IPN BASED SUSTAINED RELEASE HYDROGEL BEADS OF CARBOXYMETHYL ALGINATE CROSSLINKED WITH Ca^{2+}/Al^{3+} CONTAINING MET AND PCM FOR MICROBIAL INFECTIONS.

THESIS SUBMITTED IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHARMACY (PHARMACEUTICS)

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CERTIFICATE OF APPROVAL

This is to certify that Mr. RAHUL MOLLA (Class Roll No: 002211402026, and Registration No: 163668 of 2022-23) has carried out the research work on the subject entitled "**IPN based sustained release hydrogel beads of carboxymethyl alginate crosslinked with Ca2+/Al3+ containing MET and PCM for microbial infections**" independently with proper care and attention under my supervision and guidance in the Pharmaceutics Research Laboratory in the Department of Pharmaceutical Technology, Jadavpur University. He has incorporated his findings into this thesis of the same title being submitted by him, in partial fulfilment of the requirements for the degree of Master of Pharmacy from Jadavpur University. I appreciate his endeavour to complete the project and his work has reached my gratification.

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DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains a literature survey and original research work by the undersigned candidate as part of his Master of Pharmaceutical Technology studies. All information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and the results are original to this work.

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Dedicated to my Parents

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[1] A. Bala, R. Molla, S. Panja, and S. Mandal, "A Review on Applications of Biosynthesised Silver Nanoparticles A Review on Applications of Biosynthesized Silver Nanoparticles INTRODUCTION- BIOSYNTHESIS OF SILVER NANOPARTICLES-," vol. 10, no. 7, pp. 1136–1147, 2023.

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CHAPTER- 1

INTRODUCTION

1.Introduction

Drug substances i.e. active pharmaceutical ingredients are rarely administered alone, usually administered with different excipients it may include diluents, stabilizers, preservatives, thickening agents, suspending agents, emulsifiers, colouring agents, flavouring agents etc to maintain the bulkiness of the dosage formulation with required functions.

Dosage forms actually act as a vehicle to transport the various drug substances to the site of action. These dosage formulations are administered through different routes including oral, nasal, rectal, topical, vaginal and parenteral. But the oral routes have been utilized as a main route of administration for a decade. The oral gavage route is considered the preferred route for administering drugs due to its significant advantages related to others[1]. Oral administration is a far more patient-friendly route with practical as well as psychological benefits. The vast majority of pharmaceutical compounds currently available on the market that are intended for human use are orally administered. In fact, these orally administered compounds account for up to 90% of total pharmaceutical compounds intended for human use[2]. The oral route of delivery is currently the golden standard in drug manufacturing, where it is believed to be the safest, most convenient, and cost-effective drug delivery method. The advantages also include self-medication invasive side treatment, and ease of administration leading to a high level of patient compliance. Orally given dose forms have a local effect in the throat, mouth, or gastrointestinal tract, as well as systemic effects following absorption from the mouth or gastrointestinal tract. The oral dosage forms are generally divided into two major categories, depending on the dosage form's physical state i.e., solid oral dosage forms like tablets, powders, or capsules, and liquid dosage forms including solutions, emulsions, syrups, and suspension.

The development of a successful oral control release drug delivery dosage form requires an understanding of three aspects:

- The anatomic and physiologic characteristics of the gastrointestinal tract

- Physiochemical, pharmacokinetic, and pharmacodynamic characteristics of the drug and
- Dosage form characteristics
- evaluation

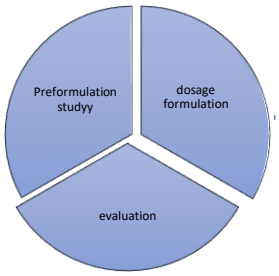


Figure 1: Conventional dosage formulation development

1.1 Historical aspect of novel drug delivery system.

Patients with chronic diseases are increasing day by day. The situation necessitates the use of drugs for a longer period and taking a lot of medicine simultaneously, which may lead to a decrease in patient compliance. This problem is serious for drugs that have a biological half-life because they must be taken more frequently. To overcome such problems, different types of novel drug delivery systems have been developed that are capable of releasing the drug gradually over a long period of time, thereby reducing the dose frequency. In this regard, scientists are trying to formulate novel drug delivery systems employing the micro-capsulation technique as one of the methods of formulation development for various dosage forms. The history of controlled release technology can be roughly divided into three time periods. From 1950 to 1970 is the period of sustained drug release. In a number of systems containing hydrophobic polymers and wax, drugs are fabricated into dosage forms with the aim of sustaining drug levels and hence drug action for an extended period of time. However, a lack of understanding of anatomical and physiological barriers-imposed impediments on the development of efficient delivery systems.

The period from 1970 to 1990 was involved in the determination of needs to control drug release and to understand the barriers to various routes of administration. Post-1990 is the modern era of control release technology and represents the period in which an attempt at drug optimization is emphasized. The drug delivery system should deliver a drug at a rate dictated by the needs of the body over a specified period of treatment. This idealized objective points to the two aspects most important to their drug delivery, namely

1. Refers to targeting a drug at a specific organ or tissue.
2. To control the rate of drug delivery to the target tissue.

1.1.1 Benefits of novel drug delivery system.

1. Convenience in dosing
2. Higher patient compliance
3. Better utilization of drugs
4. Reduced adverse effects
5. Improved efficacy

1.2 Conventional oral dosage forms

Conventional oral dosage forms refer to pharmaceutical formulations that are designed to be administered via the oral route (by mouth). These dosage forms are intended to deliver medications into the body through the gastrointestinal tract, where they can be absorbed into the bloodstream and distributed to their target sites. The conventional oral dosage form might be liquid (e.g., emulsions, solutions, and suspension), gaseous (e.g., anaesthetics), solid (e.g., tablets, capsules), and semisolid (e.g., creams, gels, and ointments).

In most cases of conventional dosage forms, the dosing interval is much shorter than the half-life of the drug, resulting in a number of limitations:

1. Unless the dosing interval is relatively short, depending on the biological half-life of the drug, large peaks and valleys in the drug level will occur.
2. Success with this approach is dependent on patient compliance with the dosing regimen. Numerous studies have documented that lack of complaint is an important reason for drug therapy's efficiency or failure.
3. During the early periods of dosing, there may be insufficient drug to generate a favorable biological response, which may be a significant problem in certain disease states.
4. For drugs with short biological half-lives, frequent dozing is needed to maintain relatively constant therapeutic levels.

There are two ways to overcome such a situation:

- Development of new, better, and safer drugs with long half-lives and large therapeutic indices.
- Effective and safer use of existing drugs through concepts and techniques of controlled and targeted drug delivery systems.

1.3 The sustained release concept.

Sustained-release dosage forms provide medication over an extended period. Control release, however, denotes that the system is able to provide some actual therapeutic control, whether of a temporal or spatial nature or both. In other words, the system attempts to control the drug concentration in the target tissue; often, this is blood serum. In general, the goal of a sustained-release dosage form is to maintain therapeutic blood or tissue levels of the drug for an extended period of time. This is generally accomplished by attempting to obtain a zero-order release from the dosage form. Sustain release systems generally do not attain this type of release; they usually try to mimic zero water release by providing drugs in a slow, first-order fashion. Controlled release, although resulting in a "zero-order" delivery system, may also incorporate methods to promote localization of the drug on the active side.

1.3.1 Sustained release dosage form.

These preparations provide the immediate dose required for the normal therapeutic response, followed by the gradual release of the drug in amounts sufficient to maintain the therapeutic response for a specific extended period of time (usually between 8 and 12 hours). The major advantage of this category is that, in addition to the convenience of reduced frequency of administration, it provides levels that are devoid of the peak and valley effect characteristic of the conventional intermittent dosage regimen.

1.3.2 Controlled release dosage form.

The purpose of the controlled release system is to deliver a constant supply of the active ingredient, usually at a zero-order rate, by continuously releasing, for a certain period of time, an amount of the drug equivalent to the amount eliminated by the body. An ideal controlled drug delivery system is one that delivers the drug at a predetermined rate, locally or systemically, for a specific period of time.

1.3.3 Delayed release dosage forms.

The drug is released at a later time after administration. The delayed action is achieved by the incorporation of a special coat, such as an enteric coating, or other time barriers, such as the formaldehyde treatment of soft and hard gelatine capsules. The purposes of such preparations are to prevent side effects related to the drug's presence in the stomach and protect the drug from degradation in the highly acidic pH of the gastric fluid.

1.3.4 Site specific dosage forms:

Advances in medical device technology, such as the development of fully implantable infusion system, now allow for site-specific drug delivery. Direct infusion of medication into the target organ or blood vessels supplying those organs goes directly to the site in the body that needs it most. Site specific drug delivery allows for higher drug accumulation at the site of action, which can often reduce the side effects, increase quality of life of the patients, and in some cases extend lives.

1.3.5 General principle of sustained release system:

The concept of a sustained release system is to deliver a constant supply of the active ingredient, usually at a zero-order rate, by continuously releasing, for a certain period of time, an amount of the drug equivalent to that eliminated by the body. The system usually delivers a very small amount of the truck at more frequent intervals.

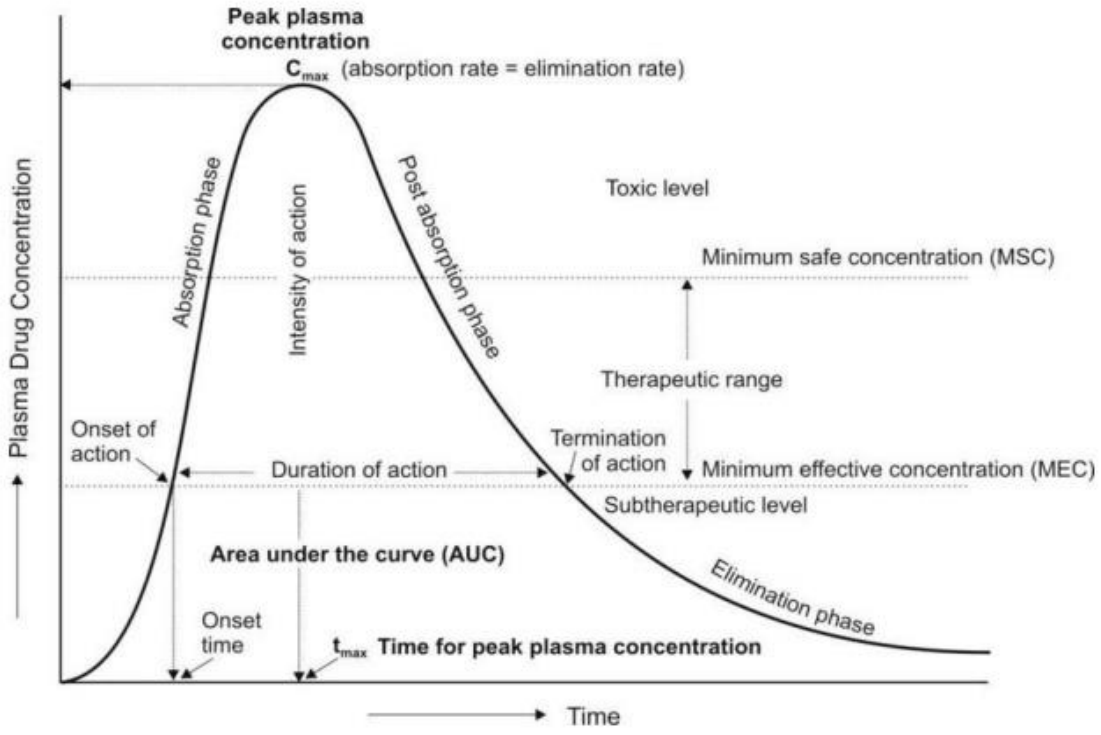


Figure 2: Plasma drug concentration profiles for conventional tablet formulations, a sustain release formulations and a zero-order controlled released formulation.

1.3.6 Objective, advantages, disadvantages of controlled/sustained drug delivery systems:

1.3.6.1 Main objective of sustain release formulation.

The formulation is designed in such a way that the minimum effective plasma concentration (MEC) level of the drug should be attained quickly, and thereafter the rate of entry of the drug into the body should equal the rate of total elimination or inactivation of the drug from the body. As a result, the plasma drug concentration curve will run parallel to the time axis, just above the MEC level. Following are examples of some of the advantages associated with sustain-release formulations:

1. The patient will get an uninterrupted therapeutic response for a prolonged period.
2. The toxicity associated with peak plasma concentration and the chances of drug resistance associated with the deep ineffective plasma drug concentration would be diminished.
3. The frequency of the drug administration is reduced; therefore, complaints are made to the patient as well as the nursing staff.
4. A much smaller amount of the drug is essential for the entire course of therapy. On the other hand, multi-dose conventional delivery systems are wasteful.

1.3.6.2 Advantages:

All controlled or sustained release products share the common goal of improving drug delivery therapy over that achieved with their non-controlled counterparts. This improvement in drug delivery is represented by several potential advantages, as below:

1. Avoid patient compliance problems.
2. Employ less total drug.
3. Minimize all local side effects.
4. Minimize or eliminate systemic side effects.
5. Obtain less potentiating or a reduction in drug activity with chronic use.
6. Minimize drug accumulation with chronic dosing.
7. Improve efficiency in treatment.
8. Cure or control the condition more promptly.
9. Improve the control of the condition and reduce fluctuations in drug levels.
10. Improve the bioavailability of some drugs.

1.3.6.3 Disadvantages:

Usually, the amount of drugs in a sustained release dosage is 3–4 times the amount, and if a dosage form is used improperly, for example, by chewing instead of swallowing, the patient would receive an overdose.

1. Improper formulations may result in excessive dosage, or the drug release may not be complete.
2. In the event of accidental failure of the product, an effective antidote may be difficult to employ.
3. Sustained-release dosage forms are sometimes more expensive because of the technology involved in producing the formulation.
4. R. medication should not be used with persons known to have impaired or erratic gastro-intestinal absorption or kidney troubles.
5. Drugs having a long biological half-life or not suitable for presentation in sustained release forms, for example, digitoxin
6. There is little control in the hands of the physician as far as dose variation is concerned.
7. It is difficult to formulate an ideal sustained-release dosage form.
8. Unpredictable or poor in vitro-in vivo correlation.
9. Increase first-pass clearance.

1.4 Gastro-retentive drug delivery system:

Gastroretentive drug delivery is an approach to prolonging gastric residence time, thereby targeting site-specific drug release in the upper gastrointestinal tract (GIT) for local or systemic effects. Gastroretentive dosage forms can remain in the gastric region for long periods and hence significantly prolong the gastric residence time (GRT) of drugs. Over the last few decades, several gastroretentive drug delivery approaches have been designed and developed, including high-density (sinking) systems that are retained in the bottom of the stomach, low-density (floating) systems that can cause buoyancy in gastric fluids, mucoadhesive systems that cause this bioadhesion to stomach mucosa, unfoldable, extendable, or swellable systems that limit emptying of the dosage forms through the pyloric sphincter of the stomach, superporous hydrogel systems, magnetic systems, etc. The current review deals with various gastroretentive approaches that have recently become leading methodologies in the field of site-specific orally administered controlled release drug delivery systems.

1.4.1 Advantages of Gastro-retentive Drug Delivery Systems

1. Enhanced bioavailability:

The bioavailability of riboflavin CR-GRDF is much higher than that of non-GRDF CR polymeric formulations. There are various processes associated to medication absorption and transit in the gastrointestinal system that function in concert to determine the magnitude of drug absorption.

2.**Enhanced first-pass biotransformation:**

The pre-systemic metabolism of the tested compound may be significantly increased when the drug is presented to the metabolic enzymes (cytochrome P450, specifically CYP3A4) in a sustained way as opposed to by a bolus input, similar to the increased efficacy of active transporters with capacity limited activity.

3.Sustained drug delivery/reduced frequency of dosing:

Sustained and slow input from CR-GRDF may result in flipflop pharmacokinetics and permit lower dosing frequency for medicines with relatively short biological half-life. This trait is linked to increased patient compliance, which enhances therapy.

4.Targeted therapy for local ailments in the upper GIT:

Prolonged and sustained medication administration from GRDF to the stomach may be useful for local therapy in the stomach and small intestine. Therapeutic medication concentrations can be achieved locally while systemic concentrations are modest as a result of drug absorption and distribution.

5.Reduced fluctuations of drug concentration:

In comparison to instant release dosage forms, continuous drug input after CRGRDF treatment results in blood drug concentrations within a tighter range. As a result, oscillations in pharmacological effects are reduced, and concentration-dependent adverse effects associated with peak concentrations can be avoided. This property is especially important for medications with a limited therapeutic index.

6.Minimization of fluctuations in drug concentration:

It enables the evoked pharmacological impact of medicines that activate different types of receptors at varying doses to be more selective.

7.Reduced counter-activity of the body:

In many circumstances, the pharmaceutical response that interferes with the body's natural physiologic processes causes a rebound activity that reduces drug activity. Slow drug delivery into the body has been proven to reduce counter-activity, resulting in improved drug efficiency.

8. **Extended time over critical (effective) concentration:**

For certain medications having non-concentration dependent pharmacodynamics, such as etalactam antibiotics, the clinical response is connected with the period of time over a key therapeutic concentration rather than the peak concentration. The sustained mode of administration allows for the prolonging of time above a critical concentration, which increases pharmacological effects and therapeutic results.

9.**Minimized adverse activity at the colon:**

The drug's retention in the GRDF in the stomach reduces the amount of drug that reaches the colon. As a result, unwanted pharmacological actions in the colon may be avoided. The reason for GRDF formulation of beta-lactam antibiotics, which are only absorbed from the small intestine and whose presence in the colon results in the development of microbial resistance, is provided by this pharmacodynamic feature.

10.Site specific drug delivery:

A floating dose form is a viable option, particularly for medicines with limited absorption sites in the upper small intestine. Controlled, gradual drug administration to the stomach gives adequate local therapeutic levels while limiting systemic exposure to the drug. This lowers the adverse effects of the medication in the bloodstream. Furthermore, the increased gastrointestinal availability provided by a site-directed administration device may minimize dose frequency.

1.4.2. Disadvantages of Gastro-retentive Drug Delivery System:

Unsuitable for drugs with solubility or stability issues in the GI tract.

Drugs that irritate the stomach mucosa are likewise not appropriate.

The high turnover rate of gastric mucus poses significant challenges for bio adhesive systems, and irritants to the gastric mucosa are likewise unsuitable.

Drugs that absorb selectively in the colon, such as corticosteroids. To float and perform efficiently, floating medicine delivery systems require a high fluid level in the stomach.

Unsuitable for medications that require an unstable, acidic environment, Erythromycin, for example.

1.4.3 Basic gastrointestinal tract physiology:

The stomach is a hollow organ that is part of the gastrointestinal system, and it is responsible for functions including the formation of chyme, synthesis of proteins necessary for vitamin absorption, microbial defences, and propagates the peristaltic reflex[3]. The peristalsis of the stomach mixes and grinds food with the secretions of the stomach, turning food into simplified liquid form. The liquefied bulk is transported to the small intestine for further digestion. Gastric acid secretion, peristaltic propulsion, and other physiologic functions of the stomach are finely controlled by the integration of the enteric nervous system, parasympathetic nervous system, and the secretion of various neurohormonal molecules (i.e., gastrin, HCl acid, intrinsic factor, bicarbonate, mucus, etc.). The human anatomy categorizes the stomach into three main parts: fundus, body, and pylorus (antrum). The proximal portion is referred to as the fundus, and the body functions as storage for undigested food. The centrum provides the main site for mixing motions and acts as a gastric emptying pump through propeller actions[4].

Both the fasting and feeding states cause gastric emptying. However, the two states vary in their patterns of motility. This phenomenal series of electric events takes place in cycles via the stomach and intestine every 2 to 3 hours. There is a phenomenon called the inter-digestive myloelectric cycle, or migrating myloelectric cycle (MMC), which is divided into four phases as given by Wilson and Washington. The four phases are enumerated below and also shown

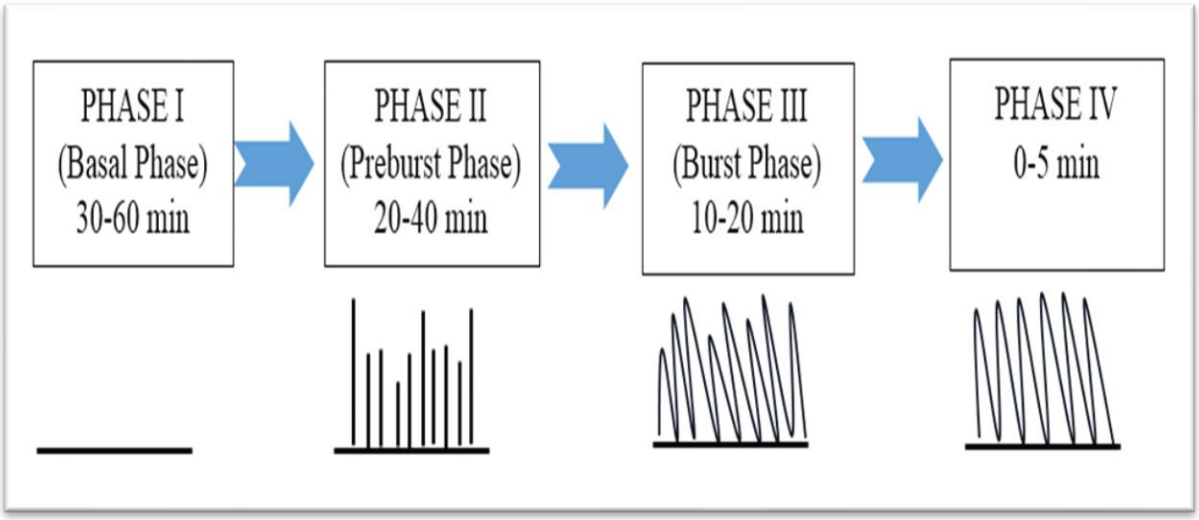


Figure 3:Motility pattern of GIT

Phase I: The basal phase lasts for 30 to 60 minutes with rear contraction and is characterized by a lack of secretory, electrical, and contractile activity.

Phase II: The pre-burst phase lasts for 20 to 40 minutes with intermittent contractions, during which contractile motions increase in frequency and size.

Phase III: The burst face lasts for 10 to 20 minutes with intense and regular contractions for a short period, termed housekeeper waves that sweep of undigested food.

Phase IV: this phase lasts for 0-5 minutes and it is the transition period between phase III and phase I.

Upon food being ingested, the stomach moves very fast to the fed state. It's termed the digestive motility pattern and constitutes regular peristalsis as in phase II of the state of fasting. This incredibly reduced food (sized to less than 1mm) propels food towards the pylorus. The gastric emptying rate is delayed during the fed-state onset of MMC, causing a slowdown in the gastric emptying rate.

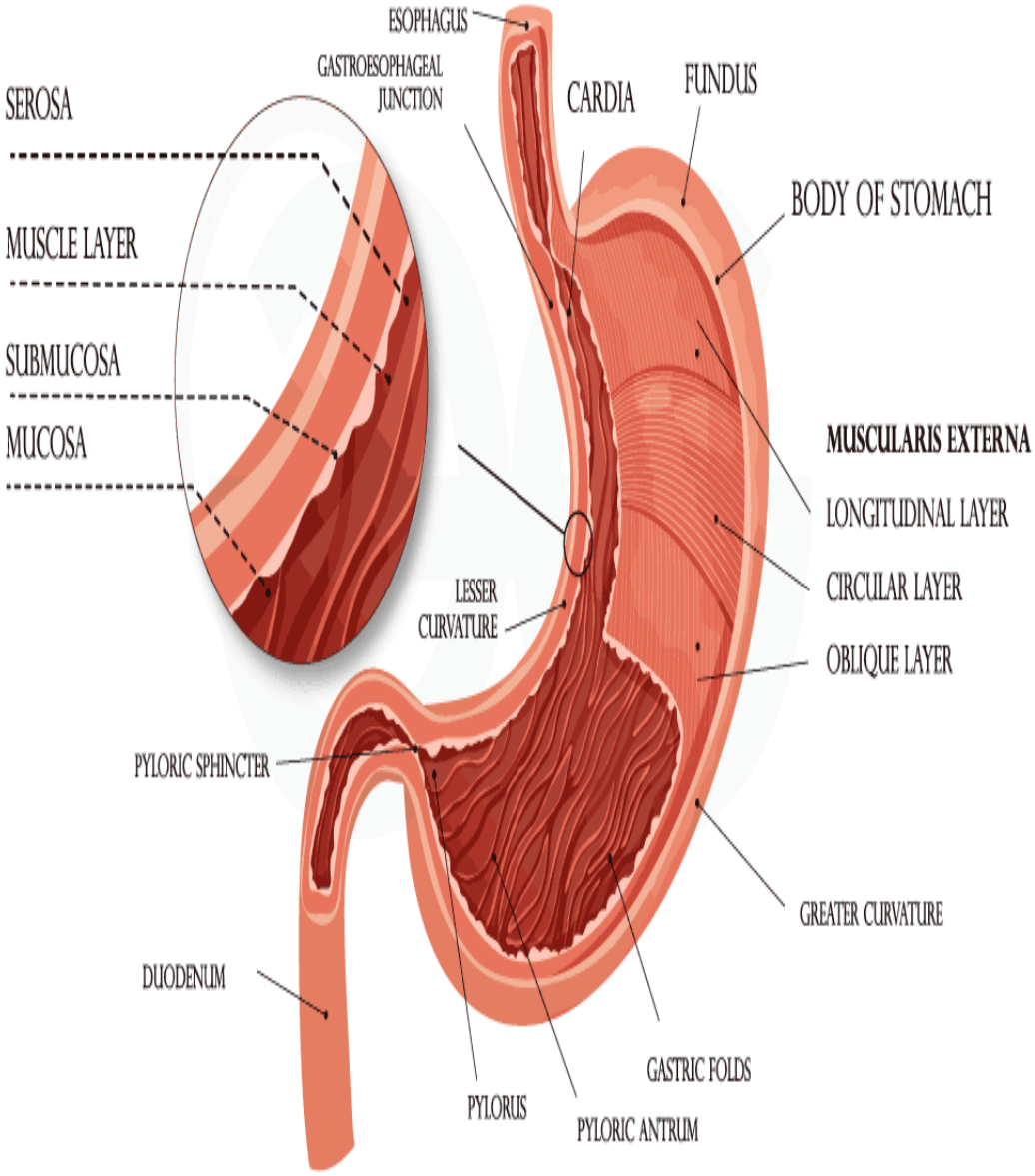


Figure 4: Structure of stomach

1.4.4 Classification of gastro retentive drug delivery system:

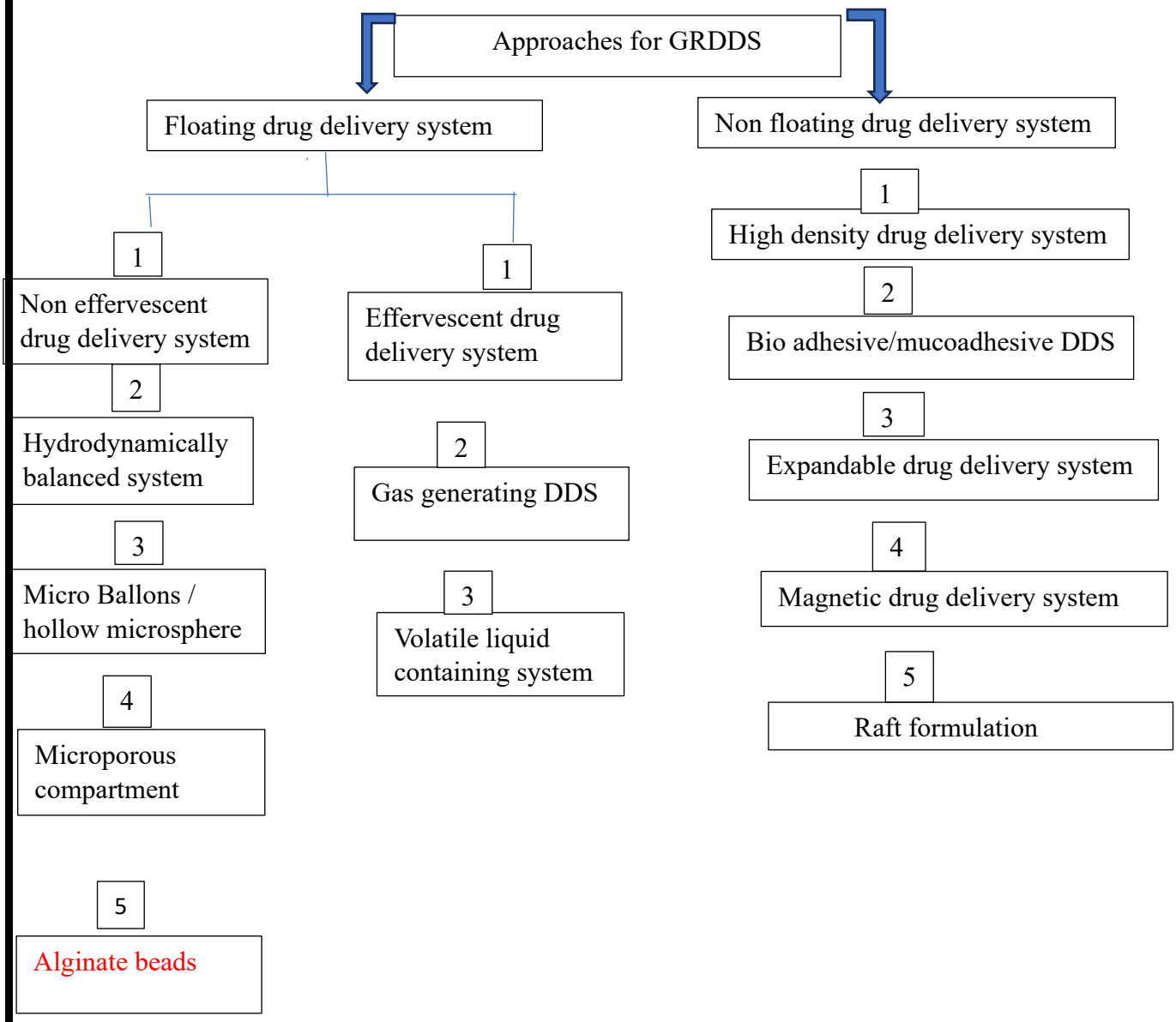


Figure 5: Approach for GRDDS

1.4.5 Non floating drug delivery system:

1.4.5.1 High density (sinking) drug delivery system:

The formulation's density exceeds the density of normal stomach content. The materials boost density to 1.5-2.4 gm/cm³. The GI transit time of pellets can be extended from 5.8 to 24 hours

depending on density. However, the efficiency of this method in humans has not been demonstrated, and no formulation has been commercialized.

1.4.5.2 Bio adhesive or mucoadhesive drug delivery system:

The gastric retention time has extended by adhering the bio adhesive system for gastric mucous membrane. The delivery system's adhesion to the stomach wall prolongs residence time, enhancing bioavailability. Chemicals used for mucoadhesion include gliadin, Carbopol, lecithin, chitosan, polycarbophil, and carboxymethyl cellulose. Novel adhesive materials produced from bacteria fimbrae or synthetic counterparts have also been tested for adhesion to the gut. However, the gastric mucoadhesive force is insufficient to resist the propulsion force of the stomach wall. Another disadvantage of this sort of system is the continual production of mucus and dilution of the gastric content. Many researchers have experimented with a synergistic method involving floating and a bioadhesion system.

A. Magnetic microsphere:

This type of drug delivery system is very important because it helps to localize the drug at the desired site of action. To treat ailments, a focused pharmaceutical delivery system is necessary. With magnetic targeting, more widely available medications can be substituted with less medical intervention. Magnetic carriers allow the materials used to make magnetic microspheres to react magnetically to a magnetic field. Magnetic microspheres are made by combining medications that dissolve in water (lipophilic medications also need dispersion agents) with 10 nm magnetite (Fe_3O_4) particles in an aqueous solvent of the matrix material[5]. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres, such as chitosan, alginate, dextran, etc., and the different types are Therapeutic magnetic microspheres are used to deliver chemotherapeutic agents to liver tumours. Drugs like proteins and peptides can also be targeted through this system[6].

B. Expandable System:

These systems have the ability to enlarge and stay in the stomach for prolonged periods of time. These are frequently presented in the form of capsules that include a folded and compressed dosing form. The dosage form expands and the capsule shell breaks down in the stomach environment, making it unable to pass through. Drug distribution that is maintained and under control can be accomplished by employing the right polymer.

C. Raft forming systems:

To achieve sustained drug delivery, these systems are built with gel-forming polymers and effervescent excipients. Because they provide a barrier between the oesophagus and the stomach, these systems are good at achieving a localised impact. As a result, the device can be used to treat peptic ulcers and gastroesophageal reflux disease. When these systems come into touch with stomach fluid, they swell and create a viscous cohesive gel, resulting in the formation of a continuous layer known as a raft. The antacid raft forming method, which uses sodium alginate as a gel forming polymer, sodium bicarbonate, and acid neutralizer as gas generating agents, was also recently created. The raft floats on the gastric fluid due to CO2 production, which reduces the system's bulk density. The raft can float on the gastric fluid for several hours and release the medicine continuously. These rafts are particularly useful for delivering antacid medications. Because of their low mechanical strength, these systems are exposed to MMC.

1.4.6 Floating Drug Delivery System:

1.4.6.1 Mechanism of floating drug delivery system:

Floating drug delivery systems (FDDS) float in the stomach for a long time without slowing down the gastric emptying rate since they have a lower bulk density than gastric fluids. As depicted in Figure, the medication is gradually released from the body at the required pace while the system is floating on the contents of the stomach. To maintain the dose form consistently buoyant on the surface of the meal, however, a minimal amount of floating force (F) is also necessary in addition to the minimal stomach content needed to achieve the buoyancy retention principle. The literature has established a specific method for calculating resultant weight to evaluate the dynamics of the floating force. The equipment works by continually measuring the force equivalent to F (as a function of time) required to keep the submerged object submerged. If F is on the positive side, the object floats better, as seen in Figure No. 5. This equipment helps to optimise FDDS in terms of the stability and lifespan of the created floating forces, avoiding the negative effects of unanticipated intragastric buoyancy capacity changes[7].

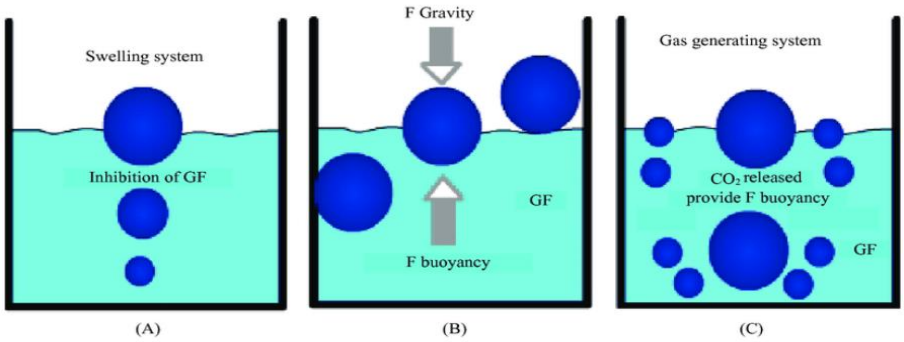


Figure6: Mechanism of floating drug delivery system

1.4.6.2 Effervescent system:

A. Gas generating System:

The main process at work in this system is the formation of CO2 gas as a result of the reaction of sodium bicarbonate, citric acid, and tartaric acid. The gas created reduces the density of the system, causing it to float on the stomach juices. Salts and citric/tartaric acid emit CO2, which becomes trapped in the system's jellified hydrocolloid layer, lowering its specific gravity and causing it to float over the chime. The system is made up of a sustain release pill as the seed, which is surrounded by a double layer. The inner layer is a bubbly layer comprising sodium bicarbonate and tartaric acid. The outer layer is a PVA shellac-containing swellable membrane layer.

B. Volatile liquid containing system:

These have an inflatable chamber that holds a liquid, such as ether or cyclopentane, which gasifies at body temperature and causes the chamber in the stomach to inflate. These systems osmotically regulate a floating system with a specified hollow unit. The system has two chambers, the first containing the medicine and the second containing the volatile system.

1.4.6.3 Non- effervescent system

A. Hydrodynamically balanced system:

It is a medication formulation containing gel-forming hydrocolloids designed to remain buoyant in the stomach contents. Because drug delivery systems have a lower bulk density than gastric fluids, they can float in the stomach for an extended period of time without altering the gastric emptying rate. The medicine is gently released from the system while the system is

floating on the gastric contents at the desired rate. Following the system's slow release at the desired rate. The residual system is emptied from the stomach once the medication is released. As a result, GRT increases and variations in plasma medication concentrations are better controlled.

B. Micro balloons:

Micro balloons (Hollow microspheres) are, strictly speaking, empty spherical particles with no core. These microspheres are often free-flowing powders made of proteins or synthetic polymers, with a size of fewer than 200 micrometres. To construct a hollow inner core in micro balloons loaded with medicine in their outer polymer shell, innovative technologies such as solvent evaporation are used. The medication and an enteric acrylic polymer mixture are dissolved in an ethanol/dichloromethane solution, which is then put into an agitated solution of Poly Vinyl Alcohol (PVA) that is thermally regulated at 40℃. After the emulsion has solidified into a stable state, the organic solvent is removed from the mixture by raising the temperature under pressure or by constant stirring. Dichloromethane evaporates in the droplet of dispersed polymer to form the gas phase inside the hollow interior cavity of the polymer microsphere.

C. Microporous compartment:

The drug reservoir is contained inside a microporous compartment with pores along its top and bottom walls in this arrangement. The delivery system floats over the gastric fluid, which enters through the aperture, dissolves the medicine, and transports the dissolved drug to the stomach and proximal part of the small intestine for absorption.

D. Alginate beads:

Calcium alginates that have been freeze dried have been used to create multi-unit floating dosage forms. Spherical beads of about 2.5 mm diameter can be made by dropping sodium alginate solution into an aqueous solution containing calcium chloride. These beads are separated and dried by air. As a result, an aporous system forms, which remains buoyant in the stomach.

Figure7: Alginate beads

1.5 Mucoadhesive Microsphere:

Since mucosal locations are easily accessible and have minimal enzymatic activity, they make an appealing non-invasive alternative for quick, regulated drug administration for both local and systemic application. To achieve the best possible therapeutic result, it is crucial to choose the right therapeutic agent, polymer, and drug carrier based on the pathophysiological state of the mucosa. A molecular weight of less than 400 to 500D, aqueous solubility of 1 mg/ml, a logP value in the range of 1 to 2, and a daily dose not to exceed 10 mg are the characteristics of an ideal medication candidate. The chemical makeup, surface tension, charge on the surface, molecular weight, rate of hydration, and concentration of polymers are important factors that influence how long drug delivery systems stay at the application site[8]. Mucoadhesive microspheres can be tailored to adhere to mucosal tissue, including those found in the eye, nasal cavity, urinary tract, and gastrointestinal tract, thus offering the possibility of localised as well as systemic controlled release of the drug[9].

1.5.1 Method of microsphere preparation:

- Coacervation method
- Spray drying method
- Emulsion solvent diffusion technique
- Multiple emulsion method
- Ionic gelation method
- Hydroxyl appetite (HAP) microspheres in sphere morphology

- Emulsion solvent evaporation technique
- Emulsion cross linking method[10]

1.5.1.1 Ionotropic gelation method:

Ionotropic gelation is based on the ability of polyelectrolytes to cross link in the presence of counter ions to form hydrogels. Since, the use of alginates, gellan gum, chitosan, and carboxymethyl cellulose for the encapsulation of drug and even cells, ionotropic gelation technique has been widely used for this purpose. The natural polyelectrolytes in spite, having a property of coating on the drug core and acts as release rate retardants contains certain anions on their chemical structure. These anions forms meshwork structure by combining with the polyvalent cations and induce gelation by binding mainly to the anion blocks. The hydrogel beads are produced by dropping a drug-loaded polymeric solution into the aqueous solution of polyvalent cations[11]. The cations diffuse into the drug-loaded polymeric drops, forming a three-dimensional lattice of ionically crossed linked moiety.

Figure 8: Preparation of calcium-alginate crosslinked beads by ionotropic gelation method

1.5.1.2. Factors affecting ionotropic gelation method:

1.Polymer and crosslinking electrolyte concentration:

The concentration of the polymer and electrolyte plays a significant role in the ionotropic gelation methods for the formulation of beads. Both should be concentrated in a ratio determined by the number of crosslinking units. The type and concentration of electrolytes have an impact on the percentage of entrapment efficiency. Different types polymers like cationic and anionic polymers have been employed in formation of hydrogel system. The stability and mechanicals strength of the hydrogel systems depend on the concentration of the polymers and nature of polymers. Another important parameter affect strength and stability of hydrogel beads is gelling agents. Ionotropic gelation is a method based on a reaction between a polymer solution and gelling agent. As a result, it is possible to compound semi-solid gel which has optimal physicochemical and pharmacological parameters[12]. Both the various monovalent, divalent, or trivalent cations, as well as the concentration of the chosen agent, have an impact on the gelation rate degree of derivatization, drug encapsulation efficiency , drug loading, beads size , circularity, mucoadhesive properties , ability to swell in aqueous environments[13]. The choice of the ion affects not only the gelation rate, as shown by Kulkarni et al., who also confirmed the influence on the beads stability. Crosslinking of sodium alginate is more effective for multivalent cations—calcium, magnesium, and zinc ions give more stable beads than monovalent sodium and potassium ions[14].

2.Temperature:

Temperature plays a crucial role in ionotropic gelation during the formation of alginate beads by affecting viscosity, ion diffusion rates, gel strength, reaction kinetics, solubility, and the stability of encapsulated materials. Careful optimization of temperature is essential to achieve desired bead characteristics for specific applications. Higher temperature decreases the viscosity of polymer solution which affect the droplet formation. And the higher temperature increases the diffusion rate of calcium which leads to faster gelation even sometimes extreme temperature causes premature gelation or uneven crosslinking and results in the formation of fragile hydrogel beads[15].

3.pH of crosslinking solution:

The crosslinking solution's pH is a significant consideration during formulation since it has an impact on the reaction rate, bead shape, and size.

4.Drug concentration:

Drug concentration has a major influence on the effectiveness of drug entrapment, so the ratio of drug to polymer in the beads must be correct. If the ratio is outside of the acceptable range, gelisphere density may increase along with their size and shape.

1.5.2 Advantages of mucoadhesive microsphere system:

Advantages of Multi particulate Drug Delivery system

- Enhanced absorption capacity.
- Extension of patent protection
- Adaptable
- Lower chance of irritation locally
- No chance of dosage dumping.
- Expected reproducibility and brief duration of stomach occupancy.
- Combining pellets with various compositions or release patterns is simple.
- Boost equilibrium.
- Boost patient compliance and comfort.
- Less variation both within and between subjects[16]

1.6 Alginate hydrogel beads as potential candidate in drug delivery system:

Several efforts have been done to achieve targeted delivery of drugs using various delivery approaches such as nanoparticles, liposomes, ethosomes, gugglosomes, nanocomposites, etc. for effective treatment of diseases. Likewise, hydrogels have gained considerable attention as an excellent carrier in targetable devices of therapeutic agents, bioadhesive devices, and controlled release devices. The unique characteristics of hydrogels such as the ability to retain water, biocompatibility, controllable swelling behaviour, etc. Have enhanced their utilization, particularly in drug delivery systems. They can be easily moulded due to their highly porous structure by controlling the density of crosslinkers in the gel matrix. Also, their porous nature facilitates drug loading into the gel matrix and ultimately releases the drug in a rate-dependent manner based on the diffusion coefficient of macromolecules or small molecules through the gel network. The drug delivers through hydrogel formulation slowly elute, maintains high drug

concentration in target and surrounding tissue for a substantial period duration and thus it can be used.

A. **Hydrogel based drug delivery via the oral route:**

Oral drug delivery is useful for a wide range of therapeutic agents for the treatment of both local as well as systemic diseases. However, many drugs cannot easily deliver through this method such as small molecular drugs. For instance, peptide and protein drugs suffer acidic denaturation, being subjected to enzymatic degradation, poor stability, less solubility, and absorption in the gastrointestinal tract (GIT)[17] . Hydrogels can be the alternative for smart oral delivery of small, hydrophobic, and macromolecular agents with molecular weights nearly 400Da to 30 KDa[18]. They help achieve site-specific delivery of therapeutic molecules by protecting them through the complex environment of GIT. Drugs particularly proteins and peptides when delivered through conventional methods faces barriers including (a) poor permeability when absorbed through the epithelial membrane into the bloodstream and (b) inactivation due to denaturation of the drug in the presence of digestive enzymes and acidic pH of GIT [19]. To achieve site-specific delivery of drugs, transportation of various drug molecules via different drug delivery systems has been closely examined in different compartment of GIT system. Among various compartments, the small intestine acted as key site for achieving maximum absorption of a drug owing to the presence of large surface area and availability of microvilli, specialized cells, associated micro-vessels that allows efficient transportation of drugs to the bloodstream in a short transit time. For example, M cells exhibit low lysosomal activity and high transcytosis capacity, capable of transporting foreign substances from the intestinal lumen to immune cells present in lamina propria. They have been investigated for optimal site-specific delivery of antigen, vaccines, DNA, proteins, or peptides. Colon has also been evaluated as an optimal delivery site for both local and systemic drug delivery due to the lower activity of proteolytic enzymes than the small intestine. However, drug delivers to colon faces additional barriers than that of the small intestine such as potential interference by faecal matter, longer transit time and bacterial activity [20].

B. Hydrogel based drug delivery via the rectal and vaginal route:

Several drugs have been delivered through rectal and vaginal routes, although patient acceptability varies due to discomfort arises from administered dosage forms. This route is primarily utilized for local treatment of conditions (e.g., haemorrhoids, vaginal infections, vaginal candidiasis, etc.) associated with the rectum and vagina [21]. Additionally, drugs

absorbed from the rectum and vaginal route exert local and systemic effects with slight metabolism, hence these routes can be useful for the delivery of drugs bearing high first-pass metabolism. Unfortunately, drugs administered in conventional dosage forms like suppositories possess some limitations such as short residence time, messiness, and leakage. These shortcomings of conventional dosage forms have been overcome by the use of hydrogels that exhibit desirable retention, distribution, and release characteristics [22]. Most of the proposed delivery systems that have been explored via rectal and vaginal routes are mucoadhesive hydrogels which provide appropriate contact between dosage form and vaginal or rectal mucosa, thereby enhancing the proper delivery of drug at an appropriate concentration to the local area [23]. Mucoadhesive polymers can also help to extend drug release by increasing the residence time of hydrogels delivered through the rectum and vagina. Several mucoadhesive polymers such as chitosan, Carbopol, etc. Have been investigated for their enhanced retention effects on pharmacokinetic parameters. For instance, a Carbopol based mucoadhesive hydrogel loaded with an antimalarial drug (artesunate) was investigated by Gaudin et al. This two-compartment hydrogel preparation was found to improve not only sustained-release behaviour and mucoadhesiveness but also the stability of artesunate when compared with current formulations[24]. Another effort has been made by Xu et al., 2017 in the fabrication of modified chitosan-based hydrogel loaded with sulfasalazine which exhibited localized and sustained drug release resulting in less production of sulfasalazine toxic metabolites. In addition, Ibrahim et al., 2012 fabricated an in-situ gelling systems containing Pluronic's F-127 and F-68 based hydrogel loaded with metronidazole for the effective treatment of bacterial vaginosis by prolonging the residence time of drug in vaginal mucosa. Singh et al., 2014 developed an interesting bio gel by exploring mucoadhesive, thickening, and water-soluble properties of Carbopol-934 in association with the complexation of sorbitan monostearate-sesame oil organogel for the effective delivery of metronidazole in the treatment of bacterial vaginosis [25]. Hydrogels have demonstrated significant potential to overcome limitations pertaining to conventional preparations and the rectal or vaginal route. By the use of specific polymers, it is possible to develop stimuli-responsive polymeric hydrogel systems with more mucoadhesiveness and therapeutic efficiency [21].

C. Hydrogel based injectable drug delivery:

The first invention based on injectable hydrogels for drug delivery was done by Elisseeff et al., 1999 [26]. They fabricated ultraviolet and visible light radiation responsive polyethylene-based hydrogel system containing chondrocytes (protein or albumins) and polyethylene glycol

dimethacrylate for their transdermal delivery. In chemotherapy, adverse effects exerted by injectable hydrogels were minimized via regulating the rate release of drugs and drug loading, degradation, and swelling behaviour of hydrogels [27]. Such control over the rate of release of drugs has also been applied to injectable hydrogels used in immunotherapy and regenerative medicine. Also, injectable hydrogels provide an excellent platform for the delivery of therapeutic agents as compared to conventional hydrogels. Recent studies on hydrogels indicated that high drug entrapment and site-specific delivery of multiple therapeutic agents and other substances such as maltose-binding proteins can be achieved by employing multi-functional injectable hydrogels for the effective treatment of cancer and other diseases [28]. In addition, injectable hydrogels have also been used as a scaffold or carrier for smart delivery of cells, bioactive moieties like enzymes, proteins, drugs, etc. for the treatment of Parkinson's disease, diabetes, microbial infection, and different types of cancer such as lung, ovarian, lymphoma, colon and breast cancer [29].

D. Hydrogel based drug delivery via topical route:

A topical drug delivery system facilitates intimate contact of the active drug with and through the skin. Topical administration is the most preferred route for localized delivery of active molecules due to its affordability and convenience particularly in the case of skin cancers [30]. From the past decades, several topical delivery systems have been developed such as creams, aerosols, emulsions, and powders. Among them, hydrogels have exhibited various advantages owing to their tunable features viz. controllable swelling behaviour, sustain release, and low degradation rate. Till today, a broad range of hydrogel systems has been synthesized for their potential usage in the treatment of skin conditions. For example, the Bioadhesive hydrogel system which was developed for cosmetological and dermatological issues tends to increase the residence time of the drug at the site of application and minimizes the frequency of using the product to the skin surface. Parente et al., 2015 fabricated a hydrogel system by combining xantham gum and carbomer homopolymer type C for the treatment of cellulite [31]. Likewise, a self-adhesive hydrogel patch was developed by using carboxymethylcellulose and sodium polyacrylate for the effective delivery of triclosan in the treatment of acne. In addition, several peel-off hydrogel masks have also been utilized in sensitive as well as normal skincare due to their cooling and soothing effects. Silk sericin-loaded carboxymethylcellulose-based hydrogels are used to synthesize these types of masks. Huang et al., 2019 developed a microcapsule embedded hydrogel patch for enhancing permeability of diclofenac sodium through the skin

which in response to ultrasound stimulus enhanced its therapeutic effects in local tissue injury [24].

E. Hydrogel based drug delivery via the ocular route:

In the ocular delivery of drugs, several physiological obstructions such as low permeability of cornea, effective tear drainage, and eye blinking prevent successful delivery of drugs into the eye. Thus, conventional eye drops readily get eliminated from the eye and show limited absorption of drugs resulting in poor ophthalmic bioavailability. Poloxamers are widely used as thermogelling polymers for the development of an efficient ophthalmic delivery system for drugs [32]. A combination of Pluronic derivatives and other polymers like methylcellulose, hydroxypropyl methylcellulose, carboxymethylcellulose, and polyethylene glycols have been used to develop hydrogel systems. An in-situ gelling system containing timolol and pilocarpine loaded xyloglucan-based hydrogel was prepared by Peppasa et al., 2000] for their sustained ocular delivery to overcome the limitations of conventional delivery systems. Hydrogels tend to enhance the residence time of any active moiety at the delivery site allowing it to diffuse through several layers of the eye thereby increasing their bioavailability. Also, increased viscosity of hydrogels plays a major role in withstanding the clearance of any formulation and prevent it from being washed due to blinking of eyes. Polymer-like chitosan helps the formulation to remain at the delivery site for a longer period due to its inherent mucoadhesive property [210]. Cellulose derivatives are also one of them which increase the viscosity of a formulation and prevent its rapid elimination from the ocular surface.

CHAPTER – 2

LITERATURE REVIEW

2. Literature Review

2.1 Sodium alginate beads were prepared by crosslinking with calcium chloride containing antihypertensive drug valsartan for the treatment of hypertension to get extended-release effect. The preparation (SA 3%, CaCl2 10%) was the most suitable pharmaceutical formula for valsartan beads. Selected beads achieved a high percentage of swelling in phosphate buffer solution pH=6.8. Entrapment efficiency was found to be as 80%. Prepared beads followed the Korsmeyer-Peppas release mechanism, and eventually obtained extended-release beads which slowed drug release for 24h, Calcium chloride concentration affected the cross-linked network, which increased the rate of swelling and the rate of release of the sparingly soluble drug valsartan. The solubility of valsartan was improved when it was formulated in the form of beads due to its greater release in the alkaline media that is similar to the intestine, which is the appropriate media for the dissolution of valsartan[33].

2.2 Hydrogel beads were by formulated by crosslinking sodium alginate and soyabean protein nanofibers in calcium chloride solution. The hydrogel beads had pH-responsive properties. The WC and hardness of the prepared hydrogel beads peaked at SNF/SA = 7:3 and CaCl2 concentration of 0.1 mol/L, while the swelling of the hydrogel beads reached the maximum value. In this experiment, a pH-responsive hydrogel bead based on soybean protein nanofiber (SNF) and sodium alginate (SA) was prepared by simple ionic crosslinking. It provides a theoretical and experimental basis for the future application of plant protein nanofibers as pH-responsive hydrogel materials [34].

2.3 Novel biopolymer hydrogels composed of sodium alginate (SA) and the oxidized sodium alginate (OSA) with an interpenetrating polymer network (IPN) structure were prepared by a combination of chemical and ionic cross-linking approaches. By using SA as the main raw material, OSA was obtained using sodium periodate. The gelation process of the IPN hydrogels was the formation of dual network: one gelatin network cross-linked by OSA with dihydrazide and another SA network cross-linked by Ca^{2+}. The factors which affect its swelling properties were discussed. The best swelling ratio of the beads was 2300, and the swelling property was greatly affected by pH and preparation condition. The stability and swelling behaviour of OSA/SA gel beads were better improved than the pure SA/Ca^{2+} system. The OSA/SA hydrogel beads with the pH sensitivity and stability will be a good candidate for site-specific controlled drug release carrier[35].

2.4 Paracetamol was entrapped in alginate beads gelled with calcium or zinc at 0·1, 0·34 or 0·7M. The payloads were of the order of 60–70% w/w which represented an entrapment yield of >75%. The release of drug from the beads was observed in three media; water, Simulated Gastric Fluid USP without pepsin (SGF) and 0·1% trisodium citrate solution. Release was slowest in water and was complete within 4–5 h. The zinc beads released more slowly than calcium beads prepared at the same molar concentration of cation. Complete release of drug from the alginate gel beads in SGF occurred within 2 h and was unaffected by the cation type and concentration. Except for beads prepared from 0·1M zinc, paracetamol was released rapidly in the citrate solution. All release profiles could be described by first-order kinetics with half-lives which ranged from 25–73 min. Due to the rapid release in acidic conditions, it is unlikely that alginate beads loaded with a relatively water-soluble drug will provide satisfactory prolonged release orally[36].

2.5 Zhao Qing Li et al. prepared calcium-alginate beads, which involved pouring an aqueous alginate solution into a calcium salt solution to act as a crosslinker. It was investigated how several factors, such as solution concentration, flow rate, and the distance between the orifice tip and the surface of the cross-linking solution, affected the morphology of the prepared beads. The researchers looked at how several factors affected the weight, diameter, and gelling rate. The results showed that the flow velocity and concentration of sodium alginate solutions had a significant impact on the form of alginate beads in the hydrated state[37].

2.6 Paracetamol loaded mucoadhesive microspheres were formulated using sodium alginate and different concentration of Xanthan gum and Guar gum by ionic gelation technique. Six formulations were prepared and evaluated for relevant parameters. Percentage yield is found between 52.34±0.58% to 84.21±0.21% in all formulations. The surface morphology of microspheres was characterized by SEM; it was discrete, spherical in shape and showed free flowing properties. The mean particle size of microspheres significantly increases and it was the range between 37.05±0.05µm to 45.29±0.06µm. Among all the formulations, XG-III showed a high entrapment efficiency is 94.80±0.54% and highest percentage sorption in distilled water is observed. The in-vitro drug release studies revealed that XG-III is controlled and found to be 77.04±0.22% at the of the dissolution studies. The mechanism of drug release was evaluated using the linear regression coefficient. Stability studies of selected

mucoadhesive microspheres showed good results. It could be also concluding that the all the formulations were shown satisfactory results and suitable for potential therapeutic uses[38].

2.7 Interpenetrating network (IPN) beads of sodium carboxymethyl xanthan (SCMX) and sodium alginate (SAL) were prepared by ionotropic gelation process using AlCl3 as a cross-linking agent. The effect of different formulation variables like total polymer concentration, gelation time, concentration of cross-linking agent, and drug load on the extent of release of ibuprofen (IBP), a non-steroidal anti-inflammatory drug, was examined. The formation of IPN structure was examined using Fourier Transform Infra-red (FTIR) analysis and the compatibility of the drug in the bead was evaluated through FTIR, X-ray diffraction (XRD) and Differential Scanning Calorimetry (DSC) analyses. While increase in the concentration of total polymer, gelation time, and drug load decreased the drug release in both acidic (pH-1.2) and phosphate buffer (PB) solution (pH-6.8), increase in the concentration of cross-linking agent tended to increase the drug release. However, from all the formulations, the drug release in acidic medium was considerably slow and a maximum 14% of the loaded drug was released in 2 h. Complete drug release was achieved in PB solution within 210 to 330 min depending upon the formulation variables. The study indicated that the IPN beads of SCMX and SAL could be a suitable dosage form to minimize the drug release in acidic medium and to control the drug release in PB solution[39].

2.8 Trimetazidine was entrapped in calcium alginate beads prepared with sodium alginate by the ionotropic gelation method, using calcium chloride as a crosslinking agent. The drug was incorporated by two methods, sequential and simultaneous methods. Beads produced by the former method had higher drug entrapment. The beads were evaluated for drug entrapment, particle size and release characteristics in enzyme-free simulated gastric and simulated intestinal fluid. The drug entrapment in the sequential method increases with increased CaCl2 and polymer concentration but decreased with higher drug concentration. In the simultaneous method, drug entrapment was higher with polymer and drug concentration increase and rose to a certain extent with increase in the concentration of CaCl2 and after further increase it decreased. Drug release was directly proportional to the polymer concentration for the drug-loaded beads prepared by both the methods. Increase in CaCl2 concentration retarded the drug release in the sequential method whereas for the simultaneous method the retardation in drug release occurred up to a certain concentration of CaCl2. The drug concentration exhibits a drug-loading dependent effect on the release behaviour in both the method[40].

2.9 Chitosan coated sodium alginate beads were formed using an electrospray to obtain spherical beads with size below 1.5mm. The encapsulation efficiency (EE) of the uncoated beads decreased with increased gelation time to reach 5±1.32% at 60 min. Chitosan coating enhanced EE to 50-76% depending on chitosan type and concentration. EE significantly improved to 99.0±1.1% by saturating the gelation bath with paracetamol. *In vitro* taste masking test and *in vivo* palatability evaluation using 12 human volunteers demonstrated that dry chitosan-coated paracetamol alginate beads were superior to the wet ones for taste masking, which was similar to the 30 marketed paracetamol suspension and even better in aftertaste evaluation. This indicates that the microencapsulation in alginate with further chitosan coating can compensate for the usage of sweetening and flavouring agents. This helps to formulate paediatric dosage forms with minimal undesired excipients [41].

2.10 The IPN hydrogel beads were prepared with more than one polymer bring forth better mechanical strength in contrast to a single polymeric-based network hydrogel system. This hydrogel bead of hydrophilic sodium alginate (SAL), the concentration of which ranges from 1.5 to 2.0% w/w, and xanthan gum (XAG) polymer, whose concentration ranges between 0.5 and 1.0% w/w, has been prepared to control the drug release profile. An ionotropic gelation technique with the crosslinking agent, calcium chloride at 2.5–7.5% w/w concentration, was adopted to prepare the IPN hydrogel bead drug carrier. The prepared hydrogel bead was studied for viscosity analysis of prepared composite dispersion, particle size, drug entrapment, swelling functions, and in vitro drug dissolution. An increase in xanthan gum quantity levels resulted in increased viscosity of prepared composite dispersions and hence the increased mean diameter of produced IPN hydrogel beads. Increased crosslinker concentration showed a slightly smaller IPN hydrogel bead mean diameter and increased encapsulation of loaded drug to about 88 to 91% glipizide. The in vitro drug dissolution was observed to be slower with increased xanthan gum polymer and calcium ion crosslinker concentration, which extended the drug release to 14h [11].

2.11 Sodium alginate microsphere beads were formed by ionotropic gelation technique using three different concentrations (5,10,15%) of sodium alginate polymer and two different types of crosslinking agents ($CaCl_2$ and $BaCl_2$). it reported that the microsphere beads prepared with $BaCl_2$ showed better % drug entrapment efficiency, good flow properties and better % drug release as compared to the microsphere beads gelled with $CaCl_2$. Particularly microsphere

beads gelled with 10% $CaCl_2$ showed better results as compared to other formulation with different concentration of $CaCl_2$ while the formulation crosslinked with 5% $BaCl_2$ showed effective results compared to other formulation with $BaCl_2$ as crosslinking agent[42].

2.12 Double-crosslinked sodium alginate-chitosan hydrogel beads were formulated with different ratio of polymers, crosslinked with $CaCl_2$ and glutaraldehyde. It was reported that the increased in chitosan amount in formulation caused a decreased in porosity and improved in gel fraction due linkage with glutaraldehyde. Based on the lowest swelling ratio at pH 1.2 and the highest swelling ratio at pH 7.4, a hydrogel composition of SALG/CS of weight ratio of 75/25 was chosen for optimization by varying the $CaCl_2$ concentration from 2 to 10% for the loading of MTZ and its controlled release at pH 1.2, 6.8 and 7.4[43].

2.13 Hydrogel beads containing metronidazole were prepared using eudragit, chitosan and sodium alginate as polymer by ionotropic gelation technique crosslinked with calcium chloride and glutaraldehyde. The value of angle of repose of formulation found to be within the range of 25˚, indicating very good flow properties for the microspheres. Mean diameter of Glutaraldehyde based microspheres found to be 680 ± 0.68 to 768±0.38µm and increasing average particle size of microspheres due to increasing in polymer ratio along with crosslinking agent. It was reported that the drug entrapment efficiency of the prepared microsphere up to 72.95 ± 0.56% with increasing the polymer concentration and was also found that the entrapment efficiency was increased up to 75.21 ± 0.4 % with addition of glutaraldehyde. The results of drug loading found to be increased from 13.72 ± 0.48% to 25.04 ± 0.39% of microsphere with increasing the amount of polymer as well as using the cross-linking agent. It was also found that the drug release was increased from 75.35% to 93.26% at the end of 24h [44].

2.14 Spherical hydrogel beads were formed by pouring the sodium alginate and metronidazole dispersion dropwise using 18G needle into calcium chloride solution due to crosslinking between cationic crosslinking agents and anionic carboxylic acid group of the polymer alginic acid. Size of the beads increases with the increase in the concentration of polymer at 10% fixed concentration of calcium chloride. Average beads size was found to be in the range of 1.954mm to 2.064mm. Drug entrapment efficiency ranged from 76% to 84%. It was observed that an increase in the amount of polymer, increases drug entrapment efficiency due to increased space for drug molecules to be retained throughout a larger cross-linked network of calcium alginate.

It was also observed that increasing of the curing time decrease the drug entrapment efficiency which may be due to the solubility of Metronidazole in water. It was noticed that different batches of the formulation were able to release up to about (92 to 98) % drug after 6 hours under study [45].

2.15 Metronidazole loaded alginate beads were prepared by crosslinking with calcium chloride. It was found that the %DEE was in the range of 81-96% and this was found lessened with decreasing the polymer concentration in the formulation and increment of curing time. The average beads size was found to be in the range of 1.4-1.9mm. In the acidic medium, the swelling ratio was measured 200% in 30 min, whereas in the alkaline medium, it was found to be augmented to the utmost of 1400% of swelling ratio within 2 h [46].

2.16 Sodium alginate beads containing metformin hydrochloride were formed by crosslinking with calcium chloride via ionotropic gelation techniques. Three natural polysaccharide copolymers e.g. Okra gum (OKG), Gellan gum (GLG), and Hydroxypropyl methylcellulose (HPMC) were added to alginate in a ratio of 1:1 to investigate their effect on mucoadhesion feature, drug encapsulation efficacy (EE %), and in-vitro drug release. It was reported that the MET-loaded OKG-alginate microsphere showed greater %EE (90 ± 3.28%), and better sustain release pattern over 10hrs compared to microsphere prepared with ALG-GLG and ALG-HPMC blend. Thus, the addition of isolated OKG to sodium alginate was manifested as a prospective controlled drug release polymer-blend in the formulation of sustained-release MET-ionically gelled microspheres for oral administration with expected enhanced bioavailability and better patient compliance due to a decreased dosage interval [47] .

2.17 Amoxicillin loaded alginate beads were prepared by ionotropic gelation method using calcium chloride as crosslinker. The effect of concentration of polymer (sodium alginate, poloxamer) and crosslinking agent ($CaCl_2$) on beads properties have been evaluated. It was reported that the %EE was improved with increment of conc of calcium chloride, %EE was found to be as 28.69±2.45 %,36.93±2.825 and 44.70±0.59% at 1%,5% and 10% of $CaCl_2$ solution respectively. It was also reported that the %EE was improved with increment of SA concentration in the formation due to increase in the viscosity of the dispersion. Poloxamer was incorporated in the formulation to improve the %EE, it was reported that the %EE was found to be 65.16±1.51%, 71.07±3.76%, and 76.40±3.27% at PL concentration of 5,10 and 15%[48] .

2.18 Metronidazole loaded gellan gum-based hydrogel beads were formulated using trivalent ion Al^{+3} as crosslinker. The Metronidazole loaded microspheres were prepared taking sodium alginate, gellan gum as excipients along with maleic anhydride, aluminium chloride as cross-linking agents. The evaluation processes of prepared metronidazole microspheres were done by invitro release study, microscopic analysis and swelling index. Each of the formulations shows good entrapment efficiency with the maximum entrapment 85.8±5.63% was governed by the F1 formulation while F2 formulation confirms 82.3±4.72% drug entrapment. The formulations provided the controlled release delivery pattern of Metronidazole [49].

2.19 Chitosan treated alginate beads gelled with calcium chloride were prepared and evaluated to deliver metronidazole in the treatment of *H. pylori* induced peptic ulcer. The drug entrapment efficiency (%), the percent of floating beads and the time for 80% of the drug to be released (T80%) were the responses evaluated. DEE increased from 22.76 to 79.08% by increasing the MZ: Na alg. ratio from 1:1 to 4:1, respectively. Due to the statistically non-significant difference (t-test, PN0.05) in DEE observed between the MZ: Na alg. ratio 3:1 and 4:1 respectively, the smaller ratio (3:1) was chosen in all the next formulae. It was found unexpectedly that increasing CaCl2 concentration (1, 2.5, 5 and 7.5% w/v) decreased the drug loading in the beads (77.03, 74.6, 70.62 and 65.8%, respectively). Formulating MZ as floating alginate beads with the use of 0.5% κ-carrageenan, 0.4% chitosan and 5% magnesium stearate, had a 92.09% DEE, immediate buoyancy for all beads with 100% drug release after 4 h [50].

2.20 Sodium alginate-based floating hydrogel beads were formulated by crosslinking with calcium chloride for sustain release of famotidine. It was reported that the beads without CaCO3 sank immediately in 0.1 N HCl, while beads containing CaCO3 showed excellent floating ability and further increase in the ratio of CaCO3: Alginate resulted in a decrease in the entrapment efficiency of Famotidine in floating beads. They reported beads containing CaCO3: Alginate in 1:1 ratio was found to be best batch in terms of in-vitro drug release with 97% release after 24 hrs as well as floating time that is 24 hrs[51].

2.21 UV first order derivative spectrophotometric method were developed with subsequent validation by using ICH guidelines for the determination of Ofloxacin and Metronidazole by using 0.1N hydrochloric acid as the solvent in combined dosage form. They reported for the selection of analytical wavelength, 10 µg/ml solution of Metronidazole was scanned in the

spectrum mode from 400 nm to 200 nm by using 0.1N Hydrochloric acid as blank. The first order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured at 293.6 nm. The proposed method is simple, precise, accurate and rapid for the determination of Ofloxacin and Metronidazole in combined dosage[52].

2.22 Sagar Kishore Savale, conducted a study on the formulation and evaluation of metformin HCl microbeads by the ionotropic gelation method. He also evaluated the drug's encapsulation efficiency, drug content, particle size distribution, and release pattern. Better sustained release activity is seen when CMC (carboxymethylcellulose) and sodium alginate are combined. The values for drug content and encapsulation efficiency are within the pharmacopoeia's permitted range. The maximum percentage of drug release, 71.15%, was shown by in vitro dissolution experiments to occur within 4 hours[53].

2.23 Charde MS et al. reviewed the sustained release of sodium alginate beads with their many morphological characteristics and characterization methods, including ionic gelation and emulsion gelation, swelling characteristics, buoyancy, and other morphological traits. The properties of in-vitro release were also included. The enhanced adaptability and flexibility of dose formulations is one benefit of employing sodium alginate beads as drug delivery vehicles, according to the study. Alginate beads were successfully produced using the ionotropic gelation technique. It was found that the loose network of beads prevents a large amount of drug leakage through the holes during the gelation process. The additional HPMCK-100m polymer postpones the release of the drug, which may last for up to 12 hours. The particle drug delivery system took on a more spherical shape as cross-linking agents like aluminium chloride were added at higher concentrations. Particulate spheres, which are hydrophobic and less soluble in aqueous solutions, can address the problem of curcumin medications low bioavailability[54].

2.24 Sudha C. Angadi et al. have done research on how to accomplish controlled release of amoxicillin in the stomach environment. Composite blend microbeads of sodium alginate with sodium carboxymethyl cellulose incorporating magnesium aluminium silicate particles and enteric coated with chitosan have been developed by the ionotropic gelation technique. In order to characterise the composite beads, X-ray diffraction (XRD), DSC, and Fourier transform infrared (FTIR) spectroscopy were used. XRD was used to research drug distribution, DSC was used to evaluate thermal stability, and FTIR was used to assess the structure of the drug-

loaded formulations. Scanning electron microscopy (SEM) was used to examine the beads' surface morphology. The range of 745-889 m was the size distribution of drug-loaded beads as determined by a particle size analyser. The encapsulation efficiency of the beads ranged pretty substantially, from 52 to 92%. According to equilibrium swelling of the beads measured in water and in vitro amoxicillin release in pH 1.2 media, drug release is dependent on the composition of the polymer mix, the number of magnesium aluminium silicates, and the degree of enteric coating[55].

2.25 Kubo W et al. investigated the oral sustained delivery of paracetamol from formulations including sodium alginate-gellan gum and in situ gelling, as well as information on the formulation's encapsulation effectiveness. They established that the oral administration of aqueous solutions of either gellan gum (1.0%, w/v) or sodium alginate (1.5%, w/v) containing calcium ions in complexed form resulted in the formation of gel depots in the stomachs of rabbits and rats. The calcium ions were released in the acidic environment, which is what caused this. In vitro testing revealed a diffusion-controlled release of paracetamol from the gels over a 6-hour period. Following oral administration of the liquid formulations, paracetamol's bioavailability from the gels that naturally formed in the stomachs of rabbits was comparable to the bioavailability of a readily accessible suspension containing the same quantity of paracetamol[56].

CHAPTER- 3

AIM AND OBJECTIVES

3. Aim

For years, significant efforts have been made to develop oral sustained-release drug delivery systems that incorporate various drugs for prolonged activity. Every day, new ways are used to broaden the applicability of pharmacological formulations. Extensive research is being conducted to construct hydrogel beads for sustained release DDS with various modifications, as well as the use of various additives and other polymers.

The primary goal of this study is to develop and evaluate a hydrogel-bead drug delivery system based on alginate and carboxymethyl alginate for sustained release. Paracetamol and metronidazole will be incorporated within hydrogel beads system for the treatment of microbial infection and to eradicate all types of gram-positive and gram-negative microorganisms.

3.1 The Objectives are:

Development of Simultaneous spectroscopic method: Firstly, different pH solutions [83] were prepared such as– pH 1.2, 7.4, neutral 7 (DDW). In those solutions required amount of pure API of Metronidazole and Paracetamol were dissolved individually. These solutions were diluted in a concentration range of 2 to 10 µg/ml and checked for their absorbance maxima in UV-spectrophotometer. Metronidazole showed its lambda max at 320nm [84] whereas for Paracetamol it was at 243nm [85]. The absorbances of Metronidazole and Paracetamol were determined at 320nm and 243nm for all the concentrations in all of the pH solvents. These absorbances were plotted against concentration to obtain the standard curve of Metronidazole and Paracetamol in different pH medias, and the effect of different pH on them was observed. The main difference between different pH media observed by the UV-spectrophotometer was an additional peak near 206-210nm in case of pH 1.2, and 7.2, no such peaks was observed in case of neutral pH, which may be due to the noise of the instrument due to the presence of buffer [86]. These studies were repeated more than 3 times, in 3 different instruments in different environmental conditions throughout the year to validate the method.

- **Validation of method:** The simultaneous spectrophotometric methods will be validated as per the the parameters of ICH guidelines like linearity, intraday and interday precision, LOD, LOQ.

- **Sodium alginate concentration alone is optimized for bead preparation**:

  The beads will be prepared using the ionotropic gelation process, with the goal of optimizing parameters such as alginate solution preparation time, crosslinking time between sodium alginate and calcium chloride, and bead curing time. The major goal is to determine the lowest concentration of sodium alginate capable of generating beads and the greatest concentration capable of forming beads clearly and extruding them via the syringe.
- **Calcium Chloride concentration optimization for bead production**:

  In addition to determining the optimum concentration capable of producing beads, the ideal concentration of Calcium Chloride is also calculated.
- **Bead preparation and characterization**:

  The beads will be prepared using the Ionotropic Gelation method, and further characterization such as drug entrapment efficiency, particle size analysis, drug release, etc. will be carried out.

However, there is no prior report on IPN hydrogel beads of carboxymethyl alginate and sodium alginate for a drug release study. The objective of this present work is to construct IPN hydrogel beads of carboxymethyl alginate and sodium alginate to compare drug release study with the hydrogel beads of only homopolymer as well as to compare crosslinking potency between calcium chloride and aluminium chloride on drug release behaviour from the hydrogel bead. In this present work, Ca-CMA, Al-CMA, Ca-Alg, Al-Alg and IPN hydrogel beads were constructed by ionotropic gelation with Ca^{+2} and Al^{+3} ions. In vitro drug release studies were conducted in simulated gastric fluid (pH 1.2) as well as in simulated intestinal fluid(pH7.4). the formation of IPN hydrogel beads were examined by FTIR study, and the compatibility and stability of drugs in formulated beads were evaluated through FTIR, XRD, DSC analysis.

CHAPTER- 4

MATERIALS AND METHODS

4.1 Materials:

a. Paracetamol (Purchased from Loba Chemie Pvt.Ltd, Maharashtra, India),

b. Metronidazole (Gift samples obtained from Holden Medical Laboratories Pvt.Ltd, Nashik, India),

c. Sodium alginate (Loba Chemie Pvt.Ltd, Maharashtra, India),

d. Monochloroacetic acid (Loba Chemie Pvt.Ltd, Maharashtra, India),

e. sodium hydroxide pellets (Loba Chemie Pvt.Ltd, Maharashtra, India),

f. Calcium chloride (Loba Chemie Pvt.Ltd Maharashtra, India),

g. Aluminium chloride (Loba Chemie Pvt.Ltd, Maharashtra, India),

h. Potassium chloride (SRL Pvt, Ltd),

i. Disodium hydrogen phosphate (Merck Life science Pvt. Ltd.),

j. Potassium dihydrogen phosphate (Merck Life science Pvt. Ltd.),

k. Hydrochloric acid (Merck Life science Pvt. Ltd.),

l. Glacial acetic acid (Merck Life science Pvt. Ltd.),

m. Trisodium orthophosphate dodecahydrate (Merck Life Sciences Pvt. Ltd.),

n. Double distilled water.

o. E. coli,

p. S. aureus.

4.1.1 Sodium alginate polymer :

Source:

Brown algae (pheophyta) belonging to the genera "Macrocystis, Laminaria, Ascophyllum, Alario, Ecklonia, Eisenia, Nercocystis, Sargassum, Cystoseira, and Fucus" are rich in alginic acid and its salts (Ca, Mg, Na, and K). The most significant are specimens of Fucus known as wracks and species of Laminaria known as kelps or sea tangles.

Chemistry:

Sodium alginate, an anionic, hydrophilic biopolymer, is a polysaccharide composed of different ratio of D-mannuronic acid(M) and L-guluronic acid (G) residues, which are arranged in GG, MM block interspersed with MG blocks[57]. Mannuronan C-5 epimerase is responsible for controlling the M/G ratio in algae[58]. While the average molecular weight of the hydrocolloids determines their viscosity, the distribution of M- and G-units and the counterions present determine the gelling properties of alginate hydrocolloids. Gels with a high M/G-ratio are typically soft and malleable, whereas those with a low M/G-ratio are typically hard and rigid[59]. The composition and extent of the sequences and the molecular weight determine the physical properties of the alginates. The molecular variability is dependent on the organism and tissue from which the alginates are isolated. For example, alginates prepared from the stipes of old *L. hyperborea* kelp contain the highest content of α-L-guluronic acid residues while alginates from *A. Nodosum* and *L. Japonica* have low content of α-L-guluronic acid blocks. As polymunnronic acid was found to dominate tissues of young algae; in older plants it is transformed into polyguluronic acid by the enzyme C5-epimerase. Two possible sources of the hydrogel polymer sodium alginate (SA) include bacteria and brown seaweed. Its great biocompatibility and biodegradability make it useful for targeted distribution of medications and proteins, as well as for tissue engineering. It is commonly used as a thickening, stabilizing, and gelling agent in various food products, pharmaceuticals, and industrial applications. Sodium alginate is known for its ability to form gels in the presence of calcium ions, making it a popular ingredient in food products such as ice cream, salad dressings, and jellies[60]. It is also used in the textile industry for printing and dyeing fabrics, as well as in wound dressings and dental impressions in the medical field. Sodium alginate is generally recognized as safe for consumption and has no known adverse effects when used in moderate amounts. However, calcium alginate beads have poor mechanical stability in physiologic environment. To avoid this problem, IPN hydrogel beads of sodium alginate has been constructed for controlled release of drug from the formulation for prolong period of time.

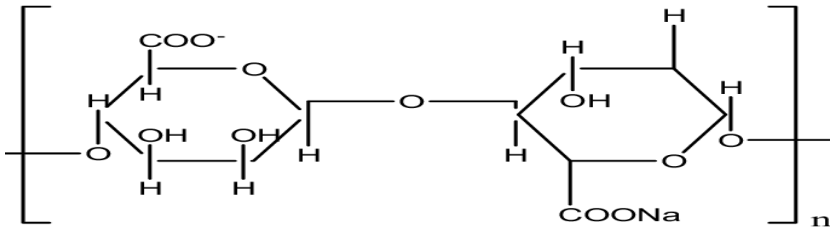


Figure 9: Structure of sodium alginate

Properties:

Table 1: Physical properties

Form	Powder
Colour	White to yellow fibrous powder
Odor	Odourless
pH value	6-8
Nature	Natural polymer
Density	1.603g/cm^3
pka	3.4-4.4
Melting point	99℃
Solubility	Slowly soluble in water, practically insoluble in ethanol
Taste	Almost tasteless

Rationale for selecting sodium alginate for the construction hydrogel beads as sustained release dosage forms: In sodium alginate ,available carboxyl group imparts anionic nature and water soluble derivatives that has been widely used for stabilizing ,gelling and sustain release properties[61].

4.1.2 METRONIDAZOLE

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

Molecular formula: C6H9N3O3

Structural formula:

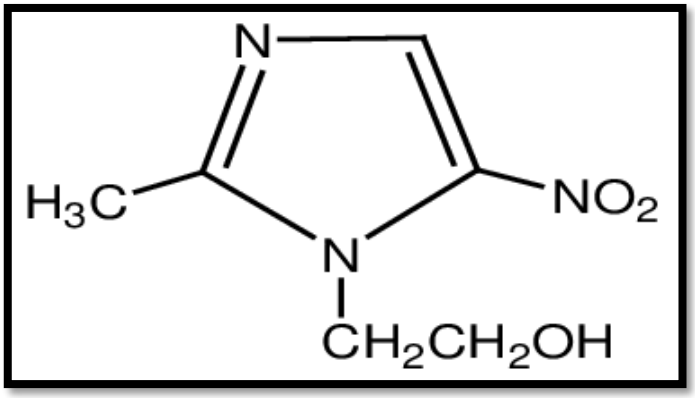


Figure 10: Structure of Metronidazole

Melting point: 159-161℃

Molecular weight: 171.15 gm/mol

Odour: Odourless powder

pKa: 15.42 (Strongest acidic), 2.81 (strongest basic)

Half-life: 8 hours

Solubility: a). Acetic acid 0.1M clear faintly yellow b). Water solubility

Pharmacotherapeutic group: Nitroimidazole derivatives

Mechanism of action:

The unique spectrum of activity of metronidazole against obligately anaerobic micro-organisms has prompted numerous studies on its mode of action. The drug has a low molecular weight -171gm/mol and readily penetrates all cell membranes of both aerobic and anaerobic micro-organisms; however, susceptible anaerobic organisms have a much higher rate of uptake. The accumulation of metronidazole in a susceptible organism is mediated through a reduction of the parent compound to what is presumed to be the reactive intermediate(s). The intercellular concentration of metronidazole decreases, thus causing a concentration gradient that enables more of the parent compound to enter the cell. The parent molecule is not active, but the reduction products are responsible for the antimicrobial and mutagenic activity of metronidazole. Reduction takes place at the 5-nitro group; it has been postulated to be either a four- or six-electron process. The process is thought to be mediated by a ferredoxin system (in most cases; there are exceptions) in anaerobic microorganisms, which is operative at an extracellular oxidation-reduction potential of approximately -400 mv. Since most aerobic and facultative organisms do not generate these low intracellular oxidation-reduction potentials, this explains the selective activity of metronidazole against anaerobic microorganisms. The reduction products have never been isolated but have been postulated to include amines and hydroxylamine. Such compounds have been shown to be convertible into species that can

interact with macromolecules and are known to disrupt DNA.'7- la The exact mechanism of cell death is not fully understood[62].

Pharmacokinetic properties

Absorption:

Metronidazole is readily absorbed following administration by mouth and bioavailability is 90-100%. Peak plasma concentrations of approximately 5µg/ml and 10µg/ml are achieved an average of 1-2 hours after single doses of 250mg and 500mg respectively. Some accumulation and consequently higher concentrations occur when multiple doses are given. Absorption may be delayed, but is not reduced overall, by administration with food[63].

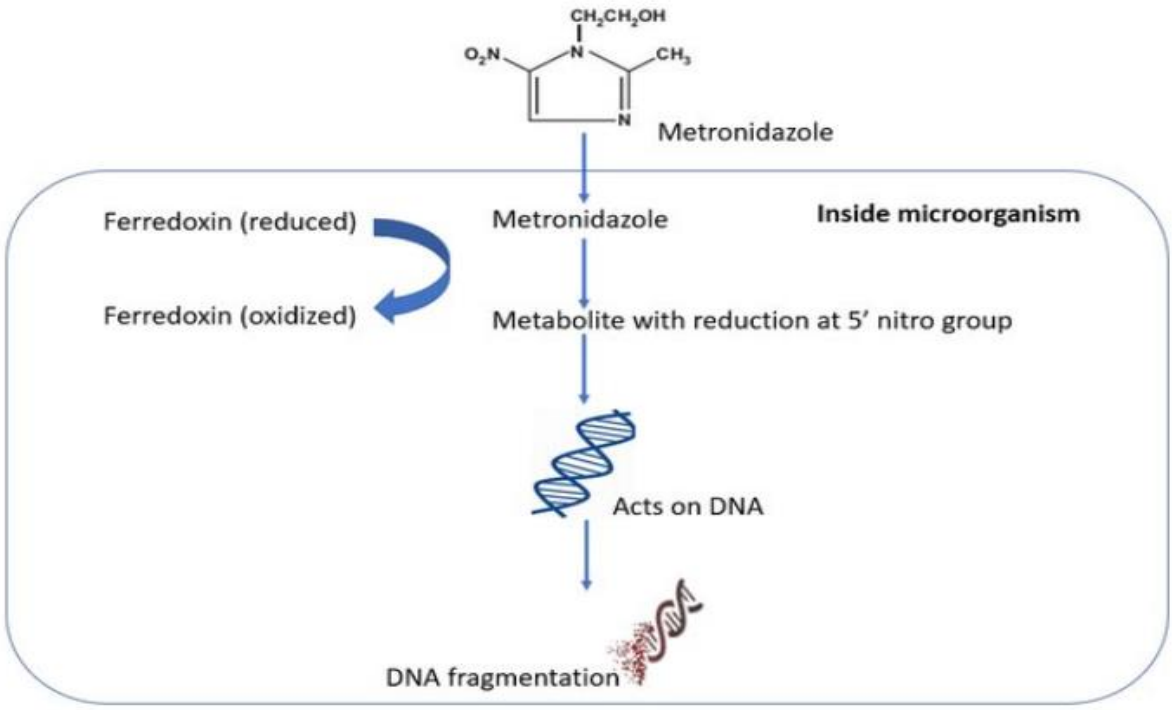


Figure 11: Mechanism of Metronidazole

Distribution:

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

Biotransformation:

Metronidazole is metabolised in the liver by side-chain oxidation and glucuronide formation. The plasma elimination half-life of metronidazole is about 6-9 hours; that of the hydroxy metabolite is slightly longer. The half-life of metronidazole is reported to be longer in neonates and in patients with severe liver disease[63].

Elimination:

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

Indications:

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

Contraindications:

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

Adverse reactions:

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

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

4.1.3 PARACETAMOL

Paracetamol (N-Acetyl-para-aminophenol), is an analgesic, antipyretic medicine that is available in the market as over the counter (OTC).

Molecular formula: $C_8H_9NO_2$

Structural formula:

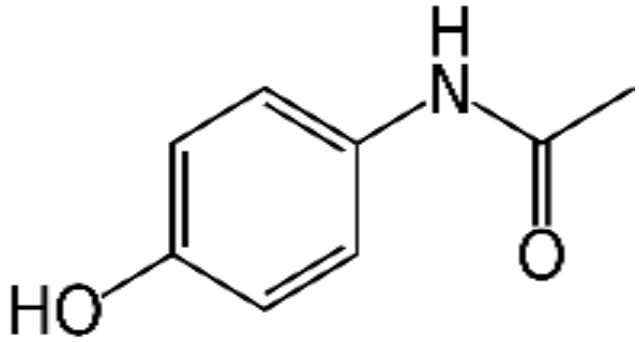


Figure 12: Structure of Paracetamol

Chemical name: N-Acetyl-para-aminophenol

Melting point: 169℃

Molecular weight: 151 gm/mol

pka value: 9.5

Half-life: 1-3 hours

Odour: Odourless

Solubility:

Sparingly soluble in cold water. Soluble in boiling water (1:20), ethanol (1:10), solutions of alkali hydroxides, methanol, dimethyl formamide, ethylene dichloride, acetone, ethyl acetate. Insoluble in pet. ether, pentane and benzene.

Pharmacotherapeutic group: Anilides

Mechanism of action:

The therapeutic effects of acetaminophen and non-steroidal, anti-inflammatory drugs (NSAIDs) are brought about by their ability to inhibit the enzyme cyclooxygenase (COX) also known as prostaglandin H2 synthase (PGHS) or prostaglandin-endoperoxide synthase. This enzyme catalyses the rate limiting step of prostaglandin synthesis from arachidonic acid. There are two main isoforms of cyclooxygenase, COX-1 and COX-2 COX-1 is constitutively expressed in most tissues and is involved in "housekeeping" processes. COX-2, in general, is the inducible isoform and is induced in response to inflammatory and physiologic stimuli. COX-1 and COX-2 can be inhibited to varying degrees by NSAID S. The therapeutic effects of NSAIDs have been suggested to be due to the inhibition of COX-2 and the unwanted side effects (e.g. gastrointestinal toxicity) to the inhibition of COX-1. At therapeutic doses, most NSAIDs inhibit both isoforms though they may vary in their relative potencies. Paracetamol is a weak inhibitor of both COX-1 and COX-2, but a good inhibitor of COX-3, which is a variant of COX-1[64].

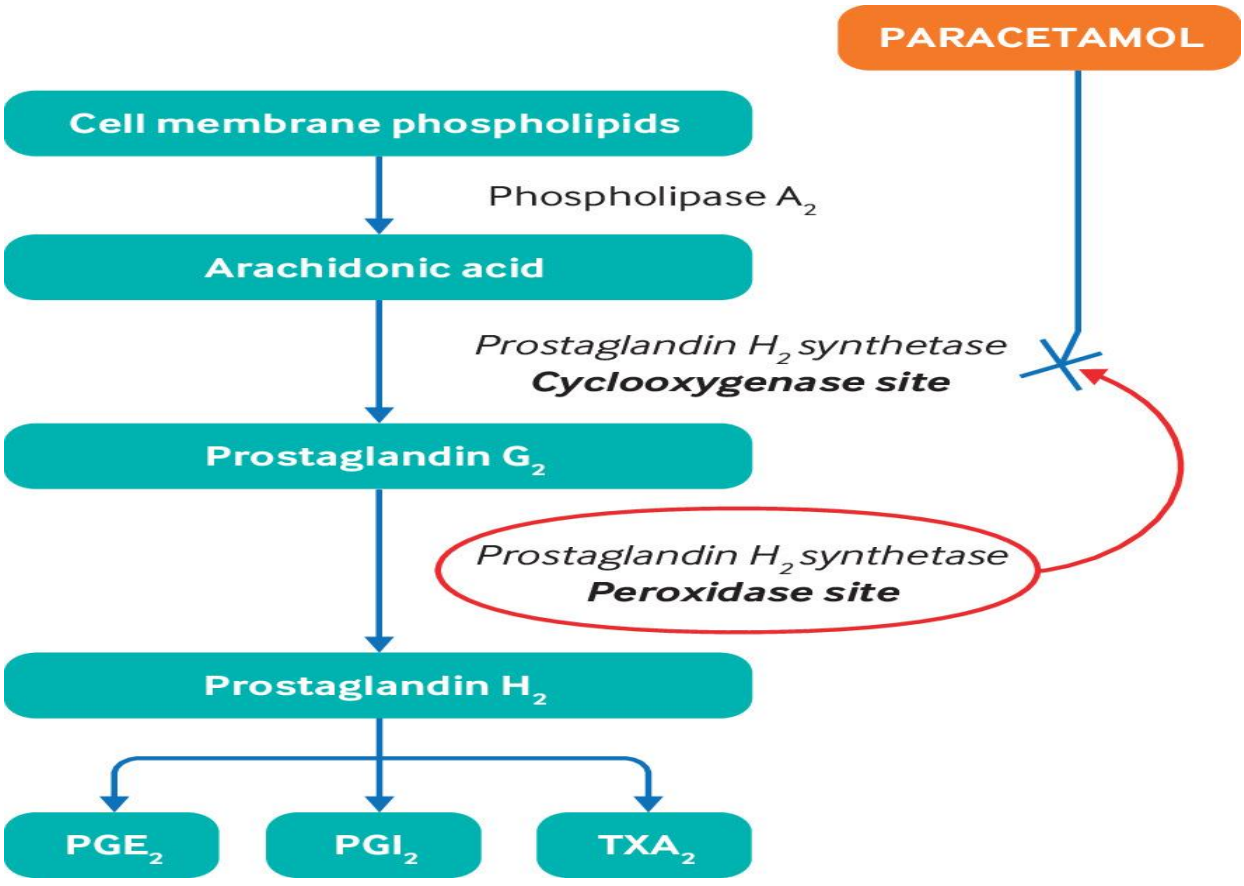


Figure 13: Mechanism of action of Paracetamol

Pharmacokinetic properties:

Paracetamol is almost completely absorbed from the gastrointestinal tract and rapidly and uniformly distributed in most tissues. It is not extensively bound to plasma proteins (<20%). The microsomal enzymes of the liver metabolize major portion (80-85%) of acetaminophen. The major metabolites are the sulphate and glucuronide conjugates. Around 85-95% of the dose is excreted in urine within 24 hours while 2-5% is excreted unchanged[65].

Adverse reactions: Rare and usually mild. Hypersensitivity reactions occur occasionally. Blood disorders and acute pancreatitis have been reported after prolonged usage. Acute over-dosage is quite common and can be extremely serious, causing severe liver damage and sometimes acute renal tubular necrosis. The antidote is N-Acetylcysteine. Long term chronic usage may lead to renal and liver damage.

Dose: 500mg-1gm.

4.2 Methods:

4.2.1 Preparation of pH 1.2 hydrochloric acid buffer:

At first, 0.2M potassium chloride (KCl) solution was prepared. To prepare that we have to take 14.91gm KCl in a 1000ml volumetric flask and add sufficient amount of double distilled water to dissolve it completely. After dissolving the KCl rest amount of double distilled water was added to the flask to make the volume up to 1000ml. By this method 0.2M KCl was prepared. [Molecular weight of KCl is 74.55g/mol (39.090g/mol of potassium +35.5g/mol of chloride) so to prepare 0.2M KCl we will need 14.91g KCl.]

Secondly, we need to prepare 0.2M Hydrochloric acid solution. For that we need to take 7.2ml of concentrated Hydrochloric acid and pour it dropwise in a 1000ml volumetric flask containing some double distilled water. When the two liquids are mixed completely rest amount of double distilled water was added to flask to make the volume up to 1000ml. With this procedure we have prepared 0.2M HCl. [Molecular weight of HCl is 36.5g/mol (1g/mol of hydrogen +35.5g/mol of chloride, so to prepare 0.2M HCl we will need 7.2g hydrochloric acid. The specific gravity (density relative to the density of water) of hydrochloric acid solution is 1.18 g/ml. So, 7.2 ml of hydrochloric acid solution is taken, then 7.2 ml/(1.18 g/ml)= 6.101ml HCl is taken.]

Finally, 50ml of the prepare 0.2M KCl solution and 85ml of the prepared 0.2M HCl solution was taken in a 200ml volumetric flask and rest of the volume was made up using double distilled water to prepare pH 1.2 hydrochloric acid buffer according to Indian Pharmacopoeia (2018, vol-1, page- 885) [66].

4.2.2 Preparation of pH 7.4 phosphate buffer:

To start with some amount of double distilled water was taken in a 1000ml volumetric flask. Thereafter Precisa Electronic balance model XB 600M/C, Switzerland was used to accurately weigh 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride. Then these compounds are added to the double distilled water in the volumetric flask one by one. The flask was vigorously shaken for a few minutes to completely dissolve the substances. Finally, when the components are dissolved completely double distilled water is added to the volumetric flask up to the mark of 1000ml. Lastly the preparation of pH 7.4 phosphate buffer saline was prepared according to Indian Pharmacopoeia (2018, vol-1, page- 889) [66].

4.2.3 Determination of λmax of MET and PCM in pH 1.2 and pH 7.4: Accurately weighted MET and PCM were dissolved in separately in 0.1N HCL (pH 1.2) and Phosphate buffer (pH 7.4). Suitable concentrated solutions for both preparations were prepared and scanned under UV-Vis spectrophotometer between 200 to 400 nm against corresponding blank buffer. The λmax of paracetamol in 0.1N HCl (pH 1.2) and Phosphate buffer (pH 7.4) is found to be 243 nm for both the buffer. The λmax of metronidazole in 0.1N HCl (pH 1.2) and in phosphate buffer (pH 7.4) was found to be 320nm for both the buffer. The λmax 243 nm was used for determination of unknown concentration of paracetamol and λmax 320 nm was used for determination of unknown concentration of metronidazole.

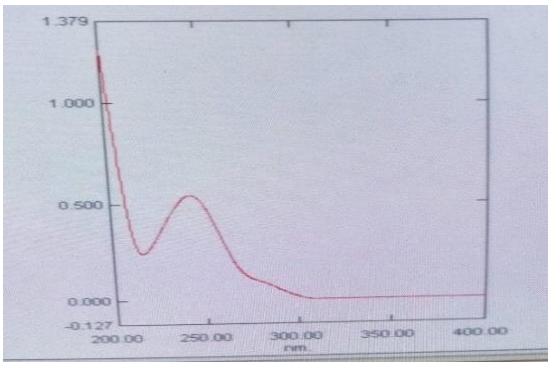


Figure 14: λmax of paracetamol in pH 1.2 and pH 7.4

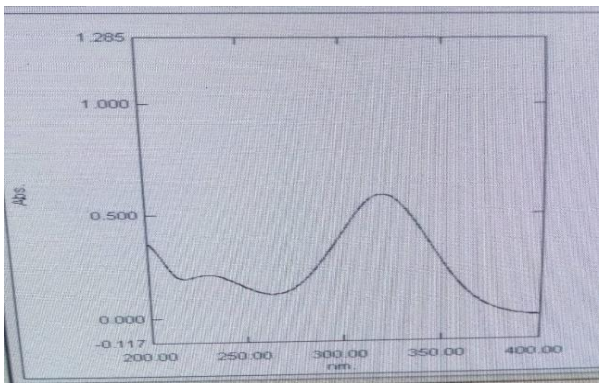


Figure 15: λmax of metronidazole in pH 1.2 and pH 7.4

4.2.4 Preparation of stock solution of MET and PCM in 0.1N HCl (pH 1.2) and phosphate buffer pH 7.4: Accurately 10 mg of MET and PCM were dissolved individually in a 100ml volumetric flask. The volume was made up to the mark with 0.1N HCl (pH 1.2) buffer and phosphate buffer (pH 7.4) to get standard stock solution having conc. of 100µg/ml of both API each.

4.2.5 Preparation of standard curve of PCM in 0.1N HCl (pH 1.2) and phosphate buffer (pH 7.4):

From the stock paracetamol solution, different solutions with known paracetamol concentrations like 2,4,6,8 and 10µg/ml solutions were prepared for both 0.1N HCl (pH 1.2) and phosphate buffer (pH 7.4). All the paracetamol solutions were subjected to UV-Vis spectrophotometric for the determination of absorbance at 243 nm. The different drug concentrations taken were plotted against the corresponding absorbance separately in 0.1N HCl and phosphate buffer (pH 7.4). The calibration curves were prepared by linear regression analysis, and the mathematical equations were drawn.

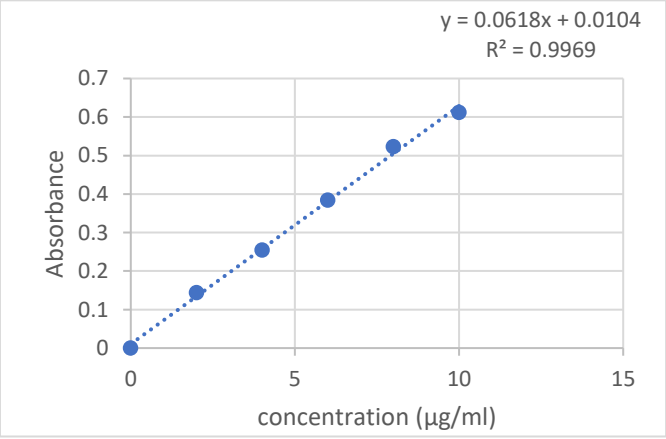


Figure 16: Calibration curve of paracetamol in pH 1.2

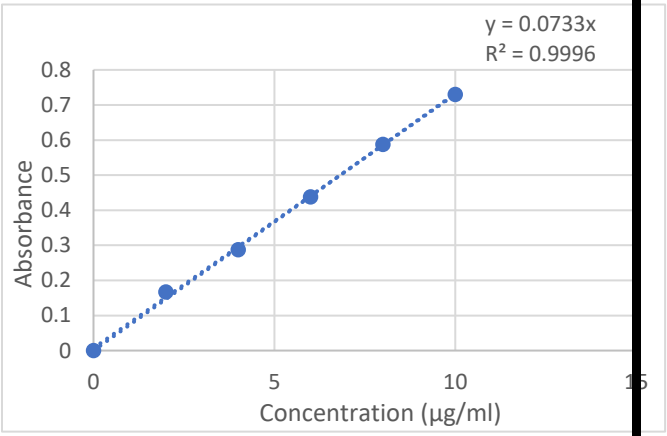


Figure 17: Calibration curve of paracetamol in pH 7.4

Table 2: Concentration and corresponding absorbance of paracetamol in 0.1N HCl (pH 1.2) and in phosphate buffer (pH 7.4)

Drug concentration (µg/ml)	Absorbance (nm) in pH 1.2	Equation
2	0.144	y= 0.0618x + 0.0104 R^2= 0.9969
4	0.254	
6	0.384	
8	0.523	
10	0.612	
Drug concentration (µg/ml)	**Absorbance (nm) in pH 7.4**	**Equation**
2	0.167	y= 0.0733x + 0.0104 R^2= 0.9996
4	0.288	
6	0.438	
8	0.588	
10	0.730	

4.2.6 Preparation of standard curve of MET in 0.1N HCl (pH 1.2) and phosphate buffer (pH 7.4): From the stock metronidazole solution, different solutions with known metronidazole concentrations like 2,4,6,8 and 10µg/ml solutions were prepared for both 0.1N HCl (pH 1.2) and phosphate buffer (pH 7.4). All the metronidazole solutions were subjected to UV-Vis spectrophotometric for the determination of absorbance at 320 nm. The different drug concentrations taken were plotted against the corresponding absorbance separately in 0.1N

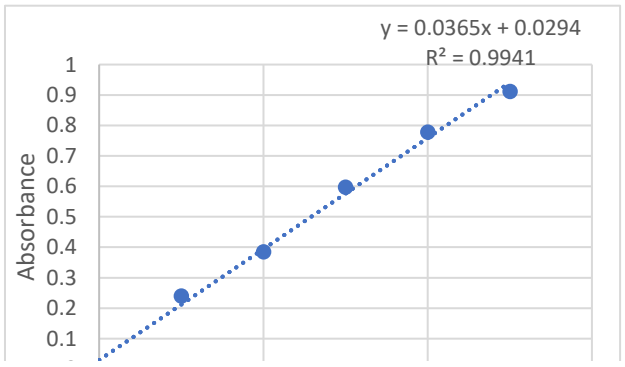


Figure 18: Calibration curve of metronidazole in pH 1.2

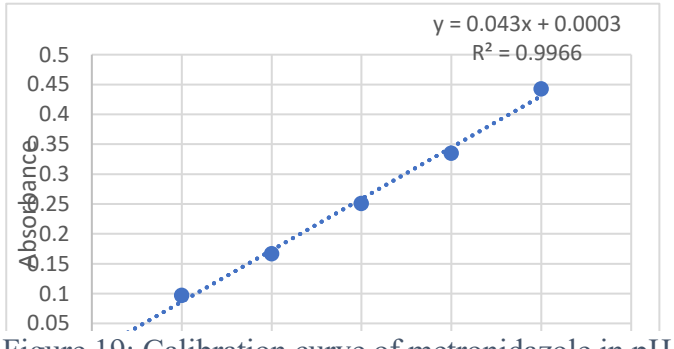


Figure 19: Calibration curve of metronidazole in pH 7.4

HCL and phosphate buffer (pH 7.4). The calibration curves were prepared by linear regression analysis, and the mathematical equations were drawn.

Table 4: Concentration and corresponding absorbance of metronidazole in 0.1N HCl (pH 1.2)

Drug concentration (µg/ml)	Absorbance (nm)	Equation
2	0.24	y= 0.0365x + 0.0294 R^2= 0.9941
4	0.385	
6	0.597	
8	0.778	
10	0.911	

Table 5: Concentration and corresponding absorbance of metronidazole in phosphate buffer (pH 7.4)

Drug concentration (µg/ml)	Absorbance (nm)	Equation
2	0.097	y= 0.043x + 0.0003 R^2= 0.9966
4	0.167	
6	0.251	
8	0.335	
10	0.443	

4.2.7 Preparation of beads:

Sodium alginate powder was accurately weighed and added in required amount of double distilled water. Then it was kept on a magnetic stirrer at 500rpm for 1 hour or until the powder is completely dispersed in the system homogeneously. The addition of paracetamol and metronidazole was done in this step. Another calcium chloride solution was prepared in distilled water for crosslinking of the beads. The alginate solution was poured in a syringe with 22G needle and it was dropwise added to the calcium chloride solution with constant stirring of 500rpm. The beads were formed instantly. They were cured for 5mins in the calcium chloride solution, then strained and rinsed to wash of excess crosslinking agent. And dried in a petri dish for overnight or sometimes occasionally used a hot air oven [40].

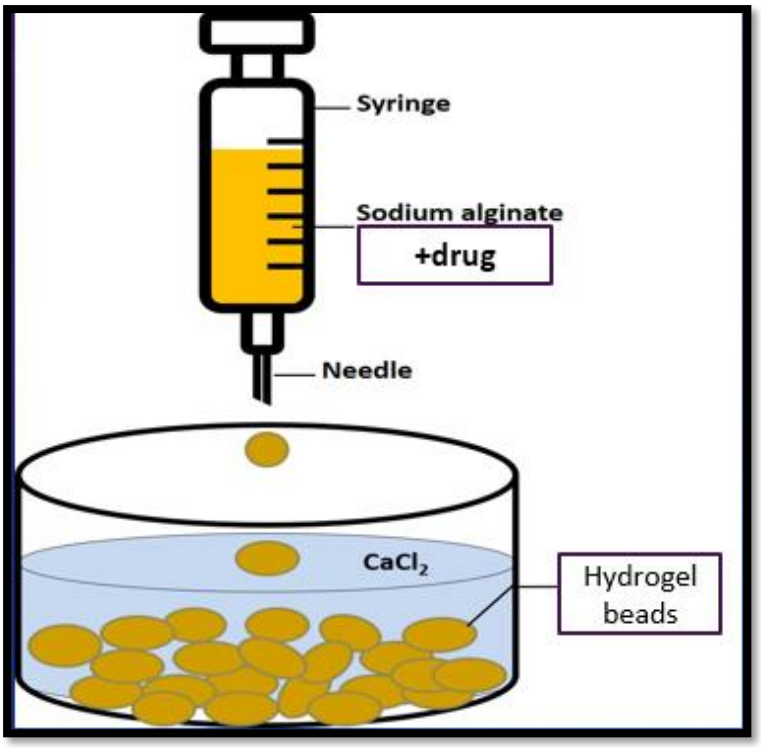


Figure 20: Ionotropic gelation

4.2.8 Characterization of the beads:

4.2.8.1 Size and shape:

Minimum 100 beads were taken and the diameter of the beads were measured using a slide caliper (Digimatic Caliper, model CD-6" CS, Mitutoyo Corporation, Japan). Mean diameter and standard deviation of the beads were calculated [41]. Sphericity factor is usually used to describe morphology of the beads, particles having sphericity factor less than 0.05 is usually considered as spherical in shape. Sphericity factor can be calculated by using following equation (Eq1),

$$\text{Sphericity factor (SF)} = D_{max} - D_{min} / D_{max} + D_{min} \ldots\ldots. 1$$

Here D_{max} is the beads maximum diameter and D_{min} is the beads minimum diameter.

4.2.8.2 Drug Entrapment Efficiency (DEE):

100 mg of dried beads of each formulated batch were dispersed in phosphate buffer solution (pH 7.4) and kept for 1h. Then the above swelled beads were crushed using mortar and pestle

and kept in a mechanical shaker for 2hours under vigorous shaking in order to disintegrate the alginate matrix and to release the drug encapsulated with the hydrogel beads[67]. The solution was filtered using Whatman's filter paper and the drug content was determined using UV spectrophotometer at 243nm and 320nm for paracetamol and metronidazole respectively. The paracetamol content was measured using a calibration curve prepared with known drug concentration in the range of 2-10µg/ml with R^2=0.999 and metronidazole content was determined using a calibration curve prepared with known concentration in the range of 2-10µg/ml with R^2=0. 9966.

Encapsulation efficiency was calculated by using the following equation (eq2)

$$\%Drug\ entrapment\ efficiency = \frac{practical\ amount\ of drug}{theoritical\ amount\ of\ drug} \times 100 \ldots\ldots\ldots 2$$

4.2.8.3 Swelling study:

50mg of dried drug free hydrogel beads were immersed in 25ml of 0.1N HCl solution (pH 1.2) at 37°C. The beads were removed by filtration and blotted with tissue paper properly to remove the water from the beads surface and weighed. The percentage of swelling of the beads were determined by using below equation (eq3),

$$\%swelling\ index = \frac{W2-W1}{W1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

W_1= weight of dry beads

W_2= weight of swollen beads

The percentage of swelling of the various batches of hydrogel beads in phosphate buffer solution (pH 7.4) was determined in the same way.

4.2.8.4 In vitro drug release study:

Drug release from the beads were checked by using the USP dissolution type 2 (paddle) apparatus. The baskets were filled with 750ml pH 1.2 hydrochloric acid buffer. The dissolution

apparatus was plugged in and the heater was started. When the temperature of the vessels turned 37±0.5ºC, accurately weighed amount of 100mg beads were added to the baskets and the rotation of the paddles were turned on. After 2 hours 250ml of trisodium orthophosphate dodecahydrate solution was added to each basket for changing the pH of the solution to 7.4 by continuous dissolution method mentioned in the Indian Pharmacopoeia (2018, vol-1, page-306) [66]. Samples of 10ml were withdrawn every 5, 10, 15, 30, 45, 60, 90, 120, 180mins for upto 8 hours and 10ml of fresh appropriate buffers were added to maintain the sink condition. The samples were then tested for their absorbances to check the cumulative percentage release of drug from the beads [68], [69].

4.2.8.5 Powdered X-ray diffraction analysis:

It is a non-destructive technique that provides detailed information about the crystallographic structure, chemical composition, and physical properties of a material. It is based on the constructive interference of monochromatic X-rays and a crystalline sample. An X-ray instrument contains three main items: an X-ray source, a sample holder and an XRD detector. The X-rays are produced by the source illuminate the sample. It was then diffracted by the sample phase and entered the detector. By moving the tube or sample and detector to change the diffraction angle (2θ, the angle between the incident and diffracted beams), the intensity was measured, and diffraction data were recorded. Depending on the geometry of the diffractometer and the type of sample, the angle between the incident beam and the sample can be either fixed or variable and is usually paired with the diffracted beam angle [70], [71].

The experimental parameters were-

Drug loaded beads were measured for their X-Ray diffraction pattern by using X-Ray diffractometer (D8, Bruker, Germany). With Transmission mode- 2θ scale of 5-42º, with an exit slit of 0.6mm. The step size taken was 0.02º, with a counting time of 0.3sec/step, and voltage 45Kv, current 30 mA. The values were detected and recorded. Finally, the graph was plotted against 2θ degrees vs Intensity. Amoxicillin trihydrate and Acetaminophen in their pure form and a formulation containing both the drugs were evaluated to check their structure and if any impurities are present in the substances [72].

4.2.8.6 Scanning Electron Microscope (SEM) Analysis:

Scanning electron microscopy or SEM is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. The instrumentation is configured with mainly 4 parts- A electron source, lenses, scanning coil, detector. Firstly, a layer of carbon tape is stick on top of two SEM stubs. Two batches of beads were selected for evaluation in Scanning electron microscopy, one batch with blank beads of the polymer and one batch with both the drugs with the polymer. Then a few sphere-shaped beads were selected from both the batches and mounted on the carbon paper tape on top of the SEM stubs. Then the beads were coated with gold in HITACHI MC1000 Ion Sputter Coater. Gold coating thickness was 6nm. Coating parameters were 40mA for 8 seconds. After the coating the SEM stubs containing the beads were installed in the SEM machine, for which we used- HITACHI SU3800 Scanning Electron Microscope with EDS (Energy Dispersive Spectroscopy) facility. Suitable images of the beads were selected [73], [74].

4.2.8.7 Fourier Transform Infrared Analysis:

FTIR spectra of SCMA, PCM, MET, SAL and physical mixtures of both drugs and polymers were recorded in a FTIR spectrophotometer (Perkin- Elmer, model Spectrum RX-1, UK). Each sample was mixed with KBr and converted into disc at 100 kg pressure using a hydraulic press. The spectra were recorded within 4000-400 cm-1 wave numbers. Similarly, the FTIR spectra of Paracetamol and metronidazole drug loaded IPN beads were recorded.

4.2.8.8 Differential Scanning Calorimetry (DSC):

Thermogravimetry Differential Thermal Analysis also known as TG/DTA is a technique mainly used to check purity of a compound by exposing the material to heat. DTA or differential thermal analysis is used to measure the temperature change between a specimen and a reference when both are subjected to the same amount of heat. DTA can be used to determine the temperatures of endo and exothermic events, and to show phase transitions. Whereas TGA or thermogravimetric analysis measures weight gain or loss as a material is heated to understand vaporization, absorption, decomposition, and more. TGA can also be used to determine the organic and inorganic content of materials, and to study the kinetics and thermodynamics of

decomposition reactions. Effectively a combination of a DSC and a TGA, this instrument allows easy correlation of data for samples up to over 1500° C as both tests are be run concurrently. Collecting both the DTA curve showing delta temperature and the weight loss allows a greater understanding of what happens in the material.

Experimental conditions were-

The thermal characteristics of the beads were determined by Thermogravimetry Differential Thermal Analysis (TG/DTA) (Pyris Diamond TG/DTA, Perkin Elmer, Singapore), and compared with those obtained using both blank beads and drug as raw material. 3-10mg sample was taken and kept in an open top T-zero aluminium pan. The temperature was kept at 25-250 ^{o} C with an increase rate of 10 ^{o} C/min. A constant air flow of dry N_2 gas was administered throughout the experimentation. The data was detected by the detector and the graph was plotted against Temperature (^{o}C) vs %weight. Amoxicillin trihydrate and Acetaminophen in their pure form and a formulation containing both the drugs were evaluated to check for their in determining purity and composition of materials, drying and ignition temperatures of materials and knowing the stability temperatures of compounds. The data obtained in DTA is used to determine temperatures of transitions, reactions and melting points of substances. With the change of temperature and time if any changes of the mass of the compound is observed or not can be determined with this method [72], [75].

4.2.8.9 In vitro antimicrobial activity assessment:

The developed hydrogels were screened for antibacterial activity by the plate diffusion test in Mueller-Hinton agar plates. Gram-positive bacteria Staphylococcus aureus ATCC 29213 and gram-negative bacteria Escherichia coli ATCC 25922 were used. Fresh cultures of both strains suspended in sterile 0.9% salt solution to optical density 108 bacteria/mL and equivalent 0.5 McFarland turbidity were inoculated on the plate. After air drying at room temperature for 15 min, each well, 6 mm in diameter, was created by aspiration of the agar using the back of a sterile Pasteur pipette. The prepared gel formulations containing MTZ or MTZ SLNs equivalent to 0.1 g were added in each well. The plates were then incubated at 37◦C for 24 h. Each assay was performed in triplicate. The zones of inhibition were measured using a caliper, RS PRO, China, which read to 0.1 mm. The control was taken as the formulation of the HEC gel without MTZ[76].

4.2.8.10 Data treatment of Release kinetics:

It was observed that the drug release from alginate beads depends on the test method as well as the media used in the experiment [77]. Differences between the release profiles obtained using various dissolution tests were observed. These results suggest the necessity of knowledge about the underlying in vivo processes and the need to translate this research to the in vitro test systems [78]. The obtained release profiles were analysed with zero-order, first-order kinetics as well as Higuchi [79], [80] and Korsmeyer-Peppas [81], [82].

- **Zero-order model**

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation:

$$Q_0 - Q_t = K_0 t$$

Rearrangement of the equation yields:

$$Q_t = Q_0 + K_0 t$$

Where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and K_0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time [83], [84].

Application: This relationship can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low soluble drugs in coated forms, osmotic systems, etc. [85].

- **First order model**

This model has also been used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug which followed first order kinetics can be expressed by the equation:

$$\frac{dC}{dt} = -Kc$$

Where K is first order rate constant expressed in units of time^{-1}.

This equation is also expressed as:

$$logC = logC_0 - \frac{Kt}{2.303}$$

Where C_0 is the initial concentration of drug, k is the first order rate constant, and t is the time [84], [86]. The data obtained are plotted as log cumulative percentage of drug remaining vs. time which would yield a straight line with a slope of (-K/2.303).

Application: This relationship can be used to describe the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices [87], .

- **Higuchi model**

The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Higuchi in 1961 [80]. Initially conceived for planar systems, it was then extended to different geometrics and porous systems [88]. This model is based on the hypotheses that-

(i) Initial drug concentration in the matrix is much higher than drug solubility;
(ii) Drug diffusion takes place only in one dimension (edge effect must be negligible);
(iii) Drug particles are much smaller than system thickness;
(iv) Matrix swelling and dissolution are negligible;
(v) Drug diffusivity is constant; and
(vi) Perfect sink conditions are always attained in the release environment.

Accordingly, Higuchi model expression is given by the equation:

$$f_t = Q = A\sqrt{D(2C - C_s)C_s t}$$

Where Q is the amount of drug released in time t per unit area A, C is the drug initial concentration, Cs is the drug solubility in the matrix media and D is the diffusivity of the drug molecules (diffusion coefficient) in the matrix substance.

This relation is valid during all the time, except when the total depletion of the drug in the therapeutic system is achieved. To study the dissolution from a planar heterogeneous matrix system, where the drug concentration in the matrix is lower than its solubility and the release occurs through pores in the matrix, the expression is given by the following equation:

$$f_t = Q = \sqrt{\frac{D\delta}{\tau}}(2C - \delta C_s)C_s t$$

Where D is the diffusion coefficient of the drug molecule in the solvent, δ is the porosity of the matrix, τ is the tortuosity of the matrix and Q, A, Cs and t have the meaning assigned above. Tortuosity is defined as the dimensions of radius and branching of the pores and canals in the matrix. In a general way it is possible to simplify the Higuchi model [80] as (generally known as the simplified Higuchi model):

$$f_t = Q = K_H \times t^{\frac{1}{2}}$$

Where, K_H is the Higuchi dissolution constant. The data obtained were plotted as cumulative percentage drug release versus square root of time [87].

Application: This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs [80], [89] .

- **Korsmeyer-Peppas model**

Korsmeyer et al. (1983) derived a simple relationship which described drug release from a polymeric system equation. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer Peppas model [82].

$$\frac{M_t}{M_\infty} = Kt^n$$

Where Mt / M∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices.

In this model, the value of n characterizes the release mechanism of drug as described in Table 1. For the case of cylindrical tablets, 0.45 ≤ n corresponds to a Fickian diffusion mechanism, 0.45 < n < 0.89 to non-Fickian transport, n = 0.89 to Case II (relaxational) transport, and n > 0.89 to super case II transport [90]. To find out the exponent of n the portion of the release curve, where Mt / M∞ < 0.6 should only be used. To study the release kinetics, data obtained

from in vitro drug release studies were plotted as log cumulative percentage drug release versus log time.

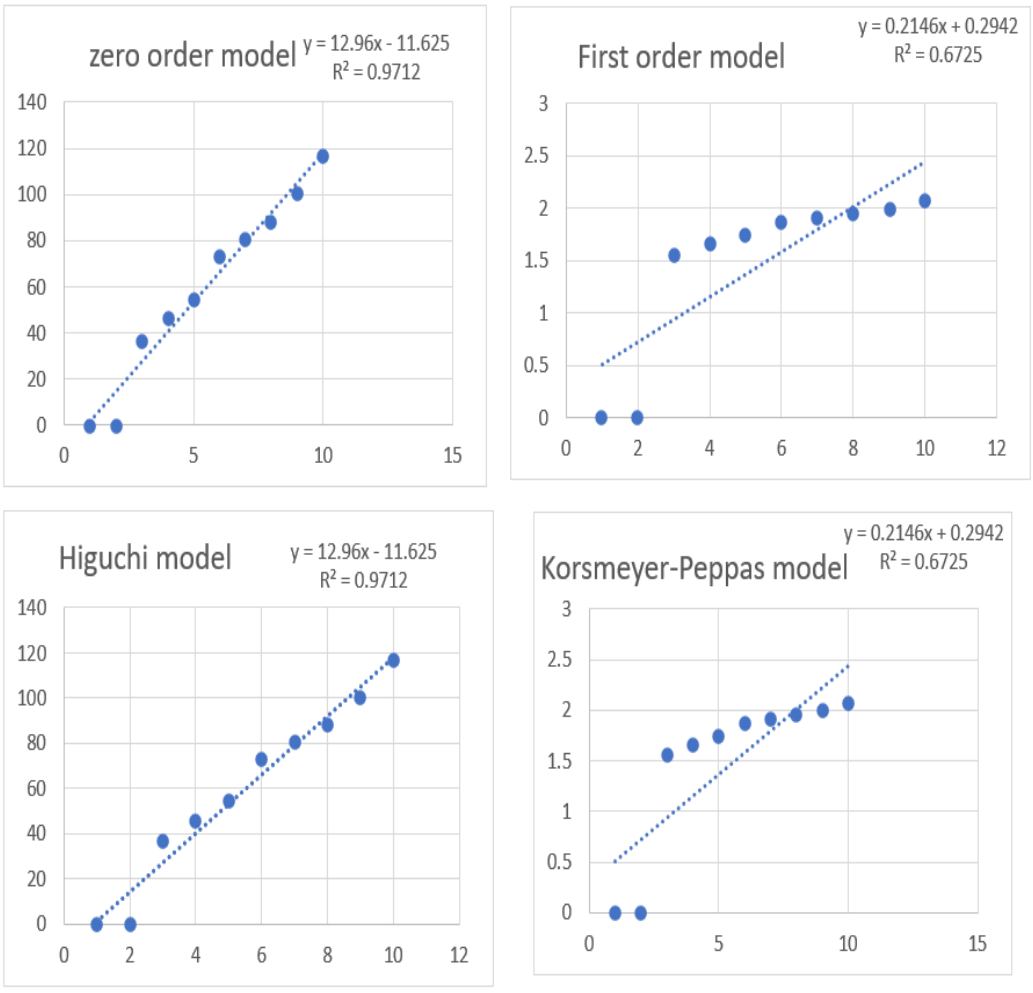


Figure 21: Drug release kinetics model

CHAPTER- 5

METHOD DEVELOPMENT

5. Simultaneous spectrophotometric method development and validation

The market is currently offering a wide range of combination dose forms, and the quantity is growing daily. Due to their improved potency, various actions, speedier relief, fewer side effects, and higher patient acceptability, these multicomponent formulations are becoming more and more popular. It is therefore intended that these formulations satisfy all requirements for their efficacy, safety, and quality. Only if many analytical methods are available for their determination can this be accomplished.

The most common OTC drug, PCM, used for pain relief and fever that has no anti-inflammatory properties. Its intricate method of action has not yet been fully understood[91]. Compared to non-steroidal anti-inflammatory medicines (NSAIDs), paracetamol is better tolerated and has fewer gastrointestinal adverse effects because it has less peripheral action on prostaglandins[92]. Paracetamol is often found in combinations with other drugs such as (Paracetamol+Etodolac)[93], (Parcetamol+Diclofenac)[93], (Paracetamol+Ibuprofen)[93], (Paracetamol+Domperidone)[93], (Paracetamol+eclofenac)[93], (Paracetamol+Caffein)[93], (Paracetamol+Aspirin)[93], (Paracetamol+Piroxicam)[93] in the management of cold, more than 600 (OTC) allergy medicine, pain reliever, sleep aids and other products. Metronidazole, an antiprotozoal drug is widely used in the treatment of invasive amoebiasis. Chemically it is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol. This agent has antibacterial, antiamoebic, antiprotozoal, antiparasitic, and antitrichomonal properties[94]. MET acts at low concentrations as a bactericidal agent. The majority of anaerobic (but not aerobic) bacteria, including bacilli, cocci, and gram-ve, gram+ve (*Clostridium diicile*, *Gardnerella vaginalis*), as well as several protozoa and capnophilic organisms including *Trichomonas vaginalis*, *Entamoeba histolytica*, and *Giardia lamblia*, are all within its area of activity. It has been used in clinical settings for a number of years and is the first-choice medication for the treatment of Crohn's disease, anaerobic infections, parasitic infections, *Helicobacter pylori*, and *Acne rosacea*. Metronidazole is available in the market as combination with other API such as (metronidazole+amoxicilin)[95],(norfloxacin+metronidazole)[96],(ofloxacin+metronidazole)[97],(metronidazole+nalidixic acid)[93]. Both the drugs are available in Indian pharmacopeia[98], and United state pharmacopeia[99].

Assessment of paracetamol, either by itself or in conjunction with other medications carried out by spectrophotometric method, HPLC[97], TLC[100], HPTLC[101], LC-MS[102], FTIR[103], Amperometric determination and micellar electrokinetic chromatographic method.

Many analytical procedure have been explained in the literature for the estimation of Metronidazole individually and along with other drugs in different pharmaceutical dosage forms[96] [104],. In our previous work we have carried out the simultaneous estimation of metronidazole in combination with norfloxacin[96]. Since the combination of these two medications is not recognized by any standard pharmacopoeia, there is no reported technique for estimating the dosage forms of metronidazole and paracetamol together. There are no publications about the simultaneous HPLC or spectrophotometric determination of paracetamol and metronidazole in combination that can be found in the literature review. This present work deals with uncomplicated, errorless, meticulous and reasonable method for the assessment of paracetamol and metronidazole in tablet dosage forms by UV Spectrophotometric method.

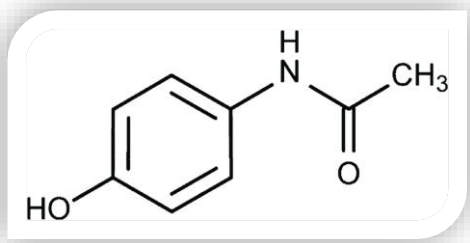


Figure 20: Paracetamol

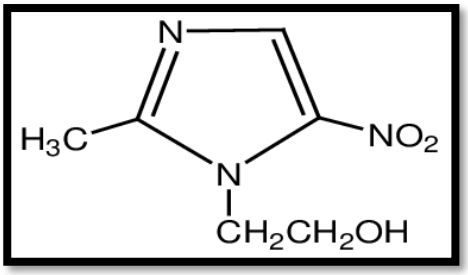


Figure 21: Metronidazole

5.1 Material and Methods:

5.1.1 Material: PCM API (Loba Chemie Pvt. Ltd.), MET (gifted by Holden medical laboratories Pvt. Ltd. Sinnar, Nashik. Double distilled water (from our lab). Digital weighing balance Lab India UV-3200 Double Beam Spectrophotometer, Shimadzu UV-2450 UV-Visible Spectrophotometer, UV Probe software.

5.1.2 Methods:

5.1.2.1 Preparation of stock solution of MET and PCM in green solvent: Accurately weighed portions of MET and PCM (10mg) were dissolved individually in a 100ml volumetric

flask. The volume was made up to the mark with double distilled water to get standard stock solution having conc. of 100µg/ml of both API each.

5.1.2.2 Preparation of standard curve of MET and PCM: The above standard stock solution was made at a conc. of 10µg/ml and 10 ml of the solution was scanned in the range of 200-400nm to determine the λ-max of both API. From the stock solution 2, 4, 6, 8, 10ml solutions were taken in 10ml volumetric flask and dilution was done using the double distilled water up to 10ml to prepare working conc. of 2, 4, 6, 8, 10µg/ml solutions. These conc. were then checked in UV- spectrophotometer at λmax 320nm and 243nm for MET and PCM respectively.

Table 6: Absorbance of PCM And MET for Standard curve

Concentration (µg/ml)	Absorbance (PCM)	Absorbance (MET)
2	0.196	0.075
4	0.390	0.137
6	0.568	0.194
8	0.709	0.255
10	0.906	0.326
Regression coefficient (R^2)	0.9977	0.9983

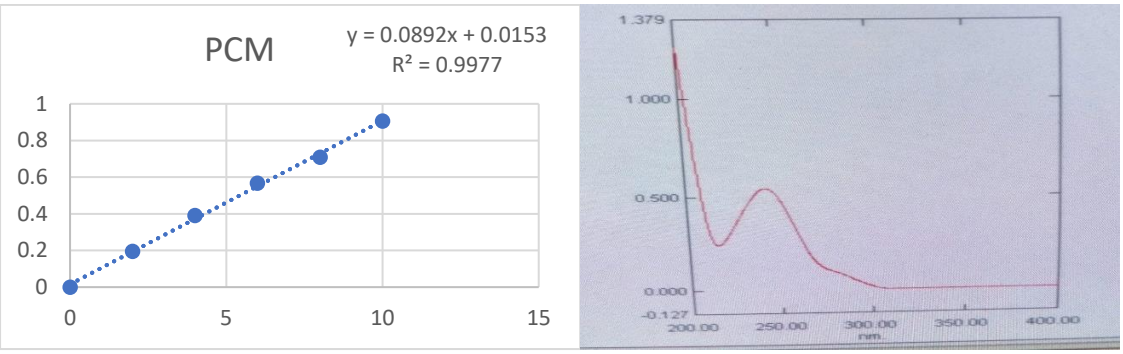


Figure 22: Calibration curve of paracetamol with UV scanned copy

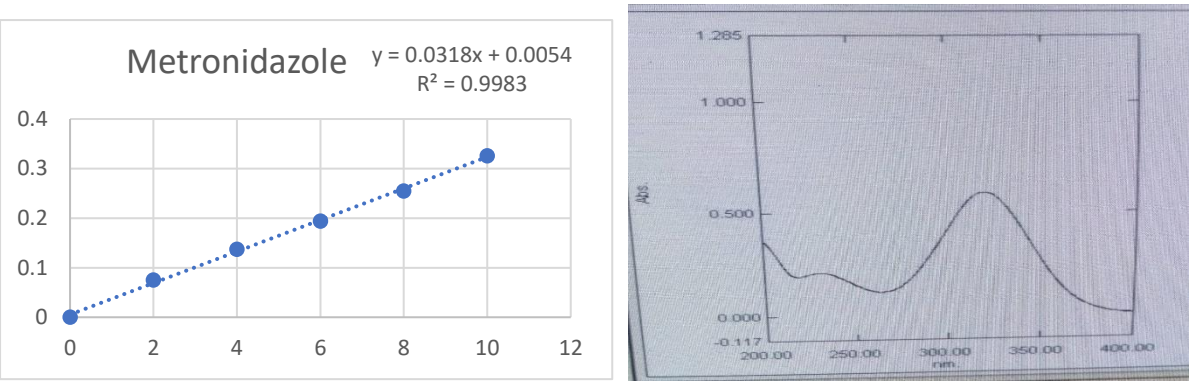


Figure 23: Calibration curve of metronidazole with UV scanned copy

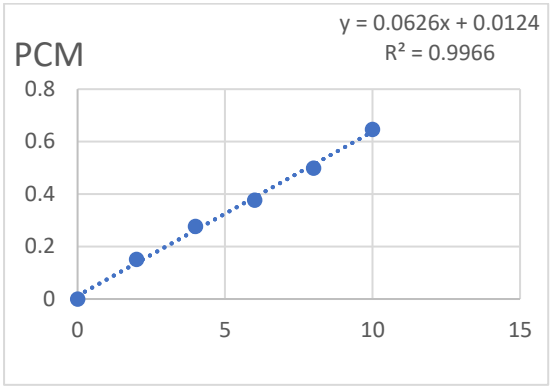
5.1.2.3 Preparation of stock solution for PCM and MET combined in green solvent: 10 mg of each API were taken in a 100 ml volumetric flask and make up the volume up to the mark using double distilled water to get a stock solution of concentration of 100µg/ml.

5.1.2.4 Preparation of standard curve of combination of PCM and MET:

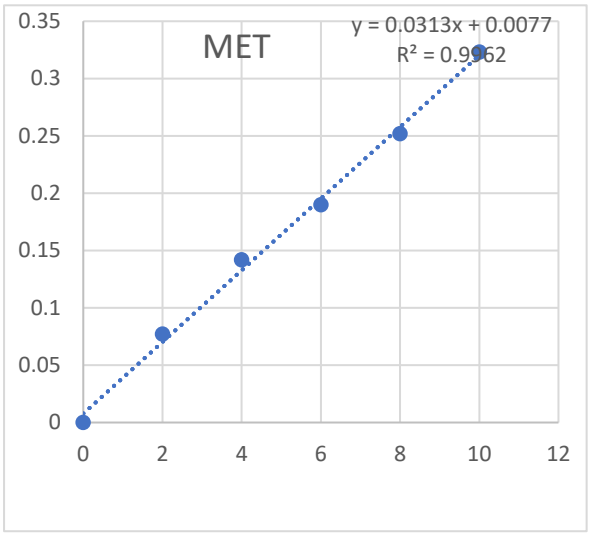
From the above stock solution, 10ml solution was transferred into a 100ml volumetric flask and make up the volume up to the mark using double distilled water to get final concentration of 10µg/ml of both API. From this stock solution working solutions were prepared by taking 2, 4, 6, 8, 10ml in 10ml volumetric flasks and dilution was done using green solvent to get the working concentrations containing 2,4,6,8,10µg/ml of each drug. Then the absorbance was checked in both 243nm and 319nm.

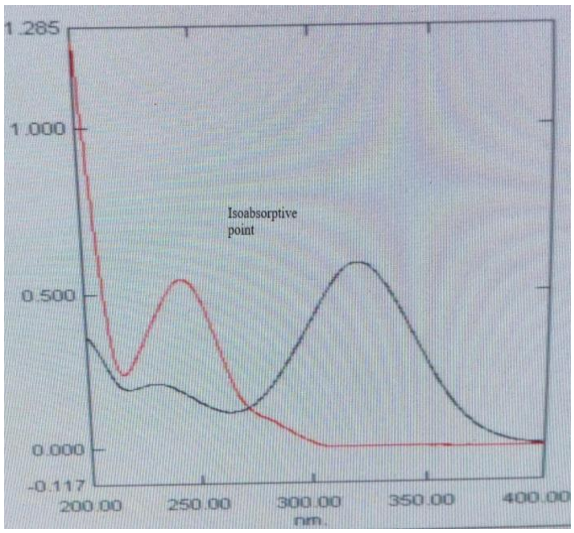
Table 7: Absorbance of combined PCM And MET for Standard curve at individual absorbance max and at 'isoabsorptive' point

Concentration (µg/ml)	PCM absorbance (243nm) in mixture	MET absorbance (319nm) in mixture	Isoabsorptive Absorbance (264nm) in mixture
2	0.151	0.077	0.083
4	0.277	0.142	0.154
6	0.377	0.190	0.219
8	0.499	0.252	0.284
10	0.647	0.323	0.369
Regression coefficient (R^2)	0.9966	0.9962	0.9979



A





B C

Figure 24: A- Absorbance of PCM in mixture at 243 nm, B- Absorbance of MET in mixture at 320 nm C- Isoabsorptive Absorbance at 264nm in mixture

5.1.2.5 Simultaneous estimation of Combined PCM and MET:

Two Spectrophotometric methods were used here for the assessment of both API and their combined form.

5.1.2.5.1 Simultaneous equation method: After determination of standard curve both the drugs absorbance maxima were found 243nm and 319nm for PCM and MET respectively as in IP.

Calibration curve was plotted by taking absorbance on Y axis and conc. on X axis at individual wavelength and regression coefficient was found.

Simultaneous equations used for both drugs concentration calculation by solving these.

C x = (A1 aY2 - A2 Ay1) / (aX1 aY2 - aX2 aY1) …….1

C y = (aX1 A2 - aX2 A1) / (aX1 aY2 - aX2 ay1) ……2

Where C x and C y represents the amount of PCM and MET in gm/100ml in their prepared solution.

A1 and A2 are absorbance of mixture at 243nm and 319nm respectively

aX1 and aX2 are absorptivity of paracetamol at 243nm and 319nm

aY1 and aY2 are the absorptivity of metronidazole at 319nm and 243nm respectively.

5.1.2.5.2 Absorbance Ratio Method (Q-Analysis): Absorbance ratio method or "Q-analysis" is an analytical method to measure the ratio of two absorbance found at the "isoabsorptive" point of one component and absorbance of the other component at its λ-max.

Following the same process, then determine the "isoabsorptive point and absorbance maxima of both the drugs. As found in Figure 4 i.e. 264nm, showing iso-absorptive point and same as before at 243nm and 319nm absorbance maxima for PCM and MET respectively.

The Q Absorbance Ratio Method Equation used to calculate concentration of both the drugs,

$^{C}_{PCM} = Q_M - Q_y/Q_x\text{-}Q_y * A_1/ax1$…….3

$C_{MET} = Q_M – Q_X/Q_X\text{-}Q_Y * A_2/ay1$ …...4

A_1 and A_2 are the absorbances of the mixtures at 243nm and 264nm and ax1 and ax2, ay1 and ay2 are absorptivities E (1%, 1 cm) of paracetamol and metronidazole at 243nm and 264nm and $Q_M = A_2/A_1$ $Q_Y = ay_2/ay_1$, $Qx = ax_2/ ax_1$.

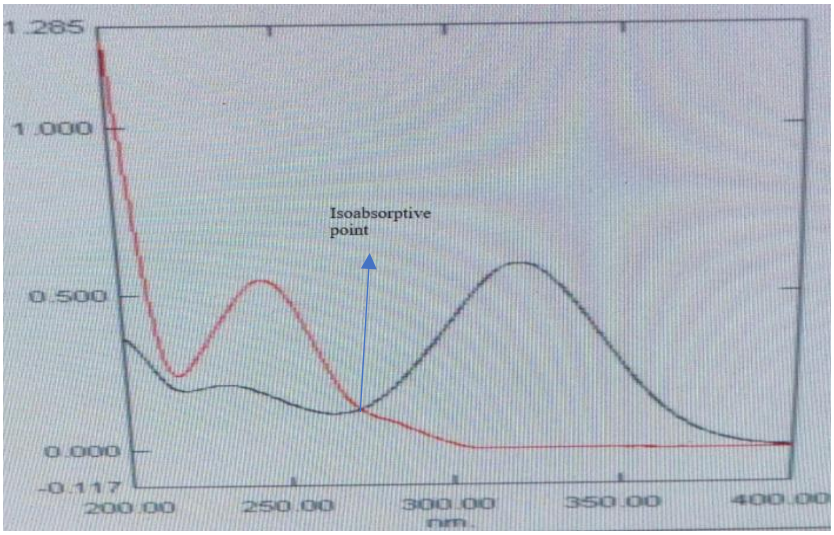


Figure 25: Overlain absorption spectra of PCM and MET

5.2 Analysis of marketed formulation: To determine the quantity of paracetamol and metronidazole in existing formulation, required volume of suspension was taken equivalent to 10mg of both the marketed formulation in 100ml of volumetric flask and add required volume of double distilled water to get desired conc. Subsequent dilution was done to prepare a concentration of 10µg/ml solution of paracetamol and metronidazole.

For Method I, the absorbances i.e. A1 and A2 were checked at 243 nm and 319 nm respectively and amount of two API in the dosage's forms were calculated using above equation (1) and (2)

For method II, the absorbances i.e. A1 and A2 were noted at 264 nm (iso-absorptive point) and 319 nm (λ-max of metronidazole) respectively and ratio of absorbance were determined i.e. A2/A1. Relative amount of two API in the sample was measured using mentioned equation (3) and (4).

5.3 Validation of method: Both the above methods were validated according to the parameters of ICH guidelines like linearity, intraday and interday precision, LOD, LOQ[105].

5.3.1 Range and Linearity: Range and linearity are important parameter to consider when performing simultaneous estimation of pharmaceutical dosage formulations.

The different std conc of paracetamol and metronidazole from 2-10 µg/ml was made to study the linearity of both API. Linearity was assessed regarding slope and R^2.

5.3.2 Precision: The precision of a measurement is determined by comparing the results of several measurements made of the same sample under specified conditions. For the precision investigations, both intraday and interday precision are taken into account. The precision of an analytical process is commonly represented by the variance, standard deviation, or coefficient of variation of a collection of measurements.

5.3.3 Intraday precision: To determine intraday precision, study was carried out for a solution of 10ppm and the absorbance of the sample solutions of paracetamol and metronidazole was taken three times on the same day (morning, afternoon and evening). Mean, standard deviation and % RSD was determined.

5.3.4 Interday precision: The absorbance of the sample solutions(10ppm) of paracetamol and metronidazole was checked on three alternative days and %RSD was found out.

5.3.5 Reproducibility: To evaluate reproducibility, an interlaboratory trial is used. Although a repeatability investigation is typically not necessary for regulatory submission, it should be taken into consideration when standardizing an analytical technique—for example, when incorporating the procedure into pharmacopoeias or when conducting the procedure across several locations.

5.3.6 Accuracy: The degree to which the measured values resemble the real ones is known as accuracy. Performing the recovery experiments allowed for the determination of the method's accuracy. The standard drug solution was spiked into the formulation's previously examined sample solution in order to conduct the recovery investigations.

5.3.7 Limit of detection: Limit of detection of an analytical method is usually performed to assess the sensitivity of the method. Limit of detection (LOD) can be calculated by below mentioned formula,

LOD=3.3*SD/SLOPE…...5

5.3.8 Limit of quantification (LOQ): Limit of quantification of any method is usually carried out to identify the contaminants and degradation of the products. The following formula can be used to determine LOQ in accordance with ICH recommendations.

LOQ=10*SD/SLOPE…...6

5.3.9 Ruggedness: Ruggedness of an analytical method is the degree of its reproducibility of the test results obtained under different variables like by different analyst, instrument,

laboratories or different days. It was carried out by determining the mean value, %RSD, SD etc.

5.3.10 Robustness: Depending on the type of procedure being studied, the applicability of the analytical procedure within the planned operating environment should be evaluated throughout the development phase and taken into consideration. In addition to demonstrating an analytical procedure's robustness to intentional changes in its parameters, robustness testing should, if applicable, demonstrate the stability of the sample preparations and reagents throughout the process. The robustness evaluation should be available upon request or may be included, on an individual basis, in the development data for an analytical technique.

5.4 Result: The solution of Paracetamol and metronidazole (2-10 µg/ ml) was scanned from 200-400 nm for determination of λ-max. The maximum absorbance of Paracetamol and metronidazole was obtained at 243 nm and 319 nm respectively.

5.4.1 Linearity and Range: The linearity of both the drug i.e. paracetamol and metronidazole were found to be in the range of 2-10 µg/ml. The data for linearity of paracetamol and metronidazole is shown in table 3. The calibration curve of paracetamol and metronidazole is shown in the fig 1,2 respectively.

5.4.2 Intraday precision: The intraday precision in terms of % RSD for paracetamol was found to be 1.613% at 243 nm and for metronidazole, it was found to be .700 at 319 nm.

5.4.3 Interday precision: The interday precision of paracetamol and metronidazole in terms of % RSD was found to be 4.03 % for paracetamol at 243 nm and 3.05% for metronidazole at 319 nm respectively.

5.4.4 LOD and LOQ: The value of limit of detection (LOD) for paracetamol at 243 nm was found to be 0.546 and for metronidazole at 319 nm, LOD was found to be 0.240.

The LOQ for paracetamol at 243 nm was found to be 1.65 and for metronidazole at 319 nm was found to be 0.729.

5.4.5 Accuracy: Recovery studies are usually carried out to evaluate the ability of the method to give accurate results after spiking the sample solution at 50%, 100%, 150% with the standard drug solution. The % recovery of paracetamol and metronidazole was given in the table 4. The % recovery of paracetamol and metronidazole was found in the range of 93-102.6% and 95.6-

107%. For most residue analytical method guidelines, an acceptable recovery range is 70 to120% with means in between 70 to 120% or 70 to 110% (depending on the regulatory guideline) at each fortification level[106].

Table 8: Data for recovery study of PCM and MET

Method	**Concentration in sample (µg/ml)**		**Concentration added (µg/ml)**		**Amount of drug(µg/ml)**		**Amount found(µg)**		**%RSD**		**% Recovery**	
A	PCM	MET	PCM	MET	PCM	MET	PCM	MET	PCM	MET	PCM	MET
	2	2	1	1	3	3	3.08	2.974	0.89	1.63	102.6	99.16
	2	2	2	2	4	4	4.04	4.28	0.441	1.72	101.08	107
	2	2	3	3	5	5	5.09	4.955	0.902	1.50	101.8	99.1
B	2	2	1	1	3	3	2.8	2.96	2.98	1.63	93.3	98.6
	2	2	2	2	4	4	2.95	2.89	0.679	1.72	98.3	96.3
	2	2	3	3	5	5	2.79	2.87	2.14	1.50	93	95.6

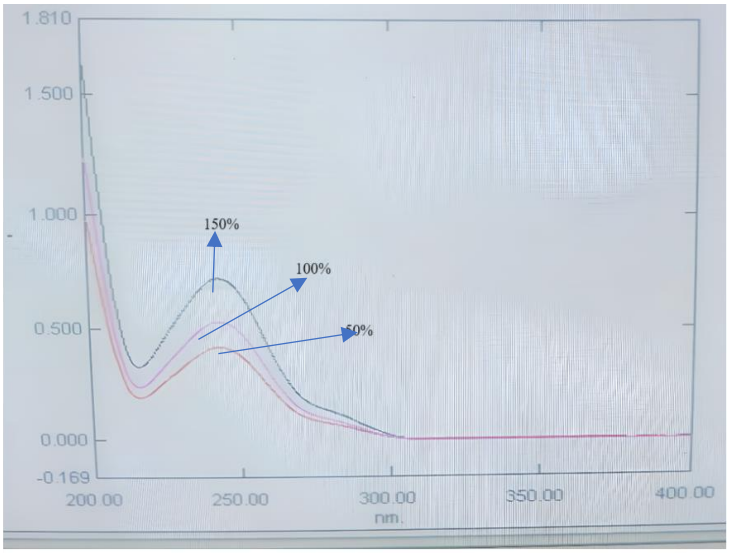


Figure 26: Recovery study of PCM

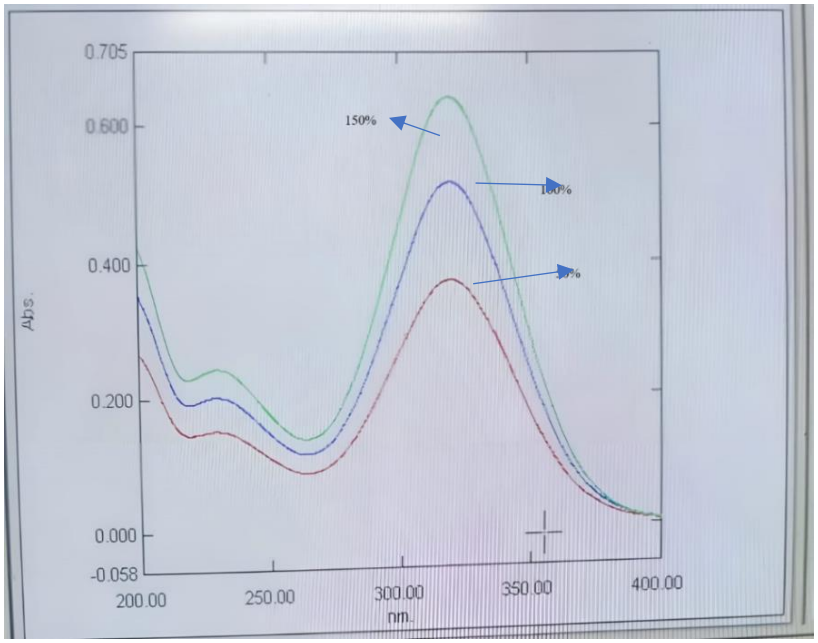


Figure 27: Recovery study of MET

Table 9: Regression analysis data and summary of validation parameter for the current method

Parameters	Paracetamol	Metronidazole
Wavelength (nm)	243	320
Beer's law limit (µg/ml)	2-10	2-10
Regression equation	Y=0.0892x + 0.0153	Y=0.0318x+.0054
Slope	0.0892	0.0318
Intercept	0.0153	0.0054
Correlation coefficient (R^2)	0.9977	0.9983
Intraday (n=3) (%RSD)	1.613	0.700
Interday (n=3) (%RSD)	4.03	3.05
LOD (µg/ml)	0.546	0.240
LOQ (µg/ml)	1.65	0.729

The suggested techniques for routinely determining the amounts of paracetamol and metronidazole in tablet formulation were found to be quick, easy, and accurate. Recovery studies were conducted in order to examine the validity and reproducibility of the suggested procedures. The linearity, accuracy, precision, specificity, and repeatability of the procedures were all validated. The simultaneous estimate of paracetamol and metronidazole in a combination dose form can be accomplished with success using both the approaches.

CHAPTER-6

SYNTHESIS OF CARBOXY METHYL ALGINATE FROM SODIUM ALGINATE

6. Synthesis of carboxy methyl alginate from sodium alginate

Sodium alginate was derivatized to sodium carboxymethyl alginate having O-carboxymethyl substitution of following the method reported previously[107]. In brief, the process of producing sodium carboxymethyl alginate which comprises suspending required quantity of sodium alginate in an ice-cold deionized aqueous solution of not more than 60% w/v sodium hydroxide. Then the dispersion was kept at 5-8 ℃ for 1h. After that the resulting solution was mixed with 1.5 gm of monochloroacetic acid solution and the temperature was raised slowly to 15-18℃, after 30 mins, temperature was increased to 75℃ and maintained for additional 30 mins. Wetted mass was collected and washed with 20ml 80% methanol three times and maintained pH at neutral by glacial acetic acid and dried at 50-60℃, dried product was milled, washed with 80% methanol and redried[107].

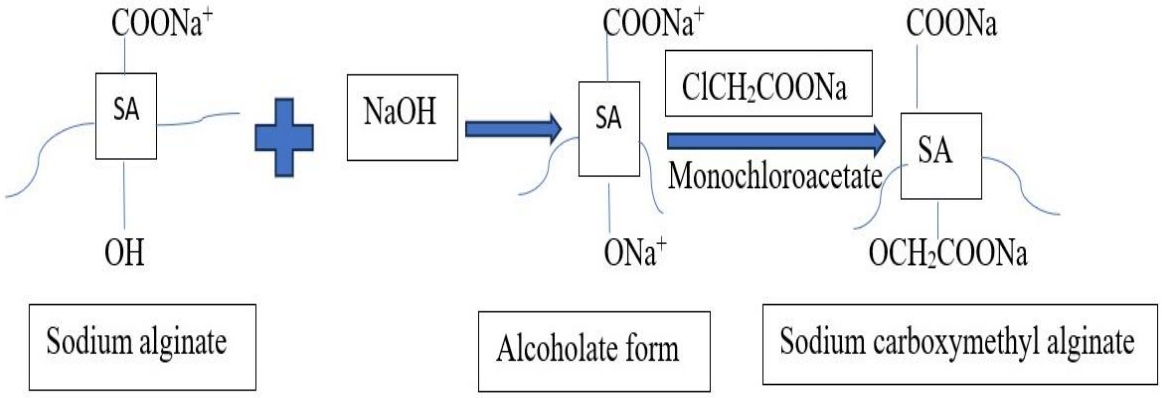


Figure 28: Schematic presentation of carboxymethylation of sodium alginate

6.1 Characterization of sodium carboxy methyl alginate:

6.1.1 Sodium alginate chemical modification: Chemical modification has been done by treating the commercially available sodium alginate with concentrated sodium hydroxide in presence of lower aliphatic alcohol and reacting the resulting alginate with monochloroacetic acid (MCA) to get carboxymethyl alginate. Carboxymethylation of sodium alginate by this method has shown that the number of carboxyl group per C_6 was enhanced about 50% over the natural uronic carboxyl content in sodium alginate[107]. Carboxymethylation imparts more

anionic nature to sodium alginate which make it compliant to interact with metal ion to form water insoluble hydrogel[108].

6.1.2 Degree of substitution: The degree of carboxymethylation of sodium alginate was determined using acid–alkaline titrimetric. In brief, aqueous dispersion of carboxy methyl alginate (0.4%, w/v) was refluxed with sodium hydroxide (0.5N, 30 ml) at 40°C. 2 drops of phenolphthalein indicator were added to the above dispersion and then excess alkali was back titrated against 0.4 N HCl until the endpoint was reached. The consumed volume of HCl was recorded. At the same time, a blank titration of sodium alginate(unmodified) was done. Then the degree of substitution was determined using following equation[109].

$$W = \frac{(V1-V2)*M*N}{Weight\ of\ sample\ (g)*1000} \times 100 \ldots\ldots\ldots 1$$

$$\text{Degree of substitution} = \frac{190*W}{100*M-[(M-1)*W]} \ldots\ldots 2$$

Where V1 and V2 are the volumes of titrant used for unmodified polymer and modified polymer, M is the molecular weight of mono chloroacetic acid, N is the normality of HCl solution, and 190 represents the molecular weight of MG (Mannuronic and guluronic residues) repeating unit, and W is the % ester substitution.

6.1.3 Acid neutralization test: An accurate volume (5 mL) of the 0.2M carboxy methylated alginate suspension was measured into a 25 ml beaker. The suspension was then transferred into a 250 ml beaker and made up to 70 ml with distilled water and stirred for one minute. An accurate volume of 30 ml of 0.1 N HCl was pipetted into the suspension whiles stirring for 15 mins. The excess HCl was titrated with 0.5 N NaOH (VS) to attain a threshold pH of 3.5. The number of milliequivalent (mEq) of acid consumed per gram of antacid was calculated

The acid neutralizing capacity (ANC) was calculated using Equation 3 1Total mEq = (30 x N HCl) – (V NaOH x N NaOH) ……. Equation 3

Where N HCl and N NaOH are the normalities of HCl and NaOH, respectively, and V NaOH is the volume of NaOH used for the back titration.

6.1.4 Rheological property: Viscosities of 2% w/v dispersion of sodium alginate and sodium carboxymethyl alginate were determined using a rotational viscometer (RHEOPLUS/32 V3) with spindle no M3 at different shear rate.

6.1.5 FTIR: FTIR spectra of sodium carboxy methyl alginate was recorded in a FTIR spectrophotometer (Perkin- Elmer, model Spectrum RX-1, UK). Sample was mixed with KBr and converted into disc at 100 kg pressure using a hydraulic press. The spectra were recorded within 4000-400 cm-1 wave numbers.

CHAPTER- 7

DEVELOPMENT AND EVALUATION OF IPN BEADS CONATINING PCM-MET (RESULT AND DISCUSSION)

7.1.1 Degree of substitution:

The degree of carboxymethylation of sodium alginate was determined using acid–alkaline titrimetric. It was found that the degree of substitution of modified sodium alginate (CMA) was found to be as 1.106±0.5649 as mentioned in the table no 1.

Table 10: Degree of substation of the modified sodium alginate (sodium carboxy methyl alginate)

Strength of NaOH	Total volume	Volume of 0.4N HCL consumed for blank	Volume of 0.4N HCL consumed for modified alginate	Degree of substitution	Mean± SD
0.5N	30ml	9	8	0.9588	
0.5N	30ml	8	7.5	0.5	1.106±0.5649
0.5N	30ml	9.5	8	1.86	

7.1.2 Acid neutralizing capacity (ANC):

The ANC of both sodium alginate and sodium carboxy methyl alginate were determined against 0.1 N HCl and it was found that the ANC of both were found in the range of 5ml to 12 ml. Sodium carboxy methyl alginate reached the neutralization point after addition of 5 ml of 0.02 M sodium carboxy methyl alginate solution whereas the sodium alginate did not attain neutralization point after addition of 5ml of 0.02M sodium alginate solution , it only reached up to pH 3.45 is mentioned in the table 2. So, it clearly showed that CMA achieved the neutralization point at a faster rate. So, we can conclude that the modified sodium alginate (CMA) has the higher acid neutralization capacity.

Table 11: Comparison of ANC between sodium alginate and CMA

Time	Added buffer (CMA) in ml	Changed pH by CMA	Added buffer (SA) in ml	Changed pH by SA
5	0.5	1.91	0.5	00
10	1	2.83	1.5	1.25

15	1.5	3.89	1.5	1.31
30	2	4.47	2	1.46
45	2.5	4.71	2.5	2.15
60	3	4.84	3	2.33
75	3.5	4.95	3.5	2.45
90	4	5.45	4	3.15
105	5	7.05	5	3.45

7.1.3 Rheological properties:

The rheological behaviour of sodium alginate and sodium carboxy methyl alginate has been represented in the below table 3. The apparent viscosity changed significantly when different shear stresses were applied, it was observed that the higher the shear, the lower the viscosity. It was also found that the viscosity of sodium carboxymethyl alginate was always lower than that of sodium alginate. The marked shear thinning behaviour of sodium alginate may be explained by conformational status of polymer molecule. The nature of the interaction among sodium alginate molecule in aqueous solutions is not certain, although both hydrogen bonding and ionic interactions are believed to be involved. In salt free solution, viscosity is built through the entanglement of random polymer coil, to the extent allowed by mutual repulsion of the negatively charged side chains[110]. However, introduction of carboxymethyl group into sodium alginate causes the chain ionization to a greater extent and prevents association due to columbic repulsion. This repulsion reduces the effective ionic interaction to some extent thereby reducing the viscosity of sodium carboxy methyl alginate and consequently, the rheogram of sodium carboxy methyl alginate solution remains below that of sodium alginate.

Table 12: Comparison of viscosity at different shear rate between sodium alginate and sodium carboxy methyl alginate

Shear rate	Viscosity of sodium alginate (poise)	Viscosity of carboxymethyl alginate (poise)
0.00999	7.35	1.75
0.0146	6.99	1.35
0.0215	6.48	1.13
0.0.0316	6.08	1.28

0.0.0464	5.72	1.78
0.0681	5.46	0.845
0.1	5.27	1.11
0.147	4.96	0.924
0.215	4.7	0.906
0.316	4.49	0.903
0.464	4.29	0.918
0.681	4.08	0.906
1	3.89	0.902
1.47	3.72	0.887
2.15	3.5	0.857
3.16	3.31	0.85
4.64	3.1	0.827
6.81	2.89	0.811
10	2.69	0.789

7.2 Preparation of Hydrogel Beads:

The required amount of PCM and MET were dispersed in dispersion of SA, SCMA, or a definite mixture of the polymers. The above uniform dispersion was poured in the calcium chloride, aluminium chloride solution using 22G flat-tip hypodermic needle and allowed to form hydrogel beads for a definite period of time. Formed hydrogel beads were collected by filtration, washed with deionized water to remove the free crosslinking agents on its surface, dried at room temperature and kept in a desiccator until used.

Few formulation variables were considered which are-

a. **Concentration of Sodium Alginate**- 2%, 2.5%, 3% w/v
b. **Drug amount-**
    - Paracetamol- 100, 150 mg.
    - Metronidazole- 50, 150 mg.
    - Combination- Metronidazole: Paracetamol – 50:100.
c. **Concentration of crosslinking agent**- 1%, 2% w/v

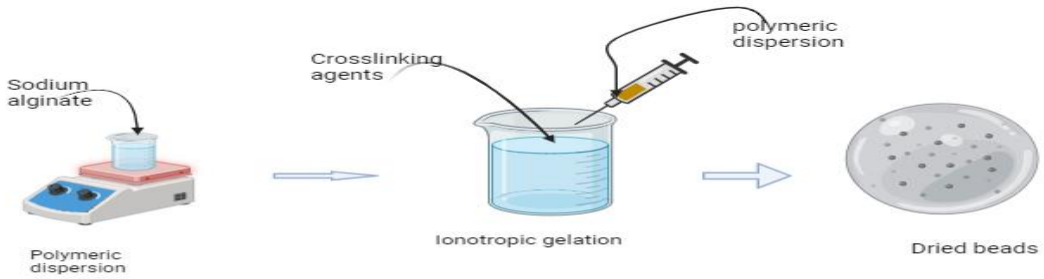


Figure 28: Schematic diagram of preparation of sodium alginate beads

Table 13: Formulation table

Formulation code	SA (%) + CSA (%)		$CaCl_2$ (%) + $AlCl_3$ (%)		Drug (mg)		% yield	% DEE		DC	
					PCM	MET	82.6%	MET	PCM	MET	PCM
C1	2	1	0	1	100	50	85.3%	34.3	49.3	82.6	82.6
C2	2	1	0	2	100	50	77.3%	25.0	32.3	85.3	85.3
C3	2.5	0.5	0	2	100	50	94.6%	29.9	44.6	87.27	87.27
C4	3	0	0	2	100	50	88%	27.7	41.02	92.72	92.72
C5	2	1	1	0	100	50	92%	35.9	42.28	88	88
C6	2	1	2	0	100	50	90%	36.5	39.8	98.66	98.66
C7	2.5	0.5	2	0	100	50	95.3%	50.9	59.2	94.6	94.6
C8	3	0	2	0	100	50	96.6%	64.1	76.1	96.7	96.7

7.2.1 Particle size analysis:

The particle size of the prepared paracetamol and metronidazole loaded calcium alginate-carboxymethyl alginate (CA-CMA) IPN beads was measured using a digital vernier caliper (Make: Mitutoyo Digmatic Caliper), with an LCD display with 0.01-mm readability. It is a useful tool to measure spherical and cylindrical objects and extensively used in pharmaceutical

industrial sector. The beads were randomly selected for particle size measurement and the mean diameter of the beads of each batch was recorded as per the equation.

$$X=\sum(Xi)/N$$

where X is the mean bead diameter, X_i is the individual bead diameter, and N is the number of beads selected for particle size measurement.

Table 14: Mean diameter of prepared hydrogel

Formulation code	Calcium chloride (%)	Aluminium chloride (%)	Particle size (mean diameter)
C1	0	1	2.055±2.02215
C2	0	2	2.2365±2.2070
C3	0	2	2.107±2.0869
C4	0	2	1.6085±1.5728
C5	1	0	1.029±1.00436
C6	2	0	1.116±1.0909
C7	2	0	0.997±0.981
C8	2	0	1.108±1.08575

It was clearly observed that the formulation batch containing high concentration of alginate polymer obtained larger beads compared to the batches containing lower concentration of polymer. This occurred due to the high viscosity of higher concentration of polymer leading to bigger droplet size from same needle [111]. Higher concentration of crosslinking agent also contributes to size increase of the beads, as they tend to adhere to the surface and increase its bulk [112].

7.2.2 Morphology of sodium alginate beads:

Al^{+3} crosslinked beads exhibit early stages of disintegration and a reddish-brown colour in simulated fluids. Ca-SALS crosslinked beads exhibit some stability in SGF concurrently. The shape of the Al crosslinked beads was distorted during drying due to shrinkage of the beads and the dried beads were not exactly spherical where Ca^{2+} crosslinked beads were observed as spherical in nature during and after the drying[113].

Figure 29: $CaCl_2$ gelled beads

Figure 30: $AlCl_3$ gelled beads

7.4 Drug entrapment efficiency:

Accurately 100 mg of dried bead of each formulated batches were dispersed in phosphate buffer solution (pH 7.4) and kept for 1h. Then the above swelled beads were crushed using mortar and pestle and kept in a mechanical shaker for 2 hours under vigorous shaking in order to disintegrate the alginate matrix and to release the drug encapsulated with the hydrogel beads. The solution was filtered using Whatman's filter paper and the drug content was determined using UV spectrophotometer at 243nm and 320nm for paracetamol and metronidazole respectively. The average drug entrapment efficiency was found to be in the range of 30%-76% varied according different variable like, crosslinking agent concentration, different CA, polymer concentration, incorporation of IPN, curing time etc were mentioned in the table 3.

It was found that the DEE was enhanced upon increasing concentration of polymer up to 2.5% mention in the table. It was also found that the DEE was improved with increasing concentration of crosslinking agent ($CaCl_2$) up to 2%, where there is no significant difference in % DEE with increasing in $AlCl_3$ crosslinking agent[114]. Incorporation of IPN (sodium carboxymethyl alginate) showed improved DEE% as compared to only sodium alginate polymer[11]. Different curing time (5mins,15mins and 30 minutes) have different effect on drug entrapment efficiency. Batch formulated of 5 minutes curing time has showed better %DEE as compared to 15 mins and 30 minutes due to high water solubility and leaching of the drugs into the crosslinking agent's solution[39].

Table 16: Drug entrapment efficiency of the formulated beads in pH 7.4

Formulation code	Drug entrapment efficiency	
	Paracetamol	Metronidazole
C1	34.8%	51.31%
C2	25.38%	34%
C3	30.28%	46.46%
C4	27.7%	41.02%
C5	35.9%	42.28%
C6	36.6%	39.8%
C7	50.91%	59.2%
C8	64.1%	76.1%

7.2.3 Swelling study:

One of the most important characteristics of drug carriers is their swelling behaviour, which ensures that fluid will enter the polymer matrix and release the medication as a result. The pH of the environment strongly influenced the swelling behaviour of beads. Swelling study was carried out in pH1.2 and pH 7.4 buffer using a tea bag.

Table15: Swelling index of different batches in pH 1.2 (2hrs) and pH

Formulation code	Swelling index (%) with respect to time (minutes)					
	15	30	45	60	120	180 (pH7.4)
C1	20%	35%	50%	70%	80%	170%
C2	15%	30%	40%	55%	70%	130%
C3	10%	20%	35%	40%	60%	118%
C4	25%	30%	45%	60%	75%	125%

The swelling profile of hydrogel beads prepared from sodium alginate and carboxymethylated alginate could not be obtained as it was swelled very fast and eroded within 10 minutes after introduction into pH 7.4 buffer solution. However, the swelling profile of prepared alginate beads were carried out in pH 1.2 for 2 hours and then it was continued in pH 7.4 buffer solutions are shown in the above table. Such profiles were evident in both test media exhibiting higher

swelling in 7.4 buffer than in 1.2 acid solution. This increased % swelling of sodium alginate beads in pH 7.4 may be due to the presence of carboxylate anion group in sodium alginate network. Strong electrostatic repulsive force between negative charge of carboxylate anion of sodium alginate molecule resulted in longer intermolecular distance that could be reason for higher % swelling index of sodium alginate beads in pH 7.4. But in acidic medium, there is formation of strong hydrogen bonding between carboxyl group and hydroxyl group of sodium alginate beads that leads to formation of compacted sodium alginate molecule. As a result, less amount of water can penetrate into the hydrogel beads in pH 1.2 and consequently the reduced %SI was observed.

7.2.4 In vitro drug release study:

In vitro release studies of prepared microbeads were carried out using USP type 2 dissolution (paddle method) apparatus at 50 rpm. The release studies were performed in both stomach and intestinal environment. The dissolution was measured at 37 ± 1◦C under 75 rpm speed. Accurately weighed quantity of hydrogel beads were added to 700ml 0.1N HCl (pH1.2). The dissolution test was carried out for first 2h in simulated gastric fluid then, continued in 900 ml phosphate buffer (pH7.4) for next 3hrs.

7.2.4.1 Effect of polymer concentration:

Hydrogel beads were prepared by taking different concentrations of sodium alginate (2%, 2.5%, 3% w/v). Beads containing 2% w/v polymer concentration showed less sustained release effect and beads containing 3% w/v polymer had more sustained effect in drug release. Higher concentration of polymer results in higher viscosity of the dispersion, which led to an increase in the diffusional resistance to the drug molecules moving from the organic phase to the aqueous phase, thereby increasing the amount of drug molecules entrapped into the polymer matrix of nanoparticles. This leads to the higher sustained effect of releasing drug from the hydrogel beads.

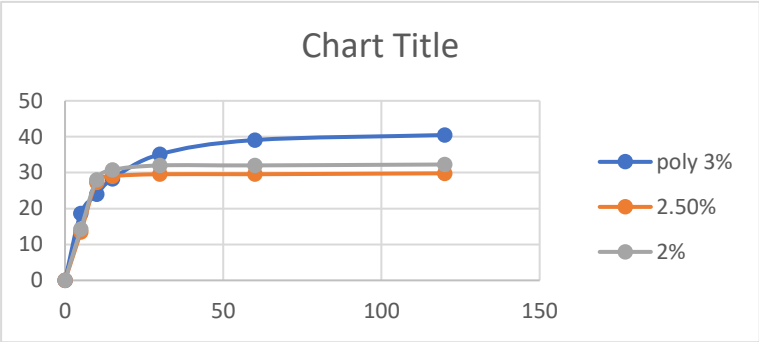


Figure 31: Effect of polymer concentration (SA)

7.2.4.2 Effect of IPN:

It was found that the drug release from Al-alginate and Al-CMA was faster compared with the IPN assisted beads. As the concentration of sodium alginate was increased, drug release decreased up to 2.5%.

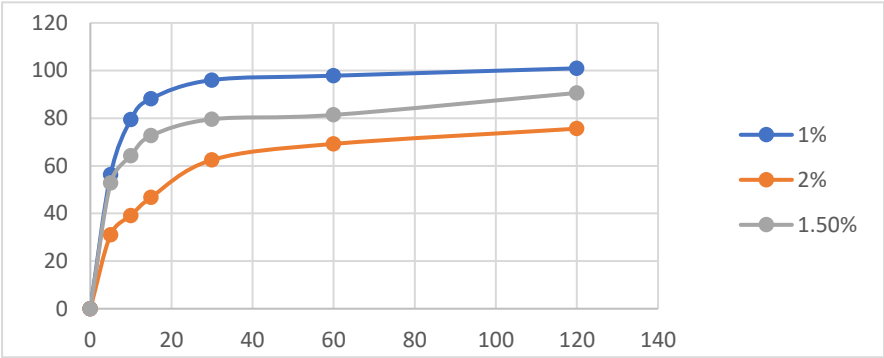


Figure 32: Effect of IPN on drug release

7.2.4.3 Effect of crosslinking agent: Drug loaded beads made up of carboxymethyl alginate, sodium alginate or different weight ratio of the two polymers were prepared by ionotropic gelation using $CaCl_2$ and $AlCl_3$ as crosslinking agents. Drug release study showed that the drug release from beads gelled with aluminium chloride was faster compared to the beads gelled with calcium chloride. Drug release from the hydrogel beads is inversely proportional to the

crosslinking agent concentration. Hydrogel beads formed with 2% concentration of aluminium chloride showed sustain release over less concentration of the same[60].

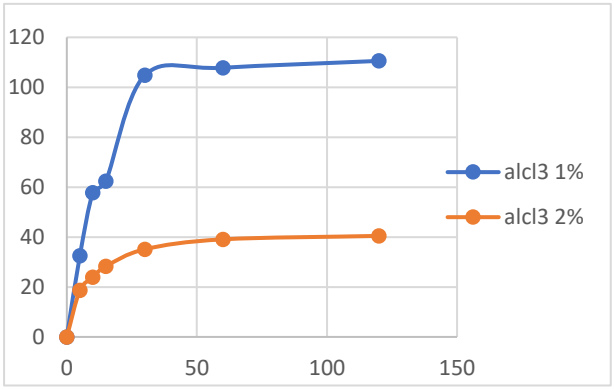


Figure 33: Effect of concentration of $AlCl_3$

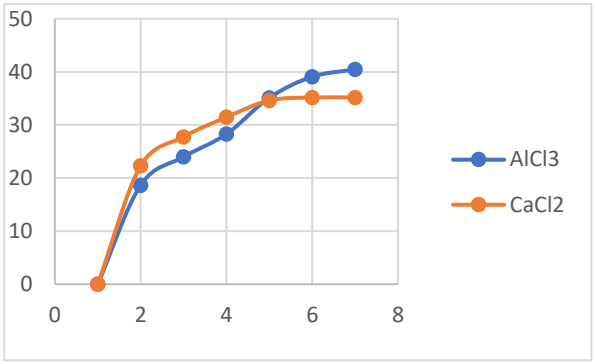


Figure 34: Comparison between $AlCl_3$ vs $CaCl_3$ on drug release

Effect of drug amount:

Drug loaded beads were prepared by taking two amounts of amoxicillin trihydrate and paracetamol- 5:3 and 1:1 ratio. 5:3 ratio showed more entrapment and more sustained effect of the drug release, whereas when 1:1 ratio was taken it shoed lesser entrapment as well as decreased sustained effect. It was found that drug releases within the 1st hour were indicative of a burst effect. This could be attributed to the highly water soluble nature of the drugs [40].

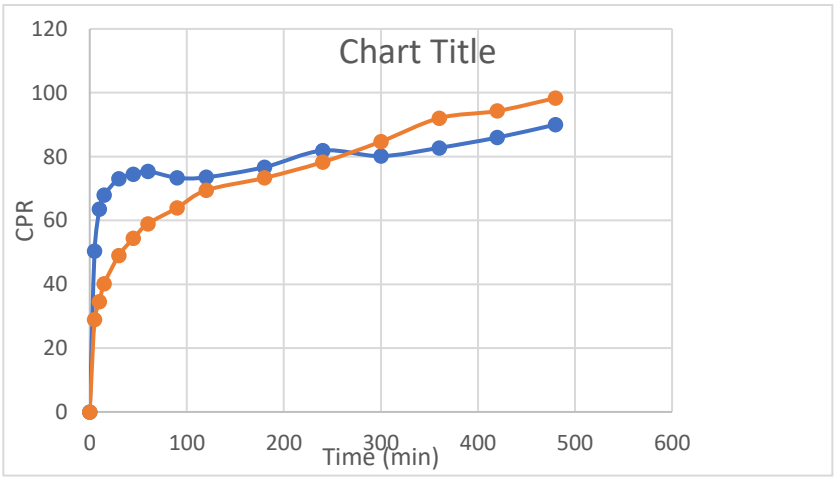


Fig- Effect of drug amount

7.3 Drug release kinetics of developed beads:

Table 6: Drug release kinetics of developed beads

Formulation code	Zero order	First order	Higuchi	Korsmeyer-Peppas
	R^2	R^2	R^2	R^2
F1	0.9632	0.5987	0.9632	0.9751
F2	0.884	0.6548	0.954	0.6548
F3	0.884	0.6598	0.884	0.6598
F4	0.8427	0.6026	0.8427	0.6026
F5	0.9712	0.6725	0.9712	0.6725
F6	0.8428	0.5997	0.8428	0.5997
F7	0.9531	0.6901	0.9531	0.6901
F8	0.8522	0.712	0.8522	0.712
F9	0.97	0.8266	0.97	0.8266
F10	0.9428	0.6837	0.9428	0.6837

All the in-vitro dissolution data were analyzed by zero order, first order and Higuchi equation for interpretation of the drug release pattern from the developed hydrogel beads. From the (Table 6) it was found that the drug release was best explained by Higuchi's kinetics up to 2 hours in 0.1N HCl followed by in phosphate buffer, as the plot showed highest linearity for beads.

7.4 In vitro antimicrobial activity assessment:

The developed hydrogels were screened for antibacterial activity by the plate diffusion test in Mueller-Hinton agar plates. Gram-positive bacteria Staphylococcus aureus ATCC 29213 and gram-negative bacteria Escherichia coli ATCC 25922 were used. Then the zone of inhibition of metronidazole (API) taken as standard and prepared hydrogel beads (powder form) containing metronidazole taken as test sample here. ZOI were measured against above

mentioned bacterial strain using slide calipers (RS PRO, China). ZOI for *E. coli* and *S aureus* was found for std- 13mm (Mean Diameter), test- 16mm (mean diameter) and std -22mm (mean diameter) test-25mm (mean diameter) respectively. It was found that the test sample have more antibacterial activity than standard sample (metronidazole (API)[76].

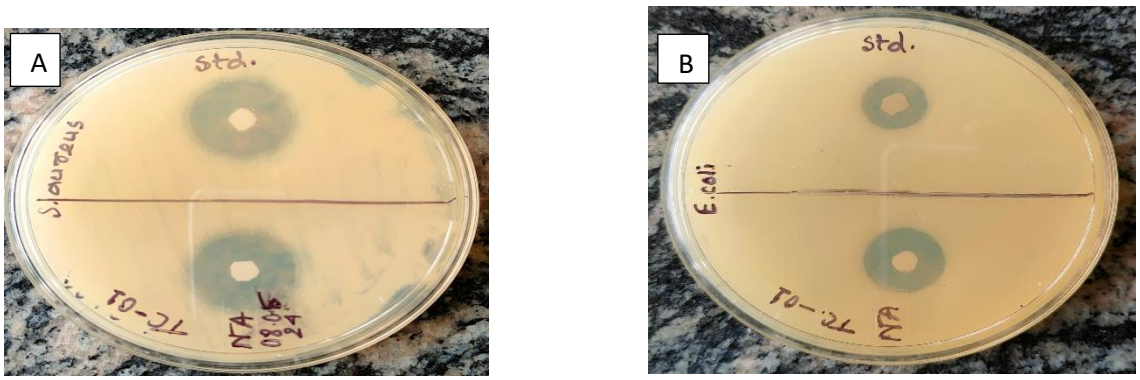


Figure 35: Antibacterial activity of metronidazole loaded beads and std metronidazole against (A) S. aureus and (B) E. coli

The effect of prepared formulation (sodium alginate and metronidazole) showed better inhibitory effect as compared to the metronidazole standard API was mentioned in table 4. The reason may be due the sodium alginate giving extra antibacterial effect[107]. It has Carboxylate group (COO-) and protonated hydroxyl(-OH) groups, which give extra electrostatic effect and hydronium interact with anionic phospholipid present on bacterial cell wall[76].This combined effect generated by both ion increases the permeability of the cell wall in bacteria, encourages release of nucleic acid, glucose, lactate dehydrogenase from cell as well as interfere transport of nutrient to cells and gradually causing bacterial death. Due to the synergistic effect of test sample (formulated beads containing sodium alginate and metronidazole), showed better inhibitory effect as compared to standard (metronidazole API) with gram negative bacterial (EC) most prominent compared to SA.

Table*:* Antibacterial activity of metronidazole loaded beads and std metronidazole against (A) *S. aureus* and (B) *E. coli*

Bacteria	Gram positive SA		Gram negative EC	
	Std (MET)	Test (beads MET)	Std (MET)	Test (MET loaded beads)
Zone of inhibition -1(mm)	24	22	13	16

Zone of inhibition -II (mm)	23	25	13	16
Zone of inhibition -III (mm)	26	22	14	17

7.5 Fourier Transform Infrared Spectroscopy (FTIR):

The overlaid IR spectra of purified sodium alginate and carboxymethyl alginate (CMA) are shown in figure 36. FTIR spectra of isolated sodium alginate and standard showed mannuronic acid functional group at wavenumber 884 cm^{-1} and the uronic acid at wavenumber 1044.95 cm^{-1}, OH functional group at wavenumber 3200-3400 cm-1, and CH2 stretching at wavenumber 2928 cm-1. The asymmetrical and symmetrical vibrations due to moiety were assigned to 1615 and 1429 cm-1, respectively, which may be attributed to the incorporation of carboxymethyl groups into the guar molecule.

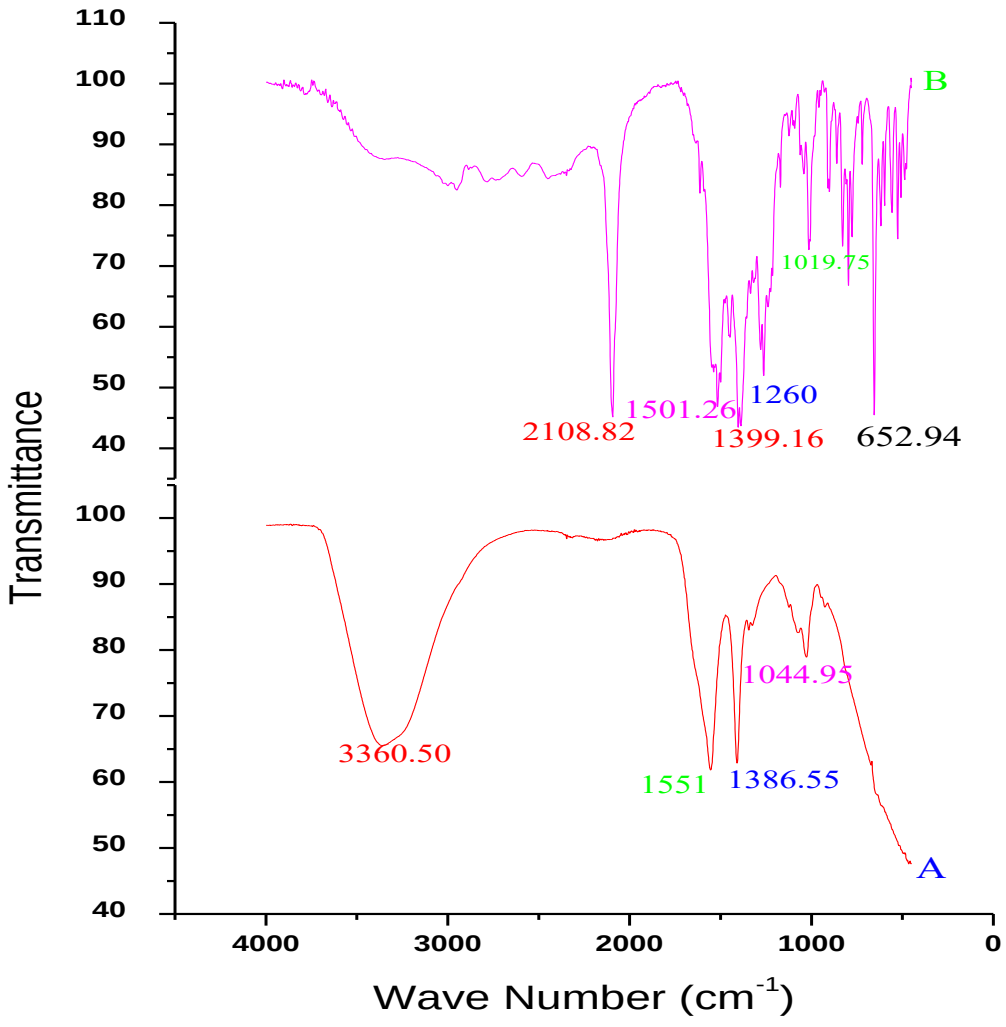


Figure 36: FTIR spectra of sodium alginate(A) and Carboxy methyl alginate (B)

The major peaks for metronidazole were observed at 1504 cm^{-1},1708 cm^{-1},2317 cm^{-1},3752 cm^{-1} and 3871 cm^{-1} for N=O, C=C stretching, CH_2 bending, OH, stretch of H bond of alcohol respective. The major peaks for paracetamol were observed at- 1670 cm^{-1}, 2317 cm^{-1}, 3727 cm^{-1}, 3859 cm^{-1} for C=O stretching of amide, CH3 stretching, OH, stretching of COOH, OH, stretch of H bond of alcohol respectively [178]. Our formulation clearly shows the major and minor peaks of amoxicillin and acetaminophen which indicates the incorporation of drugs in the beads. The appearance of peaks without any vibrations also indicates the compatibility of drugs with the sodium alginate.

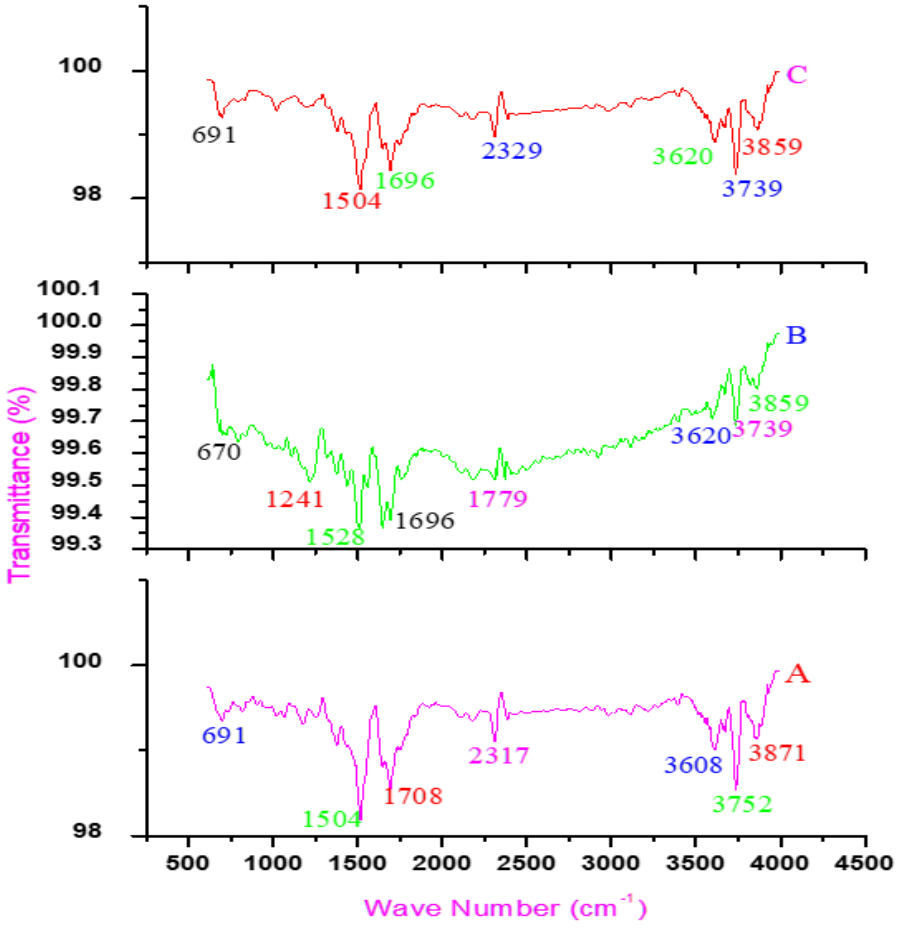


Figure 37: - FTIR analysis of (A) Metronidazole (B) Paracetamol (C) formulation containing both the drug

7.6 X-ray diffraction analysis (XRD):

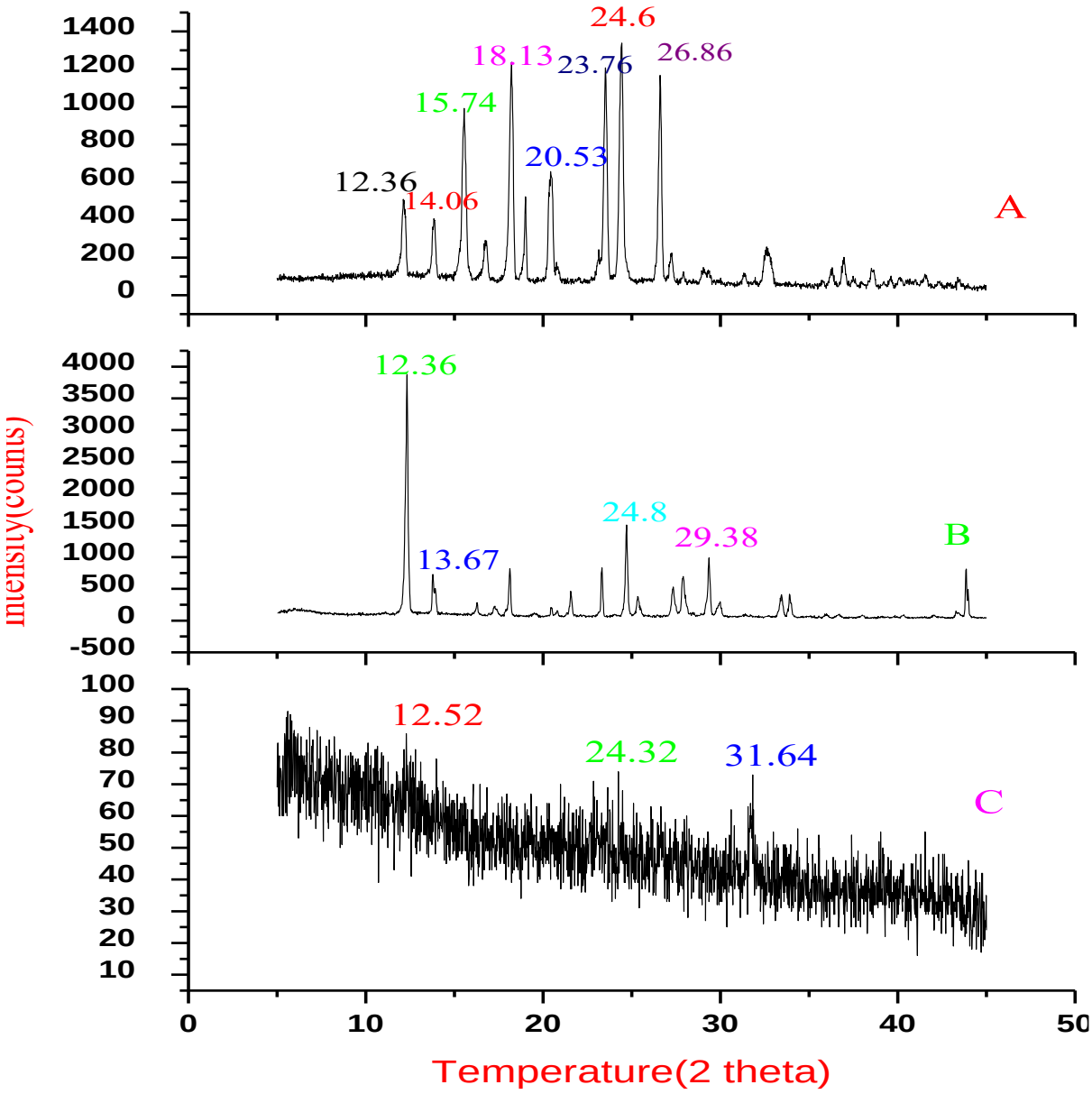


Figure 38: XRD patterns of pure (A) Paracetamol, (B) Metronidazole and (C) Drug loaded alginate beads

The diffraction patterns were obtained at 12.36,14.06,15.74, 18.13, 20.53, 23.76, 24.6, 26.86° angles for pure paracetamol and the intensities were at 489.68,420,974,1211,618,1160.93,1309 and 1170 respectively for pure paracetamol where for the metronidazole the diffraction angles were recorded at 12.36, 13.67, 24.8,29.38° and the associated intensities were found at 3853,745.85,1332 and 982.186 respectively. The peak intensities of both the paracetamol and

metronidazole in hydrogel formulation were significantly weaker (12.52,24.32,31.64) than the pure paracetamol and metronidazole. These weak intensities, suggesting a lower degree of crystallinity of drug in formulation than that of the individual drug. It indicated that the crystallinity of both drugs got reduced in the formulation and became amorphous in nature though retain a little bit crystallinity. The similar diffractograms confirmed that there was no physical instability in beads containing paracetamol and metronidazole, the drug was found to be compatible with the other ingredients of the hydrogel formulation.

7.7 Differential scanning calorimetry (DSC):

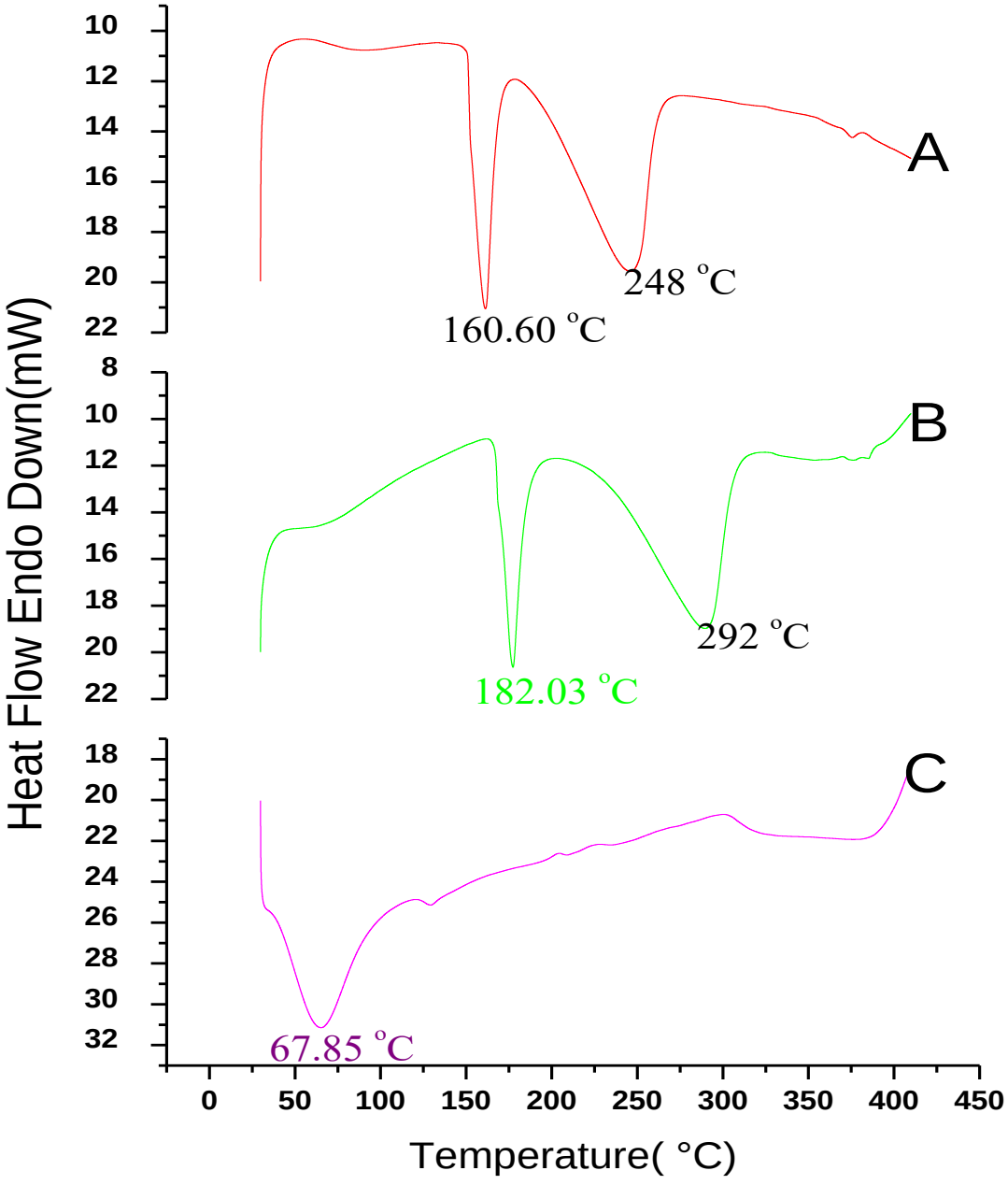
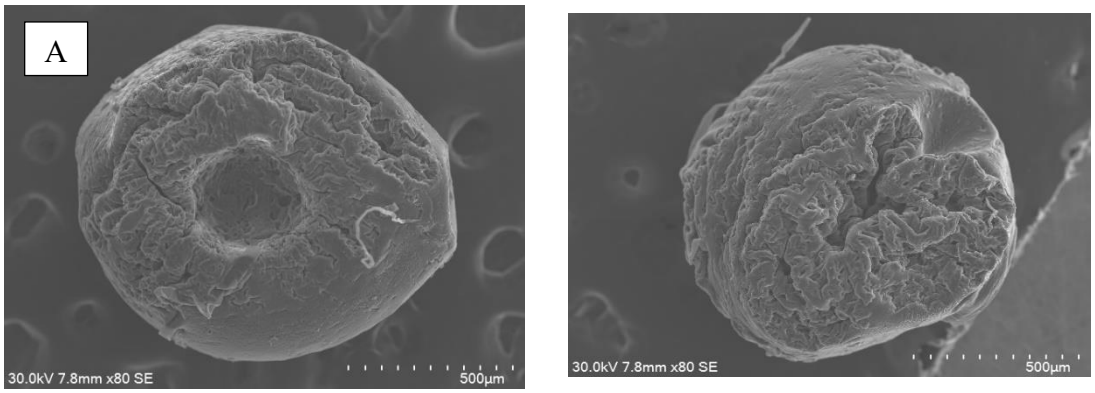


Figure 39: DSC thermogram of (A) Metronidazole, (B) Paracetamol and (C) Drugs loaded alginate beads

The thermogram shows that pure paracetamol (B) and metronidazole (A) gave only one sharp endothermic melting peak at 182.03℃ and 160.60 ℃ respectively while the hydrogel formulation containing both the drugs gave only one sharp melting peak at 67.85℃. It was noticed that the drugs loaded formulation gave only one sharp melting peak at 67.85℃, which is significantly lower than the any of the loaded drugs. The reduced endotherm of tablet formulation indicates that the crystallinity of the drug decreased in the formulation and tends to the formation of amorphous nature and also the moisture content of the formulation got increased.

7.8 Scanning electron microscopy (SEM):

The surface morphology of the prepared beads was studied by scanning electron microscopy (SEM) and the SEM photographs are depicted in the figure 11. SEM photographs of the blank beads compared with drug loaded beads show a difference in surface morphology. The SEM monograph of blank beads (Figure 1[A]) showed a regular smooth surface on the beads surface whereas the SEM monograph of drug loaded beads (Figure 11[B]) showed an irregular rough surface with dispersed drug particles. The surfaces of these beads were appeared to have rough with characteristic large wrinkles and cracks, as it was evident from the SEM photographs. This might be caused by partly collapsing the polymeric gel network during drying. Moreover, few polymeric debris and drug crystals were seen on the bead surface. Presence of polymeric derbies on the bead surface could be due to the simultaneous gel bead preparation and formation of the polymer-blend matrix; whereas the presence of drug crystals on the bead surface might be formed as a result of their migration along with water to the surface during drying.



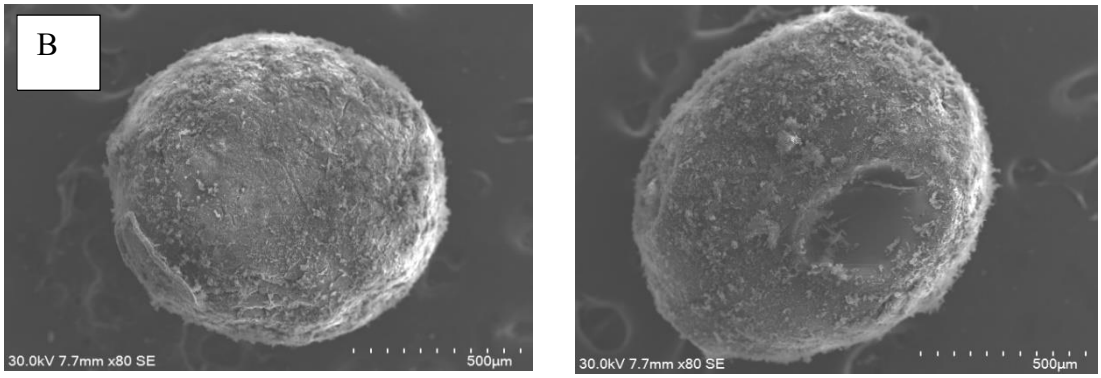


Figure 40: SEM photograph of blank alginate bead (A) and drug loaded bead (B)

CHAPTER -8
CONCLUSION

Conclusion:

After performing the above experimentation, it is concluded that formation of sustained release hydrogel beads containing metronidazole and paracetamol was successfully conducted and their evaluation tests produced desirable results. Our study was divided into two parts- method development and formulation development. A simultaneous equation method was used for development of determination of metronidazole and paracetamol from their combination.

Since the last few decades mankind has been directly and indirectly dependent on antibiotics and antipyretics for control and prevention of infectious diseases with hyperthermia, starting with common cold, UTI, RTI, stomach ulcer, skin infection etc. During the outbreak of COVID-19, the world faced a terrible pandemic situation where people were directly dependent on antibiotics and antipyretic to overcome and cure the symptoms of coronavirus infection. The aim of our experiment was to combine an antibiotic (Metronidazole) and an antipyretic drug (Paracetamol) and make a sustained release formulation of the combination to administer in microbial infection with hyperthermia.

This experiment has investigated a simple, quick, cost effective and easy technique for the estimation of Paracetamol and Metronidazole in combination by simultaneous equation method. The method was validated by ICH guidelines Q2R2. The findings suggest that the following method is linear (R2 values are >0.99), precise (%RSD values are lesser than 2%), accurate (%recovery falls under the range of 70-120%). These results have important implications for method validation and provide valuable insights into the simultaneous estimation of both the drugs separately as well as in a combination (at lambda max 264 nm). Moving forward, further research is warranted to explore the synergistic properties of the two drugs, paracetamol and metronidazole combined in a single dosage form, can be administered in bacterial infections accompanying fever like- urinary tract infection, stomach ulcer, skin infections, lower respiratory tract infection, which is not yet available in the market. Overall, this study contributes to the understanding of simultaneous estimation of metronidazole in presence of paracetamol in pure drug form, combination and marketed dosage form.

The formulation development was done by fabricating sodium alginate beads crosslinked with calcium chloride salt by dropping gel method (ionotropic gelation). The drugs were incorporated in the alginate solution for incorporation into the beads. The resulted beads were evaluated based on various parameters. The entrapment efficiency of both the drugs were in the range of 33-76% in our optimum batch (code-C8; SA-2.5%, CaCl2-2%, MET-50mg, PCM-100mg). The beads show a burst release in the first 60mins which acts as the loading dose and then it is sustained up to 8hours (release up to 99%). The swelling study concludes up to 60% (code-; SA-2.5%, CaCl2-1%, MET-50mg, PCM-100mg) swelling index in acidic pH, which is the least sweeling index. The TG/DTA studies shows decrease in temperate of formulation than pure drug which indicates incorporation of drugs in the formulation causes changes in the thermogram of the drugs. The XRD pattern confirms that drug crystallinity

changes to amorphous when formulated with alginate, as there is no sharp peak observed. The SEM image shows clear incorporation of drugs in the beads. FTIR analysis confirms that both the drugs are distinctly present in the formulation and their compatibility of the drug with the excipients. The antimicrobial study obtains zone of inhibitions which indicate the antimicrobial activity of metronidazole is still present after formulating with paracetamol and alginate. With the obtained results of the research work it was concluded that this formulation is suitable for sustaining the drug release from the hydrogel beads which may prevent degradation of metronidazole in the acidic environment of stomach, and increase patient compliance.

Due to the limited time several studies regarding this experiment are yet to be conducted. Preclinical animal study is needed to prove its synergistic activity as an antibiotic and antipyretic agent. The continuation of the work will include stability testing of metronidazole and paracetamol in the longer run, animal study for their combined effect and antimicrobial effect of any specific microorganism.

CHAPTER-9

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9.REFERENCE:

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