Form

1- Useful for drugs that are absorbed from specific sites within the GI tract such as stomach e.g. ferrous salts [11].

2- Improve absorption phase of drugs with narrow absorption window at the upper parts of GI tract i.e. less soluble in a high pH environment, may have poor colonic absorption or disturb normal colonic bacteria [12].

3- Improve the pharmacotherapy of the stomach through local drug release and thus less fluctuation in plasma drug level due to continuous releasing of the drug, e.g. systemic antacids: aluminum hydroxide [13].

4- Extending drug delivery in the GI tract by controlling gastric residence time (GRT) and thereby improving bioavailability and reducing side effects by overcoming the physiological adversities like inability to restrain and locate within the desired region. This is due to variable gastric emptying and motility in humans which normally averages 2-3 hr through the major absorption zone, i.e., stomach and upper part of the intestine that can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose [14].

5- Effective for sparingly soluble and insoluble drugs. As the solubility of a drug decreases, the time available for drug dissolution becomes less suitable and thus the transit time becomes a significant factor affecting drug absorption. It is found that gastroretentive drug delivery system provides continuous, controlled release at the absorption site with dose reduction, e.g. acyclovir, metformin, baclofen [15].

6- Gastroretentive drug delivery system is considered much better alternative than other formulations or novel dosage forms like nanoparticle, microspheres and liposome that can also be used for controlled release effect, by improving drug absorption through the stomach. That is by controlling delivery for longer duration and thus reducing the frequent dosing of such drug as a result improve patient compliance [16].

1.2. Biological Aspects of Gastroretentive Dosage Form (GRDF)

1.2.1. Stomach

The gastrointestinal tract is divided into three main regions as shown in Figure 1.2:

1. Stomach.
2. Small intestine: duodenum, jejunum and ileum.
3. Large intestine [17].

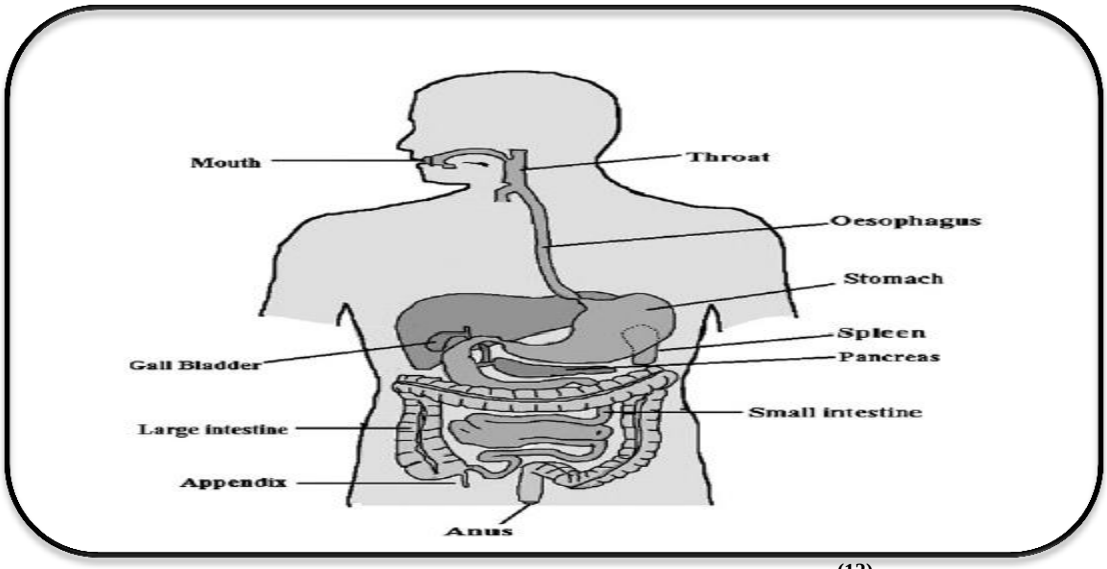


Figure 1.2: Gastrointestinal Tract Anatomy [12]

The walls of GI tract, from stomach to large intestine, have the same basic arrangement of tissues, from outside to inside. The exception is for the stomach that has three different smooth muscle layers which are responsible for performing the motor functions of the GI tract, i.e. gastric emptying and intestinal transit [18] as shown in Figure 1.3.

The stomach is divided into 3 parts:

a) Fundus: Also called proximal stomach, which exerts pressure on the gastric contents by pressing them towards the distal region.

b) Body: The central part, acts as a reservoir for undigested materials.

c) Pylorus or antrum: Also called distal stomach, which acts as a site of mixing motions to propel gastric contents for emptying [19].

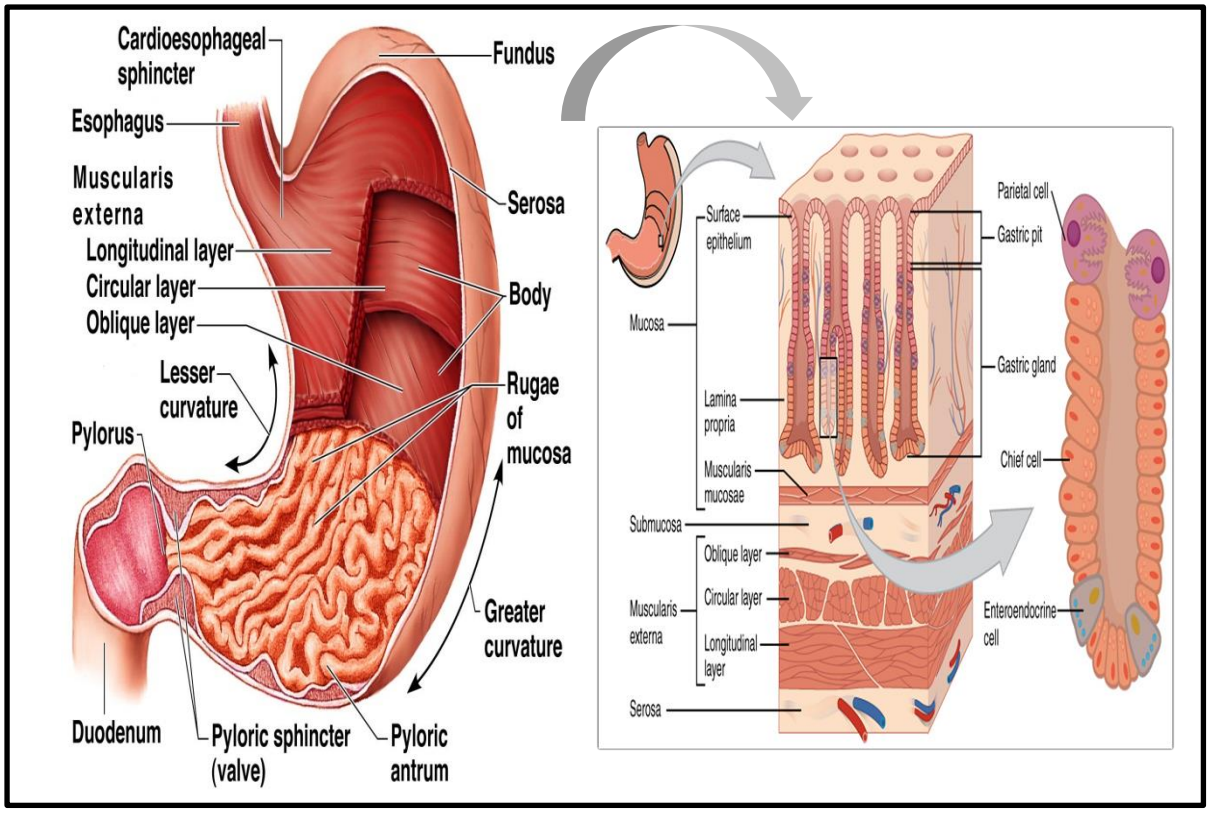


Figure 1.3: Stomach Anatomy [17]

The contraction of gastric smooth muscle serves two basic functions:

- ❖ Ingested food is crushed, grounded and mixed to form chyme.
- ❖ Chyme is forced through the pyloric canal into the small intestine in a process called gastric emptying [20].

1.2.2 Salient Features of Upper GIT

The characteristic features of upper GIT is shown in Table 1.1:

Table 1.1: Features of Upper GIT

Section	Length (m)	Transit time (hr)	PH	Microbial count	Absorbing surface area (m^2)	Absorption pathway
Stomach	0.2	Variable	1 - 4	<103	0.1	P, C, A
Small intestine	6-7	3 ± 1	5 - 7.5	103-1010	120-200	P, C, A, F, I, E, CM

P – Passive diffusion; C – Aqueous channel transport; A – Active transport; F – Facilitated transport; I – Ion-pair transport; E – Entero-or pinocytosis; CM – Carrier mediated transport.

Concerning the stomach:

Gastric pH: Fasted healthy subject 1.1 ± 0.15 while in fed healthy subject 3.6 ± 0.4 and may rise to 6 in the presence of water and food.

Volume: Resting volume (collapsed state) is about 25-50 ml while after meal the volume of distention may reach to 1500 ml.

Gastric secretion: About 60 ml of acid, pepsin, gastrin, mucus and some enzymes are secreted.

Effect of food on Gastric secretion: About 3 liters of secretions are added to the food during gastro intestinal transit time [21].

1.2.3 Physiological Factors Effecting Drug Absorption

1.2.3.1 Gastric Motility

The motility of the stomach is mostly contractile, controlled by a complex set of neural and hormonal signals. Thus gastric motility comes from smooth muscle cells integrating a large number of inhibitory and stimulatory signals which causes food grinding into smaller particles, mixing with gastric juices, forward and backward movements of gastric contents and emptying, with all of the actions occurring together [22, 23].

There are two marked differences between gastric motility:

a) In the fasting state; the motoric activity termed interdigestive myoelectric motor complex (IMMC) or migrating myloelectric cycle (MMC) which is a series of electrical events happening every 2-3hr., also this cycle of peristaltic movements generated to clear the stomach and the small intestine of indigested debris, swallowed saliva and sloughed epithelial cells

b) In the fed state; the digestive mode comprises continuous contractions. These contractions result in reducing the size of food particles (< 1 mm), which are propelled towards the pylorus in suspension form. During the fed state onset of MMC is delayed resulting in slowdown of gastric emptying rate [24].

1.2.3.2 Gastric Emptying Rate

Passage of drug from stomach to the small intestine is called gastric emptying and it occurs during fasting as well as fed states. It is the rate limiting step for drug absorption because the major site for absorption is the intestine. Generally rapid gastric emptying increases bioavailability of the drug. Delayed gastric emptying promotes the dissolution of the poorly soluble drugs and useful for the drugs that is majorly absorbed from stomach or proximal part of intestine [25].

In general the rate of gastric emptying depends mainly on viscosity and volume. However, increase in acidity slows down gastric emptying time. In case of elderly persons, gastric emptying is slowed down. Generally females have slower gastric emptying rates than males. Stress increases gastric emptying rates while depression slows it down [26].

MMC is further divided into following 4 phases as in Figure 1.4:

1. Phase I (basal phase): It lasts from 30 - 60 minutes with rare contractions.
2. Phase II (preburst phase): It lasts for 20 - 40 minutes with intermittent action potential and contractions. As the phase progresses the intensity and frequency also increases gradually.
3. Phase III (burst phase): It lasts for 10 - 20 minutes including intense and regular contractions for short period. It is due to this wave that all the undigested material is swept out of the stomach down to the small intestine. It is also known as the housekeeper wave.
4. Phase IV: It is a period of transition from phase III and phase I and last for 0 - 5 minutes [27].

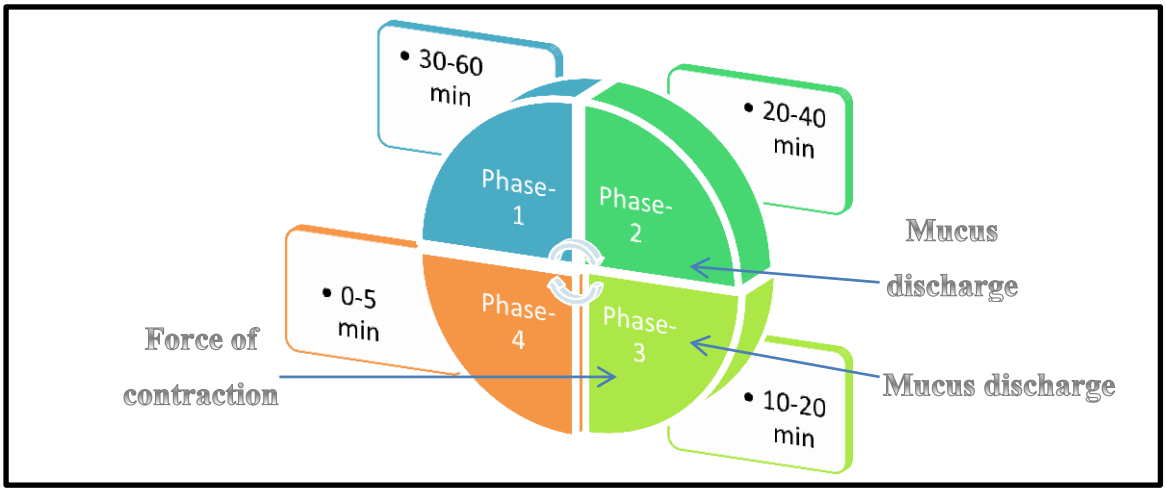


Figure 1.4: Gastrointestinal Motility Pattern [27]

1.3. Factors Controlling Gastroretentive Drug Delivery System (GRDDS)

Various factors considered to affect gastric retention time (GRT) that has impact on the development of gastroretentive dosage forms and prolong the dosing intervals and thus improve patient compliance, these factors can be classified into:

❖ *Factors related to the dosage forms*

- Size of the dosage form

To allow the dosage form to pass through the pyloric valve into the small intestine the particle size should be in the range of 1 to 2 mm. In most cases, the larger the dosage form the greater will be the GRT [28].

- Shape of dosage form

Ring-shaped and tetrahedron-shaped devices have a better gastric residence time as compared to other shapes [28].

- Density of dosage form

The density of the dosage form affects the gastric emptying rate. A buoyant dosage form having a density of less than that of the gastric fluids (1.004g/ml). Thus the dosage unit is retained in the stomach for a prolonged period since it is away from the pyloric sphincter [28].

❖ *Factors related to food intake and its nature*

- Fed & unfed state

Gastric motility is higher in fasting conditions which depicts less gastric retention time [29].

- Nature of food

Usually the presence of food in the gastrointestinal tract and feeding of indigestible polymers or fatty acid salts can change the motility pattern of the stomach to a fed state. Thus decreasing the gastric emptying rate improves the gastric retention time of the dosage form & will increase absorption of drugs by allowing its stay at the absorption site for a longer period of time [29].

- Calorie content

The rate of gastric emptying primarily depends on the caloric contents of the ingested meal. It does not differ for proteins, fats, carbohydrates as long as their caloric content is the same. Generally an increase in acidity, osmolarity, and caloric value slows down gastric emptying [30].

- Frequency of feed

Higher the frequency of taking food, longer will be the gastro retention time [30].

❖ *Patient related factors*

- Gender & Age

Gastric emptying rate may differ in male & female. Generally the gastric emptying in women was slower than in men. Elderly people, especially those over 70 years have a longer gastroretentive time. Thus gastric emptying time is slowed down [31].

- Posture

Gastric retention time can vary between supine and upright patient states. In the upright position, the floating systems floated to the top of the gastric contents and remained for a longer time, showing prolonged GRT. But the non-floating units settled to the lower part of the stomach and underwent faster emptying as a result of peristaltic contractions. However, in supine position, the floating units are emptied faster than the non-floating units of similar size [31].

- Concomitant drug administration

Administration of anticholinergic drugs like atropine increases gastric residence time by contracting GI smooth muscles thus decreases tone, amplitude and frequency of the peristaltic contractions. While drugs like metoclopramide decreases gastric residence time by stimulating GI smooth muscle and thus augments acetylcholine release and sensitizes muscarinic receptors [31].

❖ *Disease state*

Diseases like gastroenteritis, pyloric stenosis and diabetes shows an increase in gastric residence time. In the case of partial or total gastrectomy and duodenal ulcers there is a decrease in gastric residence time [32].

❖ *Volume of the GI fluid*

The volume of liquids administered affects the gastric emptying time. When the volume is large, the emptying is faster. Cold fluids delay gastric emptying while warmer fluids fasten gastric emptying [32].

❖ *Effect of gastrointestinal fluid*

On comparison between the floating and non-floating units, it was concluded that regardless of their sizes the floating units remained

buoyant on the gastric contents protected from the peristaltic waves during the digestive phase, while the non-floating units stayed close to the pylorus and were sink, thus they are subjected to propelling and retropelling waves of the digestive phase [32].

1.4. Requirements for Gastric Retentive Dosage Form

One of the key issues is that the dosage form should achieve gastric retention by:

1- Satisfying factors like density, size and shape of dosage form in the stomach.

2- It must be able to withstand the forces caused by peristaltic waves in the stomach, constant contractions and grinding.

3- It must resist premature gastric emptying.

4- Furthermore, once its purpose has been served, it should easily leave the stomach [33].

1.5 Approaches for Gastric Retentive Dosage Form

Several technological approaches have been made in the last decade to develop a dosage form that increases the retention of an oral dosage form in the stomach. These approaches for GRDDS are shown diagrammatically in Figure 1.5 and schematically in Figure 1.6

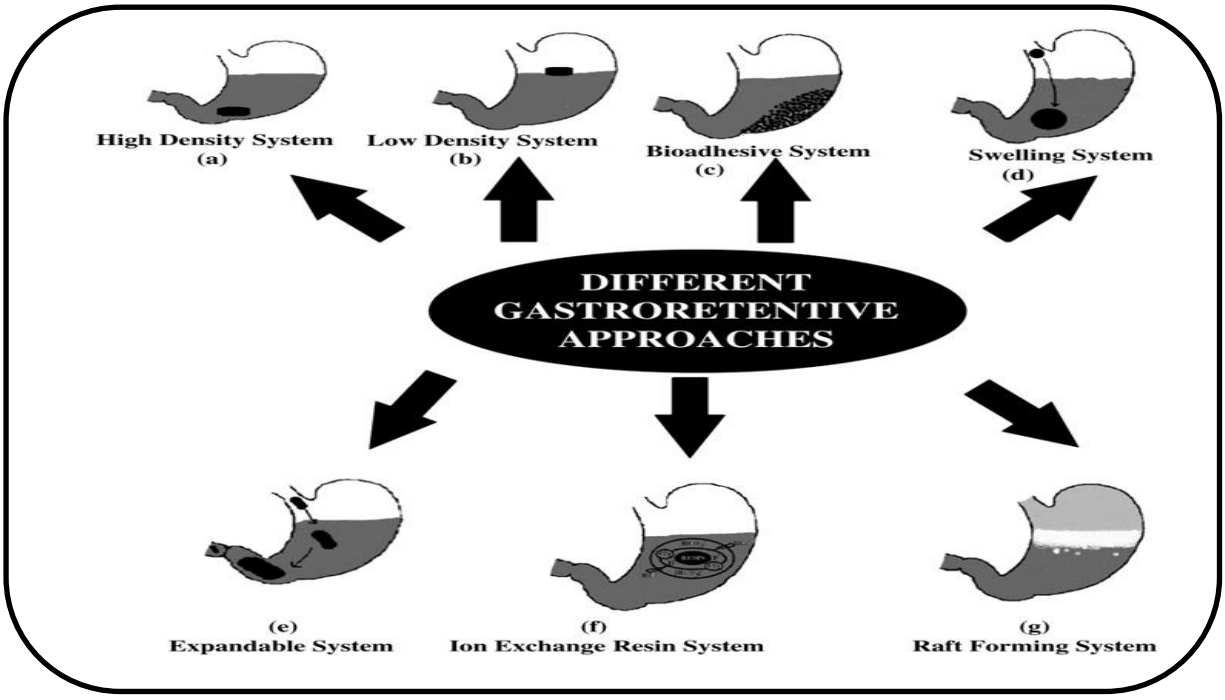


Figure 1.5: Different Approaches of Gastroretentive Drug Delivery System [12]

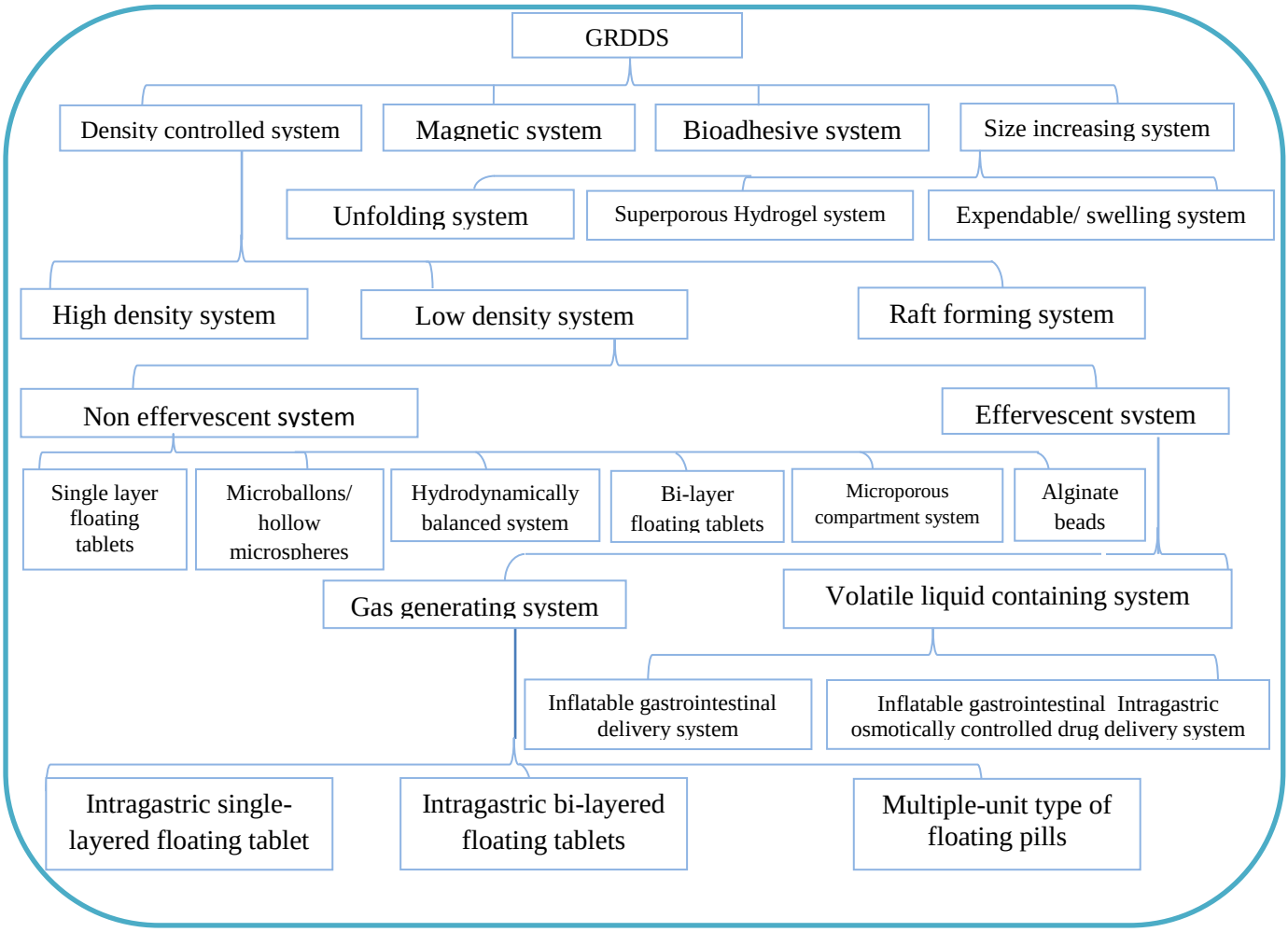


Figure 1.6: Various Approaches Used for Gastroretentive Drug Delivery System [34]

1.5.1 Size Increasing Systems

Retention of dosage form in the stomach can be achieved by increasing its size above the diameter of pylorus (13 mm) (even during the housekeeper wave). Initially, the dosage form should be of small size to facilitate swallowing but after coming in contact with the gastric fluid, it should increase in size quickly to avoid the premature gastric emptying. After a definite time interval, the system should be cleared from the stomach [34]. The size increasing system can be achieved by the following:

❖ *Expandable/Swellable Systems*

This dosage form is retained in the stomach for a long period of time referred to as "plug type systems" because they tend to remain lodged at the pyloric sphincter. Thus three arrangements are required:

a- Oral intake swallowed in a small configuration.

b- Expanded to a size that prevents their passage through the pylorus as shown in Figure 1.7.

c- Finally after the drug release at a predetermined time, it becomes ready for evacuation since the device is no longer can attain or retain the expanded configuration. This is because the system will lose its integrity as a result of a loss of mechanical strength caused by abrasion or erosion or will burst into small fragments when the membrane ruptures as a result of continuous expansion in addition it may erode in the presence of gastric juice [35].

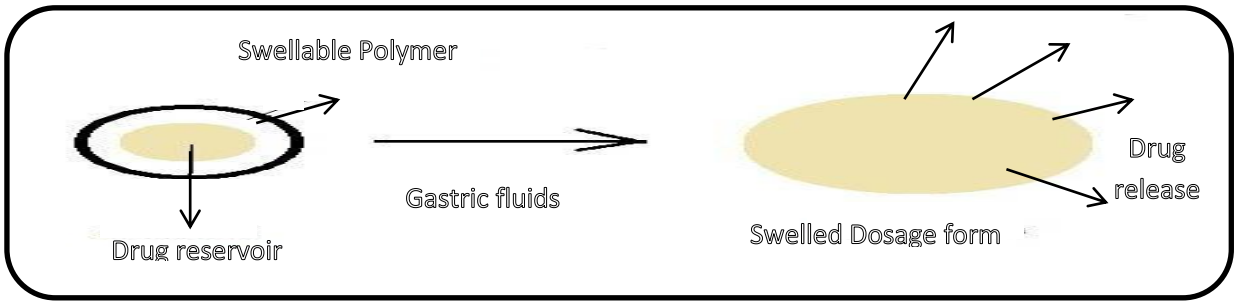


Figure 1.7: Drug Release from Swellable Systems [18]

❖ *Superporous Hydrogels Systems*

Although these are swellable systems but they differ sufficiently from the conventional types. Where conventional hydrogels with pore size ranging between 10 nm and 10 μm has very slow process of water absorption and require several hours to reach an equilibrium state during which premature evacuation of the dosage form may occur. While the superporous hydrogel, have an average pore size >100 μm which swell to an equilibrium size within a minute, due to rapid uptake of water by capillary wetting through numerous interconnected open pores. Moreover, they swell to a large size (swelling ratio 100 or more) and intended to have sufficient mechanical strength to withstand pressure by gastric contraction [36] as shown in Figure 1.8.

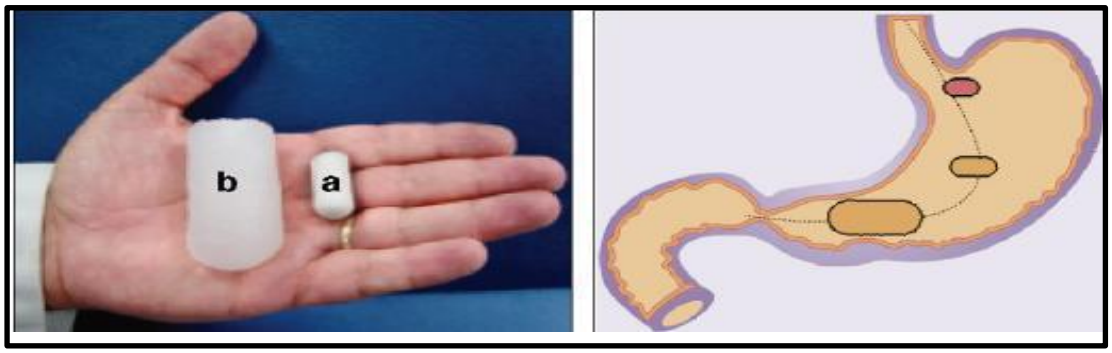


Figure 1.8: Schematic Illustration of the Transit of Superporous Hydrogels, on the Left (a) in its Dry State (b) in its Water-Swollen State. On the Right; the Transit of Superporous Hydrogel [18]

❖ *Unfolding Systems*

This system contains several unfolding geometrical shapes as shown in Figure 1.9, such as tetrahedron, ring, clover leaf, disk, string and pellet/sphere that increases in the dimensions ultimately and prevents passage through the pylorus. For convenient uptake the dosage form should be packed tightly into a gelatin capsule which will dissolve in the stomach and the device unfold or open out to achieve extended configuration after dissolution of capsules shell. These systems consist of

at least one erodible polymer and a drug that is dispersing within the polymer matrix necessary for prolonged gastroretentive time [37].

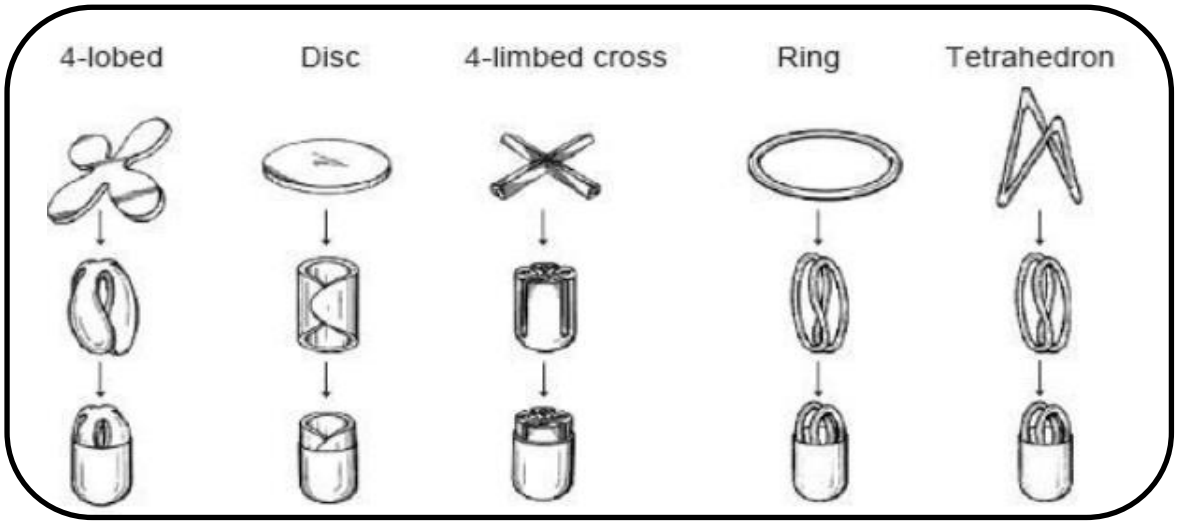


Figure 1.9: Different Geometric Forms of Unfolding Systems [18]

1.5.2 Bioadhesive/ Mucoadhesive Systems

The term bioadhesion is defined as an adhesion to a biological surface i.e. mucin and / or gastric epithelial (mucosal) surface and thus extend the GRT of drug delivery system in stomach [38]. Mucoadhesive controlled release systems increase the effectiveness of the drug by maintaining the drug concentration within therapeutic level, inhibiting the dilution of drugs in body fluids, and allowing targeting and localization of drugs at specific site. The duration of contact and intimacy between polymer-drug particles and mucosal surface is increased by mucoadhesion with the biological membrane [39].

1.5.3 Magnetic Systems

This dosage form contains small internal magnet incorporated inside the core or matrix of the system and an external magnet placed on the abdomen over the position of the stomach. But the external magnet must be positioned with a degree of precision that might compromise patient compliance [40].

1.5.4 Density Controlled Systems

1.5.4.1 High Density Systems

Sedimentation has been employed as retention mechanism for high-density systems that are small enough to be retained in the folds of stomach body near the pyloric region. This approach involves formulation of dosage forms with the density that must exceed density of normal stomach content (~ 1.004 g/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. The materials increase the density by up to 1.5-2.8 g/cm^3 [41].

1.5.4.2 Raft Forming Systems

It is an advanced revolution in oral controlled drug delivery that has received much attention for the delivery of the drug for gastrointestinal infections and disorders. The raft forming system is one of the approaches which involve the formulation of effervescent floating liquid, which has been assessed for sustaining drug delivery and targeting. The mechanism of the raft forming system involves the formation of a viscous cohesive gel in contact with gastric fluids, where in each portion of the liquid swells forming a continuous layer called a raft. This layer floats on the gastric fluid because it has bulk density less than the gastric fluid so the system remains buoyant in the stomach over its content without affecting the gastric emptying rate but it prevents the reflux of gastric content into the esophagus by acting as a barrier between the stomach and the esophagus [42].

1.5.4.3 Low Density Systems/Floating Systems

Floating drug delivery system is one of the important approaches to achieve gastric retention and to obtain sufficient drug bioavailability. Such delivery system is desirable for drugs with an absorption window in

the stomach or in the upper small intestine [43]. Floating systems have bulk density lower than that of the gastric fluid, and thus remain buoyant in the stomach without effecting gastric emptying rate for a prolonged period of time. While the system floats over the gastric contents, the drug is released slowly at the desired rate, which results in increased GRT and reduced fluctuation in plasma drug concentration [44].

1.5.5 Merits and Demerits of GRDDS

The advantages and disadvantages of different GRDDS approaches are presented in Table 1.2.

Table 1.2: Merits and Demerits of Different GRDDS Approaches

Approaches	Merits and Demerits
Expandable system	**Merits:** Small in size and can be easily swallowed, also increases in size to prevent passing through pylorus for prolonged stay in the stomach [45]. **Demerits:** Time consuming, difficulty in formulation, not widely used [46].
Superporous hydrogel system	**Merits:** Fast swelling property, high swelling ratio, good mechanical strength and short swelling time **Demerits:** Weak mechanical properties [47].
Bioadhesive/ Mucoadhesive system	**Merits:** Improves patient compliance, excellent accessibility, rapid onset of action, reduce the frequency of dosing, rapid absorption. **Demerits:** Bioadhesion is difficult to maintain due to rapid turnover of mucin in GIT, occurrence of local ulcerous effects due to prolonged contact of the drug in stomach [48].
Magnetic system	**Merits:** Extending gastric retention of drugs in the stomach. **Demerits:** Not widely used because external magnet should be positioned with high degree of precision [49].
High density system	**Merits:** Density higher than gastric fluids so retained in the antrum part of the stomach and capable of withstanding its peristaltic movements thus allows the release of drug for a prolonged period of time [50]. **Demerits:** Not marketed because difficult to manufacture with high amount of drug (>50%) also difficult to achieve a density of about 2.8 g/cm^3 [51].

Approaches	Merits and Demerits
Raft forming system	**Merits:** • Forms a low density viscous layer on gastric contents so more surface area that lead to more drug release and improve therapeutic efficacy. • Improve patient compliance by once a day therapy and its action is within seconds for long duration. **Demerits:** • Stability problem that will lead to change in the pH on prolonged storage also chemical degradation or microbial degradation [52, 12].
Low density system/ Floating Systems	**Merits:** • Better compliance so widely used, no dose dumping, and improve efficacy due to sustained release of drugs by reducing the frequency of dosing. • Floats on gastric fluid that release drug slowly thus improve drug absorption because of increasing GRT that enhance bioavailability and reduce irritation. • Specific drug delivery for drugs that are absorbed and local action through the stomach e.g. ferrous salts and antacids [53]. **Demerits:** • Buoyancy cannot be predicted that may cause obstruction in the GI tract producing irritation. • Float requires high level of fluids in the stomach with a minimum of full glass of water (200-250 ml). • Not taken before going to bed because gastric emptying occurs randomly and highly dependent on the diameter and size in supine position [54, 55].

1.6 Floating Drug Delivery System

1.6.1 Classification of Floating Drug Delivery System

Based on the mechanism of buoyancy two distinctly different technologies have been utilized for the development of floating drug delivery system including:

1.6.1.1 Non Effervescent System

The non-effervescent floating drug delivery system is based on the mechanism of swelling of polymer in the GI tract. Non-effervescent floating dosage forms use a gel forming or swellable cellulose type of hydrocolloids, polysaccharides, and matrix forming polymers. The formulation methods of such dosage forms involve mixing of the drug

with a polymer, which swells upon contact with gastric fluid due to air trapped by the swollen polymer that confers buoyancy to these dosage forms after oral administration. In addition it maintains a relative integrity of the shape and a bulk density less than one within the gastric environment. The so formed swollen gel-like structure acts as a reservoir and allows sustained release of drug through the gelatinous mass. The excipients widely used in these systems are hydroxypropylmethylcellulose (HPMC), sodium alginate and calcium chloride [56]. There are many types of non-effervescent system including:

❖ *Hydrodynamically balanced systems (HBS)*

These systems contain drug mixed with gel-forming hydrocolloid polymer administered in a gelatinous capsule to remain buoyant on the stomach content [57]. The capsule shell dissolves in contact with water and the mixture swells to form gelatinous barrier, which imparts buoyancy to dosage form in gastric juice for a long period. Continuous erosion of the surface allows water penetration to the inner layers maintaining surface hydration and buoyancy of the dosage form [58], as shown in Figure1.10.These systems contain one or more gel forming hydrophilic polymers like (HPMC) which is most commonly used, hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), sodium carboxymethylcellulose (NaCMC), agar, carrageenan and alginic acid [59].

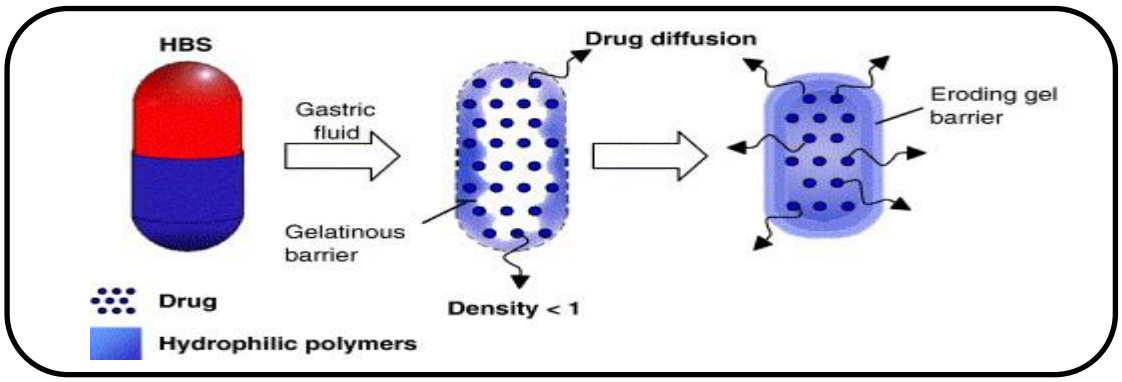


Figure 1.10: Hydrodynamically Balanced System (HBS) [59].

❖ *Microballoons/Hollow microspheres*

Microspheres are defined as homogeneous, monolithic particles in the size range of about 0.1-1000 μm and are widely used as drug carriers for controlled release. They float on the stomach contents, and then adhere to the mucous linings as the stomach empties as shown in Figure1.11. Commonly used polymers to develop these systems are polycarbonate, cellulose acetate, calcium alginate, eudragit S, agar, low methoxylated pectin [(60)].

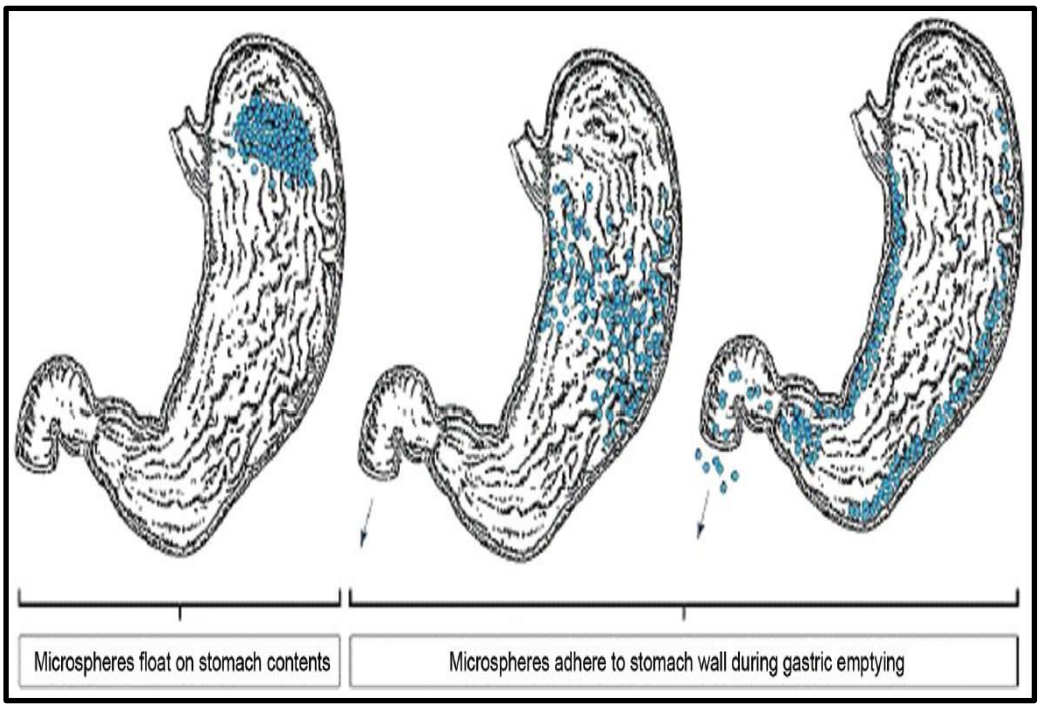


Figure 1.11: Mechanism of Retention of Microspheres in Human Stomach [(60)]

❖ *Alginate beads*

To develop a floating system based on cross-linked beads, spherical beads of approximately 2.5 mm in diameter can be formulated by using Ca^{2+} and sodium alginate. In this approach generally, sodium alginate solution is dropped into an aqueous solution of calcium chloride and causes the precipitation of calcium alginate. These beads are then separated and dried by air convection and freeze drying, leading to the

formulation of a porous system, which can maintain a floating force for over 12 hr. These beads improve GRT for more than 5.5 hr [61].

❖ *Microporous compartment system*

This approach is based on the principle of the encapsulation of a drug reservoir inside a microporous compartment with pores along its top and bottom walls as shown in Figure 1.12. The peripheral walls of the device were completely sealed to prevent any direct contact of the gastric surface with the undissolved drug. In the stomach the floatation chamber containing entrapped air causes the delivery system to float in the gastric fluid. Gastric fluid enters through the aperture, dissolves the drug and causes continuous transport of the drug across the intestine [62].

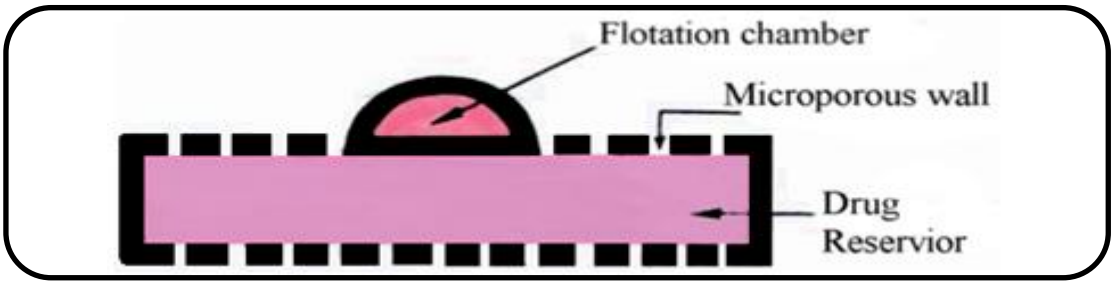


Figure 1.12: Microporous Compartment Model [62]

❖ *Floating tablets*

☞ *Single layer floating tablets.*

They are formulated by uniform mixing of a drug with low density gel-forming hydrocolloid enteric materials such as cellulose acetate phthalate and hydroxyl propyl methyl cellulose, which swells in contact with gastric fluid and maintains specific gravity less than one [63].

☞ Bi-layer floating tablets

A bi-layer tablet contains two layers, one is an immediate release layer which releases loading dose from the system while the other is a sustained release layer which releases dose by absorbing gastric fluid to form an impermeable colloidal gel barrier on its surface, and maintains a

specific gravity less than unity and thereby remains buoyant in the stomach [64].

1.6.1.2 Effervescent System

❖ *Gas-generating Systems*

Floatability achieved when the system reached stomach and came in contact with gastric fluids, then entrapment of liquid in the gelled hydrocolloid layer matrices prepared with swellable polymers such as methylcellulose (MC) and HPMC. The reactions occur between carbonate/bicarbonate salts and citric/tartaric acid to liberate CO_2 gas at body temperature, thus decreasing its specific gravity making it to float in the stomach and release the drug slowly at a desired rate [65].

Effervescent substances incorporated in the hydrophilic polymer, and CO_2 bubbles are trapped in the swollen matrix as in Figure 1.13 [66].

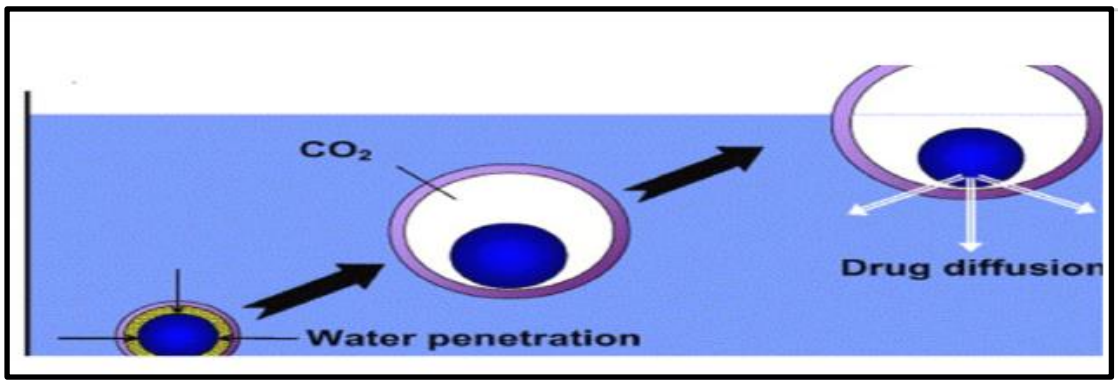


Figure 1.13: Gas Generating System [66]

There are many types of gas generating system including: intragastric single-layered floating tablet [67], intragastric bi-layered floating tablets [68] and multiple-unit type of floating pills [69].

❖ *Volatile liquid containing system.*

This device is a controlled floating system which increases GRT and sustained the release of the drug. It contains a hollow deformable unit that can be transformed from a collapsed to an expanded position and returned to collapse position after an extended period. The deformable

unit consists of two chambers separated by an impermeable, pressure responsive, movable bladder. The first chamber loaded with the drug and the second chamber loaded with the volatile liquid shown in Figure 1.14 [70]. There are several types of volatile liquid containing system including inflatable gastrointestinal delivery system [71] and intragastric osmotically controlled drug delivery system [72].

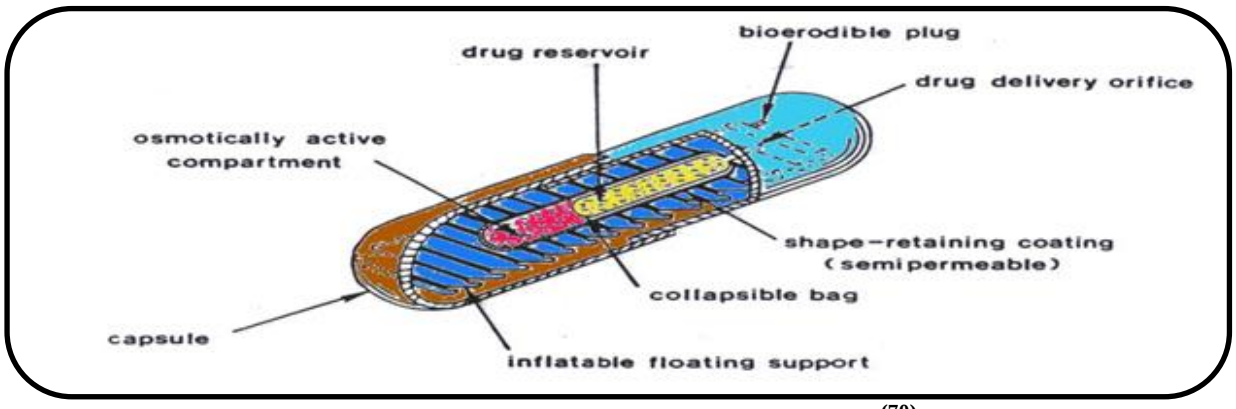


Figure 1.14: Volatile Liquid Containing System [70]

1.7 In-Situ Gelling Systems

In-situ is a Latin word which means 'In its original place or in position' [73]. Extensive researches focused on the development of new drug delivery systems with improving efficacy and bioavailability together, thus reducing dosing frequency to minimize side effects. As a progress, they design in-situ forming polymeric delivery systems sparked by the advantages of easy administration, accurate dose as well as prolong residence time of drug in contact with mucosa compared to conventional liquid dosage form, improved patient compliance and comfort [74].

In-situ gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. Smart polymeric systems represent promising means of delivering the drugs; these polymers undergo sol-gel transition upon administration [75]. Gels are an intermediate state of matter containing both solid and liquid

components. The solid component comprises a 3D network of inter connected molecule or aggregates which immobilizes the liquid continuous phase. Gels may also be classified (based on the nature of the bonds involved in the 3D solid network): chemical gels arises when strong covalent bonds hold the network together and physical gels when hydrogen bonds, electrostatic and Vander walls interaction maintain the gel network [76].

Hydrogels are aqueous gel having high molecular weight, hydrophilic, cross-linked polymers or copolymers that form a 3D network in water. These gels have been shown to combine significantly longer residence time with increased drug bioavailability. The hydrogels are polymers which have the ability to absorb and retain large amounts of water and biological fluids; in addition, they swell and induce a liquid-gel transition [77].

Gastroretentive floating In-situ gel refers to a polymer solution of low viscosity which upon coming in contact with the gastric fluids; undergoes change in polymeric conformation and a viscous strong gel of density lower than the gastric fluids is produced. The gelation can be triggered by temperature modulation, pH change, and ionic crosslinking. Insitu gels can be administered by oral, ocular, rectal, vaginal, injectable and intra-peritoneal routes [78, 79].

1.7.1 Approaches of Designing In-situ Gel System

I) physically induced in-situ gel systems

A- Swelling: In situ formation occurs when material absorbs water from surrounding environment and expands to give the desired space. Example of substance is myverol 18-99 (glycerol mono-oleate), which is polar lipid that swells in water to form liquid crystalline phase structures. It has

some bioadhesive properties and can be degraded in vivo by enzymatic action.

B- Diffusion: This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. N-methyl pyrrolidone (NMP) has been shown to be useful solvent for such system [80].

II) Chemically induced in-situ gel systems

A- Ionic crosslinking: Certain ion sensitive polysaccharides such as iota carrageenan, gellan gum(Gelrite®), pectin, sodium alginate undergo phase transition in presence of various ions such as k^+ , Ca^{2+}, Mg^{2+}, Na^+ [81]. In-situ gel formation involves administration of aqueous liquid solutions, once administered they form gel under certain conditions involve the use of gelling agent which can form a system that contain the dispersed drug and other excipients. The gelling of this system is achieved by using polymer solutions such as gellan gum & sodium alginate triggered by ionic complexation that contains divalent-ions complexed with Na-citrate which breakdown in acidic environment of stomach to release free divalent ions (Ca^{2+}) due to change in pH. The free Ca^{2+} ions get entrapped in polymeric chains thereby causing cross linking of polymer chains to form matrix structure causes the in situ gelation of orally administered solution as shown in equation [82]:

$$\text{Sodium citrate} + NaHCO_3 + CaCl_2 \longrightarrow \text{Ca. citrate}$$

$$\text{Complex} \xrightarrow{\text{Acidic Environment}} Ca^{2+} + COO^-$$

In-situ gel involves formation of double helical junction zones by aggregation of double helical segments to form dimensional network by complexation with cations& hydrogen bonding with water. While the system is floating in the stomach the drug is released slowly at the desired

rate from the system. After release of drug, the residual system is emptied from the stomach [83].

B- Enzymatic crosslinking: In-situ gel formation catalyzed by natural enzymes. For example, cationic pH-sensitive polymers containing immobilized insulin and glucose oxidase can swell in response to blood glucose level releasing the entrapped insulin. Thus adjusting the amount of enzyme controls the rate of gel formation, which allows the mixtures to be injected before gel formation [84].

C- Photo-polymerization: A solution of monomers such as acrylate or other polymerizable functional groups and initiator can be injected into tissue site and the application of electromagnetic radiation used to form gel designed to be readily degraded by chemical or enzymatic processes or can be designed for long term persistence in-vivo. Typically; long wavelength ultraviolet and visible wavelengths are used, while short wavelength ultraviolet is not used because it has limited penetration of tissue and biologically harmful [84].

III) In-situ gel formation based on physiological stimuli

A- Temperature dependent in-situ gelling: These hydrogels are liquid at room temperature (20ºC-25ºC) and undergo gelation when contact body fluids (35ºC-37ºC), due to an increase in temperature. This approach exploits temperature-induced phase transition. Some polymers undergo abrupt changes in solubility in response to increase in environmental temperature (lower critical solution temperature, LCST) and formation of negative temperature sensitive hydrogel in which hydrogen bonding between the polymer and water becomes unfavorable, compared to polymer–polymer and water–water interactions. Also an abrupt transition occurs as the solvated macromolecule quickly dehydrates and changes to a more hydrophobic structure. Alternatively,

some amphiphilic polymers increase LCST, where self-assembles in solution show more micelle packing and gel formation because of polymer– polymer interactions when temperature is increased for e.g. crosslinked N-isopropylacrylamide-co-butylmethacrylate {P(NIPAAm-co-BMA)} polymer. A positive temperature- sensitive hydrogel has an upper critical solution temperature (UCST), such hydrogel contracts upon cooling below the UCST and swell at high temperature for e.g. polymer networks of poly (acrylic acid) (PAA) and polyacrylamide (PAAm) or poly (acryl amide-co-butyl methacrylate) have positive temperature dependence of swelling [78, 85].

B- pH dependent in-situ gelling: Polymers containing acidic or alkaline functional groups that respond to changes in pH are called pH sensitive polymers. The pH is an important signal, which can be addressed through pH-responsive materials. Gelling of the solution is triggered by a change in pH. The polymers with a large number of ionizable groups are known as polyelectrolytes. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups. For example: carbomer and its derivatives as anionic polymer [86].

1.7.2 Mechanisms of Drug Release from In-situ Gel System

1- Diffusion- controlled mechanism:

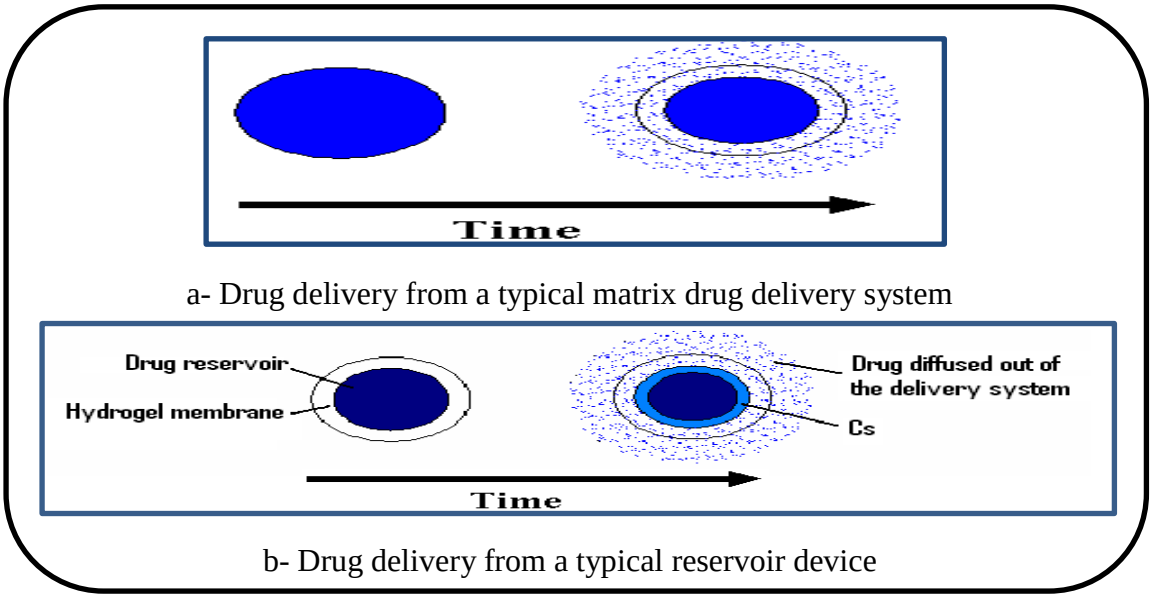
a- Matrix system: The active agent is homogenously dispersed as a solid into a hydrogel inert bio-degradable polymers matrix as in Figure 1.15a. The release of drug depends on:

1- Diffusion of water into the matrix followed by the dissolution of the drug and finally the diffusion of the dissolved drug from the matrix.

2- Polymers interact with drugs leading to modulate the release of the drug.

3- Thickness of the hydrated matrix is considered as the diffusional path length of the drug. If we consider the polymer matrix to be inert and the drug release is diffusion-controlled, then the release rate of the drug could be described by Higuchi equation [87].

b- Reservoir devices: The drug is contained in a core (often termed as reservoir) which is surrounded by a rate-controlling polymeric membrane of hydrogel which allows the diffusion of drug as shown in Figure 1.15b. As the system comes in contact with water, water diffuses into the system and dissolves the drug, and then drug transport (from the core through the external polymer membrane) occurs by dissolution at one interface of the membrane and diffusion driven by a gradient in thermodynamic activity. Drug transport can be described by Fick's first law, if the activity of the drug in the reservoir remains constant and infinite sink conditions are maintained, then the drug release rate may be continued to be constant since it depends on the membrane permeability and it will be independent of time, thus zero-order kinetics can be achieved. Once drug is exhausted, the release becomes concentration dependent following first order kinetics. These kinds of drug delivery systems are mainly used to deliver the active agent by oral routes [88].



a- Drug delivery from a typical matrix drug delivery system

b- Drug delivery from a typical reservoir device

Figure 1.15: Drug Delivery System a- From Typical Matrix b- From Typical Reservoir Device [87]

2- Swelling-controlled mechanism [89]

a- Solvent activated system: It occurs when diffusion of drug is faster than hydrogel swelling. When a hydrogel is placed in an aqueous solution, water molecules will penetrate into the polymer network that occupy some space, and as a result some meshes of the network will start expanding, allowing other water molecules to enter within the network. But, swelling is not a continual process; the elasticity of the covalently or physically cross-linked network will counter-balance the infinite stretching of the network to prevent its destruction. For example the release of drugs from (HPMC) hydrogel is commonly modeled using this mechanism. If the drug delivery system is a true swelling-controlled system then it is described by Ritger and Peppas equation as shown in Table 1.3:

Table 1.3: Types of Swelling Drug Delivery System

Delivery systems	Mechanism of release
Fickian system	Fickian diffusion
Anomalous transport	Fickian diffusion and polymer relaxation
Case II transport	Polymer relaxation
Super case II transport	Plasticization at gel layer

b- Osmotic swelling: For hydrogels, the total swelling pressure of gel could be related to volume fraction, relaxed volume of network, and cross-link density while it is independent on gel pH and swelling time [90].

3- Chemically-controlled mechanism

It can be categorized according to the type of chemical reaction occurring during drug release within a delivery matrix into:

a) Pendant chain system is the most common reaction where the drug is covalently attached to a polymer backbone. The bond between the drug and the polymer is labile and can be broken by hydrolysis or enzymatic degradation and then the drug release.

b) Erodible drug delivery system where the release of the drug is controlled by the dissolution during surface-erosion or bulk-degradation of the polymer backbone then the drug diffuses from erodible systems.

Depending on whether diffusion or polymer degradation controls the release rate, the drug is released following different mechanisms; if erosion of polymer is much slower than diffusion of the drug through the polymer, then drug release can be treated as diffusion controlled process. While if diffusion of the drug from the polymer matrix is very slow, then polymer degradation or erosion is the predominate mechanism, for example hydrophobic erodible polymers [91, 92].

1.7.3 Criteria of Drugs Suitable for In-situ Gel Drug Delivery System [93]

• Drugs that act primarily in the stomach like misoprostol.

• Drugs that are primarily absorbed from the stomach like amoxicillin trihydrate.

• Drugs those are poorly soluble at alkaline pH like verapamil HCl and diazepam.

• Drugs with a narrow window of absorption like levodopa and cyclosporine.

• Drugs which are rapidly absorbed from the GIT like tetracycline.

• Drugs that degrade in the colon like ranitidine and metformin.

• Drugs that disturb normal colonic microbes like ampicillin.

1.7.4 Criteria of Drugs Unsuitable for In-situ Gel Drug Delivery System [94]

• Drugs that have very limited acid solubility e.g. (phenytoin).

• Drugs that suffer instability in the gastric environment e.g. (erythromycin) or solubility problem in GIT for e.g. (phenytoin).

• Drugs intended for selective release in the colon e.g. (5- amino salicylic acid and corticosteroids).

• Drugs that are absorbed along entire GIT, which under go first-pass metabolism e.g. (nifedipine, propranolol).

1.7.5 Polymers of In-situ Gel System

1.7.5.1 Polymers Selection for In-situ Gel System [95]

The polymers selection for preparation of in-situ gel drug delivery system should be soluble, biologically compatible, biodegradable, having good drug polymer linkage, good mechanical strength and inert.

1.7.5.2 Classification of Polymers of In-Situ Gel System [96, 97]

Polymers used for in-situ gel system can be classified according to:

1. Interaction with water: This include soluble polymers (e.g. polyethylene glycol (PEG)), cellulose based polymers (e.g. HPMC) and hydrocolloids (e.g. carrageenan, sodium alginate).
2. Natural polymers: This includes polymers (e.g. gellan Gum).
3. Bio-stability: This includes biodegradable polymers (e.g. chitosan).

1.7.5.3 Polymers Used in this Study

Sodium Alginate (Na Alginate):

It is a linear polysaccharide extracted from brown seaweed consists chiefly of the sodium salt of alginic acid, which is a mixture of polyuronic acids composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues joined by 1,4-glycosidic linkage as shown in Figure 1.16.

Gelation of dilute solutions of sodium alginate takes place upon contact with simulated gastric fluid; when divalent cations (usually calcium ions) interact ionically by a co-operative process involving consecutive blocks of guluronic residues in the α -l-guluronic acid (G) blocks of the alginate chain, resulting in the formation of a three-

dimensional network that is usually described by an 'egg-box' model. It is the ion exchange process between sodium and calcium ions that is supposed to be responsible for the swelling and subsequent degradation of sodium alginate in the colon [98].

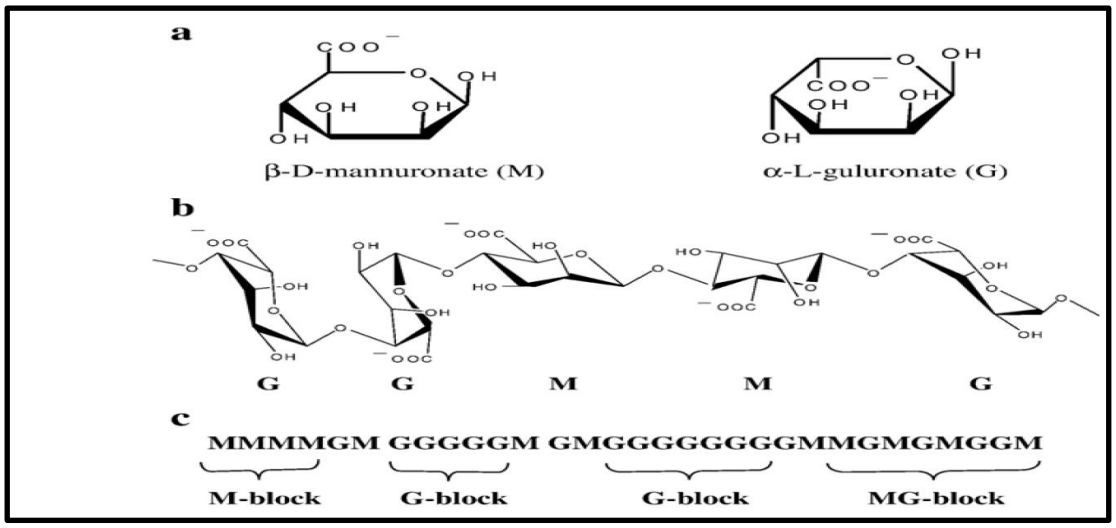


Figure 1.16: Structure of Sodium Alginate [99]

Sodium alginate applied pharmaceutically as a water soluble polymer so useful in SR liquid preparations for oral administration, act as a stabilizing agent; viscosity-increasing agent, as a hydrogel systems for delivery of proteins and peptides, as tissue engineering matrices, as both a binder and disintegrant in tablet formulations and as a diluent in capsule formulations [99].

Gellan Gum:

(Gelrite®) It is an anionic, deacetylated extracellular linear polysaccharide with a tetra saccharide repeating unit of one α-L-rhamnose, 1β-D-glucuronic acid and 2β-D-glucose obtained from cultured solution of Pseudomonas species as shown in Figure 1.17.

In an ion-free aqueous medium; the polymer chains form double helices, resulting in a fluid that has a viscosity close to that of water. In the presence gel-promoting cations (K^+, Mg^{2+}, Ca^{2+}, and Na^+), portion of the helices associates and the cation mediated aggregates cross-link the gel network. A rapid gelling can be expected upon contact with the

mucosa since, even at low polymer concentrations, small quantities of ions sufficient for the formation of a strong gel within GIT [100, 101].

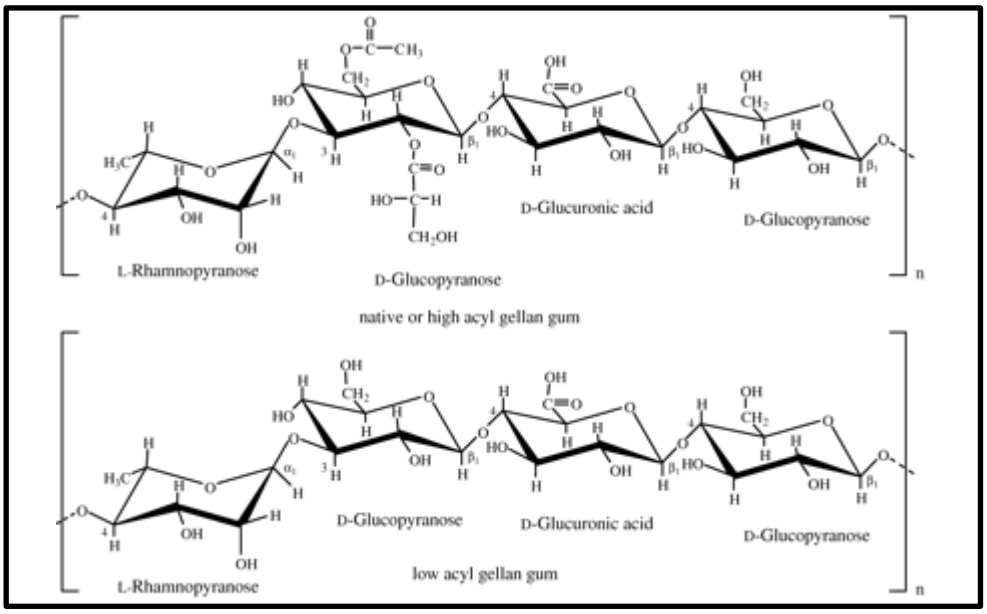


Figure 1.17: Structure of Gellan Gum [101]

Gellan gum can be applied pharmaceutically as a water soluble polymer acts as a potential carrier for different oral floating sustained delivery dosage forms. As a thickening or gelling agent thus can produce very hard, non-elastic gel and thermally reversible gel and as a good film former because it is chemically inert to most biological growth media additives and have excellent stability, flexibility and high clarity [102].

Iota Carrageenan (*i*-carrageenan):

Carrageenan is a sulphated linear polysaccharide of D-galactose and 3, 6-anhydro-D-galactose obtained by extraction of certain red seaweeds of the Rhodophyceae class. The carrageenans are divided into three families as shown in Figure 1.18. λ -Carrageenan (lambda-carrageenan) is a non-gelling polymer, *i* -Carrageenan (iota-carrageenan) is a gelling polymer and *k* -Carrageenan (kappa-carrageenan) is a strongly gelling polymer which has a helical tertiary structure that allows gelling. [99].

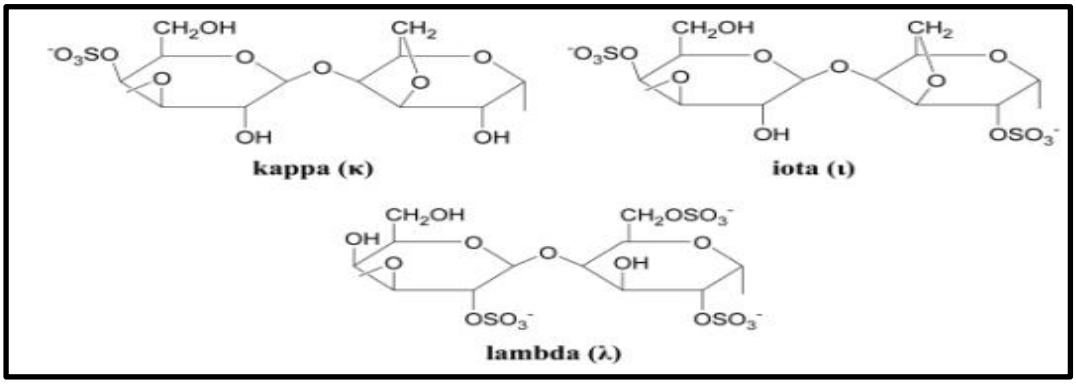


Figure 1.18: Structure of Carrageenan Families [99]

Application of carrageenan included as excipient in pharmaceutical industry, for example, as polymer matrix in oral extended release tablets. Moreover, carrageenan has strong negative charge; thus it has been used as a gelling agent/viscosity enhancing agent for controlled drug release and prolonged retention. Furthermore, carrageenan has been used for tissue regeneration with therapeutic bio-macromolecules and for cell delivery [103].

Hydroxypropyl Methyl Cellulose (HPMC):

Hydroxypropyl Methyl Cellulose (HPMC) as a partly O-methylated (OCH_3) and O-(2-hydroxypropylated) (OCH_2CH (OH) CH_3) cellulose conforming to the limits for the various types of HPMC as in Figure 1.19. It is available in several grades that vary in viscosity (50-100000 cps) and extent of substitution (OCH_3) either E or K. Molecular weight is approximately 10000–1500000 [104].

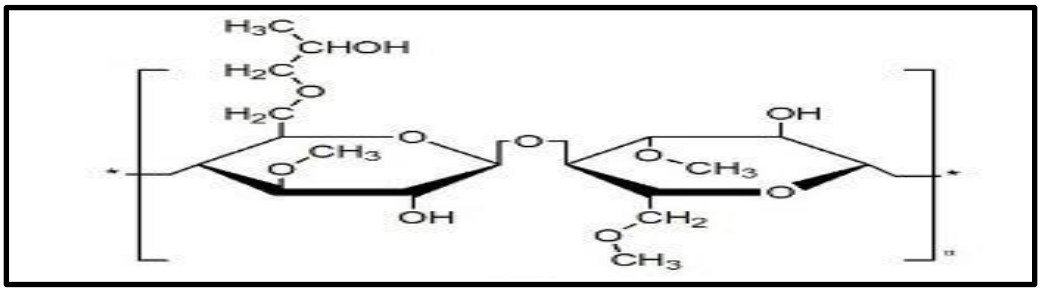


Figure 1.19: Structure of HPMC [104]

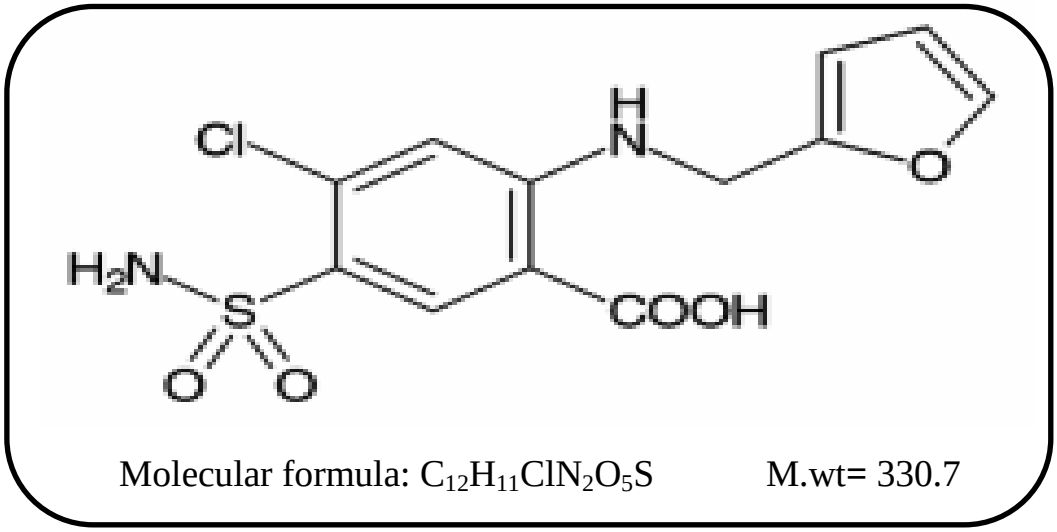
It is widely used in oral, ophthalmic, nasal, and topical pharmaceutical formulations as coating agent, controlled-release agent, dispersing agent, dissolution enhancer, extended-release agent, film-

forming agent, modified-release agent, release-modifying agent, solubilizing agent, stabilizing agent, sustained-release agent, thickening agent and viscosity-increasing agent [99].

1.8 Drug under Investigation: Furosemide

1.8.1 Chemical Structure of Furosemide

Furosemide chemical structure is benzoic acid, 5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl amino] [105] as shown in Figure 1.20.



Molecular formula: $C_{12}H_{11}ClN_2O_5S$ M.wt= 330.7

Figure 1.20: Chemical Structure of Furosemide [106]

1.8.2 Physicochemical Properties

It has a white or slightly yellow color, in the state of solid-crystals and solid-powder. It is odorless and practically insoluble in water (<0.1 mg/mL); very slightly soluble in chloroform; slightly soluble in ether; freely soluble in acetone; dimethylformamide; methyl alcohol and solutions of alkali hydroxides (freely soluble in dilute alkali solutions) and insoluble in dilute acids. It is slightly soluble in ethanol, soluble in methanol and DMSO [105]. It is weakly acidic and has a pK_a 3.8 (carboxylic acid) also its commercial solutions giving a pH 7.0-10.0 [107, 108]. Its melting point is 206 °C [109]. It is labeled in class IV of the Biopharmaceutical Classification System (BCS) [110].

1.8.3 Biopharmaceutical & Pharmacological Considerations

Furosemide absorption is fairly rapid from the gastrointestinal tract and the peak serum concentration C_{MAX} occurs within 60-90 minutes. The elimination half-life is relatively short (0.5-2hr). Absorption of furosemide after oral use is erratic and is subjected to large inter- and intra-individual variation; it is influenced by the dosage form, underlying disease processes and by the presence of food. The bioavailability in healthy persons is approximately from 50% to 70%. In patients, the bioavailability can be reduced to 30%, as in nephrotic syndrome [(111)].

Although it has very good permeability from the stomach and upper GI tract region but its bioavailability is poor and variable due to its poor solubility in gastric fluid (5–20 Mg/ml). Though it has good solubility in the intestinal fluid but due to its poor permeability through intestinal region makes its absorption very small [(112)].

Furosemide is about (97-98%) bound to plasma protein and is mainly excreted in the urine, largely unchanged. The effectiveness of furosemide as a diuretic depends upon its reaching to site of action (renal tubules) unchanged. About one-half to two-thirds of an intravenous dose or one-quarter to one-third of an oral dose are excreted unchanged, the difference being largely due to the poor bioavailability from the oral route. Furosemide crosses the placental barrier and distributes into breast milk. Urinary excretion may be reduced in renal impairment due to reduced renal blood flow and reduced tubular secretion also the proportion of free (unbound) furosemide is higher in patients with heart disease, renal impairment and cirrhosis of the liver [(113, 114)].

The most common adverse effect of furosemide is the fluid and electrolyte imbalance including hypovolaemia, hyponatraemia, hypokalaemia, and hypochloraemic alkalosis, particularly after large doses or prolonged use [(106)].

1.8.4 Mechanism of Action

Furosemide acts primarily by inhibiting active reabsorption of sodium and chloride ions in the ascending limb of the loop of Henle. Urinary excretion of sodium, chloride, potassium, hydrogen, calcium, magnesium, ammonium, bicarbonate and possibly phosphate is increased. The resulting low osmolality of the medulla inhibits the reabsorption of water by the kidney [(115)].

1.8.5 Therapeutic Uses and Dose

Furosemide is a high ceiling loop diuretic. It is primarily used for the treatment of hypertension; it is the first-line agent with edema caused by (Congestive Heart Failure) CHF. It is also used for hepatic cirrhosis, renal impairment, nephrotic syndrome, in adjunct therapy for cerebral/pulmonary edema where rapid diuresis is required (IV injection), management of severe hypercalcemia in combination with adequate rehydration and useful in the treatment of hyperkalemia [(116)].

Dose in Adult: By mouth, in edema: 20 - 80 mg given as a single dose. The same dose can be administered 6 to 8 hrs later or the dose may be increased 4-6 times daily. Resistant hypertension: The usual initial dose is 80 mg, usually divided into 40 mg twice as day [(117)]. While dose in children: By mouth, in edema: Neonate: 0.5–2 mg/kg every 12–24 hours child 1 month–12 years: 0.5–2 mg/kg 2–3 times daily; higher doses may be required in resistant oedema; maximum 12 mg/kg daily, not to exceed 80 mg daily. Child 12–18 years: 20–40 mg daily, increased in resistant oedema to 80–120 mg daily [(118)].

1.8.6 Marketed Dosage Form of Furosemide

Tablets: furosemide 20 mg, 40 mg, 500 mg.

Oral solution: furosemide, 20 mg/5 ml, 40 mg/5 ml, 50 mg/5 ml.

Injection: furosemide 10 mg/ml [(119)].

1.9 Some Recent Research Works on Furosemide

Nowadays pharmaceutical industry progress to yield novel designed techniques to improve solubility of Class IV BCS drugs. One of them is the complexes of β-cyclodextrin and the two solid forms of furosemide were prepared using kneading and freeze-drying methods. It was observed that this novel supramolecular binary complex significantly increased the solubility of furosemide in the simulated gastric fluid, which resulted in a rise in the bioavailability of this formulation after oral administration [(120)].

Another technique is the self-nano emulsifying drug delivery system (SNEDDS) which is a novel drug delivery system utilized to improve the water solubility, permeability and ultimately bioavailability of furosemide. Remarkable increase in dissolution was observed for the optimized SNEDDS when compared with the plain marketed Furosemide[(121)].

The Technique of novel drug solubilization platform (solid nanodispersion) SNDs represents a significant improvement over current enabling technologies such as nanocrystal and spray-dried dispersion. It is prepared by a simple co-grinding of furosemide and solvent-free process. It was able to increase the furosemide free fraction available for oral absorption [(122)].

A Technique of a gastroretentive dosage form suitable for controlled drug release of a drug with narrow therapeutic windows consists of a furosemide loaded polymeric film made up of a bilayer of immediate (IR) and controlled release (CR) layers folded into a hard gelatin capsule. The capsule was shown to unfold and swell under acidic conditions and provide IR of drug over 1 hr and CR for up to 12 hr in acidic medium resulted in optimum drug release, bioadhesion and mechanical properties[(123)].

A new oral solid dosage form of furosemide that improves its release in preferential absorption region (stomach) prepared by including the drug in the mesoporous silica material SBA-15 obtaining an inorganic–organic compound, and the results showed a remarkable dissolution rate improvement in comparison to the crystalline drug and to the marketed product Lasix® [124].

The technique of preparation of sustained release cellulose acetate microcapsules by coacervation phase separation technique and phase separation was prepared to enhance bioavailability and reduce the short half-life problem of Furosemide [125].

Aim of the Study

The aim of this study is to formulate a gastroretentive floating in-situ gel (sol-gel) system of furosemide to control the release and further to improve its absorption and bioavailability. This can be achieved through studying different related factors and application of in-vitro/ in-vivo evaluations of gastroretentive property for the prepared formula.

2. Experimental Work

2.1 Materials

Materials used in this study were listed in Table 2.1.

Table 2.1: Materials Used in the Study

Material	Company
Calcium Chloride	Gainland chemical company- UK
Diethylether	S D Fine-Chem Limited- India
Fructose	Thomas Baker-India
Furosemide	Samara drug industry-Iraq
Gellan Gum	Provizer pharma-India
Glycerol	Gainland chemical company - UK
HPMC 5 cp	Provizer pharma-India
HPMC K100M	Provizer Pharma-India
HPMC K4M	Provizer pharma-India
Hydrochloric acid	Hopkin & Williams- UK
Iota Carrageenan	Provizer pharma-India
PEG 6000	Sigma chemical co. (Aldrich)-USA
Propylene Glycol	Samara drug industry-Iraq
Sodium Alginate	Avonchem-UK
Sodium Benzoate	British drug house (BDH)-UK
Sodium Bicarbonate	Scharlau-Germany
Sodium Citrate	Panreac-Spain
Sodium Methyl Paraben	Samara drug industry-Iraq
Sodium Propyl Paraben	Samara drug industry-Iraq

2.2 Instruments

The instruments used in this study were listed in Table 2.2.

Table 2.2: Instruments Used in this Study

Instrument	Manufacturer
Dissolution Apparatus	Copley- UK
Electronic Balance	Kern ALS 220-4N- Germany
Flame Photometry	Jenway 8515- Germany
FTIR Spectroscopy	Shimadzu 8400S-Japan
Gel Strength Apparatus	Locally Modified
Magnetic stirrer	Dragon Lab- USA
Melting Point Apparatus	Stuart SMP 30- UK
Oven	Memmert- Germany
pH Meter	WTW-INO LAB- Switzerland
Sonicator	Elma- Germany
U.V. spectrophotometer	Shimadzu 1650 pc-Japan
Viscometer	Brookfield-DVE- USA
Water Bath	Copley- Uk

2.3 Methods

2.3.1 Characterization of Furosemide

2.3.1.1 Determination of Furosemide Melting point

The melting point of furosemide was determined by capillary tube method according to the USP. A sufficient quantity of furosemide powder was introduced into the capillary tube to give a compact column of 4-6 mm in height. The tube was introduced in electrical melting point apparatus and the temperature was raised. The melting point was recorded, which is the temperature at which the last solid particle of furosemide in the tube passed into liquid phase [105].

2.3.1.2 Determination of UV Absorption (λ max) of Furosemide

Furosemide stock solutions of (1mg/100ml) in 0.1N HCl (pH 1.2) and (0.6mg/100ml) in distilled water were prepared, the solutions scanned by UV-visible spectrophotometer at the range of 200-400 nm, and the λ max of the drug was determined.

2.3.1.3 Determination of Calibration Curves of Furosemide

Calibration curves of furosemide in the gastric fluid (GF) 0.1N HCl (pH 1.2) and in distilled water were obtained by preparing serial dilutions from stock solutions (1mg/100ml) and (0.6mg/100ml) respectively. Samples were analyzed spectrophotometrically at the determined λ max. The measured absorbance value of each sample was plotted versus concentration to obtain the standard calibration curve [126].

2.3.1.4 Determination of Furosemide Solubility

The solubility of furosemide was determined in distilled water, in GF 0.1N HCl solution (pH 1.2) and in GF 0.1N HCl solution (pH 1.2) with the presence of mixed solubilizers (1% (w/v) PEG 6000, 1% (w/v) sodium benzoate, 1% (w/v) sodium citrate, 2 ml propylene glycol and

0.5ml glycerin) by using the shake-flask method at 37^{o} C, where excess amount of furosemide pure powder was taken and dissolved in the above solutions separately with continuous shaking for 24 hrs at 37^{o} C. Then, sample was taken, filtered through whatman filter paper No. 41and diluted. The diluted samples were analyzed by UV spectroscopy at the specified λ max to determine the dissolved quantity of furosemide [127].

2.3.2 Preparation of Oral Furosemide Solution to Act as In-Situ Gel

Different polymers were used to prepare furosemide to act as in-situ gelling preparation as shown in Table 2.3. The methods of preparation for the required formulas were as follows:

❖ **Using Sodium Alginate**

Sodium benzoate at concentration 1% (w/v), sodium citrate at concentration 0.75% (w/v) and PEG 6000 at concentration 1% (w/v) were dissolved in distilled water to prepare mixed solubilizers solution. The Mixture heated to 37^{o} C while stirring. Then add 0.5 ml glycerin and 2 ml propylene glycol with continuous stirring and heating until all ingredients were dissolved and mixed completely. At the same time the polymer (Na alginate) was dissolved at concentrations 0.5, 1 and 1.5 % (w/v) (F4-F6) each one separately in distilled water containing 0.25% (w/v) sodium citrate and 0.1% (w/v) calcium chloride, heating to 60^{o} C while stirring. Then mixed solubilizers solution was added to the polymer solution with continuous stirring. Finally various amounts of sodium bicarbonate (F15, F2 & F16) were added, then 0.4% (w/v) furosemide was dispersed in the resulting solution after cooling [128, 129].

Table 2.3: Composition of Different Formulas of In-situ Gel of Furosemide

Formulas code / Ingredient Name	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Furosemide (% w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Sodium benzoate (% w/v)	1	1	1	1	1	1	-	-	-	1	1
Sodium citrate (% w/v)	1	1	1	1	1	1	1	1	1	1	1
Glycerin (ml)	0.5	0.5	0.5	0.5	0.5	0.5	-	-	-	0.5	0.5
Propylene glycol (ml)	2	2	2	2	2	2	-	-	-	2	2
PEG 6000 (% w/v)	1	1	1	1	1	1	1	1	1	1	1
Calcium chloride (% w/v)	0.075	0.1	0.15	0.1	0.1	0.1	0.016	0.016	0.016	0.1	0.1
$NaHCO_3$ (% w/v)	0.5	0.5	0.5	-	-	-	-	-	-	-	-
Sodium alginate (% w/v)	1	1	1	0.5	1	1.5	-	-	-	1	1
Gellan Gum (% w/v)	-	-	-	-	-	-	0.25	0.5	0.75	-	-
Iota carrageenan (% w/v)	-	-	-	-	-	-	-	-	-	-	-
HPMC K100M (% w/v)	-	-	-	-	-	-	-	-	-	0.6	0.8
HPMC K4M (% w/v)	-	-	-	-	-	-	-	-	-	-	-
HPMC 5 cp (% w/v)	-	-	-	-	-	-	-	-	-	-	-
Na^+ methyl paraben (% w/v)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Na^+ propyl paraben (% w/v)	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018
Fructose	-	-	-	-	-	-	-	-	-	-	-
D.W. Q.S.(ml)	100	100	100	100	100	100	100	100	100	100	100

Table 2.3: To be continued

Formulas code / Ingredient Name	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22	F23	F24
Furosemide (% w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Sodium benzoate (% w/v)	1	1	1	1	1	-	-	-	1	1	1	-	-
Sodium citrate (% w/v)	1	1	1	1	1	1	1	1	1	1	1	1	1
Glycerin (ml)	0.5	0.5	0.5	0.5	0.5	-	-	-	0.5	0.5	0.5	-	-
Propylene glycol (ml)	2	2	2	2	2	-	-	-	2	2	2	-	-
PEG 6000 (% w/v)	1	1	1	1	1	1	1	1	1	1	1	1	1
Calcium chloride (% w/v)	0.1	0.1	0.1	0.1	0.1	0.016	0.016	0.016	0.1	0.1	0.1	0.016	0.016
$NaHCO_3$ (% w/v)	-	-	-	0.25	1	0.2	0.4	0.6	0.5	0.5	0.5	0.4	0.4
Sodium alginate (% w/v)	1	1	1	1	1	-	-	-	1	1	1	-	-
Gellan gum (% w/v)	-	-	-	-	-	0.5	0.5	0.5	-	-	-	0.5	0.5
Iota carrageenan (w/v %)	-	-	-	-	-	-	-	-	0.2	0.25	0.3	0.2	0.25
HPMC K100M (% w/v)	-	-	-	-	-	-	-	-	-	-	-	-	-
HPMC K4M (% w/v)	0.5	1	1.5	-	-	-	-	-	-	-	-	-	-
HPMC 5 cp (% w/v)	-	-	-	-	-	-	-	-	-	-	-	-	-
Na^+ methyl paraben (% w/v)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Na^+ propyl paraben (% w/v)	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018
Fructose	-	-	-	-	-	-	-	-	-	-	-	-	-
D.W. QS.(ml)	100	100	100	100	100	100	100	100	100	100	100	100	100

Table 2.3: To be continued

Formulas code / Ingredient Name	F25	F26	F27	F28	F29	F30	F31	F32	F33	F34	F35
Furosemide (% w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.8	1	0.4	0.4
Sodium benzoate (% w/v)	-	1	1	1	1	1	1	1	1	1	1
Sodium citrate (% w/v)	1	1	1	1	1	1	1	1	1	1	1
Glycerin (ml)	-	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propylene glycol (ml)	-	2	2	2	2	2	2	2	2	2	2
PEG 6000 (% w/v)	1	1	1	1	1	1	1	1	1	1	1
Calcium chloride (% w/v)	0.016	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
$NaHCO_3$ (% w/v)	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium alginate (% w/v)	-	1	1	1	1	1	1	1	1	1	1
Gellan Gum (% w/v)	0.5	-	-	-	-	-	-	-	-	-	-
Iota carrageenan (% w/v)	0.3	-	-	-	-	-	-	0.25	0.25	0.25	0.25
HPMC K100M (% w/v)	-	0.6	0.8	-	-	-	-	-	-	-	-
HPMC K4M (% w/v)	-	-	-	0.5	1	1.5	-	-	-	-	-
HPMC 5 cp (% w/v)	-	-	-	-	-	-	0.5	-	-	-	-
Na^+ methyl paraben (% w/v)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Na^+ propyl paraben (% w/v)	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018
Fructose(%w/v)	-	-	-	-	-	-	-	-	-	1	2
D.W. QS.(ml)	100	100	100	100	100	100	100	100	100	100	100

❖ **Using Gellan Gum**

Sodium citrate at concentration 0.8% (w/v) and PEG 6000 at concentration 1% (w/v) were used as mixed solubilizers of furosemide in gellan gum solution. Where, sodium citrate and PEG 6000 dissolved in distilled water. The mixture heated to 37^{o} C while stirring until all ingredients were dissolved and mixed completely. At the same time 0.2% (w/v) sodium citrate was dissolved in distilled water at 90^{o} C, and then adds gellan gum at concentrations 0.25, 0.5 and 0.75 % (w/v) (F7-F9) each one separately while stirring. Then mixed solubilizers solution was added to the polymer solution with continuous stirring. Finally various amounts of sodium bicarbonate (F17-F19), 0.016% (w/v) calcium chloride and 0.4% (w/v) furosemide were then dispersed in the resulting solution after cooling [128, 130].

❖ **Using combination of Sodium Alginate and Iota Carrageenan**

Sodium benzoate at concentration 1% (w/v), sodium citrate at concentration 0.55% (w/v) and PEG 6000 at concentration 1% (w/v) were dissolved in distilled water to prepare mixed solubilizers solution. The mixture heated to 37^{o} C while stirring. Then add 0.5 ml glycerin and 2 ml propylene glycol with continuous stirring and heating until all ingredients were dissolved and mixed completely. At the same time Na alginate at concentration 1 % (w/v) was dissolved in distilled water containing 0.25% (w/v) sodium citrate and 0.1% (w/v) calcium chloride, heating to 60^{o} C while stirring. Iota carrageenan solution (F20-F22) was prepared separately by dissolving at concentrations 0.2, 0.25, 0.3% (w/v) in distilled water containing 0.2% (w/v) sodium citrate and heating to 80^{o} C while stirring. Then the three prepared solutions were mixed together after cooling to 60^{o} C with continuous stirring. Finally various amounts of sodium bicarbonate were added, then 0.4% (w/v) furosemide was dispersed in the resulting solution after cooling [131, 132].

❖ **Using Combination of Gellan Gum and Iota Carrageenan**

Sodium citrate at concentration 0.6% (w/v) and PEG 6000 at concentration 1% (w/v) as mixed solubilizers were dissolved in distilled water. The mixture heated to 37^{o} C while stirring until all ingredients were dissolved and mixed completely. At the same time 0.2% (w/v) sodium citrate was dissolved in distilled water at 90^{o} C, and then 0.5% (w/v) gellan gum was added while stirring. Iota carrageenan solution (F23-F25) was prepared separately by dissolving at concentrations 0.2, 0.25, 0.3% (w/v) in distilled water containing 0.2% (w/v) sodium citrate and heating to 80^{o} C while stirring. The three prepared solutions were mixed together with continuous stirring. Finally various amounts of sodium bicarbonate, 0.016% (w/v) calcium chloride and 0.4% (w/v) furosemide were dispersed in the resulting solution after cooling [131, 133].

❖ **Using Combination of Sodium Alginate with Various Grades of HPMC**

Sodium benzoate at concentration 1% (w/v), sodium citrate at concentration 0.75% (w/v) and PEG 6000 at concentration 1% (w/v) as mixed solubilizers were dissolved in distilled water. The mixture heated to 37^{o} C while stirring. Then add 0.5 ml glycerin and 2 ml propylene glycol with continuous stirring and heating until all ingredients dissolved and mixed completely. At the same sodium alginate of concentration 1% (w/v) was dissolved in distilled water containing 0.25% (w/v) sodium citrate and 0.1% (w/v) calcium chloride, heating to 60^{o} C while stirring. HPMC K100M solution (F10 & F11) at 0.6, 0.8% (w/v) concentrations or HPMC K4M solution (F12-F14) at 0.5, 1, 1.5% (w/v) concentrations or HPMC solution 5 cp at 0.5% (w/v) concentration were dissolved separately in distilled water previously heated to 80^{o} C while stirring. The three prepared solutions were mixed together after cooling to 60^{o} C with

continuous stirring. Finally various amounts of sodium bicarbonate (F26-F31) were added, then 0.4% (w/v) furosemide was dispersed in the resulting solution after cooling [134, 135].

To all the prepared formulations; 0.02% (w/v) sodium methyl paraben and 0.018% (w/v) sodium propyl paraben were added as preservatives. In addition other formulations (F32 & F33) 0.8% (w/v) & 1% (w/v) of furosemide were used respectively. Also some formulations (F34 & F35) 1% & 2% (w/v) of fructose was added respectively as sweetening agent (Taste Masking Agent).

2.4 Evaluation of Floating In-Situ Gel Furosemide Solution

2.4.1 In-Vitro Gelation Study

2.4.1.1 Gel Strength Determination

Gel strength is indicative of the tensile strength of the gelled mass. It signifies the ability of the gelled mass to withstand the peristaltic movement. The gel strength of the formulation is an important variable dependent on the type and concentration of the polymer, combination of polymers, gas generating agent and cation source ($CaCl_2$).

The method to measure gel strength of gelled mass was modified; by using fabricated gel strength apparatus and it was done triplicate as shown in Figure 2.1. Solution of 5 ml was taken in the cylinder followed by addition of 25 ml of GF 0.1 N HCl (pH 1.2) for gelation. After gelation the HCl was drained off leaving the formed gel mass, and then the device was rested on to surface of the gel. At the free end of the device a light weight pan (4 g) was attached to which the weights were added. The gel strength was reported in terms of weight required to pass the apparatus through the formed gel mass [136, 137].

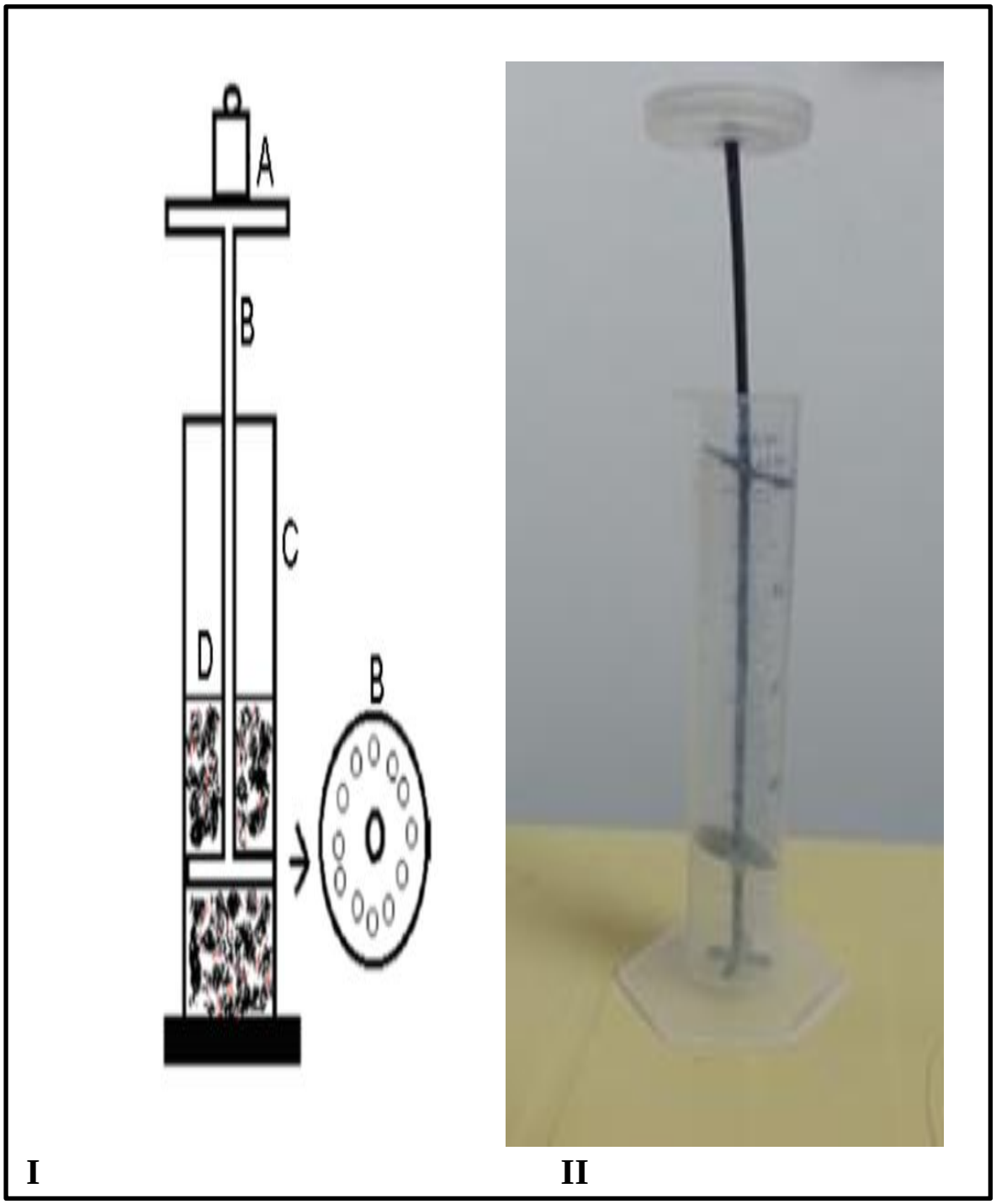


Figure 2.1: Gel Strength Measuring Device as I- Represents schematic labeled as; (A) weights; (B) device; (C) cylinder; (D) gel [137].

II- Modified gel strength measuring apparatus

2.4.1.2 Gelation Time Determination

Gelation time was evaluated visually; it was measured by placing 5ml of GF 0.1 N HCl (pH 1.2) in test tube and maintained at 37±1^{o} C. One ml of each formula was taken with pipette and transferred slowly on the surface of the fluid, as the solution come in contact with gastric fluid solution; it was immediately converted into gel like structure. The gelation time was evaluated triplicate on basis of time period for which gel formed [138].

2.4.2 Swelling Index

The percentage of swelling index of in-situ gel of the formulations was determined. In situ gel formed by putting 5 ml of each formula in a petri dish and 40 ml of GF 0.1 N HCl (pH 1.2) was added. Then 0.1N HCl solution was removed from the gel and the excess of 0.1N HCl solution was blotted out with whatman filter paper. The initial weight (W_o) of the gel was recorded, to this gel 10 ml of distilled water was added and after 60 minutes the water was decanted and the final weight (W_t) of the gel was recorded, this process was repeated for 5 hrs and the difference in the weight was calculated and reported [139]. The % weight gain (swelling index) for formulations is calculated by the following equation (1):

% Swelling index = $(W_t - W_0 / W_t) \times 100$……. (1)

Where, W_0 =Initial weight of the gel. W_t =weight gain by the gel.

2.4.3 Viscosity Measurements

The viscosity of the prepared solutions were measured out using sample of 100ml. Measurements were performed using suitable spindle number 64 and sheared at a rate of 3, 4, 5, 6, 10, 12, 20, 30, 50, 60, 100 rpm, and the temperature was maintained at 37° C. The viscosity was read directly after 30 seconds. All measurements were made in triplicate.

The rheological velocity was explained by plotting viscosity against angular velocity [140]. This method is applied for the prepared formulations and for the marketed conventional furosemide solution (20mg/5ml) (Fudesix®).

2.4.4 In-Vitro Buoyancy Study

The in vitro buoyancy study is characterized by floating lag time and total floating duration. In vitro buoyancy study was carried out triplicate using USP dissolution apparatus type II using 900 ml medium of 0.1N HCl (pH 1.2). The medium temperature was kept at 37 $\pm$ 0.5^{o} C. Accurately 10 mL of the prepared in-situ gel formulation was drawn up using disposable syringe and placed into the petri dish (4.5 cm internal diameter) and finally the petri dish containing the formulation was placed carefully in the dissolution vessel. Then the dissolution test apparatus was run at 50 rpm, this speed was slow enough to avoid breaking of gelled formulation and maintaining the mild agitation conditions believed to exist in vivo. The time the formulation took to emerge on to the medium surface (floating lag time) and the time over which the formulation constantly floated on the dissolution medium surface (duration of floating) were reported [141].

2.4.5 Density Measurement of the Gel

The prime requirement for stomach specific floating drug delivery system is the density; which is an important parameter and it should be less than the stomach fluid density (< 1.004 g/cm^3). The densities of all formulations were measured by forming gel of known volume (5 ml) in a petri dish containing 0.1N HCl. The weight of this gel was measured by using calibrated balance and accordingly the densities of formulations were calculated. Density measurement for each formulation was done in triplicate [142].

2.4.6 pH Measurement

The pH of the prepared solution for all formulations was measured by digital pH meter at 25 $\pm$ 0.5^{o} C after it is calibration using standard buffer solutions of pH 4, 7, 9 then the measurements of pH were recorded[143].

2.4.7 Determination of Drug Content

Accurately, 5 ml of liquid solution (containing 20 mg of the drug) from all formulations was taken and to which 70 ml of 0.1N HCl was added, then the sample was sonicated for 30 min until clear solution is made. The volume completed to 100 ml and filtered using whatman filter paper No. 41. From this solution, 1ml sample was withdrawn and diluted to 10 ml with 0.1N HCl. Contents of furosemide was determined spectrophotometrically at 274.2 nm using double beam UV-Visible spectrophotometer [144].

2.5 In Vitro Drug Release Study

The in vitro release of furosemide from buoyant in-situ gel solutions was studied using USP type II (paddle type) dissolution test apparatus. Five ml (containing 20 mg of furosemide) from each formulation was transferred using disposable syringe, the needle was wiped clean and excess formulation was removed from needle end. The syringe plunger depressed slowly to extrude 5 ml into a petri dish with an internal diameter of 4.5 cm already containing 10 ml of 0.1N HCl. This petri dish containing formulation was placed on the surface of the medium and plunged into a dissolution vessel containing 900 ml of 0.1N HCl (pH 1.2) without much disturbance as shown in Figure 2.2.The dissolution test apparatus was run at 50 rpm for maximum up to 5 hrs at a temperature 37$\pm$ 0.5^{o} C. This speed was slow enough to avoid the breaking of gelled

formulation and was maintaining the mild agitation conditions believed to exist in vivo. Five ml samples were withdrawn form dissolution medium with disposable syringe at predetermined time intervals of 5, 10, 15, 20, 30, 60, 120, 180, 240, 300 min and replenished with 5 ml of pre-warmed fresh medium. Samples were filtered using whatman filter paper No.41 and furosemide contents in the aliquots was determined spectrophotometrically using double beam UV-Visible spectrophotometer at a wavelength of 274.2 nm after suitable dilution. The experiments were conducted in triplicate at each time interval and the average was recorded [145].

2.5.1 Study the Effect of Variables on the Release Profile

2.5.1.1 Effect of Different Concentrations of Ion Crosslinking Agent

Formulas F1-F3 were prepared to study the effect of different $CaCl_2$ concentrations on release profile of furosemide.

2.5.1.2 Effect of Types and Concentrations of Polymers

Formulas F4-F9 were prepared to study the effect of different types and concentrations of primary polymers (Na alginate and gellan gum) on release profile of furosemide.

2.5.1.3 Effect of Different Concentrations of Gas Generating Agent

Formulas F2, F15- F19 were prepared to the study the effect of different concentrations of $NaHCO_3$ on release profile of furosemide, using Na alginate and gellan gum as primary polymers.

(A) At the Beginning of Release

(B) After 5hrs of Release

Figure 2.2: Photograph of Dissolution Vessel for Furosemide in In-Situ Gel, (A) At the Beginning and (B) After 5hrs of Release.

2.5.1.4 Effect of Combination of Polymers with or without Gas Generating Agent

- Formulas F10- F14 were prepared to study the effect of combination of various grades of HPMC (HPMC K100M and HPMC K4M) as secondary polymers with Na alginate as primary polymer in the absence of $NaHCO_3$ on release profile of furosemide.

- Formulas F20- F25 were prepared to study the effect of combination of Iota carrageenan as secondary polymer with Na alginate and gellan gum as primary polymers in the presence of $NaHCO_3$ on release profile of furosemide.

- Formulas F26- F31 were prepared to study the effect of combination of various grades of HPMC (HPMC K100M, HPMC K4M and HPMC 5 cp) as secondary polymers with Na alginate as primary polymer in the presence of $NaHCO_3$ on release profile of furosemide.

2.5.1.5 Effect of Different Drug Concentrations

Formulas F21, F32 & F33 were prepared to study the effect of different drug concentrations (0.4% w/v, 0.8% w/v and 1% w/v) on release profile of furosemide.

2.5.1.6 Effect of Different Concentrations of Sweetening Agent (Taste Masking Agent)

Formulas F21, F34 & F35 were prepared to study the effect of different concentrations of fructose on release profile of furosemide.

2.5.2 Kinetic Mathematical Modeling of Drug Release Profile

The cumulative amount of furosemide release from the prepared in-situ gel formulations at different time interval was fitted to zero order kinetics, first order kinetics, Higuchi model and Koresmeyer-Peppas model to characterize the mechanism of drug release.

Zero Order kinetic

It describes the system in which the drug release rate is independent on its concentration as shown in equation (2):

$$\mathbf{Q_t = Q_0 + K_0\, t} \quad \ldots\ldots\ldots (2)$$

Where Q_t = the amount of drug dissolved in time t.

Q_0 = the initial amount of drug in solution.

K_o = the zero order release constant.

In this way, a graph of drug dissolved fraction versus time will be linear if the previously established condition were fulfilled [146].

First Order kinetic

It describes the drug release from the systems in which the release rate is concentration dependent as described by equation (3):

$$\mathbf{\log Q_t = \log Q_0 - K_1 t/2.303} \quad \ldots\ldots\ldots (3)$$

Where Q_t = the amount of drug released in time t.

Q_0 = the initial amount of drug in the tablet and K_1 is the first order release constant [147].

In this way, a graph of the decimal logarithm of the released amount of the drug versus time will be linear [146].

Higuchi Model

It describes release model in which the fraction of drug release from the matrix is proportional to square root of time as shown in equation (4)

$$Q_t/Q_0 = K_H \sqrt{t} \quad \ldots\ldots (4)$$

Where Q_t / Q_0 = cumulative amount of drug release at time t

K_H = the Higuchi dissolution constant reflecting formulation characteristics.

In this way, a graph of the cumulative percentage drug released versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to K_H [148].

Korsmeyer-Peppas Model

It is used for better description of drug release behavior from a polymeric system [149] as shown in equation (5)

$$\log (Q_t/Q_\infty) = \log K_{kp} + n \log t \quad \ldots\ldots\ldots (5)$$

Where Q_t/Q_∞ = the fraction of drug release at time t,

k_{kp} =the constant incorporating structural and geometrical features of controlled release device and

(n) = a diffusional release exponent indicative of the drug release mechanism for dissolution.

The (n) value in equation (5) was used to determine different release mechanisms, and it is equal to the slope of line obtained by plotting log (Q_t/Q_∞) versus log t while the intercept represent log k_{kp} in the above equation [150, 151].

When (n) equal to 0.45 corresponds to Fickian diffusion, $0.45 < n < 0.89$ corresponds to anomalous (non-Fickian) diffusion, n equal to 0.89 corresponds to Case-II transport, and $n > 0.89$ corresponds to Super case-II transport [152].

2.6 In-Vivo Test for the Optimum Formula

After selection of optimum formula (F21) for its good properties (like floating lag time, floating time, viscosity, gel strength, pH and

release profile), the in-vivo diuretic activity assay test was performed with male Wister albino rats. All procedures were approved by the Appropriate Animal Care Ethics Committee in Al-Mustansiriya University; the animals were acclimatized for 7 days under standard conditions, i.e. room temperature 35 ± 1° C, relative humidity 45-55% and light/dark cycle 12/12 hr. Twelve healthy rats weighing 250 - 360 g; these rats were involved in each step, divided into 4 groups, each group consist of 3 rats placed in modified metabolic cages (each cage equipped with a wire mesh floor to allow free passage and collection of excreted material while containing the rats, also stainless steel sieves were placed on to the mesh which assured good separation of urine from feces and urine was collected in a plate) [153]. Before treatment; rats after overnight fasting and free access to water, were anesthetized in an induction chamber with ether for 5 – 10 min. Once anesthetized, the rat was removed from the induction chamber then all animals were received physiological saline (0.9% NaCl) at an oral dose of 4% body weight by oral gavage syringe to impose water and salt load [154].

Step one: Each group received same volume of distilled water in a feeding bottle (200 ml) orally and considered as control, then urine was collected from each group and measured over a periods of 1, 5 and after 24 hr. Electrolyte (Na^+, K^+) concentrations were estimated from each urine sample of each group at all-time intervals using flame photometry.

Step two: After one week recovery period for rats (with free access to water and food). The rats were fasted overnight and subsequently received commercial furosemide solution (Fudesix®) orally using gavage syringe at a dose of 10, 25, 50, and 100 mg/kg for group I, II, III and IV respectively. Urine volume and electrolyte concentrations were estimated similar to step 1.

Step three: After one week recovery period similar to that of step 2, the rats were fasted overnight and subsequently received the optimum formula (F21) orally at a dose of 10, 25, 50 and 100 mg/kg for group I, II, III and IV respectively. Urine volume and electrolyte concentrations were estimated similar to step 1 [155].

2.7 Drug-Excipient Compatibility Studies

The physicochemical compatibilities of the drug, additives and excipients were tested through mixing the drug with each excipient and mixture of them characterized by FT-IR spectroscopy from 4000 - 500 cm^{-1} using potassium bromide disc (13 mm in diameter) [156].

2.8 Accelerated Stability Studies: Effect of Temperature

This study was done at accelerated thermal conditions (40, 50 and 60^{o} C). The solutions were stored in well stoppered dark glass bottles (each containing 100 ml of the selected formula) in ovens for 12 weeks. Samples of 5 ml were taken every 2 weeks and characterized for furosemide content by measuring their UV absorbance at 274.2 nm. pH, viscosity, floating lag time and floating time were measured (at room temperature 25^{o} C) during the experiment period [157].

2.9 Statistical Analysis

Statistical analysis of formulations was done by using one-way analysis of variance (ANOVA). The difference is statistically significant when ($P < 0.05$).

3. Results and Discussion

3.1 Characterization of Furosemide Powder

3.1.1 Determination of Melting Point

The measured melting point of furosemide was found to be 210^{o} C; which is consistent with the reported range of 206 to 210^{o} C indicating the purity of drug powder [158, 159]. The melt was irreversible and produce a dark brown color.

3.1.2 Determination UV Absorption (λ max) of Furosemide

Scanning furosemide stock solution by UV spectrophotometer at 200 - 400 nm gave the spectrum that have wave length of maximum absorption (λ max) at 274.2 nm in gastric fluid solution 0.1N HCl (pH 1.2) and at 271 nm in distilled water as shown in Figure 3.1 . The result is in agreement with the reported one [112].

3.1.3 Determination of Calibration Curves of Furosemide

The calibration curves of furosemide in gastric fluid solution 0.1N HCl (pH 1.2) and in distilled water were shown in Figures (3.2 & 3.3). Straight line was obtained by plotting the absorbance versus concentration with high regression coefficient; this indicates that the calibration curves obeys Beer's law within the range of concentration used [160].

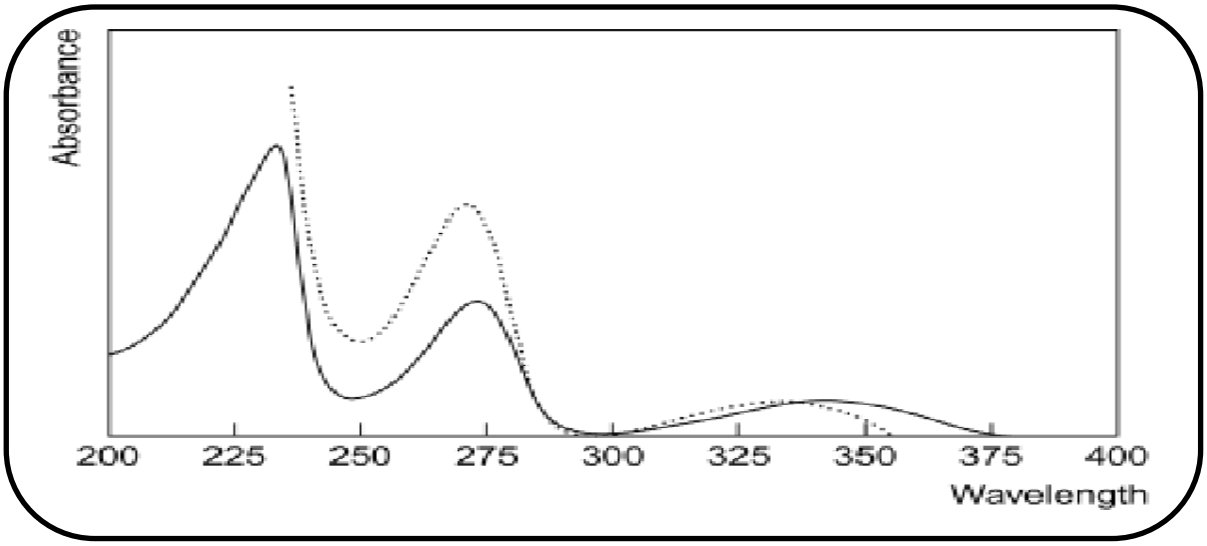


Figure 3.1: UV Spectrum of Furosemide in Gastric Fluid Solution 0.1N HCl (pH 1.2) (Solid Line) and in Distilled Water (Dashed).

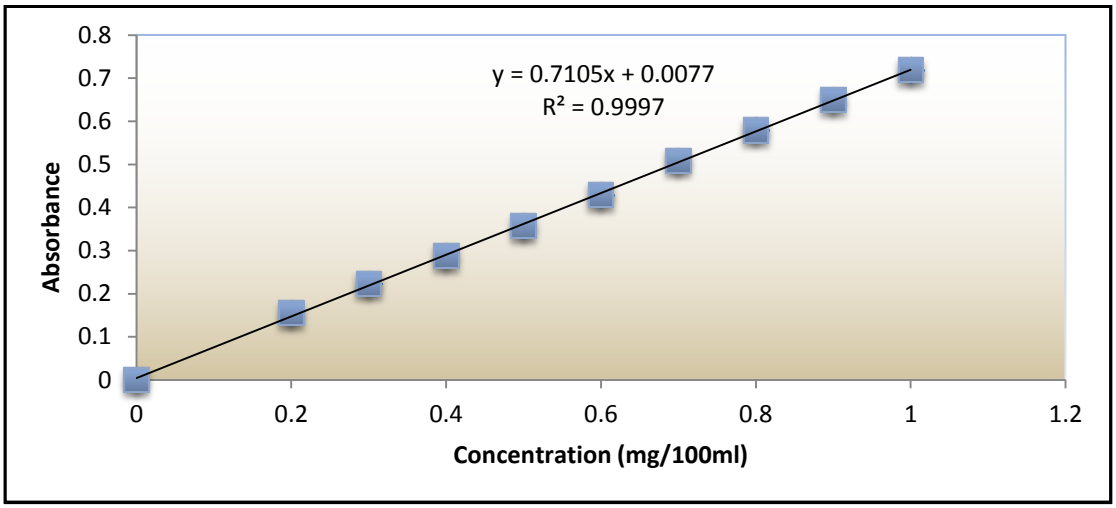


Figure 3.2: Calibration Curve of Furosemide in Gastric Fluid Solution 0.1N HCl (pH 1.2), at λ max 274.2 nm.

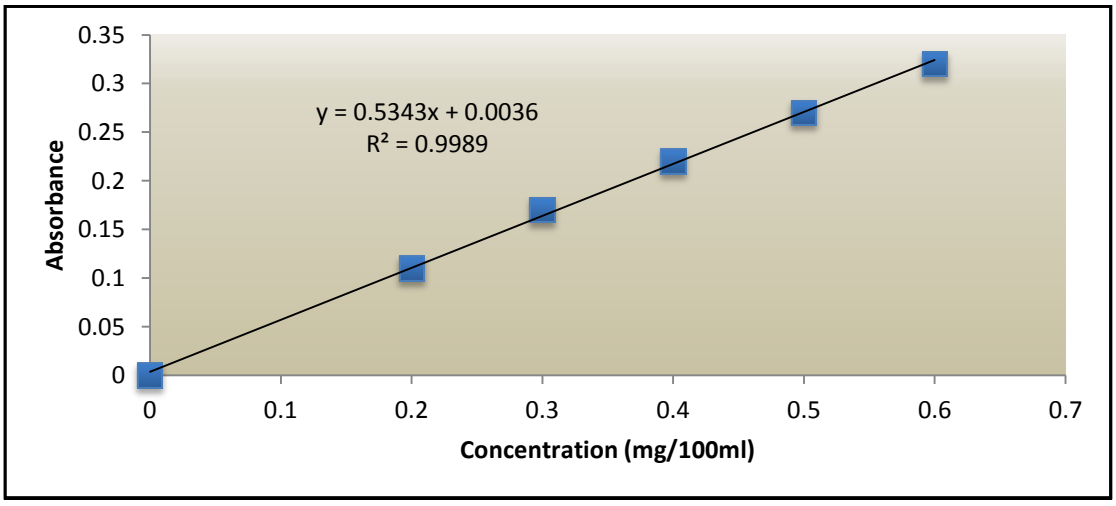


Figure 3.3: Calibration Curve of Furosemide in Distilled Water, λ max at 271nm.

3.1.4 Determination of Furosemide Solubility

The solubility of furosemide in water was found to be 0.006 mg/ml (0.6 mg/100ml) and this agreed with the reported data. In addition, the results showed that the solubility of furosemide in 0.1N HCl (pH 1.2) was 0.01 mg/ml (1mg/100ml) this is due to the acidic nature of the drug since furosemide is a weak acidic drug which in acidic medium tend to be in the unionized form (lipophilic) [5,107].

Solubility study of furosemide in 0.1N HCl (pH 1.2) with the presence of mixed solubilizers (PEG 6000 as water solubilizer, sodium benzoate and sodium citrate as hydrotropes, propylene glycol and glycerin as co-solvents) resulted in increasing solubility of furosemide to 4 mg/ml and this agreed with the reported data [161].

3.2 Evaluation of Furosemide Floating In-Situ Gel

All the formulations (F1-F35) prepared were evaluated for different parameters like: gel strength, gelation time, content uniformity, floating lag time, floating duration, pH measurement, density and swelling index, the results are summarized in Table 3.1.

Table 3.1: Evaluations of Furosemide Floating In-Situ Gel

Formula No.	Gel strength (N/m^2)	Gelation time (sec)	Content uniformity (%)	Floating lag time (sec)	Floating duration (hr)	pH	Density (g/cm^3)	Swelling index (%)
F1	6.87±0.24	11±0.05	95	90±0.07	17±0.08	8.4	0.6±0.05	46.1
F2	8.01±0.15	2±0.01	97	60±0.06	19±0.05	8.5	0.7±0.03	65.6
F3	11.72±0.1	0	90.6	30±0.12	20.5±0.07	8.6	0.98±0.1	90.2
F4	4.76±0.21	10±0.07	90	-	-	7.6	1.14±0.55	63.7
F5	5.59±0.19	6±0.11	95	-	-	7.2	1.21±0.74	75.6
F6	6.43±0.27	0	91	-	-	7.0	1.36±0.39	84.2
F7	32.50±0.44	0	90.5	-	-	7.7	1.05±0.48	8.3
F8	40.06±0.52	0	95.6	-	-	7.3	1.11±0.26	10.1
F9	45.35±0.7	0	92	-	-	7.0	1.17±0.61	12.2
F10	7.94±0.32	5±0.03	94	-	-	6.95	1.14±0.78	60.9
F11	20.18±0.42	4±0.08	92	-	-	6.93	1.36±0.66	64.4
F12	7.18±0.24	6±0.04	93	-	-	6.92	1.05±0.54	43.5
F13	10.20±0.11	3±0.02	91	-	-	6.91	1.18±0.83	67.2
F14	17.76±0.5	0	90.5	-	-	6.9	1.4±0.97	83.5
F15	9.45±0.08	0	90.5	120±0.55	20±0.08	8.2	0.99±0.35	74.5
F16	2.27±0.3	0	94	20±0.09	12±0.2	8.7	0.45±0.04	50.5
F17	8.69±0.09	0	90.7	110±0.4	22±0.11	7.8	1.01±0.8	34.3
F18	4.91±0.08	0	91	25±0.08	21.5±0.09	8.2	0.99±0.45	22.5
F19	2.65±0.25	0	92	15±0.03	20±0.1	8.6	0.98±0.64	16.4
F20	1.89±0.07	7±0.06	99.6	55±0.07	23.5±0.03	7.2	0.6±0.06	8.8
F21	10.96±0.09	2±0.01	99.9	35±0.02	24±0.01	7.5	0.8±0.02	19.7
F22	15.49±0.05	0	92	29±0.06	24±0.04	7.7	0.91±0.08	35.6
F23	1.89±0.22	10±0.09	91	100±0.35	23±0.02	7.4	0.84±0.12	13.2
F24	3.40±0.17	5±0.1	90.5	80±0.34	23.5±0.5	7.6	0.88±0.22	27.6
F25	5.67±0.28	0	90	15±0.01	24±0.12	7.8	0.96±0.17	39.1
F26	2.65±0.4	25±0.12	96	90±0.23	18±0.07	8.5	0.98±0.3	67.9
F27	10.96±0.2	20±0.23	93	150±0.7	20±0.15	8.4	0.99±0.7	58.8

Table 3.1: to be continued

Formula No.	Gel strength (N/m^2)	Gelation time (sec)	Content uniformity (%)	Floating lag time (sec)	Floating duration (hr)	pH	Density (g/cm^3)	Swelling index (%)
F28	1.89±0.35	120±0.26	99	50±0.3	19±0.21	8.2	0.91±0.07	60.2
F29	3.02±0.26	60±0.08	98	110±0.31	21±0.09	8.1	0.96±0.49	62.6
F30	6.05±0.32	30±0.12	95	170±0.45	23±0.13	8.0	0.99±0.6	64.5
F31	6.43±0.43	0	91.4	20±0.2	20.5±0.07	8.0	0.99±0.35	66.1
F32	3.02±0.4	0	91	-	-	7.45	1.84±0.77	13.2
F33	4.54±0.37	0	90	-	-	7.4	2.1±0.9	17.9
F34	2.65±0.17	20±0.05	95.2	240±0.15	20±0.4	7.5	1.005±0.7	20.8
F35	3.40±0.23	10±0.07	90.1	300±0.5	20.5±0.16	7.5	1.01±0.5	30.1

3.2.1 In-Vitro Gelation Study

3.2.1.1 Gel Strength Determination

Table 3.1 shows that the polymers (Na alginate and gellan gum) as primary polymers and the secondary polymer (iota carrageenan, HPMCK100M and HPMC K4M) play an important role in gel strength. As the concentration of Na alginate increases in (F4-F6) and concentration of gellan gum increases in (F7-F9), the gel strength increased significantly ($p< 0.05$). This is due to the fact that Na alginate and gellan gum containing both carboxyl and hydroxyl groups in their structure, so increasing their concentrations resulting more carboxylic groups ready for crosslinking, thus triggering in an increase in electrostatic interaction in polymer matrix with the induction of the formation of strong bridges between polymer units by allowing the matrix to stretch further forming rigid matrix and hence increasing the gel strength. Similar observation was found with crosslinked biodegradable alginate hydrogel floating beads for stomach site specific controlled delivery of metronidazole and verapamil HCl [162, 136].

Formulas containing HPMC K4M and HPMC K100M (F10-F14) shows satisfactory gel strength ($p < 0.05$), as the concentration of HPMC increases. Rapid formation of uniformly homogenous thick gelatinous layer and higher hydration rate achieved due to the presence of methyl and hydroxypropyl substituents that interfere with the close packing between neighboring chains leading to increase gel strength of matrices[163].

Formulas containing iota carrageenan (F20-F25) combined with Na alginate and gellan gum separately, showed significant increase in gel strength ($p < 0.05$). As the concentration of iota carrageenan increases, it produces rigid gel of 3D networks of double helices due to crosslinking of the adjacent chains in which the sulfate groups are oriented externally as it has the optimum degree of sulfation, thus increasing gel formation in the presence of divalent ions such as calcium resulting in an increase in gel strength. This observation was reported in cell delivery systems using alginate−carrageenan hydrogel beads and fibers for regenerative medicine applications [164].

Moreover increasing concentration of $CaCl_2$ (F1-F3) shows significant increase in gel strength ($p < 0.05$), the degree of rigidness of gel increases due to increasing degree of crosslinking of divalent Ca^{2+} ions with the polymer chains. This observation complying with the findings reported in pH triggered sol-gel transition system of ofloxacin for prolonged gastric retention [165].

3.2.1.2 Gelation Time Determination

Table 3.1 shows that Na alginate and Gellan gum (as primary polymers) in addition to iota carrageenan and different HPMC grades (as secondary polymers) have different effect on gelation time. The in-situ

gel formed should preserve its integrity without dissolving or eroding so as to localize the drug at absorption site for extended duration.

Formulas (F1-F3) showing that increase in $CaCl_2$ concentration which upon contact with 0.1N HCl (pH 1.2) the liquid polymeric solution should undergo a rapid sol-to-gel transition by means of ionic gelation. The composition of gastric fluid is rich in Cl^- ions; hence on interacting with $CaCl_2$ as cross-linking agent, in-situ gel formed rapidly. Formulas (F4-F6) showing that increase in concentration of Na alginate causing gelation to undergo instantly and formed good gel, this is due to internal ionotropic gelation effect of calcium on Na alginate, all these formulas (F1-F6) show significant decrease ($p < 0.05$) in gelation time, these observations complying with the findings reported in the formulation and evaluation of stomach specific in-situ gel of metoclopramide using natural, bio-degradable polymers [157].

Studying gelation time of gellan gum Formulas (F7-F9), non-significant effect was observed since gellan chains are in a random coil conformation. They rearrange in a "double helix" conformation (coil-helix transition), and the double helixes assemble leading to physical junction zones; indicating that whatever the gellan concentration is, it undergoes rapidly from sol to gel transition due to ionic interaction. This observation was reported in the in vitro and in vivo evaluation of the Gelrite® gellan gum-based ocular delivery system for indomethacin [166].

Moreover for formulas containing iota carrageenan (F20-F25), the results showed that increasing iota carrageenan concentration lead to significant effect on gelation time ($p < 0.05$) due to contact of iota carrageenan solution with 0.1N HCl (pH 1.2).Thus the dissolved polymer random coils undergo rapid transition into double helical conformation at the junctional zone depending on the cross-link with Cl^- present in gastric

fluid leading to rapid gelation with strong gel formation, this observation was shown in comparison of ion-activated in situ gelling systems for ocular drug delivery [167].

To study the effect of $NaHCO_3$ on the gelation time, (F10-F14) contain HPMC without $NaHCO_3$ and (F26-F30) containing HPMC with $NaHCO_3$ were used. It was found that gelation time retarded significantly ($p < 0.05$) due to poor cross linking of sodium ion of sodium bicarbonate and need more time to crosslink Cl^- of gastric fluid with $CaCl_2$. Thus form soft gel and less time for gel to start rupture and as a result formation of pores with time, such observation was shown in formulation and evaluation of ranitidine HCl as floating in-situ gel [168].

3.2.2 Swelling Index

The results in Table 3.1 and Figure 3.4 show that types and concentrations of primary polymers (Na alginate and gellan gum) also the addition of the secondary polymers (iota carrageenan, HPMC K100M, HPMC K4M) play important role on swelling behavior of the in-situ gel. Increasing of Na alginate concentration in (F4-F6) shows significant increase in swelling index ($p < 0.05$). Increasing concentration of Na alginate leads to high percentages of hydration, and sodium-calcium ion exchange forming insoluble calcium alginate regions, followed by solvent penetration into the gel network and these result in ease of hydration and fast swelling of Na alginate. Similar observation was found in formulation and evaluation of oral in-situ floating gel of domperidone[169]. As the concentration of gellan gum increased in the formulations (F7-F9) the swelling index increased non-significantly ($p > 0.05$). Since gellan gum gel has a quite low water uptake because of the carboxylic groups are involved in the formation of the double helices, which increases the amount of the junction zones, thus at low pH environmental conditions

0.1N HCl (pH 1.2) more stable junction zones induced because the carboxylate groups are in their acidic form and as a result the polymer chains can be closer to one another leading to the low swelling of the polymer [170].

High hydration percentages were observed with formulas containing HPMC K100M and HPMC K4M as secondary polymers and 1% (w/v) Na alginate (F10-F14). The water uptake data was correlated with the molecular weight and concentration of HPMC; as high molecular weight HPMC grades is used or increasing concentration of specific HPMC; there will be significant increase in the swelling index ($p < 0.05$) because the polymer gradually absorbs water. These findings correlated with the hydrophilicity of HPMC K100M and HPMC K4M as non-ionic cellulose derivatives water soluble polymers, the outermost hydrophilic polymer hydrates and swells and a gel barrier is formed at the outer surface. Then as the gelatinous layer progressively dissolves and/or is dispersed, the hydration swelling release process is repeated towards new exposed surfaces, thus maintaining the integrity of the dosage form with higher swelling state. This result was similar with that observed in formulation of gastroretentive drug delivery system of itopride HCl [171].

The effect of the presence iota carrageenan as secondary polymer together with Na alginate as primary polymer in formulas (F20-F22) and gellan gum (F23-F25) were studied. It was found that as the concentration of iota carrageenan increased; the swelling index increased significantly ($p < 0.05$). This is due to the presence of sulfate group in carrageenan, so as the concentration of carrageenan increased, the counter ions also increased and this contribute to stronger electrostatic repulsion between the sulfate groups and therefore the swelling of the carrageenan also increased. However, this swelling is limited due to acidic pH, since most

of the carboxylate anions present in both Na alginate and gellan gum are protonated. So the main anion-anion repulsive forces with iota carrageenan are eliminated and consequently swelling values are limited. Similar observation was found in swelling behaviour of cross-linked-carrageenan/NaCMC hydrogel and carrageenan-graft-polymethacrylamide hydrogel [172, 173].

The increase in $CaCl_2$ crosslinker concentration (F1-F3) caused in a significant increase in the swelling index ($p < 0.05$), since the formation of cross-linked networks providing an additional barrier to water penetration outside. Thus increasing concentration of the cross-linker in the delivery system provides an increase in water uptake concentration and collapsing of the gel was negligible compared to gels with low concentrations of cross-linker. Similar result was observed in floating in- situ gel based on alginate as carrier for stomach-specific drug delivery of famotidine [174].

Figure 3.5 demonstrates the swelling index of in-situ gel formulation at the beginning of addition of 0.1N HCl and after 5 hrs.

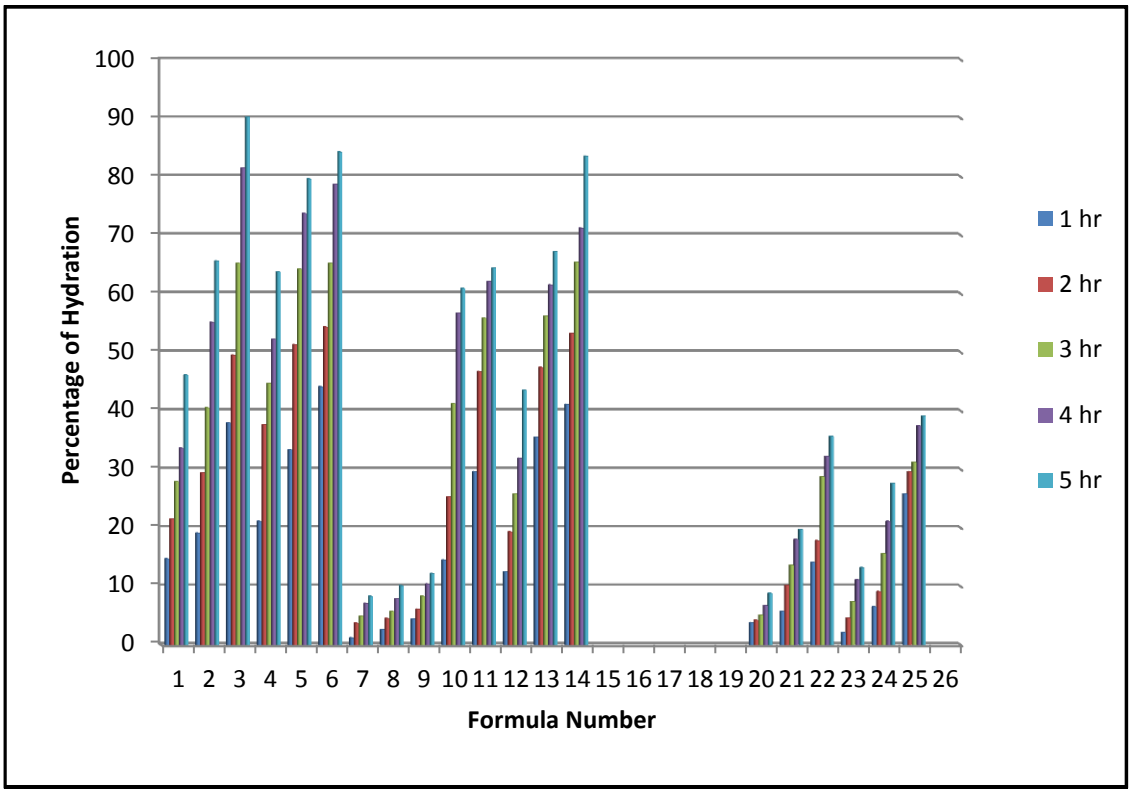


Figure 3.4: Swelling Index of Formulations

(A) At the Beginning **(B) After 5 hrs**

Figure 3.5: Swelling Index of Furosemide In-Situ Gel, (a) At the Beginning & (B) After 5 hrs

3.2.3 Viscosity Measurements

The rheological properties of the solutions are of importance in viewing of their proposed oral administration. The formulation should have an optimum viscosity that will allow easy swallowing as a liquid, which then undergoes a rapid sol–gel transition due to ionic interaction.

Table 3.2 and Figure 3.6 illustrate significant ($p < 0.05$) increase in the viscosity of the formulations (F4-F9) as the concentration of Na alginate and gellan gum were increased with shear thinning behaviour. This phenomenon is a consequence of increasing chain interaction with an increase in polymer concentration. Similar results were obtained for carbamazapine in-situ gel [175] and verapamil in-situ gel [136].

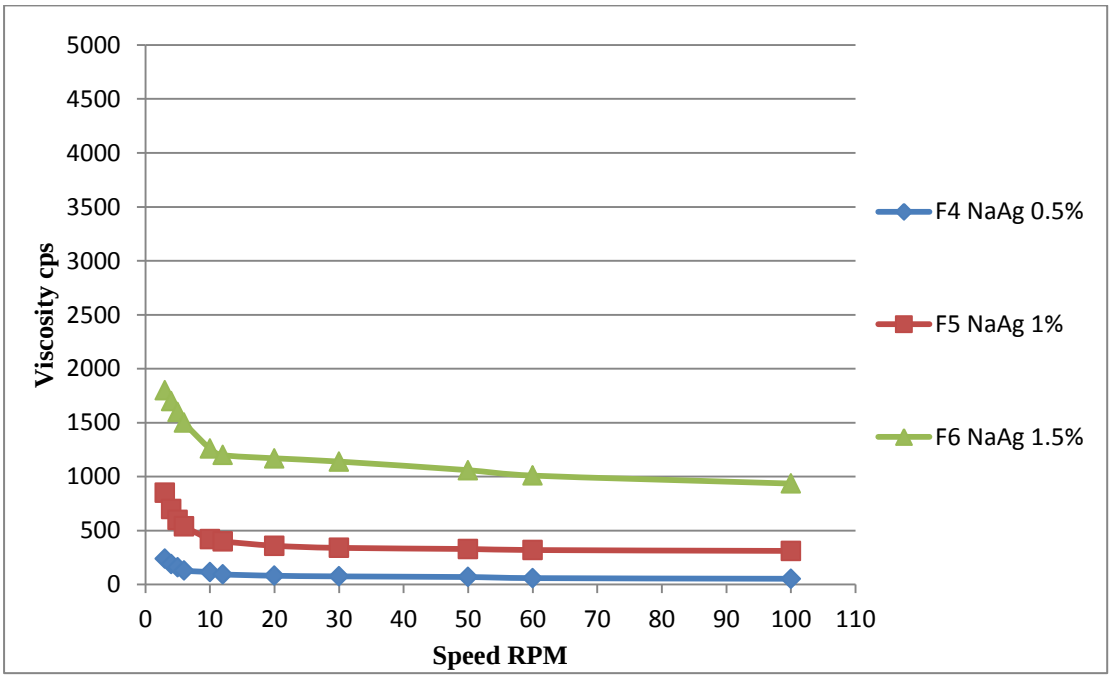
Combination of polymers in (F10-F14) containing various HPMC grades and in (F20-F25) containing iota carrageenan affect the viscosity of solution as seen in Figure 3.7. Thus increasing concentration of HPMC has significant ($p < 0.05$) effect on the viscosity with shear thinning behaviour due to increasing in polymer network crosslinking with strong elastic behaviour of the highly concentrated polymer. This result is in an agreement with reported data [176]. Also increasing iota carrageenan concentration resulted in a significant ($p < 0.05$) increase in viscosity, this result is due to strong crosslinking of iota carrageenan which result in strong elastic crosslinking polymeric network. Similar observations were seen in gel composed of iota-carrageenan [177].

Table 3.2: Effect of Shear Stress on Viscosity of Formulations

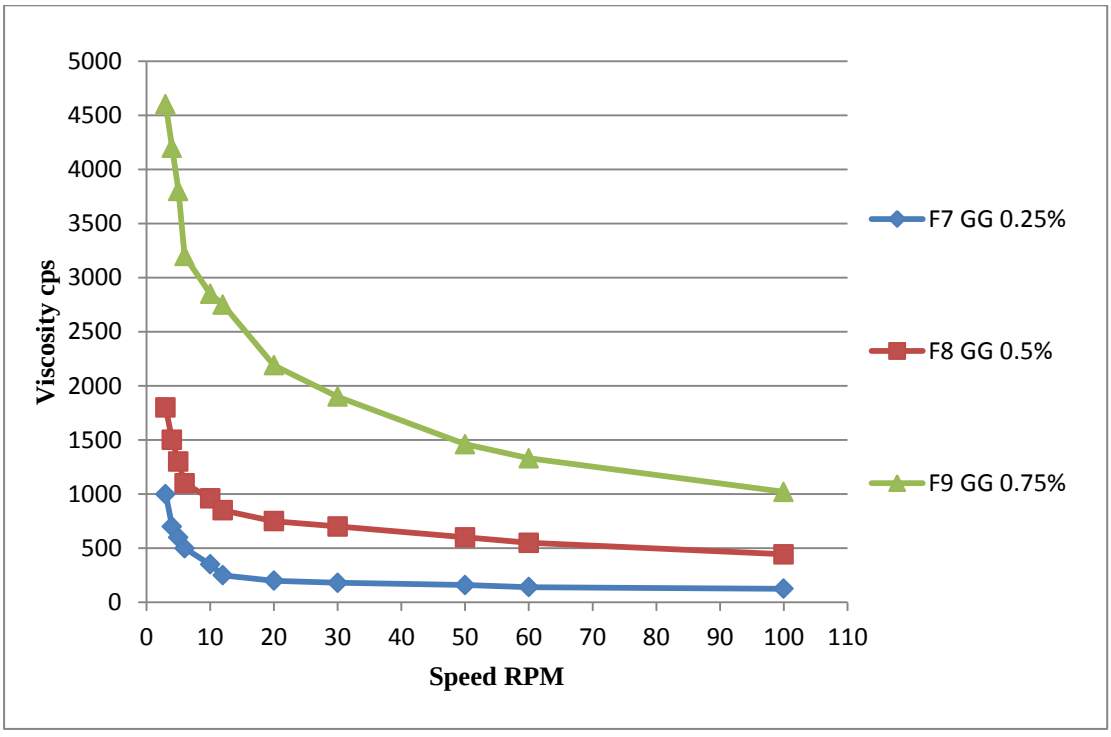
Shear Speed (RPM) / Formula No.	Viscosity(cps)										
	3	**4**	**5**	**6**	**10**	**12**	**20**	**30**	**50**	**60**	**100**
F1	860	800	700	600	480	450	420	400	380	360	330
F2	900	880	770	650	530	500	410	400	365	354	340
F3	1200	1100	1000	800	700	650	570	540	520	490	458
F4	240	190	160	130	115	95	82	76	70	64	50
F5	850	700	600	540	420	400	360	340	330	320	312
F6	1800	1700	1600	1500	1260	1200	1170	1140	1060	1010	936
F7	1000	700	600	500	350	250	200	180	160	140	126
F8	1800	1500	1300	1100	960	850	750	700	600	550	444
F9	4600	4200	3800	3200	2850	2750	2190	1900	1460	1330	1020
F10	6800	6700	6500	6300	6120	5900	5700	5520	5230	3280	2160
F11	9000	8500	8000	7500	7000	6500	5900	5400	5000	3920	3790
F12	2800	2500	2300	2100	1920	1850	1770	1640	1580	1520	1460
F13	3400	3100	2900	2700	2400	2250	2160	2080	1900	1820	1640
F14	9000	8200	7900	7500	6540	6300	5520	4900	4340	4200	3580
F15	870	800	700	600	480	450	420	380	360	340	320
F16	1000	900	800	700	600	540	450	420	380	360	350
F17	2600	2100	1700	1400	1200	1050	880	780	650	600	480
F18	3400	2800	2500	2200	1500	1350	900	840	700	620	530
F19	3800	3300	2600	2500	1700	1550	1200	1040	890	780	650
F20	1600	1400	1100	900	740	650	590	500	430	380	210
F21	1800	1500	1300	1200	840	750	690	540	460	408	290
F22	3200	3100	2700	2300	1920	1750	1500	1340	1040	930	780
F23	1800	1600	1200	1000	600	500	300	260	200	170	126
F24	2000	1600	1400	1300	720	650	510	340	230	210	150
F25	3800	3400	2800	2500	1820	1400	900	680	460	370	270
F26	11000	10300	9500	8600	7260	6800	5970	5420	4670	4190	3605
F27	16000	14700	13600	12800	10500	9950	8460	7420	5340	5079	4400
F28	5800	5400	5000	4800	4260	4150	3630	3420	3070	2999	2560

Table 3.2: to be continued

Shear Speed (RPM) / Formula No.	Viscosity(cps)										
	3	**4**	**5**	**6**	**10**	**12**	**20**	**30**	**50**	**60**	**100**
F28	13800	12700	11900	10800	9420	8500	7260	5820	4700	4180	3570
F29	15000	13200	12200	11400	10660	9850	8460	7680	6720	6170	5003
F31	1400	1300	1200	1100	950	930	900	880	840	820	758
F32	2100	1899	1730	1510	1220	1140	1100	900	680	540	366
F33	2400	2220	1900	1770	1550	1420	1310	1150	830	640	378
F34	1000	900	700	600	540	480	450	420	410	400	380
F35	1200	1100	1000	900	700	600	550	480	460	450	414
Fudesix®	210	170	150	120	94	88	82	76	70	60	54



A



B

Figure 3.6: Rheological Properties of Na Alginate (A) and Gellan Gum (B) Solutions

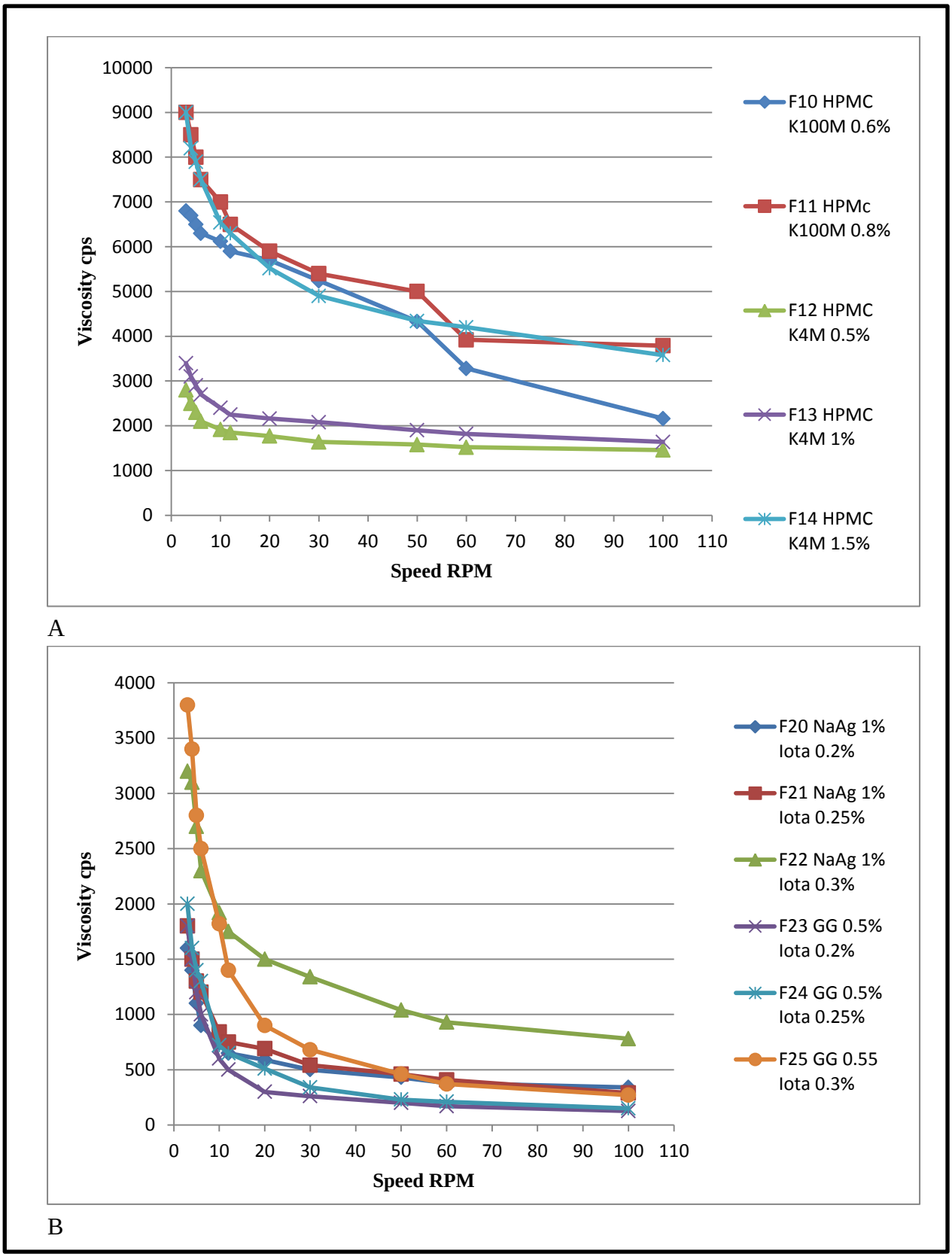


Figure 3.7: Rheological Properties of Combination of Polymers of Various Grades of HPMC (A) and Iota Carrageenan (B) Solution

Figure 3.8 shows the effect of $NaHCO_3$ on the viscosity of formulations (F2, F5, F8, F15, F16, F17, F18 and F19) with shear thinning behaviour. The formulations showing non-significant ($p > 0.05$) increase in viscosity. This is due to the fact that Na alginate and gellan gum form strong crosslinking in the polymer matrix and addition of $NaHCO_3$ decrease in the elasticity of matrix without effecting viscosity. Similar observations were seen in effects of mucokinetic drugs on rheological properties of reconstituted human nasal mucus [178].

While Figure 3.9; illustrates the effect of addition of 0.5% (w/v) $NaHCO_3$ on the viscosity of formulations (F26-F30) containing different HPMC grades. The results showed significant ($p < 0.05$) increase in viscosity of the formulations in comparison to (F10-F14) which contain same HPMC grades with no $NaHCO_3$ added. These results were due to increasing the ionic strength of the matrix composition and salting out the macromolecules due to the presence of $NaHCO_3$ that may cause shrinkage of the polymeric chains and controlling chain expansion, thus causing an increase in viscosity. These observations are in an agreement with formulation of controlled-release matrix system of high load and highly water-soluble drug niacin [179].

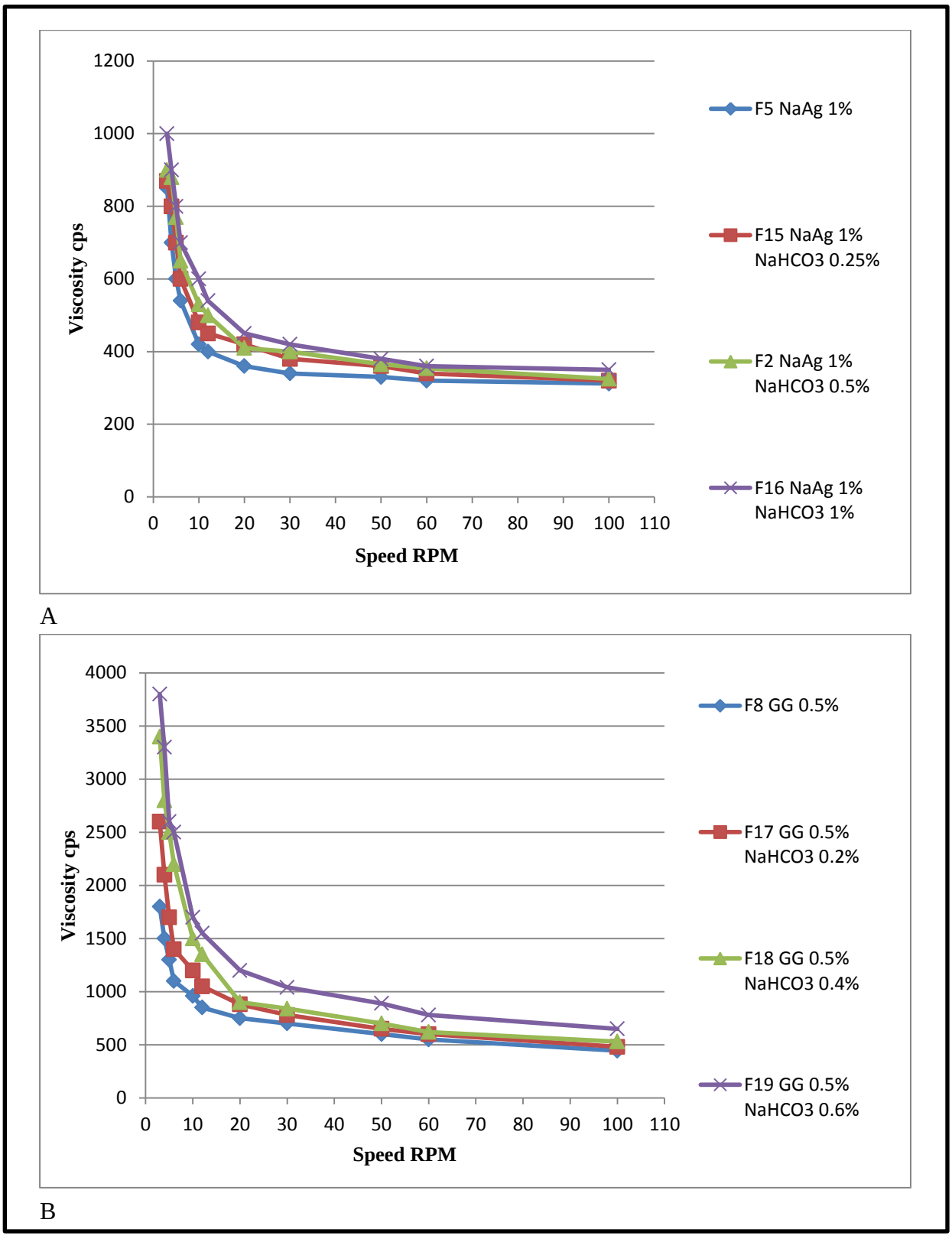


Figure 3.8: Rheological Properties of Na Alginate (A) and Gellan Gum (B) with $NaHCO_3$ and without $NaHCO_3$

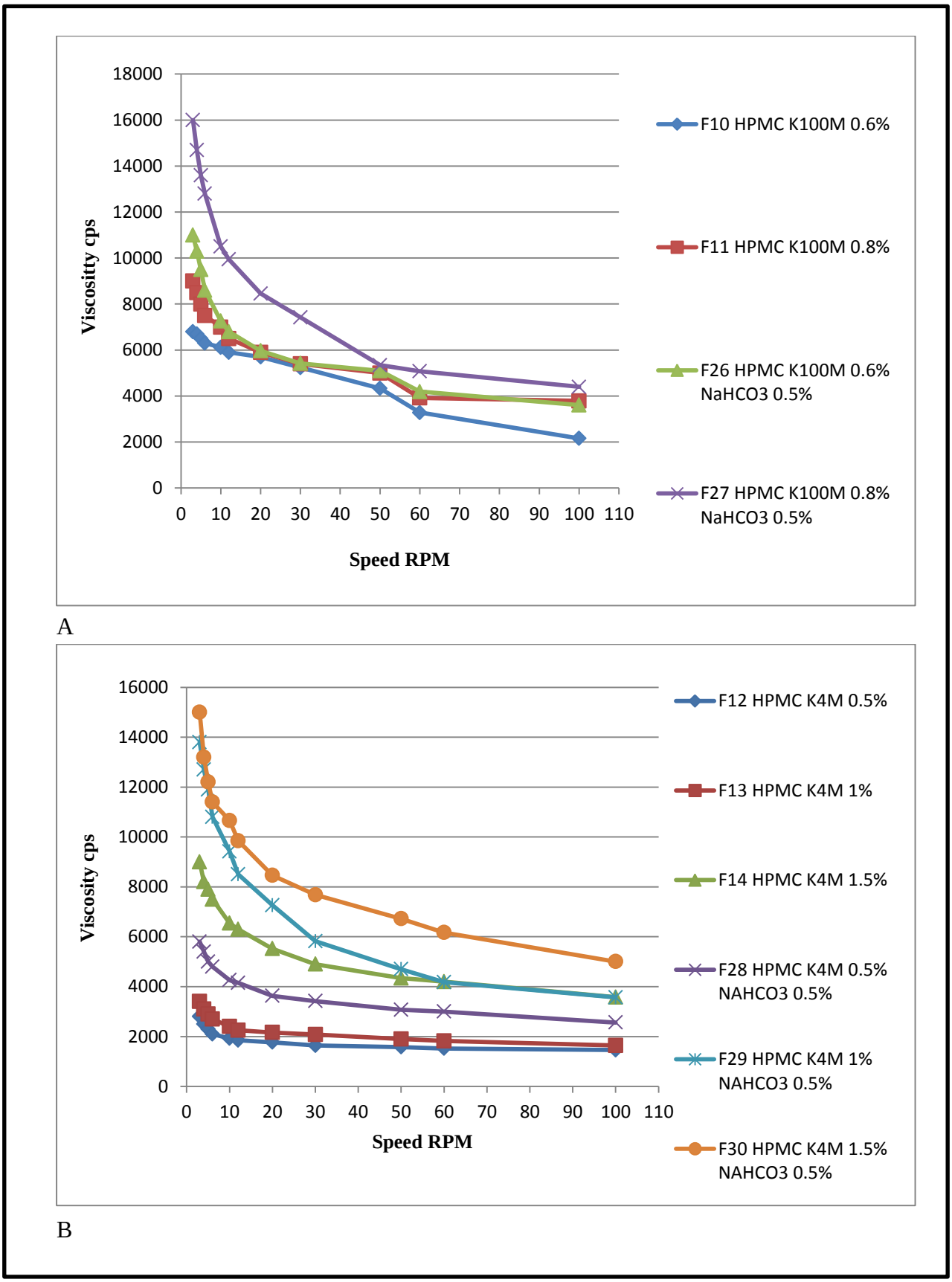


Figure 3.9: Rheological Properties of HPMC K100M (A) and K4M (B) with $NaHCO_3$ and without $NaHCO_3$

Moreover, increasing drug concentration (F21, F32 & F33) has no significant effect on the rheological properties of the polymeric solution as shown in Figure 3.10. The reason behind this is that viscosity depends mainly on concentration of polymer more than on concentration of drug, Therefore, as the drug loading is increased, the mass of insoluble drug increased and this has no significant effect on the viscoelastic, flow and textural properties of the formulations. These observations are in accordance with that reported for tetracycline-containing bioadhesive polymer networks [(180)].

In addition increasing concentration of fructose (Sweetening agent) (F21, F34 & F35) as shown in Figure 3.11, resulted in increase in viscosity non-significantly ($p > 0.05$). The reason behind this is the reduction in the intermolecular hydrogen bonding between water and polymer matrix. This resulted in depletion of water leading to enhance hydrophobe–hydrophobe interaction leading to a highly branched polymer that increases the resistance of the solution to flow freely; and therefore, increases the viscosity of the system. This observation is in agreement with in-situ fast gelling formulation for ketorolac tromethamine [(181)].

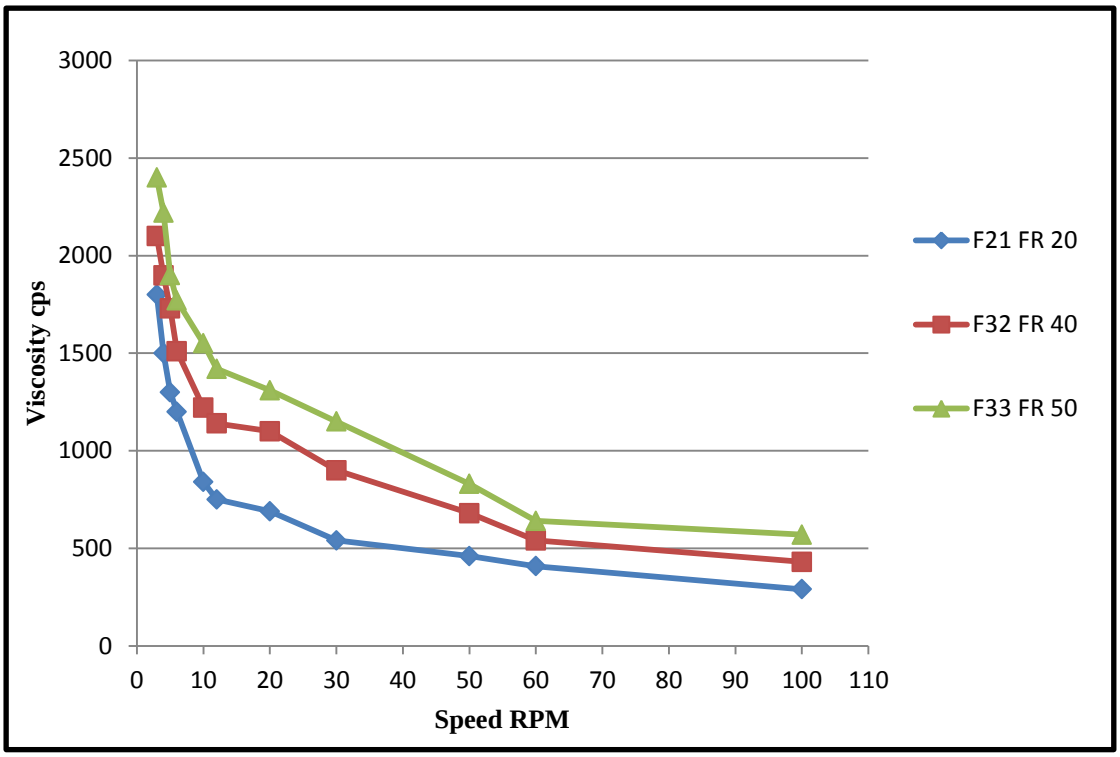


Figure 3.10: Rheological Properties of Polymeric Solution upon Increasing Furosemide Concentration

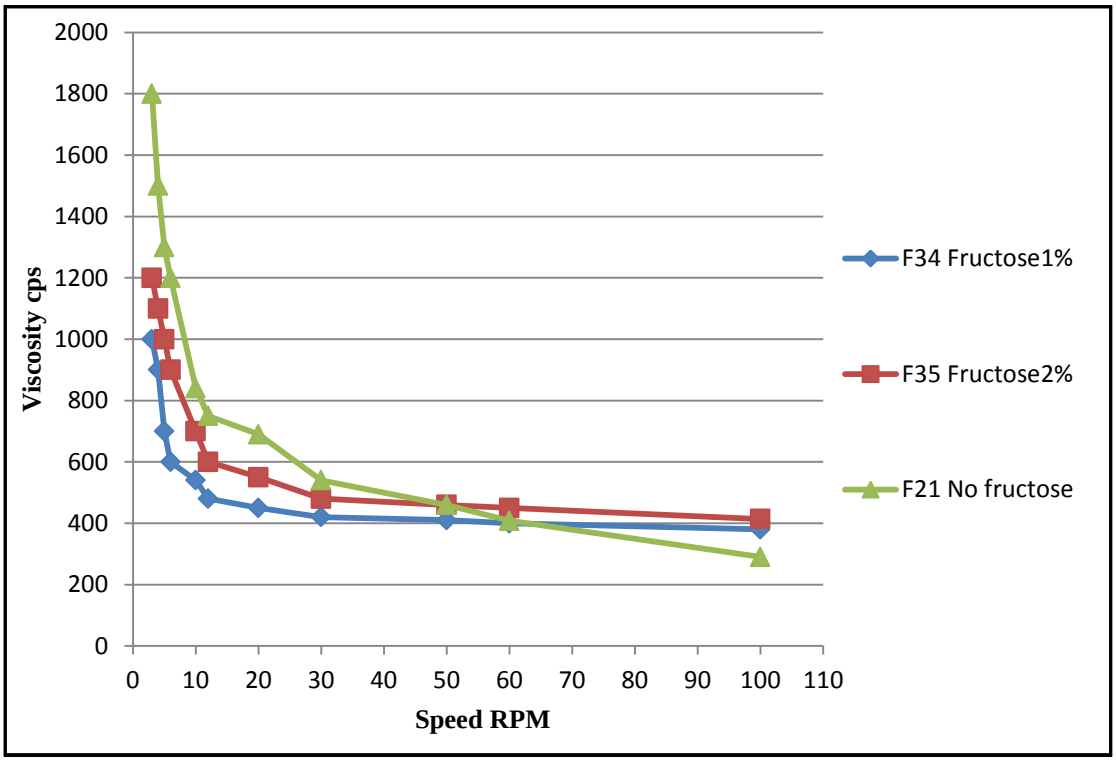


Figure 3.11: Rheological Properties of Polymeric Solution upon Increasing Fructose Concentrations

3.2.4 In-Vitro Buoyancy

The in vitro floating ability of the prepared formulations was investigated using dissolution medium 0.1N HCl (pH 1.2) shown in Table3.1. Results obtained for formulations without sodium bicarbonate (F4-F14) illustrated that these formulas were non-floating. The reason behind non floating ability is directly related to the gas content of the polymer matrix since highly dense crosslinked internal structure with no pores was prepared in the absence of gas forming agent and it was expected to retain the drug for more time and thus unable to float [182, 183].

Generally formulations containing sodium bicarbonate as a gas-generating agent maintain buoyancy due to generation of carbon dioxide in presence of dissolution medium and the combination of sodium bicarbonate and citric acid provided desired floating ability. It was observed that the gas generated was trapped and protected within the gel formed by hydration of polymer, thus decreasing the density of the formulations below 1g/cm^3 and the gel became buoyant also the gel swollen during in vitro buoyancy studies.

It was found that increasing the amount of sodium bicarbonate in (F15, F2 & F16), the floating lag time decreases significantly ($p< 0.05$). Thus in (F15) (containing 0.25% (w/v) $NaHCO_3$) showed highest floating lag time due to the generation of small amount of CO_2 gas, While (F2) (containing 0.5% (w/v) $NaHCO_3$) the amount of CO_2 was essential to achieve optimum in vitro buoyancy since these formulations containing Na alginate and the calcium ions reacted with Na alginate to produce a crosslinking 3D gel network and swollen structure that may restrict further liberation of carbon dioxide and drug molecules, with intact formed gel. Further increase in concentration of sodium bicarbonate in (F16) does not show any significant effect on floating behaviour

($p>0.05$), where, the increased amount of sodium bicarbonate causing a large amount of effervescence, which in turn resulted in pore formation, leading to rapid hydration of the polymer matrix and thereby weak gel was formed that may be removed early from stomach by peristaltic movement [184].

In formulations (F1-F3) the effect of increasing $CaCl_2$ concentration on floating ability was found to be significant ($p< 0.05$).The reason behind that is due to the formation of double helical junction zone followed by aggregation of the double helical segments to form a 3D network by complexation of Na alginate with Ca^{2+} ions. Thus as the concentration of $CaCl_2$ increased, the time taken by formulation to emerge on the medium surface (floating lag time) decreased and the duration over which formulation continuously floated (duration of floating) increased.

In case of formulations containing gellan gum (F17-F19), increasing sodium bicarbonate concentration lead to significant ($p< 0.05$) decreases in floating lag time and non-significant decrease in floating time. This is due to the gelation and crosslinking of gellan gum with Ca^{2+} ions decreased leading to the formation of a thin gel due to the liberation of high amount of carbon dioxide from the gel matrix producing more buoyant effect characterized by decrease in floating duration and floating lag time. The same observations were reported in the development and in vitro evaluation of an in-situ gelling oral liquid sustained release formulation of nizatidine [141].

Moreover, as the concentration of HPMC K100M (F26-F27) and HPMC K4M (F26-F30) was increased, the floating time increased significantly ($p< 0.05$). Also floating lag time increased due to slight increase in density and gel strength of the matrices, which entrapped CO_2

inside the jellified polymeric matrices and prevented its escape, thus inducing the gel to float. This is in an agreement with preparation and characterization of a gastric floating dosage form of capecitabine [185].

Increasing iota carrageenan concentrations in (F20-F22) containing 1% Na alginate and in (F23-F25) containing 0.5% gellan gum, resulted in decreasing floating lag time significantly ($p < 0.05$) while floating duration remain almost constant. The reason behind that is due to rapid crosslinking of polymer matrix as a result of the presence of strongly acidic sulfate groups in iota carrageenan molecule that allows a certain degree of polymer ionization in 0.1N HCl (pH 1.2) leading to the formation of insoluble gel-like layer of aggregated double helical segments that form a 3D network by complexation, and consequently slower solvent penetration into the matrices and more controlled CO_2 diffusion were achieved and thus inducing the in-situ gel to float rapidly[186, 187].

3.2.5 Density Measurement of Gel

Density is important parameter as far as the floating properties of the gastroretentive dosage form is concerned. Table 3.1 shows the density values for all the formulations. Ideally the density of the dosage form, to float on the gastric content must be less than or equal to gastric contents (~1.004 g/cm^3).

Formulations (F4-F14) were found to have an increase in the density of formulations as the polymer concentration increased and the floating behaviour was not achieved. The reason is that the polymer matrix is highly dense non-porous internal structure also these formulations do not contain $NaHCO_3$ [183].

Moreover, F1-F3 (containing 0.5% $NaHCO_3$); as the amount of $CaCl_2$ increased, the density of formulations is non-significantly

increased, since $CaCl_2$ formed double helical junction zone followed by aggregation of the double helical segments to form a 3D network by complexation, thus a rigid gel is formed. As a result, the gas generated is trapped and protected within the gel formed by hydration of polymer thus decreasing the density of the gel below gastric fluids density resulting in a buoyant gel [136].

Formulations (F15, F2 & F16) (containing 0.25%, 0.5% and 1% $NaHCO_3$ respectively and 1% Na alginate), and F17-F19 (containing 0.2%, 0.4% and 0.6% $NaHCO_3$ respectively and 0.5% gellan gum) showed significant ($p< 0.05$) effect of $NaHCO_3$ on the density of the formulation. The reason behind this is due to the formation of double helical segments to form a 3D network by complexation, thus a rigid gel is formed. As a result, the gas generated is trapped and protected within the gel formed by hydration of polymer thus decreasing the density of the gel below gastric fluids density resulting in a buoyant gel.

Formulations (F21-F31 and F34-F35); showed non-significant effect of different polymers (primary and secondary polymers) also additives on the density of the formulations [188].

As the concentration of furosemide increased in F32 & F33 (containing (0.8% w/v equivalent to 40mg/5ml and 1% w/v equivalent to 50mg/5ml), it showed significant ($p< 0.05$) increase in density. The reason behind this is that the solubility of drug within the gel systems decreased. Therefore, as the drug loading is increased, the mass of insoluble drug will increase; thus the density of the gel increased. Therefore these formulations failed to float. Same observations were seen with tetracycline-containing bioadhesive polymer networks [180].

3.2.6. pH Measurement

The pH of all formulations (F1-F35) was measured by using pH meter. The values of pH were ranged (6.9-10) as shown in Table 3.1 and these values reveal that all the formulations provide an acceptable pH according to USP [112].

The pH in (F1-F3) is in the range of (8.4-8.6) showing non-significant ($p > 0.05$) effect of increasing $CaCl_2$ concentration. The recorded results described a high pH due to the presence of sodium bicarbonate in the formulations that may had a pH of 8.3, also anhydrous $CaCl_2$ has a wide range of pH (4.5-9.2). So it may be considered as a second reason of increasing pH due to its hygroscopic property that may absorb water causing an increase in the pH of the solution.

Increasing Na alginate & gellan gum concentrations in (F4-F9) showing decrease in pH significantly ($p < 0.05$), this is may be due to the presence of high amount of carboxylate group (COO^-) in the structure of polymers which imparts acidic proprieties to the solution.

Increasing concentration of various grades of HPMC in (F10-F14) showing non-significant effect ($p > 0.05$) on pH, due to the nature of HPMC as non-ionic polymer which has a pH range of 5.0-8.0, knowing that these formulations do not contain $NaHCO_3$.

Moreover, sodium bicarbonate had significant effect ($p < 0.05$) on pH in (F15-F31). The reason behind this is due to the alkaline nature of sodium bicarbonate pH 8.3 that may raise the pH of the solution.

Increasing furosemide concentration in the formulations (F32-F33) showing non-significant effect ($p > 0.05$). The reason is due to the alkaline nature of $NaHCO_3$ that maintain the solution at a stable pH regardless of increasing the concentration of furosemide which had carboxylic acid group in its structure [189].

3.2.7 Drug Content Uniformity

The absorbance of the suitably diluted solutions was measured and the formulations were evaluated for uniform distribution of furosemide. All the readings measured in triplicate and the average of the % drug content is determined by using standard calibration curve at 274.2 nm and they found to be in the range of 90-99.9% as shown in Table 3.1 indicating that furosemide was uniformly distributed within all the formulations.

3.3 In-Vitro Drug Release Study

The prepared formulations were subjected for in vitro dissolution study in 0.1N HCl, to study the effect of different variables on percentage of drug release.

3.3.1 Study the Effect of Variables on the Release Profile

3.3.1.1 Effect of Different Concentrations of Ion Crosslinking Agent

The release profiles and the effect of the $CaCl_2$ (as ion crosslinking agent) amount on the formulations of furosemide were shown in Figure3.12. The results show that increasing concentration of $CaCl_2$ from 0.075% (F1), 0.1% (F2) and to 0.15% (F3) has significant effect ($p < 0.05$) to retard the release rate. This is related to the increase in the number of Ca^{2+} ions, which increase the crosslinking with the polymer chains thereby contributing to increase in the density of the polymer matrix and consequent increase in the diffusional path. This result is consistent with sodium alginate based in situ gelling system of meloxicam [(190)].

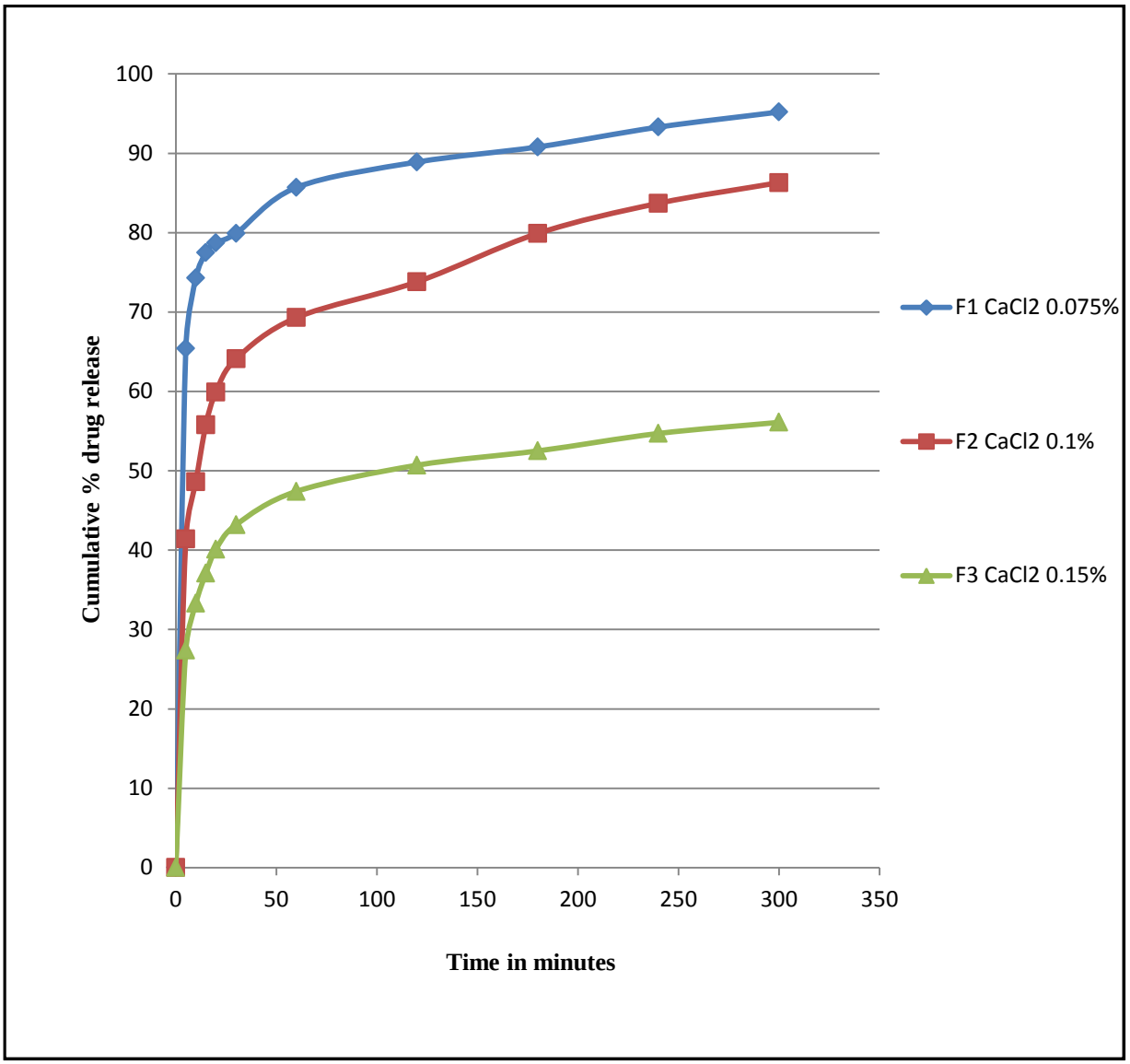


Figure 3.12: The Effect of Adding Different Concentrations of $CaCl_2$ on Release Profile of Furosemide in 0.1N HCl, at 37^o C.

3.3.1.2 Effect of Types and Concentrations of Polymers

Effect of Na alginate and gellan gum in F4-F9 and their concentrations on in-vitro drug release from floating in-situ gels is shown in Figure 3.13. A significant ($p < 0.05$) decrease in drug release was observed with increase in polymer concentration.

The release of drug from these gels (F4-F6) was characterized by an initial phase of high release (burst effect) due to water penetration into the floating insitu gel matrix and then release of drug via diffusion and dissolution. However, as gelation proceeds, the remaining drug was released at a slower rate. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics. The initial burst effect was considerably reduced with increase in polymer concentration due to increase in density of polymer matrix and thus an increase in diffusion path length through which the drug molecules have to traverse; in addition higher swelling of the Na alginate led to increase diffusion pathway.

The reason for the retarded release of drug from gellan gum gels (F7-F9) may be explained by the fact that gelation and aggregation of gellan gum occur via chemical bonding between calcium and carboxylic groups in the gellan chains. Calcium, being a hard electrophile, interacts with the carboxylate group of gellan gum electrostatically. As the concentration of gellan gum increased, more carboxylate group side chains would be available for the formation of stronger gellan-calcium network. Same results observed in floating in-situ gel of moxifloxacin HCl and in rifabutin-loaded floating gellan gum beads [191, 192].

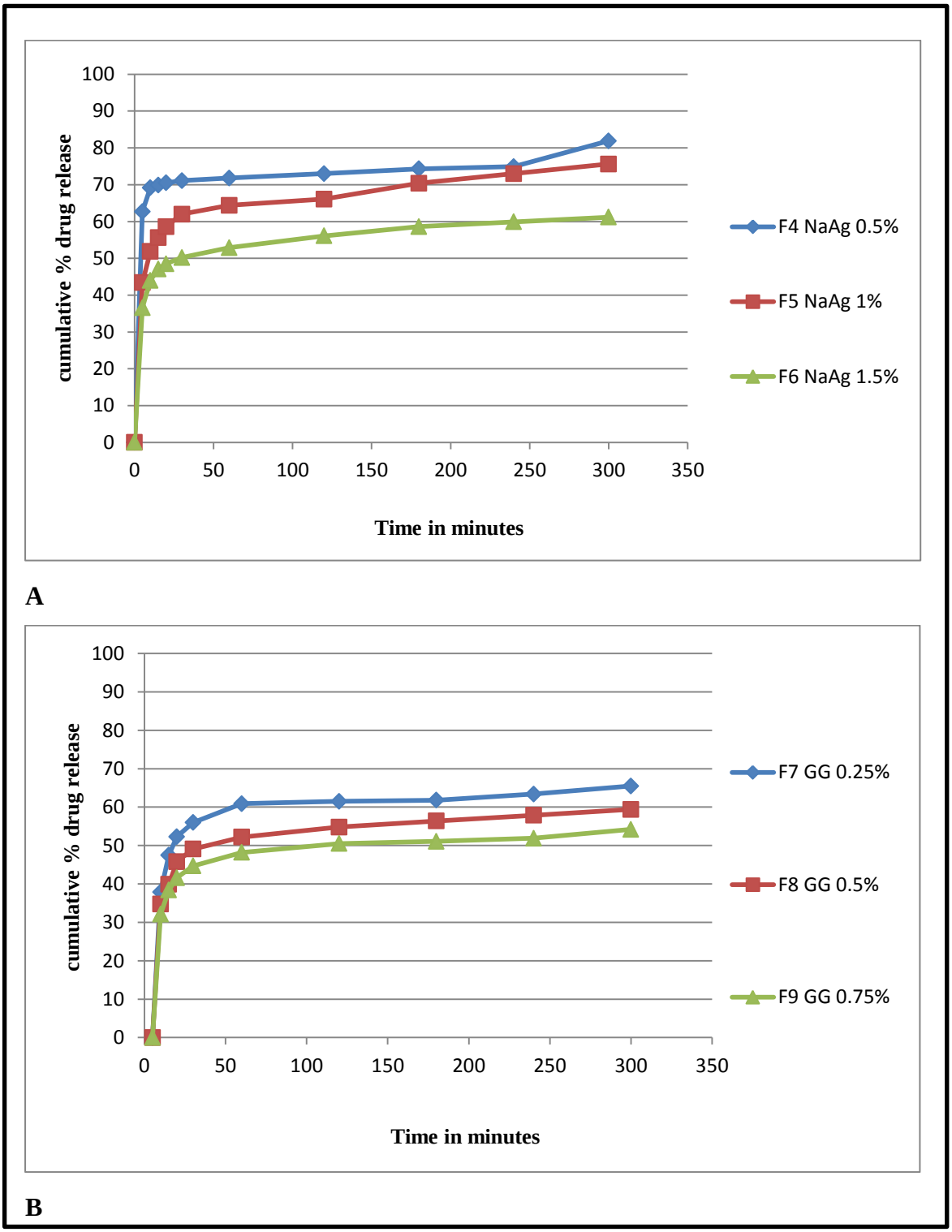


Figure 3.13: The Effect of Adding Different Concentrations of Na Alginate (A) and Gellan Gum (B) on Release Profile of Furosemide in 0.1N HCl, at 37^{o} C.

3.3.1.3 Effect of Different Concentrations of Gas Forming Agent

The effect of different concentrations of $NaHCO_3$ in (F2, F15 & F16) loaded with sodium alginate showing significant effect ($p < 0.05$) on the release profile of furosemide formulations. It is found that in the presence of small amount of gas forming agent as in F15, the release of the drug from the formulation was slower. This decrease in release is due to the fact that the highly dense internal structure of the gel prepared containing small amount of gas forming agent was found to retain the drug more effectively. The rate of drug release was found to increase with increasing weight ratios of $NaHCO_3$ as shown in Figure 3.14. This is a direct results due increasing the porosity of sodium bicarbonate containing gels. This observation is confirmed in studying the effect of effervescent agents on the formulation of famotidine loaded sodium alginate floating beads [193].

While increasing concentrations of $NaHCO_3$ in (F17-F19) loaded with gellan gum were showing non-significant effect ($p > 0.05$) on release profile of furosemide. The reason behind this is due to fixed amount of gellan gum in the formulations that form highly crosslinked polymer chain network which entrapped CO_2 within gel matrix contributing of highly dense; constant porosity structure resulting in a constant diffusional path length and a constant drug release [194].

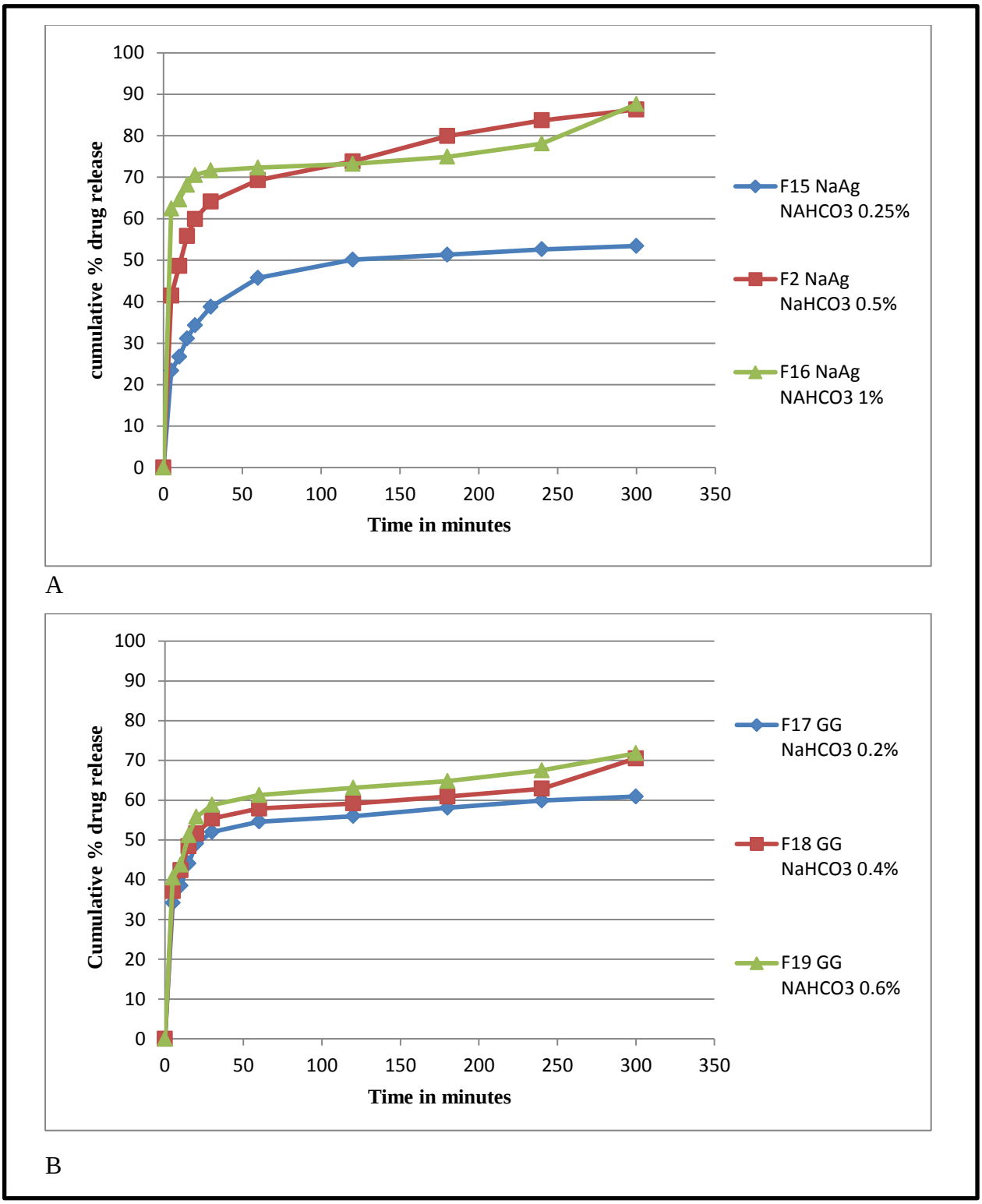


Figure 3.14: The Effect of Adding Different Concentrations of $NaHCO_3$ on Release Profile of Furosemide in 0.1N HCl, at 37° C, (A) Using Na Alginate as Primary Polymer and (B) Using Gellan Gum as Primary Polymer

3.3.1.4 Effect of Combination of Polymers with or without Gas Generating Agent

Combination of various grades of HPMC (HPMC K100M and HPMC K4M) as secondary polymers with Na alginate as primary polymer in the absence of $NaHCO_3$ in (F10- F14) resulted in significant decrease ($p< 0.05$) in release profile of furosemide as the concentration of HPMC was increased as shown in Figure 3.15.This result could be described to the formation of a thick gel structure which increased diffusion path length of the drug and hence delayed drug release from the gel matrix. The strength of gel layer increased as the polymer proportion was increased. The results are in agreement with that reported for nizatidine gastroretentive floating tablet [(195)].

Combination of various grades of HPMC (HPMC K100M, HPMC K4M and HPMC 5 cp) as secondary polymers with Na alginate as primary polymer in the presence of $NaHCO_3$ (F26- F31) has significant effect ($p< 0.05$) on drug release as shown in Figure 3.16. It was found that release from the matrix is largely dependent on the polymer swelling, drug diffusion and matrix erosion. The percentage drug release from formulations varies from 87.6 to 99.8 %. High viscosity polymer (F27 K100M 0.8% and F30 K4M 1.5%) induces formation of strong viscous gel layer; in addition to the presence of gas forming agent which is trapped and protected within the gel formed by hydration of polymer that decreased the density of the gel to become buoyant. Thus the results showed slowing down in the rate of water diffusion into the gel matrix due to the swelling structure effected by $NaHCO_3$, which may affect drug release by increasing diffusional path length. The same observations were seen in oral floating tablet of cephalexin [(188)].

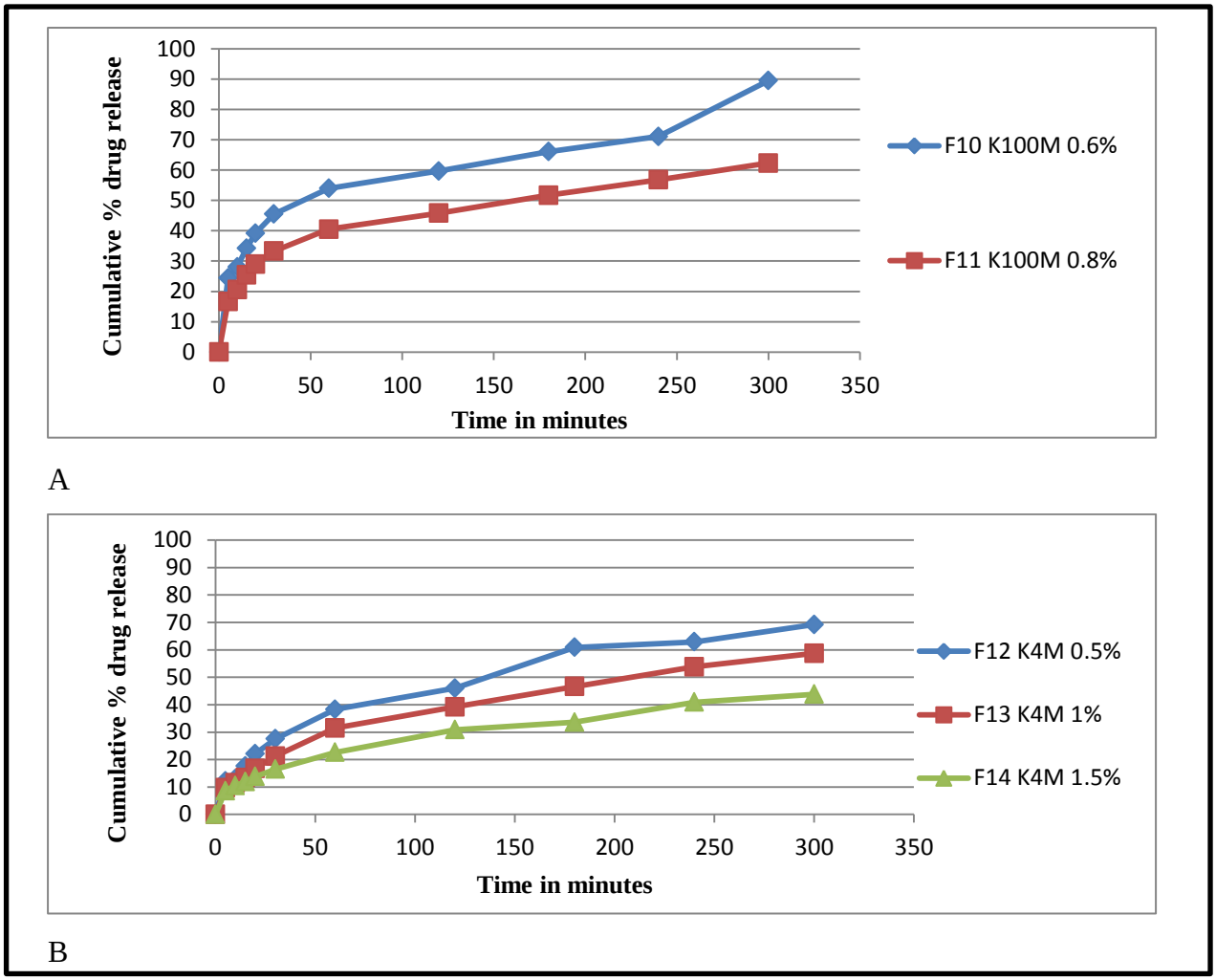


Figure 3.15: The Effect of Adding Different Concentrations of HPMC K100M (A) and K4M (B) in the Absence of $NaHCO_3$ on Release Profile of Furosemide in 0.1N HCl, at 37° C.

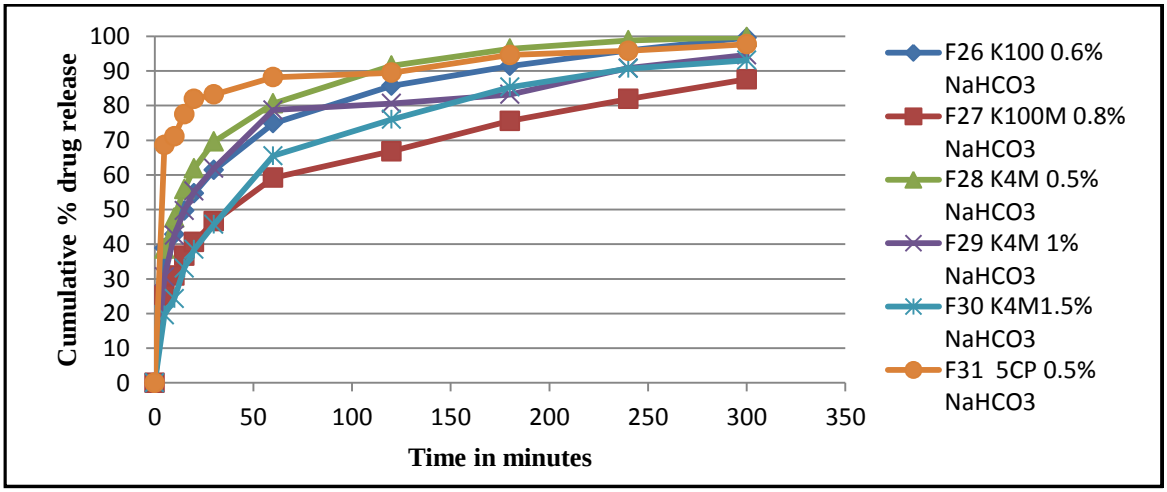


Figure 3.16: The Effect of Adding Different Concentrations of HPMC Various Grades in the Presence of $NaHCO_3$ on Release Profile of Furosemide in 0.1N HCl, at 37° C.

The prepared formulations (F20- F25) were selected to illustrate the effect of combination of iota carrageenan as secondary polymer with Na alginate and gellan gum as primary polymers in the presence of $NaHCO_3$ on release profile of furosemide. The results showed significant decrease ($p< 0.05$) in drug release as shown in Figure 3.17. The results indicating appreciable ability of iota carrageenan gels to sustain drug release due to increasing in its concentration correlating with their ability on wetting in their matrices, thus the gel matrices swell at low grade and resist erosion under the acidic conditions of the stomach maintaining constant diffusion path length forming highly crosslinked matrices with minimum porosity, this is in an agreement with observations of studying carrageenan gels for oral sustained delivery of acetaminophen [196, 164].

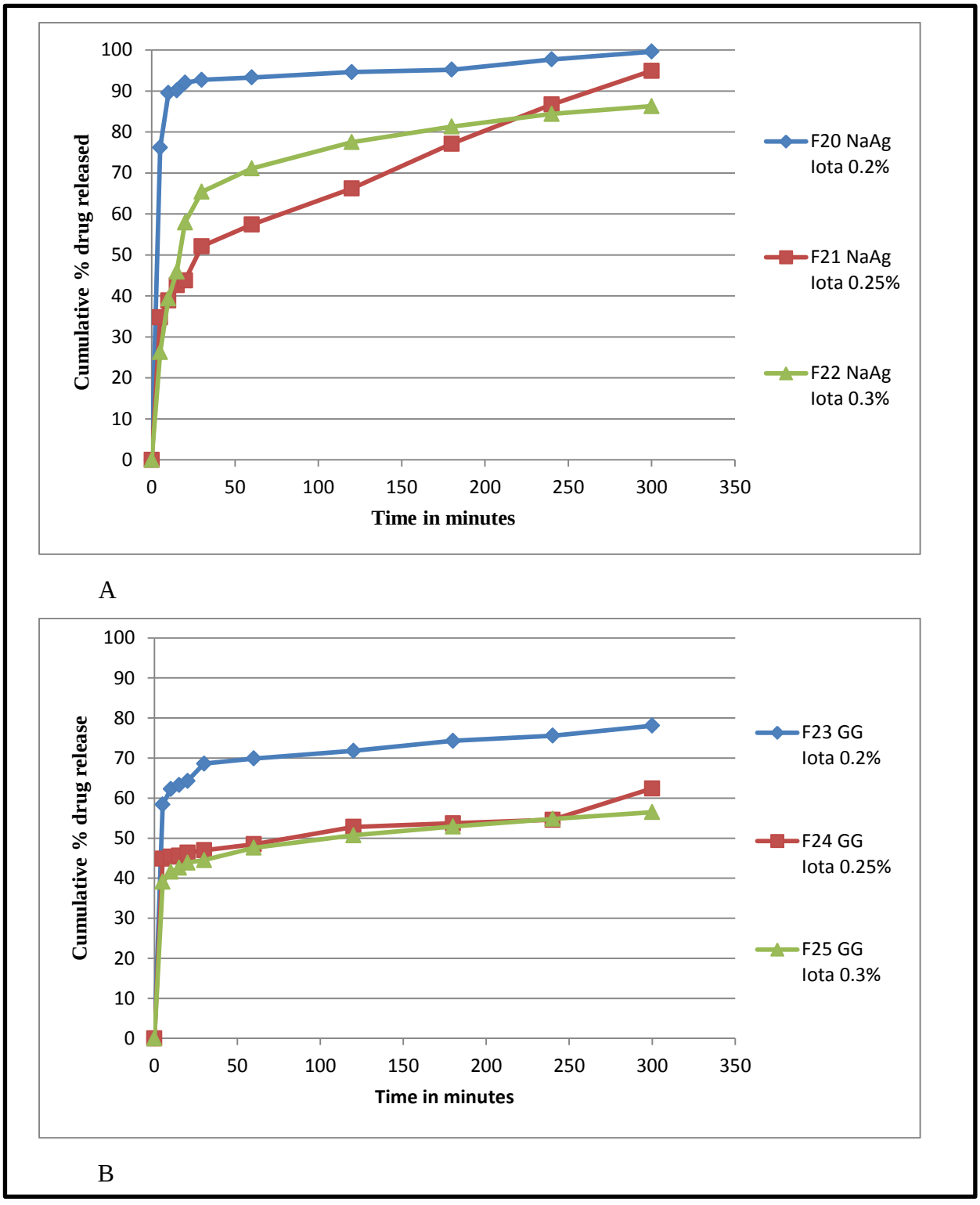


Figure 3.17: The Effect of Adding Different Concentrations of Iota Carrageenan by Using Na Alginate (A) and Gellan Gum (B) as Primary Polymers on Release Profile of Furosemide in 0.1N HCl, at 37^{o} C.

3.3.1.5 Effect of Different Drug Concentrations

Increasing the concentration of furosemide (F21, F32, F33) led to be significant ($p < 0.05$) decrease in drug release as shown in Figure 3.18. The higher loading of the drug gave more contracted gel matrix, which in turn results in longer retardation time of drug release from the formulations. That could be attributed to a more condensed carrageenan–alginate gel formed in the presence of more drug molecules interacting as strengthening additive, probably hydrogen bonding between the polymer and drug improves the morphological strength, the same results observed in natural hydrogel beads for controlled release of betamethasone [(197)].

3.3.1.6 Effect of Different Concentrations of Sweetening agent (Taste Masking Agent)

Formulations (F21, F34 & F35) illustrate the effect of different concentrations of fructose on release profile of furosemide in comparison to F21 which is without fructose. An increase in fructose concentration showed non-significant effect ($p > 0.05$) on retarding release profile of furosemide as depicted in the Figure 3.19. These results indicated that the incorporation of fructose increases the viscosity of the gel matrix, thus water penetration is reduced. Also fructose acts as a humectant to retain water in the pre-hydrated gel matrix, thus increases the duration of drug release form the formulations. This is in an agreement with in situ fast gelling formulation of ketorolac tromethamine [(181)].

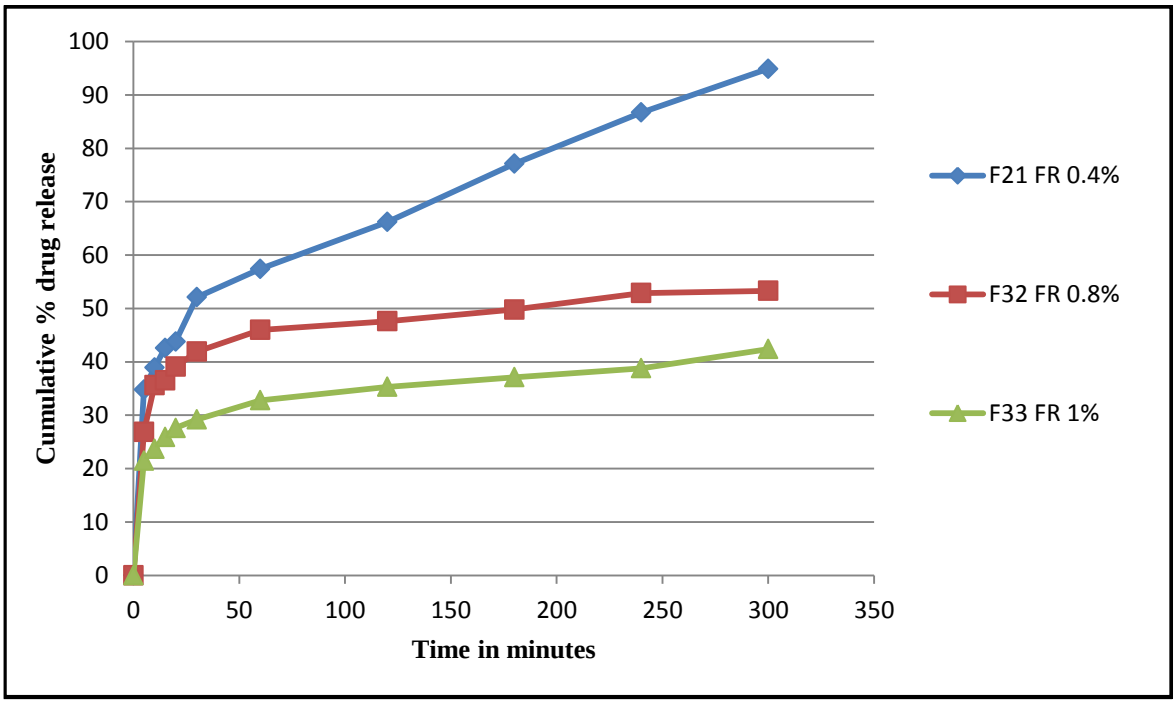


Figure 3.18: The Effect of Adding Different Drug Concentrations on Release Profile of Furosemide in 0.1N HCl, at 37° C.

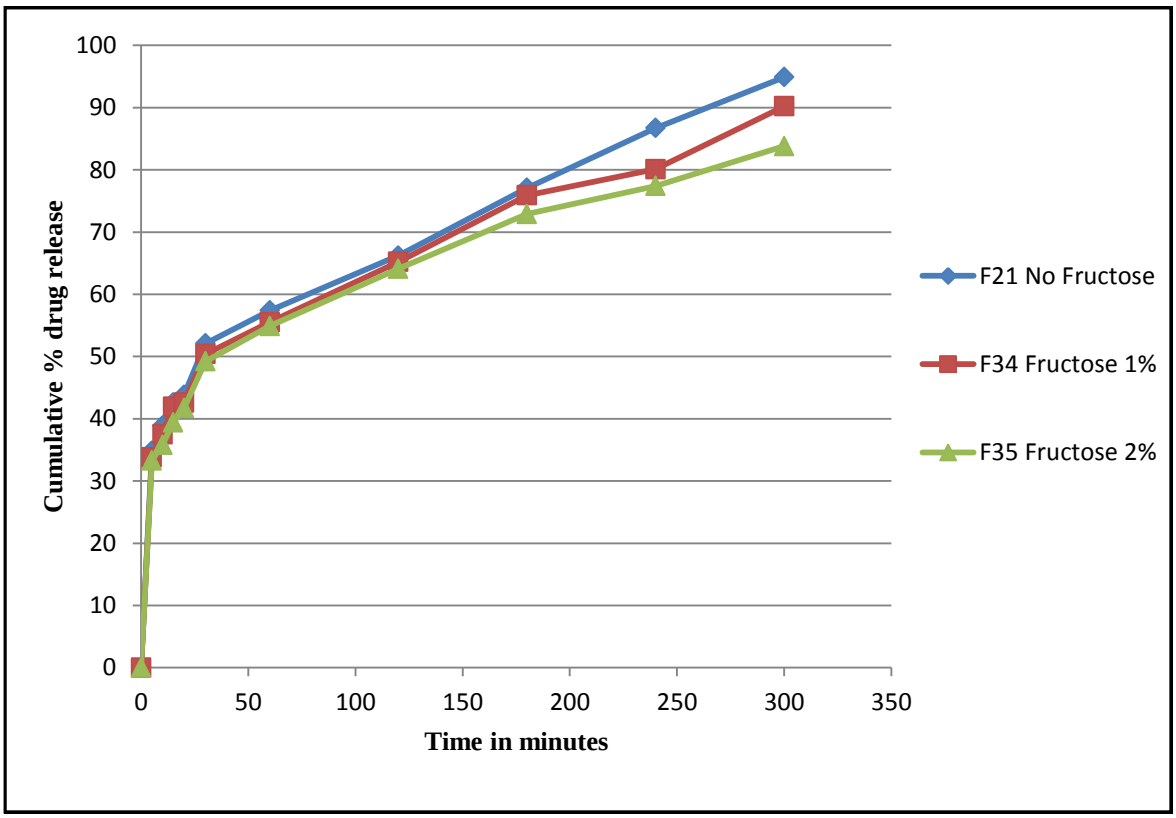


Figure 3.19: The Effect of Adding Different Fructose Concentrations on Release Profile of Furosemide in 0.1N HCl, at 37° C.

3.3.2 Kinetic Mathematical Modeling of Drug Release Profile

In-vitro release data were fitted to various mathematical models such as zero order, first order, Higuchi and Korsemeyer- Peppas model in order to understand the mechanism of drug release and the release rate from dosage forms.

Table 3.3 illustrate the correlation of dissolution data to different models of release kinetic, where best fitting to Higuchi order model was observed for most formulations, indicated by highest regression value (R^2). This result indicated that most formulations exhibit diffusion mechanism in drug release accompanied by acceptable regression value for zero order. Kinetic model which best fit zero order and Higuchi's diffusion equation were most suitable for controlled release formulation.

For Korsmyer-Pappas model, the value of release exponent (n) defines the release mechanism, the n value of all formulation found to be less than 0.5. Hence it can be concluded that drug release occurred via Fickian diffusion release; this mathematical model, also known as the Power Law, from which the rate of diffusion is much less than that of relaxation modes of the polymer-penetrant system [(146)].

Table 3.3: The Kinetic Analysis of Furosemide Formulations

Formula No.	Zero-order		First-order		Higuchi-order		Koresmeyer-peppas		
	K_O(mg h^{-1})	R^2	K_1(h^{-1})	R^2	K_H($h^{-1/2}$)	R^2	n	K_{KP}($h^{-1/3}$)	R^2
F1	0.0772	0.7703	0.0025	0.9434	1.6155	0.8851	0.0802	1.783	0.9596
F2	0.1262	0.8074	0.0019	0.9351	2.6207	0.9143	0.1655	1.5353	0.9646
F3	0.0781	0.7058	0.0006	0.8057	1.6499	0.8785	0.1604	1.369	0.9508
F4	0.0395	0.754	0.0007	0.788	0.7839	0.7789	0.0425	1.7853	0.8062
F5	0.0812	0.7593	0.001	0.8566	1.6928	0.8661	0.1146	1.5971	0.9324
F6	0.0629	0.7498	0.0006	0.8106	1.3211	0.8683	0.1081	1.5274	0.9395
F7	0.0614	0.5309	0.0006	0.6044	1.3459	0.6697	0.1094	1.5707	0.7987
F8	0.0638	0.6321	0.0006	0.6709	1.3787	0.7738	0.1203	1.5021	0.8866
F9	0.0557	0.6331	0.0005	0.6836	1.1978	0.7694	0.1137	1.4758	0.8817
F10	0.1845	0.9096	0.0023	0.8962	3.6918	0.9558	0.2908	1.1921	0.9755
F11	0.1358	0.8976	0.0011	0.95	2.8114	0.9727	0.3081	1.0338	0.9859
F12	0.1943	0.9217	0.0015	0.9691	3.9215	0.9847	0.4548	0.7336	0.9828
F13	0.1661	0.9481	0.0011	0.9775	3.3313	0.9937	0.4653	0.6248	0.9912
F14	0.1189	0.9511	0.0007	0.9715	2.3749	0.9952	0.4133	0.6142	0.9954
F15	0.091	0.736	0.0007	0.7781	1.937	0.876	0.207	1.2488	0.9563
F16	0.0606	0.8391	0.0011	0.8214	1.186	0.8424	0.0616	1.7551	0.8473
F17	0.0689	0.6501	0.0006	0.7123	0.0132	0.7279	0.1291	1.4837	0.8932
F18	0.0799	0.7377	0.0008	0.8098	0.0135	0.7785	0.1285	1.5169	0.9077
F19	0.0795	0.7097	0.0009	0.796	0.0129	0.7667	0.1238	1.55	0.9056
F20	0.0426	0.5099	0.004	0.827	0.9088	0.6088	0.0436	1.8907	0.7196
F21	0.1923	**0.9643**	0.0033	**0.9548**	3.8084	**0.9924**	0.2418	1.3483	**0.9826**
F22	0.1583	0.6767	0.0022	0.8551	3.3961	0.8173	0.2551	1.3528	0.8764
F23	0.0548	0.8326	0.0008	0.8898	1.1294	0.9273	0.0654	1.7262	0.9795
F24	0.0512	0.9478	0.0005	0.9332	0.9867	0.9223	0.0693	1.5826	0.8484
F25	0.0536	0.9168	0.0005	0.9387	1.0852	0.9852	0.0877	1.527	0.9918
F26	0.196	0.8481	0.0062	0.9361	4.0563	0.9528	0.2409	1.4188	0.9886
F27	0.1958	0.8943	0.0025	0.9831	3.9928	0.9755	0.3003	1.2074	0.9932
F28	0.1838	0.7673	0.0076	0.9853	3.8857	0.8996	0.2285	1.4696	0.9632
F29	0.1773	0.758	0.0033	0.9367	3.7323	0.8819	0.2501	1.3845	0.9365

Table 3.3: to be continued

Formula No.	Zero-order		First-order		Higuchi-order		Koresmeyer-peppas		
	K_O(mg h^{-1})	R^2	K_1(h^{-1})	R^2	K_H($h^{-1/2}$)	R^2	n	K_{KP}($h^{-1/3}$)	R^2
F30	0.2417	0.8429	0.0036	0.9806	5.0082	0.9495	0.3922	1.0478	0.9741
F31	0.083	0.7661	0.0035	0.9587	1.7338	08225	0.0842	1.7856	0.9587
F32	0.0685	0.7446	0.0005	0.7982	1.4417	0.8652	0.1441	1.385	0.9282
F33	0.0606	0.8788	0.0004	0.9027	1.2353	0.9598	0.1563	1.2278	0.991
F34	0.1783	0.9522	0.0025	0.9751	3.5531	0.9923	0.2366	1.3421	0.9873
F35	0.1651	0.9328	0.002	0.9879	3.3212	0.9907	0.2329	1.3333	0.9905

3.4 Selection of Optimum Formula

Formula (F21) was selected as optimum formula since it has a good release profile (94.6%) after 5 hr, sufficient gel strength (10.96 N/m^2) to remain in the stomach for sufficient time parallel to the time that required for dissolution study. In addition to pH value (7.7) which is within the range of furosemide solution pH as referred in USP, so there is no irritation would be expected from this formulation, in addition to acceptable properties like floating lag time (35 second), floating duration (24 hrs), gelation time (2 second), density (0.8 g/cm^3), and swelling index (19.7%). Consequently this formula was subjected to further study like in-vivo test and shelf life.

Figure 3.20 shows comparative rheological study that was done to differentiate the rheology of Fudesix ® (commercial furosemide solution) in comparison to the optimum formula (F21). The result indicated that Fudesix ® follow Newtonian behaviour thus shear stress not effecting viscosity of solution. While (F21) follows non-Newtonian or shear thinning behaviour as the viscosity change with increasing shear stress. This is due to strong crosslinking of iota carrageenan which result in strong elastic crosslinking polymeric network. Thus F21 viscosity helps to ease swallowing of the solution [176, 177].

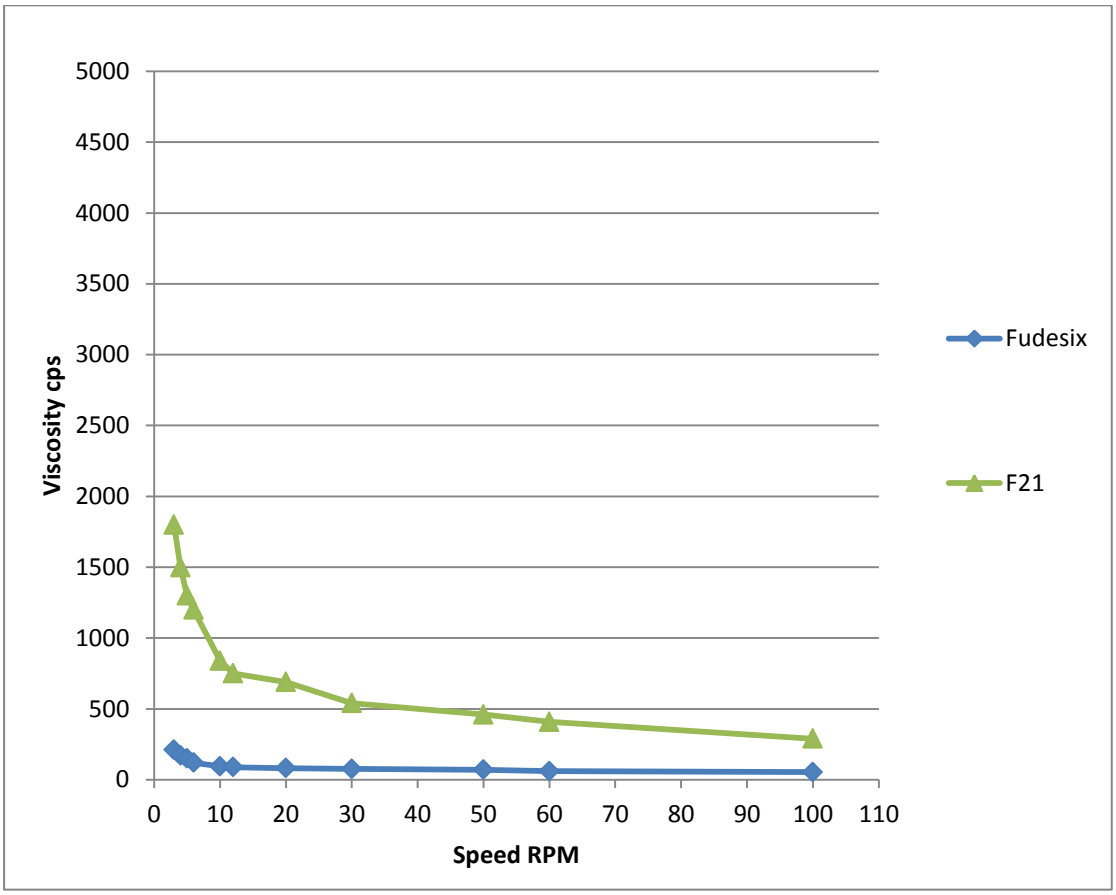


Figure 3.20: Comparative Viscosities of Fudesix ® and Optimum Formula (F21)

3.5 In-Vivo Test for the Optimum Formula

Tables (3.4-3.7) and Figures (3.21-3.23) showed that selected formula (F21) has lower excretion rate (urine volume and electrolyte concentrations) during the first hour in comparison to Fudesix; this can be related to faster onset of action of furosemide from the conventional solution. While after 5 and 24 hrs; the excretion rates of the drug from the selected formula were significantly higher than that from the conventional solution (Fudesix). That indicating the slow, continuous and prolonged mode of release of the drug from the in-situ gel preparation; that improves drug absorption from the stomach region and as a result increases its pharmacodynamic action and minimizing the counteractivity of the body after the administration due to delayed activation of the compensatory mechanisms (tolerance development)[198,199].

Moreover, the results showed dose-dependent increase in the diuretic index and saluretic index of the drug from the optimum formula which is significantly ($p < 0.05$) higher than that for the control, which indicates that the mechanism of absorption is mainly diffusion and it is agreed with the in-vitro release study and the proposed kinetic mathematical modeling [200, 155].

Table 3.4: Effect of Single Oral Furosemide Administration on Urine Volume and Electrolyte Excretion

Treatment / Group No.	Dose (mg/kg)	Urine volume (ml/24hr)	Diuretic index [a]	Na^+ (Mmol/l/24hr)	K^+ (Mmol/l/24hr)	Saluretic index [b]	
						Na^+	K^+
Control I	-	9	-	3.17	2.55	-	-
Control II	-	11.5	-	3.76	2.91	-	-
Control III	-	15	-	5.3	4.78	-	-
Control IV	-	10	-	2.93	2.63	-	-
Fudesix ® I	10	10.5	1.17	3.30	2.85	1.04	1.12
Fudesix ®II	25	12.5	1.08	4.01	3.60	1.07	1.24
Fudesix ® III	50	23	1.53	6.92	5.70	1.31	1.19
Fudesix ® IV	100	39	3.90	11.73	9.80	4.00	3.73
Optimum F21-I	10	21	2.33	6.48	5.52	2.04	2.16
Optimum F21-II	25	35	3.04	10.63	8.75	2.83	3.01
Optimum F21-III	50	73	4.86	22.81	18.25	4.30	3.82
Optimum F21-IV	100	100	10.0	30.38	25.1	10.34	9.54

[a] Diuretic index (volume treated group/ volume control group)

[b] Saluretic index (Mmol/l treated group/ Mmol/l control group)

Table 3.5: Electrolyte Excretion for Furosemide Solutions

Time / Dose (mg/kg)	Electrolyte excretion											
	Optimum formula (F21)						Fudesix ®					
	Na^+ (Mmol/l)			K+ (Mmol/l)			Na^+ (Mmol/l)			K+ (Mmol/l)		
	1 hr	5 hr	24 hr	1 hr	5 hr	24 hr	1 hr	5 hr	24 hr	1 hr	5 hr	24 hr
10	0.19	1.18	6.48	0.14	1.38	5.52	0.26	0.79	3.3	0.28	0.57	2.85
25	0.21	2.13	10.63	0.18	1.75	8.75	0.43	0.84	4.01	0.44	0.72	3.6
50	0.35	4.15	22.81	0.29	3.32	18.25	0.75	1.73	6.92	0.74	1.43	5.7
100	0.48	6.75	30.38	0.49	5.58	25.1	1.69	2.61	11.73	1.01	2.45	9.8

Table 3.6: Electrolyte Excretion for Control

Time / Group NO.	Electrolyte Excretion (control)					
	Na^+ (Mmol/l)			K+ (Mmol/l)		
	1 hr	5 hr	24 hr	1 hr	5 hr	24 hr
1	0.14	0.66	3.17	0.13	0.51	2.55
2	0.17	0.73	3.76	0.11	0.53	2.91
3	0.27	1.06	5.3	0.24	1.01	4.78
4	0.12	0.53	2.93	0.15	0.66	2.63

Table 3.7: Cumulative Urine Volume for Control and Furosemide Solutions

Dose (mg/kg) / Time	Cumulative Urine Volume (ml)											
	F21				Fudesix ®				Control			
	10	25	50	100	10	25	50	100	1	2	3	4
1	1.3	2.9	8.5	16.5	1.9	4	9.5	22	0.6	0.5	0.9	0.4
5	6.1	13.5	27.3	37.5	3.2	6.5	14	26.5	2.1	2.8	4	1.9
24	21	35	73	100	10.5	12.5	23	39	9	11.5	15	10

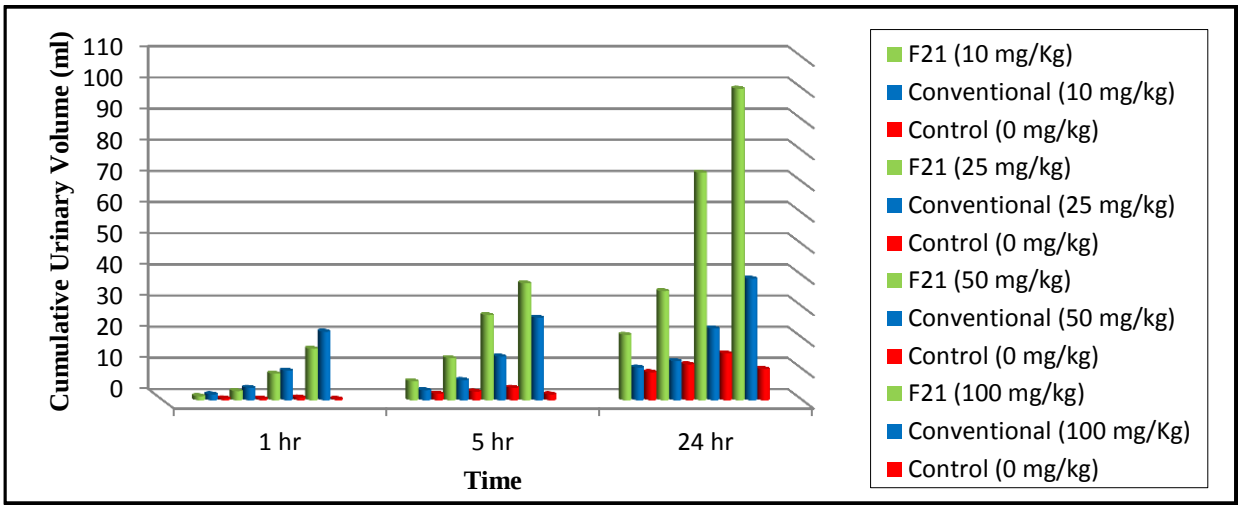


Figure 3.21: Time Course of Urinary Volume Excretion in Rats as a Comparison between Formulas F21, Conventional Solution and Control

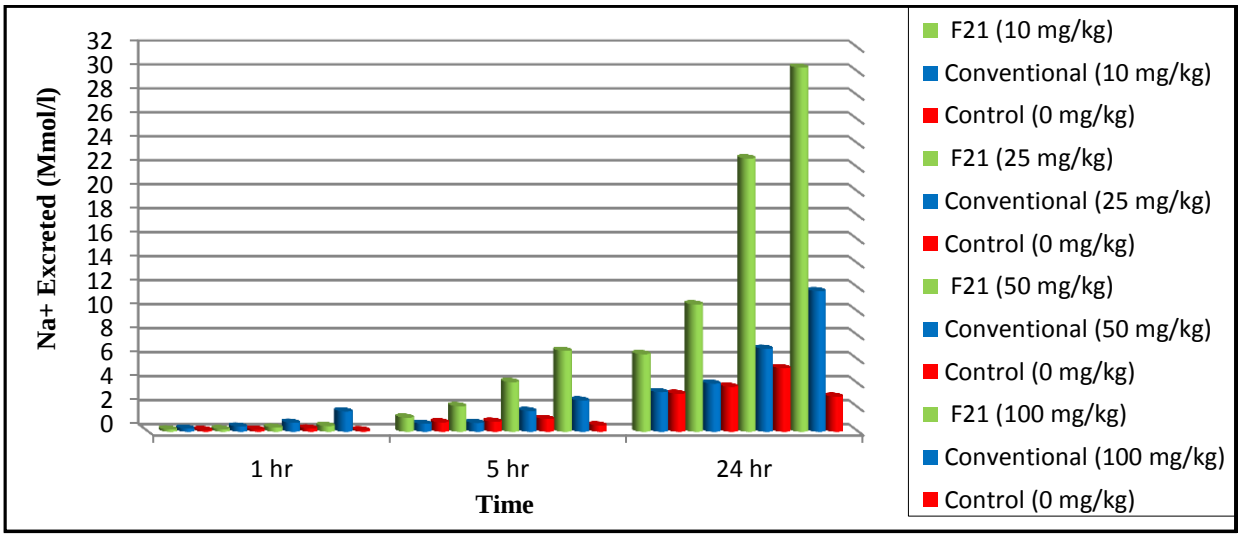


Figure 3.22: Na^+ Excretion Concentrations in Rats as a Comparison between Formulas F21, Conventional Solution and Control

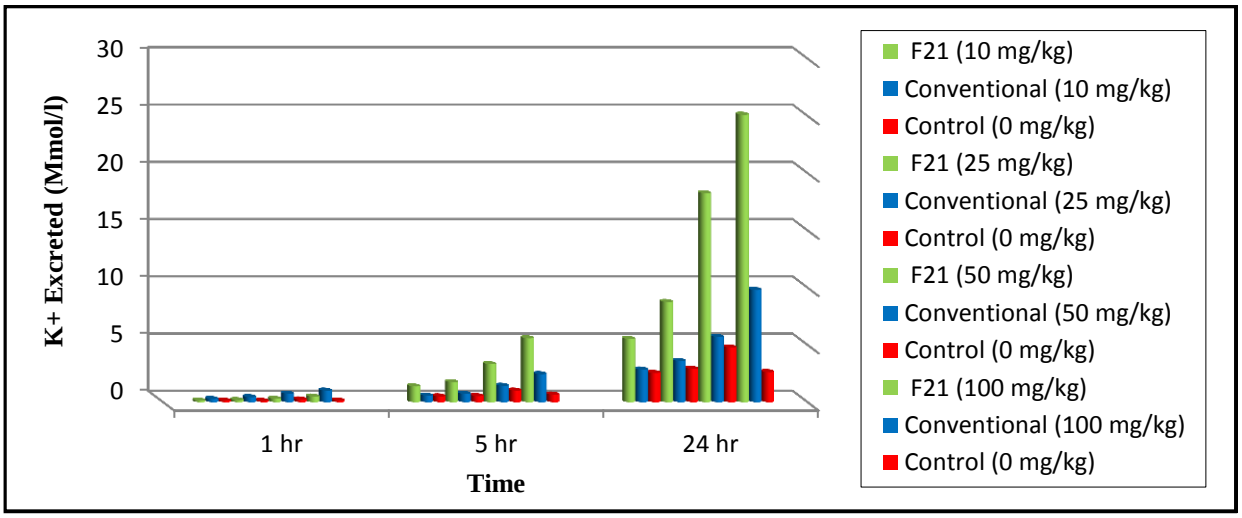


Figure 3.23: K^+ Excretion Concentrations in Rats as a Comparison between Formulas F21, Conventional Solution and Control

3.6 Drug-Excipient Compatibility Studies

The FTIR spectra for the pure furosemide powder Figure 3.24 showed characteristic absorption bands at 3399, 3351, 3284, 1674, 1595, 1566, 1325, 1260, 1150, 578 cm^{-1} which represent the following groups: stretching vibration of primary amine SO_2NH_2, stretching vibration of Ar-$NHCH_2$, asymmetric stretching vibration of the carboxyl group, C=C stretching vibration, asymmetric stretching vibration of the sulfonyl group, C-O stretching vibration interaction with in plane O-H bending and C-Cl stretching vibration [201,202].

The results in Table 3.8 showed that these bands did not change significantly in the FT-IR spectra of grinded selected formula of furosemide with excipients except the disappearance of C=O asymmetric stretching vibration of COOH near 1700 cm^{-1} and appearance of COO^- stretching vibration at 1599 cm^{-1} due to the presence of the Na^+ ion of Na benzoate as shown in Figure 3.25, and thus suggested that no interaction of the drug occurred with the excipients added.

Table 3.8: Characteristic Absorption Bands of Furosemide

Characteristic Group	Standard [201,202] cm^{-1}	Pure Furosemide cm^{-1}	Furosemide-Polymer Excipients cm^{-1}
SO_2NH_2	3398 and 3350	3399 and 3351	3398 and 3360
Ar-$NHCH_2$	3260	3284	3384
C=O	1678	1674	-
COO^-	1593	1595	1599
C=C	1560	1566	1566
S=O	1318 and 1153	1325 and 1150	1310 and 1150
C-O	1260	1260	1278
C-Cl	578	582	582

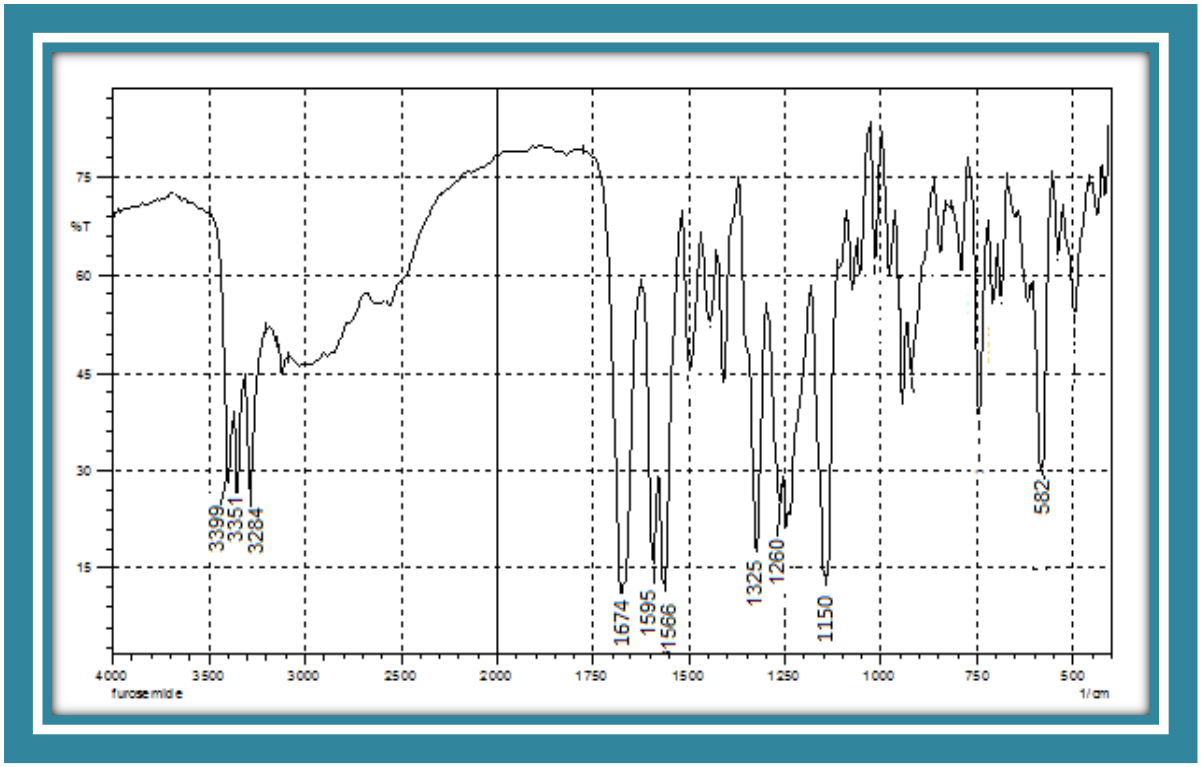


Figure 3.24: Fourier Transform Infrared Spectroscopy (FT-IR) of Pure Powder of Furosemide

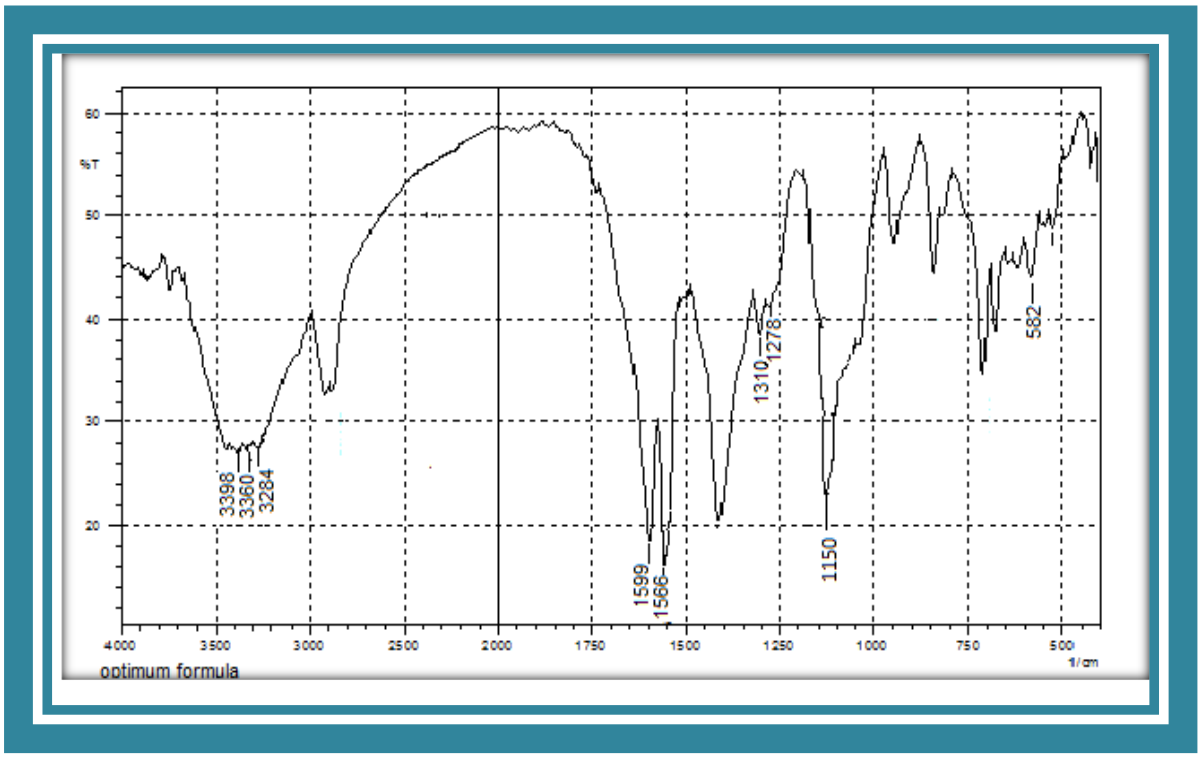


Figure 3.25: Fourier Transform Infrared Spectroscopy (FT-IR) of Optimum Formula (F21)

3.7 Stability Study

The physical stability of the selected formula (F21) was followed every 2 weeks (for 12 weeks at 25^{o} C) through measuring pH, floating lag time and floating duration, viscosity, drug content as shown in Table 3.9 and it was found that these properties did not significantly changed during the storage period.

Table 3.9: Physical Properties of the Selected Formula of Furosemide (F21) Before and After Storage at 25^{o} C

Evaluation parameter	Time period of sample (Week)						
	Initial	2	4	6	8	10	12
pH	7.5	7.5	7.5	7.45	7.45	7.4	7.4
Floating lag time (Sec)	35	35	35	32	32	31	31
Floating duration (hr)	24	24	24	24	24	24	24
Viscosity (cp) at 100 rpm	290	290	290	291	291	293	293
Drug Content (%)	99.9	99.7	99.5	99.3	99.1	98.9	98.7

Accelerated stability of the selected formula (21) was studied at three different temperatures (40, 50, and 60^{o} C) for 3 months. It was found that degradation profile follows first-order kinetics since straight line was obtained when plotting the logarithm of percent remaining versus time as shown in Figure 3.26. The degradation rate constants (K) were calculated from the slopes of the lines and shown in Table 3.10.

In order to determine the expiration date ($t_{10\%}$); Arrhenius plot was constructed to predict the degradation rate constant at 25º C (K25) as shown in Figure 3.27. The expiration date can be calculated using the following equation since the degradation of the drug follows first order kinetics [6]:

$$\mathbf{t_{10\%} = 0.105 / K_{25}} \text{ ---------- (5)}$$

Where ($t_{10\%}$) is the time required for a drug to lose 10% of its potency and it was found to be about 2.9 years or 138.4 weeks since K_{25} was equal to 7.58×10^{-4} $week^{-1}$.

Table 3.10: Degradation Rate Constants (K) of the Selected Furosemide Formula (F21) at Different Temperatures

Temperature (º C)	K ($week^{-1}$)
40	1.4×10^{-3}
50	2.1×10^{-3}
60	2.9×10^{-3}

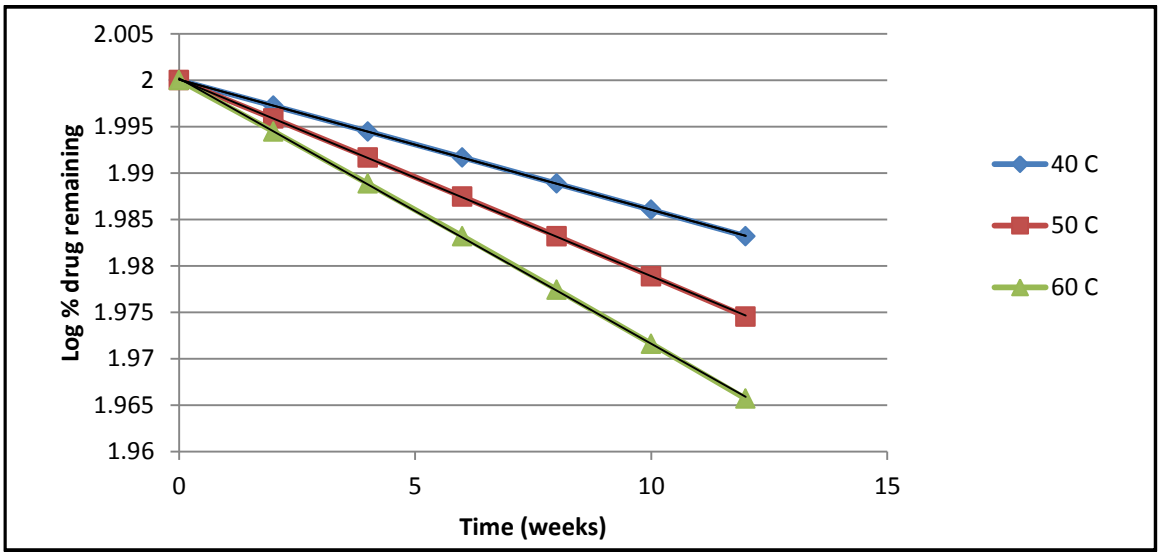


Figure 3.26: Accelerated Degradation of Furosemide in the Selected Formula (F21) at 40, 50 and 60º C

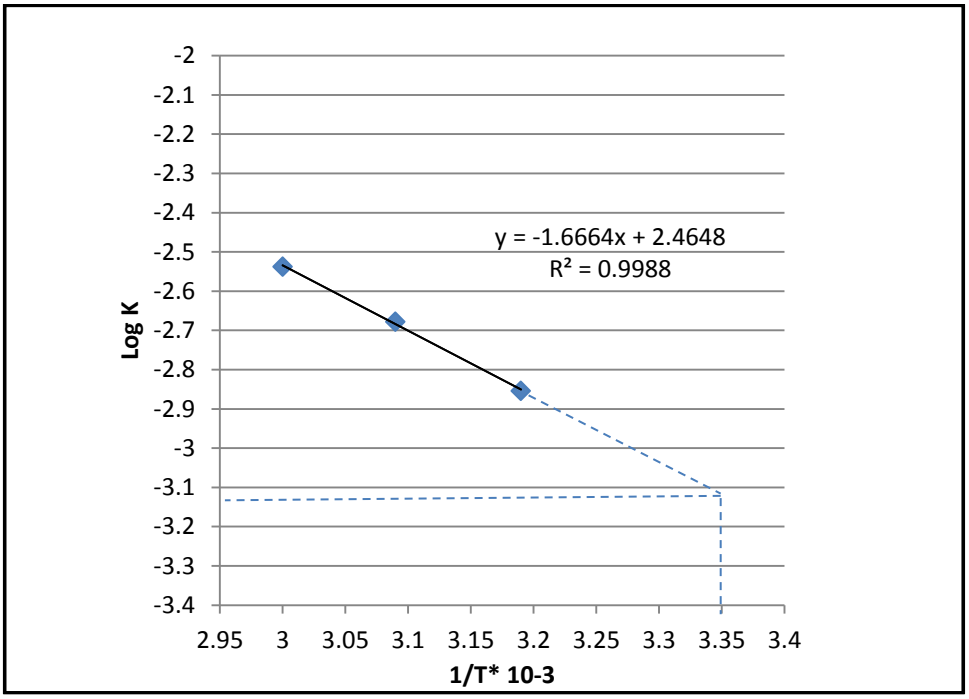


Figure 3.27: Arrhenius Plot of Furosemide in the Selected Formula for the Estimation of Expiration Date

4- Conclusions and Recommendations

Conclusions

Based on the results, the following points may be concluded:

1- Oral furosemide solution can be formulated as in-situ gel preparation by using Na alginate and iota carrageenan.

2- Viscosity of the solution increased significantly with increasing concentrations of Na alginate and iota carrageenan.

3- Gel strength increased with addition of iota carrageenan as secondary polymer.

4- Gelation time reduced significantly with increasing $CaCl_2$ concentration and it is affected by type of primary and secondary polymer.

5- Swelling index increased significantly with increasing Na alginate concentration and it is affected by type of secondary polymer.

6- Floating duration and floating lag time reduced significantly by the presence of $NaHCO_3$.

7- Significant retardation in the drug release occurs by increasing concentration of ion crosslinking agent ($CaCl_2$), in addition to increasing primary and secondary polymers concentrations.

8- Release rate increased significantly with increasing $NaHCO_3$ loaded with Na alginate while it is non-significant with gellan gum.

9- The release of furosemide retarded significantly with an increase in drug concentration.

10- The drug release retarded non-significantly when taste masking agent (fructose) was incorporated.

11- Furosemide release from the optimum formula followed Higuchi order of kinetics.

12- It was found that the best formula is (F21) that has (1% w/v) Na alginate, (0.25% w/v) iota carrageenan, (0.5% w/v) $NaHCO_3$ and (0.1% w/v) $CaCl_2$.

13- In-vivo test gave a good indication about the gastroretentive property of the selected formula in the diuretic activity of furosemide and it agreed with the in-vitro results and the proposed mathematical modeling for release kinetic.

14- The study of FT-IR showed no interaction between drug, additives and excipients of the solution.

15- Furosemide was generally stable in the selected formula and the estimated expiration date was 2.9 years at 25^{o} C.

16- We concluded that the formulation of furosemide as a floating in-situ gel can be used to increase drug absorption and patient compliance in comparison with the traditional furosemide solution in the market.

Recommendations:

1- Future studies are needed to investigate the bioavailability of furosemide in blood to confirm the effect of formulation on C max and T max of the drug.

2- Relative studies to investigate the need of reducing dose and dose frequency to minimize side effects after administration of furosemide floating gastroretentive dosage form.

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الخلاصة

ان النظام الموضعي الهلامي العائم هو واحد من الانظمة المتطورة لتقديم اشكال دوائية جديدة كي يبقى لفترات طويلة في المعدة، وهو يمثل ثورة في تطورالصناعات الدوائية التي تعطى عن طريق الفم وذلك بالسيطرة على تحرر الكميات الدوائية بالمقارنة مع الشرابات التقليدية. ويعزى سبب ذلك لكونها تطيل فترة بقاء الدواء في المواقع المعنية وهومن المقتضيات اللازمة للادوية التي لها نافذة امتصاص ضيقة في المواقع الاستيعابية مثل المعدة أو الجزء الداني من الامعاء الدقيقة، ويتحقق ذلك من خلال تقليل كثافة الهلام لكي يصبح اخف من السوائل المعدية وبالتالي سيبقى مرتفعا في المعدة دون التأثير على معدلات التفريغ حتى يساعد على تحرر الدواء ببطء وبشكل مستمر وكامل.

الفوروسيميد هو مدرر للبول وهو من ذوات السقف العالي (الذي يساعد على تخلص الجسم من الماء والاملاح بنسب كبيرة)، وهومستخدم على نطاق واسع من قبل المرضى الذين لديهم قصور القلب الاحتقاني (CHF) حيث يقوم بتخليص الجسم من المياه الزائدة، فضلا عن قابليته على خفض مستوى ضغط الدم والاورام الناتجة عن تجمع السوائل في الجسم.

وقد تضمنت هذه الدراسة تصنيع محاليل (شرابات) للفوروسيميد تعطى عن طريق الفم، والتي تتحول مباشرة الى هلام عند الاتصال مع السوائل في المعدة، وتطوف باستخدام الكثييرات الأولية مثل الصوديوم الجينيت وصمغ الجيلان بتراكيز مختلفة، فضلا عن مزج الايوتا كاراجينان وهايديدروكسي بروبيل مثيل السيليلوز (K100M وK4M) ككثييرات ثانوية وبتراكيز مختلفة.

وقد أجريت تقييمات مختلفة على جميع صيغ الهلام الموضعي ال35 وذلك لقياس قوة الهلام، ووقت تكون الهلام، ودالة الانتفاخ، المحتوى المتجانس، وتأخير وقت الطفو، والمدة التي يبقى فيها الهلام طافيا، فضلا عن دالة الحموضة والكثافة. وقد تم ايضا دراسة عدد من المتغيرات المختلفة التي تؤثر على تحرر الدواء من الهلام مثل نوع وتركيز الكثيير، دمج الكثييرات، المواد المساعدة على تحرر غاز ثنائي اوكسيد الكاربون الذي يساعد على الطوفان، المواد الرابطة المساعدة على تكوين الهلام، فضلا عن وضع تراكيز مختلفة من الدواء والمواد المحلية اللذان يعدان ايضا من المواد المؤثرة على عملية تحرر الدواء من اجل اختيارأفضل الصيغ وامثلها.

لقد وجد من ملف التعريف المستحصل لتحرر الدواء؛ أن الزيادة في تركيز الصوديوم الجينيت ككثيير اساسي ادى الى زيادة دالة الانتفاخ، قوة هلام، اللزوجة والتقليل من تحرر الدواء من الهلام، بينما اظهرت النتائج انه مع زيادة تركيز الايوتا كاراجينان ككثيير ثانوي بوجود بيكربونات الصوديوم أدى إلى الانخفاض في وقت تكون الهلام ، تأخير وقت الطفو، والكثافة وبالتالي المزيد من التأخير لتحرر الدواء. فضلا عن زيادة تركيز بيكاربونات الصوديوم الذي ادى إلى زيادة تحرر الدواء، في حين ان الزيادة في تراكيز الدواء والمادة المحلية كانتا كعاملين أديا إلى الانخفاض في تحرر الدواء.

كشفت النتائج ان الصيغة 21 التي تحتوي على (1٪) من الصوديوم الجينيت و (0.25٪) من الايوتا كاراجينان كانت من أفضل الصيغ في ما يتعلق بقوة هلام ($10.96\ N/M^2$)، ووقت تكون الهلام (2 ثانية)، وتأخير وقت الطفو(35 ثانية)، والمدة التي بقي فيها الهلام طافيا (24 ساعة)، فضلا عن دالة الحموضة (7.5)، والكثافة ($0.8\ g/cm^3$) ودالة الانتفاخ(19.7٪). التي كانت كافية لتحرر 94.9٪ من الدواء بعد 5 ساعات.

كما تم تطبيق الدراسة كأختبار في الجسم الحي (In-vivo) للصيغة المختارة (F21) ومقارنتها مع الشراب التقليدي للفيروسيمايد بأستخدام الجرذان كتطبيق حي؛ وقد أظهرت النتائج تاخرمعدل الافراز(لحجم البول وتركيزات الاملاح) خلال الساعة الاولى، بينما وجدت زيادة في معدلات الافراز بعد خمس ساعات واربع وعشرين ساعة وهذا بخلاف الذي حصل مع الشرابات التقليدية كأشارة تثبت تأثر ملف مدرر البول للفوروسيميد بخاصية بقاء الدواء في المعدة للصيغة المختارة، والتي اعطت هلاما طافيا بعد تماسها مع محتويات المعدة مما ادى الى التحسن في امتصاص الدواء والتوافر الحيوي. كما ان النتائج للاختبار في الجسم الحي كانت على توافق جيد مع دراسة وانموذج الية التحرر المختبرية.

وقد اظهر تقدير الاستقرارالمتسارع للفوروسيميد للصيغة المختارة ان تاريخ انتهاء الصلاحية يصل إلى 2.9 سنة.

واستنتج من ذلك ان تصنيع الفيروسيمايد كنظام هلامي عائم طويل الأمد في المعدة له القابلية في السيطرة على تحرر الدواء وبالتالي فأنه يؤدي الى تحسين خواص الامتصاص والتوافر الحيوي للدواء.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
الجامعة المستنصرية
كلية الصيدلة

تصييغ الفيروسيمايد كنظام معدي موضعي هلامي عائم بجرعات فموية مسيطرة التحرر

رسالة مقدمة الى فرع الصيدلانيات ولجنة الدراسات العليا في كلية الصيدلة/ الجامعة المستنصرية كجزء من متطلبات الحصول على درجة الماجستير في علوم الصيدلة (الصيدلانيات)

من قبل
أنس طارق نافع
بكلوريوس صيدلة
٢٠٠٧

بأشراف
الاستاذ المساعد الدكتور
نضال خزعل مرعي

١٤٣٦هـ ٢٠١٤م