DEVELOPMENT AND EVALUATION OF HYDROGEL BEADS FOR SUSTAINED DRUG RELEASE

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EXAMINATION ROLL NO.: M4PHP23015

REGISTRATION NO.: 160259 of 2021-22

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Thesis submitted in partial fulfilment of the requirements for the

DEGREE OF MASTER OF PHARMACY

Department of Pharmaceutical Technology

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JADAVPUR UNIVERSITY,

Kolkata- 700032

Session: 2021-2023

2023

CERTIFICATE OF APPROVAL

This is to certify that ***Bikram Roy*** *bearing Registration No:* ***160259 of 2021-22*** *has carried out the research work entitled "****DEVELOPMENT AND EVALUATION OF HYDROGEL BEADS FOR SUSTAINED DRUG RELEASE****" 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* ***MASTERS OF PHARMACY*** *from Jadavpur University. I appreciate his endeavour to do 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 literature survey and original research work by me, as part of my Master of Pharmacy studies.

All information in this document have 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 material and results that are not original to this work.

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ACKNOWLEDGEMENT

I take this opportunity to humble acknowledge the contributions of my supervisor, ***Dr. Manas Bhowmik*** *(Associate Professor), Division of Pharmaceutics, Department of Pharmaceutical Technology, Jadavpur University, Kolkata for his invaluable guidance and constant encouragement to materialize this piece of work and I express my heartfelt gratitude and thankfulness for his encouragement all through.*

This acknowledgement will be incomplete if I do not express my sincere thanks to the team of our Pharmaceutics-II Laboratory, Department of Pharmaceutical Technology, Jadavpur University.

I extend my thanks to my co-guide ***Prof. (Dr.) L. K. Ghosh*** *(Professor Jadavpur University),* ***Abhijit Changder*** *for providing polymers and excipients and also my seniors of different laboratories for their help and guidance throughout my thesis work. I am also thankful to my batch mates* ***Moumoyee Chakraborty, Saban Karmakar and Rinchen Palzong Bhutia*** *and my juniors* ***Biswadeep Das, Sagar Roy, Mahesh Kumar, Snehaneel Mitra.***

I express my thanks to AICTE, for providing the financial assistance in the form of fellowship.

At last, but not the least, I express my sincere regards to my family for their inspiration and motivation throughout my studies and all they have done for me during my studies.

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CHAPTER 1

INTRODUCTION

1. INRODUCTION.

Oral administration is the most versatile, convenient, and commonly employed route of drug delivery for systemic action. Indeed, for the controlled release system, the oral route of administration has received more attraction and success because gastrointestinal physiology offers more flexibility in dosage form design than other routes [1].

Oral controlled-release dosage forms have been developed for the past few decades due to their considerable therapeutic advantages and applications. The high level of patient compliance with oral dosage forms is due to the ease of administration and handling of these forms [2].

A controlled drug delivery system provides drug release at a predictable and controlled rate to achieve higher therapeutic efficiency with minimal toxicity. Despite tremendous advancements in drug delivery, the oral route remains the preferred route for the administration of therapeutic agents, and oral drug delivery is by far the most preferable route of drug delivery because of the low cost of therapy. Each route of administration leads to high levels of patient compliance, and the gastrointestinal physiology offers more flexibility in dosage form design than most other routes. Consequently, much effort has been put into the development of strategies that could improve patient compliance through the oral route [3].

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

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 [4, 5].

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 [6]:

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

1.2. Benefits of novel drug delivery system.

- Convenience in dosing
- Higher patient compliance
- Better utilization of drugs
- Reduced adverse effects
- Improved efficacy [7].

1.3. Conventional drug therapy.

To appreciate the value of sustained-release drug delivery in therapy, it is necessary to review some aspects of conventional dosage forms and drug therapy. 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 [8, 9].

1.4. 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 [10].

1.4.1. 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 [10].

1.4.2. 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 [10].

1.4.3. Delayed release preparation.

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 gelatin 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 [10].

1.4.4. General principle of Controlled release system:

The concept of a 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 that eliminated by the body. The system usually delivers a very small amount of the truck at more frequent intervals [11].

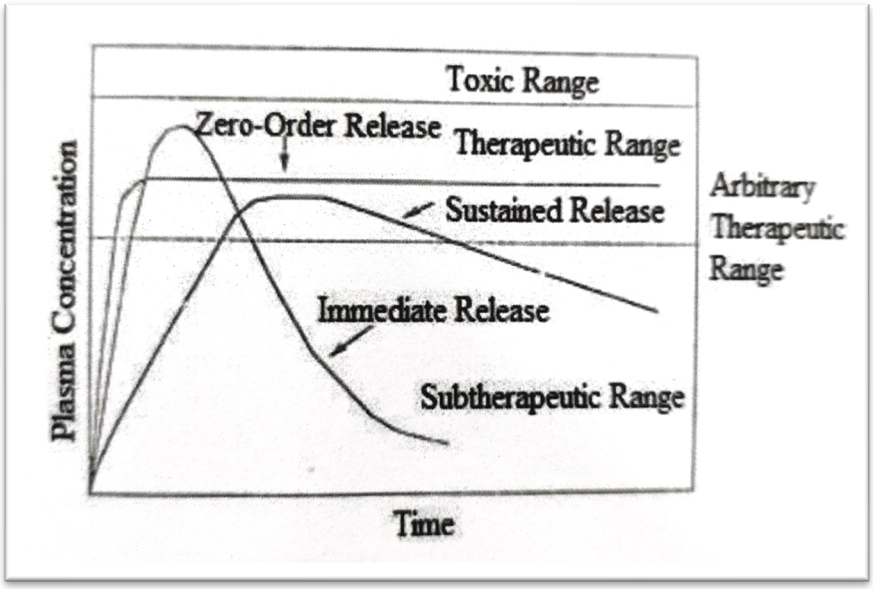


Fig. 1.1: Plasma drug concentration profiles for conventional tablet formulation, a sustained release formulation and zero order controlled released formulation.

1.4.5. Objective, advantages, disadvantages of controlled/sustained drug delivery systems.

1.4.5.1. 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 [12, 13].

1.4.5.2. 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. Minimise all local side effects.
4. Minimise or eliminate systemic side effects.
5. Obtain less potentiating or a reduction in drug activity with chronic use.
6. Minimise 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

11. Make use of special effects, for example, sustained-release aspirin for morning relief of arthritis by dosing before bedtime [12, 14].

1.4.5.3. Disadvantages.

1. 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.
2. Improper formulations may result in excessive dosage, or the drug release may not be complete.
3. In the event of accidental failure of the product, an effective antidote may be difficult to employ.
4. Sustained-release dosage forms are sometimes more expensive because of the technology involved in producing the formulation.
5. R. medication should not be used with persons known to have impaired or erratic gastro-intestinal absorption or kidney troubles.
6. Drugs having a long biological half-life or not suitable for presentation in sustained release forms, for example, digitoxin
7. There is little control in the hands of the physician as far as dose variation is concerned.
8. It is difficult to formulate an ideal sustained-release dosage form.
9. Unpredictable or poor in vitro-in vivo correlation.
10. Increase first-pass clearance [12, 15].

1.5. 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 [16].

1.5.1. Basic gastrointestinal tract physiology.

The stomach primarily aims at processing and transporting food. The stomach provides for short-term food preservation and quick consumption of relatively large meals. The primary substantial metabolism of enzymes is promoted in the stomach by proteins. 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. 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 [17].

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 in ***Fig. 1.2.***

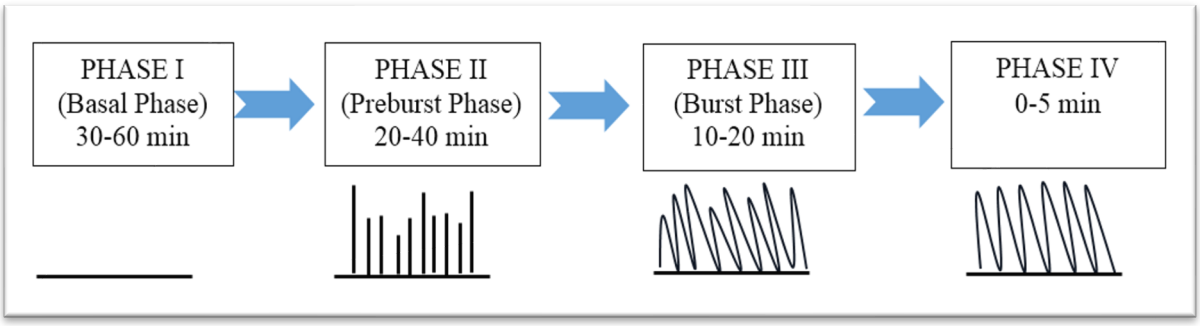


Fig. 1.2: Motility pattern of GIT.

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 is the transition period between phases III and 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 [18].

1.6. Microsphere.

Microspheres are solid, spherical particles ranging in size from 1 to 1000. They are spherical, free-flowing particles consisting of proteins or synthetic polymers that are biodegradable in nature.
There are two types of microspheres:

1. Microcapsule
2. Micro matrices

Microcapsules are those in which the entrapped substance is distinctly surrounded by a distinct capsule wall, and micro matrices are those in which the entrapped substance is dispersed throughout the microsphere matrix. Solid biodegradable microspheres incorporating a drug dispersed or dissolved through a particle matrix have the potential for controlled drug release. They are made up of polymeric, waxy, or other protective materials, such as biodegradable synthetic polymers and modified natural products [19].

1.6.1. Types of Microsphere.

1.6.1.1. Bio-adhesive Microsphere.

Adhesion can be defined as the sticking of drugs to the membrane by using the sticking properties of water-soluble polymers. Adhesion of drug delivery devices to the mucosal membrane, such as buccal, ocular, rectal, nasal, etc., can be termed bioadhesion. The term 'bioadhesion' describes materials that bind to biological substrates such as mucosal membranes. Adhesion of bioadhesive drug delivery devices to mucosal tissue offers the possibility of creating intimate and prolonged contact at the site of administration. This prolonged residence time can result in enhanced absorption, and in combination with a controlled release of the drug, it also improves patient compliance by reducing the frequency of administration. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres,

nanospheres, liposomes, nanoparticles, etc., which modulates the release and absorption of the drug. Microspheres constitute an important part of these particulate drug delivery systems by virtue of their small size and efficient carrier capacity [20].

1.6.1.2. Magnetic Microsphere.

This kind of delivery system is very important because it localises the drug to the disease site. In this case, a larger amount of freely circulating drug can be replaced by a smaller amount of magnetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres, such as chitosan, 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 [21].

1.6.1.3. Floating Microsphere.

In floating types, the bulk density is less than the gastric fluid and so remains buoyant in the stomach without affecting the gastric emptying rate. The drug is released slowly at the desired rate if the system is floating on gastric content, increasing gastric residence and causing a fluctuation in plasma concentration. It also reduces the chances of striking and dose dumping and prolongs the therapeutic effect. Drug (Ketoprofen) given through this form [22].

1.6.1.4. Radioactive Microsphere.

Radio mobilisation therapy microspheres sized 10–30 nm are larger than capillaries and get trapped in the first capillary bed when they come across them. They are injected into the arteries that lead to the tumour of interest. So these radioactive microspheres deliver a high radiation dose to the targeted areas without damaging the normal surrounding tissues. It differs from drug delivery systems in that radioactivity is not released from microspheres but acts from within a radioisotope's typical distance, and the different kinds of radioactive microspheres are emitters [23].

1.6.1.5. Mucoadhesive Microsphere.

Mucoadhesive microspheres are 1-1000 nm in diameter and consist either entirely of a mucoadhesive polymer or have an outer coating of it. The coupling of mucoadhesive properties to microspheres has additional advantages, e.g., efficient absorption and enhanced bioavailability

of the drugs due to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of the drug to the absorption site achieved by anchoring plant lectins, bacterial adhesion, antibodies, etc. on the surface of the microspheres. 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 [24].

1.6.1.6. Polymeric Microsphere.

(i) Biodegradable polymeric microspheres:

Natural polymers such as starch are used with the concept that they are biodegradable, biocompatible, and also bio-adhesive in nature. Biodegradable polymers prolong the residence time when in contact with mucous membranes due to their high degree of swelling properties in aqueous medium, which results in gel formation. The rate and extent of drug release are controlled by the concentration of polymer and the release pattern in a sustained manner. The main drawback is that, in clinical use, the drug loading efficiency of biodegradable microspheres is complex and it is difficult to control the drug release.

(ii) Synthetic polymeric microspheres:

Synthetic polymeric microspheres are widely used in clinical applications; they are also used as bulking agents, fillers, embolic particles, drug delivery vehicles, etc. and have proven to be safe and biocompatible. But the main disadvantage of these kinds of microspheres is that they tend to migrate away from the injection site, posing a potential risk of embolism and further organ damage [25].

1.6.2. Method of 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 [26].

1.6.2.1. Ionotropic gelation method.

Polyelectrolytes like sodium alginate have the ability to cross-link in the presence of opposing ions (e.g., calcium chloride solution), which results in the formation of hydrogel beads, also referred to as gelispheres. The release of drugs through gelispheres, which are spherical, crosslinked hydrophilic polymeric entities capable of substantial gelation and swelling in simulated biological fluids, is regulated by polymer relaxation. By adding a drug-loaded polymeric solution to an aqueous solution of polyvalent cations, hydrogel beads can be formed. In the drug-loaded polymeric droplets, the cations developed and created a three-dimensional lattice of ionically crosslinked moieties [27].

Fig. 1.3: Preparation of Calcium- Alginate crosslinked beads by Ionotropic gelation method.

Since naturally occurring polysaccharides acting as biopolymers are able to encapsulate numerous micro- and macro therapeutic molecules in their hydrogel network structure, ionotropic gelation is a modern method in the development of biocompatible, novel, sustained, and targeted controlled drug delivery systems. The practical application of these biopolymers is growing daily as a result of recent advances in polymer chemistry and the creation of clever, strategic encapsulation strategies. Due to the discovery of ionotropic gelation, the use of expensive and hazardous organic solvents in the micro-encapsulation process has been significantly decreased, providing an environmentally acceptable method for creating gelispheres for pharmaceutical product development [28].

1.6.2.1.1. 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.

(2) Temperature:

Temperature has an effect on both the curing time, or the time needed for crosslinking, and the size of beads produced by the ionotropic gelation process.

(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.

(5) Gas forming agent concentration:

The addition of gas-forming substances like calcium carbonate and sodium bicarbonate to the formulation to create porous gelispheres has a significant impact on the size and shape of the gelispheres. As the gas-producing agent creates porous gelispheres, it ruptures their lining and creates an uneven surface [29].

1.6.3. Applications.

1.6.3.1. Microspheres in Vaccine delivery.

An ideal vaccine must fulfil the requirements of efficacy, safety, convenience in application, and cost. Biodegradable delivery systems for the vaccine that are given by parenteral route may overcome the shortcomings of conventional vaccines. The interest in specific advantages includes:

(i) Improved antigenicity by adjuvant action

(ii) Modulation of antigen release

(iii) Stabilization of antigen [30].

1.6.3.2. Targeting using micro-particulate carriers.

The concept of targeting, i.e., site-specific drug delivery, is a well-established dogma that is gaining full attention. The therapeutic efficacy of the drug relies on its access and specific interaction with its candidate receptors. The ability to leave the pool in a reproducible, efficient, and specific manner is central to drug action mediated by the use of a carrier system [31].

1.6.3.3. Monoclonal antibodies mediated microspheres targeting.

Monoclonal antibodies targeting microspheres are immune microspheres. This targeting method is used to achieve selective targeting at specific sites. Monoclonal antibodies are extremely specific molecules. Monoclonal antibodies can be directly attached to the microspheres by means of covalent coupling. Monoclonal antibodies can be attached to microspheres by any of the following methods [32]:

(i) Non-specific adsorption and specific adsorption

(ii) Direct coupling

(iii) Coupling via reagents.

1.6.3.4. Chemoembolization.

This is an endovascular therapy that involves the selective arterial embolization of a tumour together with the simultaneous or subsequent local delivery of the chemotherapeutic agent [33].

1.6.3.5. Imaging.

The imaging size range of microspheres is an important factor in determining the imaging of particular sites using radiolabelled microspheres. The particles injected intravenously apart from the portal vein will become entrapped in the capillary bed of the lungs. This phenomenon is exploited for the scintiographic imaging of tumour masses in the lungs using labelled human serum albumin microspheres [34].

1.6.3.6. Topical porous microspheres.

Microsponges are porous microspheres with a myriad of interconnected voids of particle sizes in the range of 5–300 m. These microsponges, having the capacity to entrap a wide range of active ingredients such as emollients, fragrances, essential oils, etc., are used as the topical carrier system [35].

1.6.4. Medical applications.

(i) Release of proteins, hormones and peptides over extended period of time

(ii) Gene therapy with DNA plasmids and also delivery of insulin

(iii) Vaccine delivery for the treatment of diseases like hepatitis, influenza, pertussis, ricin toxoids, diphtheria, birth control

(iv) Passive targeting of leaky tumour vessels, active targeting of tumour cells, antigens, by intra-arterial/ intravenous application

(v) Tumour targeting with doxorubicin and also

(vi) Treatments of leishmaniasis

(vii) Magnetic microsphere can be used for stem cell extraction and bone marrow purging

(viii) Used in isolation of antibodies, cell separation and toxin extraction by affinity chromatography

(ix) Used for various diagnostic tests for infectious diseases like bacterial, viral and fungal [36].

1.6.5. Radioactive microsphere application.

(i) Can be used for radioembolization of liver and spleen tumours

(ii) Used for radio synovectomy of arthritis joints, local radiotherapy, interactivity treatment

(iii) Imaging of liver, spleen, bone marrow, lung and even imaging of thrombus in deep vein thrombosis can be done [37].

1.7. Multi-particulate systems.

As part of chronic disease therapy, many medications must be administered on a regular basis. Additionally, several times throughout the day, drugs with a relatively short half-life are given. It is necessary to develop a sustained-release dosage form to help maintain the drug's therapeutic effective concentration for a long period of time in order to get around this. The sustained release dosage form is a kind of drug delivery system designed to continually administer medication over a longer period of time after the administration of a single dose of a drug or tablet in order to extend the therapeutic impact.

The main goal of the treatment is to keep the tissue level constant while continuing to be therapeutically effective and non-toxic. The development of the proper dosage form is a crucial step in achieving, delivering, and realizing this objective. The highest drug bioavailability can be

attained by aiming for and achieving a maximum rate and extent of medication absorption. Sustained release dosage forms are considered to be the most effective way to maximize drug dispersion, which is crucial for obtaining some level of control over the therapeutic effect and lowering in vivo variability [38].

Sustained-release dosage forms are designed to prolong a medicine's duration of action, lessen changes in plasma levels, enhance drug absorption, and lessen side effects. They also seek to ensure consistent drug administration and a lower dose frequency. Sustained-release delayed drug delivery systems are able to show their clearly defined action by changing the pharmacokinetics and pharmacodynamics of pharmacologically active drug molecules delivered by novel drug delivery systems or by changing the molecular structure and physiological parameters inherent in a chosen route of drug administration [39].

1.7.1. Advantages of Multi-particulate systems.

Multi-particulates (pellets, non-peariles, etc.) are used as drug carriers in pH-sensitive, time-dependent, and microbially controlled systems for colon targeting. Tablets or capsules are used as single dosage forms; in those cases, if by any chance one tablet or capsule fails, then the whole drug system and the dosage form fail. Intersubject variation also influences the system.

But in the case of multi-particulate dosage forms like beads, microsponges, microspheres, microcapsules, and pellets, whole systems don’t fail, and inter-subject variation is reduced. Multi-particulate systems have several advantages in comparison to conventional single-unit controlled release technology, such as more predictable gastric emptying and fewer localised adverse effects than those of single-unit tablets or capsules. A multi-particulate dosage was prepared to deliver active molecules to the colonic region, which combines pH-dependent and controlled drug release properties [40].

1.8. Microbeads as potential multi-particulate system.

1.8.1. Advantages of Beads:

1. Less inter and intra subject variations in gastric transit time
2. Taste masking
3. No risk of dose dumping
4. Lesser local irritation

5. Increased solubility or dispersibility, hence quick diffusion, leading to more rapid drug release and better absorption, as having large surface area
6. Avoid risk of toxicity since they have ability to spread uniformly throughout gastrointestinal tract
7. Improves patient compliance by decreasing dosing frequency
8. Bioavailability enhances despite first pass effect because fluctuations in plasma drug concentration is avoided, a desirable plasma drug concentration is maintained by continuous drug release
9. Increased therapeutic efficiency
10. Improved stability

Hydrophilic polymers derived from plant, animal, microbial, or synthetic sources, when added to water, form hydrocolloids that disperse evenly as microscopic particles. At sufficiently high concentrations, the polymers become entangled with each other, forming loose networks that change the rheological properties of solutions. Many hydrocolloids, such as gelatin and pectin, can form gels by hydrogen bonding within and between the polymers. Formulations based on hydrocolloids may have some advantages over other sustained release formulations; for instance, different structures can be obtained upon dehydration of the hydrocolloid formulations, which can be modified by the drying conditions and formulation composition. Structural characteristics such as porosity may affect the penetration rate of the liquid into the formulations and thus modify the release pattern of the drug. Moreover, the stability and physical properties (dimensions, strength, etc.) of various hydrocolloids are affected by factors such as swelling in water, pH values, and enzymes and therefore vary in different parts of the gastrointestinal tract. Changes in the physical properties of the formulations may also lead to different drug release patterns in different parts of the gastrointestinal tract, thus providing a wide scope for their utilisation as carriers for the controlled release of drugs. The incorporation of suitable concentrations of active ingredients for therapeutic or cosmetic uses in microbeads is generally carried out by two processes:

i) Incorporation of active ingredients during the process of preparation of the microbeads
ii) Adsorption of a solution or suspension of the active ingredients in the previously crosslinked microbeads, in case the active ingredients are incompatible with the dehydration solvents [41, 42].

CHAPTER 2

LITERATURE REVIEW

2. LITERATURE REVIEW.

Attempts to sustain the release of drugs by using natural polymers started around two decades ago. Several researchers throughout the world have published their different approaches to this purpose, sometimes using natural polymers in combination with another polymer to extend the drug release time. The tremendous orientation of the pharmaceutical world towards these naturally derived polymers has become a subject of interest. So here is a brief review of some literature in this context.

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 separation 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 [43].

Poonam P et al. studied the preparation technique, namely the ionotropic gelation technique, in order to produce a pharmaceutical product with the needed properties. The basis for ionotropic gelation was the capacity of polyelectrolytes to cross-link with opposing ions to create hydrogels. The utilisation of naturally occurring polysaccharides as biopolymers has risen in emerging fields such as hydrogel sustained release formulation, offering an environmentally acceptable method of creating pharmaceutical products [44].

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 [45].

Zam W et al. *studied* the effects of polyphenols derived from pomegranate peels (Punica granatum) on loading efficiency and radical scavenging activity using sodium alginate and combinations of sodium alginate and pectin. The findings show that when microbeads were made with a single type of polymer as opposed to microbeads made with two types of polymers, the polyphenol content was lower. Additionally, there was a preferred ratio of these two polymers (2:1), which was responsible for producing the highest amount of polyphenol. While antioxidant activity remained constant, the microencapsulated particles offered polyphenols an effective defense against the phenomenon of degradation. According to in vitro release tests, sodium alginate and sodium alginate-pectin microbeads released 64.87% and 48.81% of polyphenols in simulated stomach juice, respectively. In simulated intestinal fluid, sodium alginate and sodium alginate-pectin microbeads, respectively, released 88.37% and 70.48% of polyphenols. The study's microcapsules are an intriguing food additive that can be added to functional foods. The gelled microbeads containing polyphenols extracted from pomegranate peels were formed by ionic gelation using a single polymer (sodium alginate or pectin) and different combinations of sodium alginate and pectin. These microbeads can entrap polyphenols in insufficient amounts and also protect their antioxidant activity. They can also successfully deliver them in gastrointestinal fluids. Thus, without using any organic solvent or taking any time-consuming steps in the preparation of these microbeads, it is possible to develop effective, cheap, and nontoxic microbeads that can be used as food supplements [46].

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 [47].

Aslani P et al. conducted a study on the effect of gelation conditions and dissolution media on the release of paracetamol from alginate gel beads. They also performed a dissolution study of simulated gastric fluid, and the release pattern was noted. The results indicated that three media—water, Simulated Gastric Fluid USP without Pepsin (SGF), and 0–1% trisodium citrate solution—were used to monitor the drug release from the beads. In water, release moved more slowly but was finished in 4-5 hours. In comparison to calcium beads made at the same molar cation concentration, zinc beads release more slowly. The cation type and concentration had no impact on the speed or extent of the drug release from the alginate gel beads in the SGF, which was complete in 2 hours. The paracetamol in the citrate solution was released quickly, with the exception of beads made from 0–1M zinc. With half-lives ranging from 25 to 73 minutes, first-order kinetics might be used to describe all release profiles [48].

Ramesh Babu V et al. have shown that by using the water-in-oil (W/O) emulsion process, sodium alginate (Na-Alg) and methylcellulose (MC) carbohydrate polymeric blend microspheres were created. These microspheres contained the anti-inflammatory medication nifedipine and were glutaraldehyde-crosslinked. Differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and a laser particle size analyzer were used to characterise the microspheres. The molecular-level distribution of nifedipine in the polymer matrix was validated by DSC thermograms of Na-Alg-MC microspheres that had been loaded with nifedipine. The microspheres SEM image revealed that spherical particles had formed. Important data on the characteristics of drug diffusion were obtained from research on the swelling of the microspheres. In order to understand the nature of the transport of the drug-containing solution through the polymeric matrix, release data have been analysed using an empirical equation. In pH 7.4 media, the controlled release properties of the nifedipine matrix were studied. A laser light diffraction particle size analyzer was used to examine the microspheres particle size and size distribution. For up to 12 hours, the drug was released under regulated circumstances [49].

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 [50].

Badwan AA et al. studied a sustained-release drug delivery system using calcium alginate beads. There was a study conducted to analyse the morphological characteristics as well as the release pattern of the formulations, and the conclusion drawn was that the sulfamethoxazole intake by the beads was roughly half of the integrated amount, and the mean bead diameter was 1.25mm. The USP dissolving method was used to monitor the release behaviour. Researchers examined how variables including sodium alginate, calcium chloride concentration, pH, moisture, and compression affected the release of substances. Concentrations of sodium alginate had little impact on the release [51].

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 [52].

CHAPTER 3

AIM AND OBJECTIVES

3. AIM AND OBJECTIVES.

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 gelatin for sustained release. Paracetamol will be employed as a model drug in this case. In the developed formulations, any other drug with a comparable solubility profile may be replaced.

Extensive research will be conducted on sodium alginate, gelatin, calcium chloride, and formaldehyde as base polymers, additive polymers, and crosslinking agents respectively to attain the predicted goal.

The objectives are.

- **Sodium alginate concentration alone is optimized for bead production**: The beads will be manufactured 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.
- **Gelatin concentration optimization in the presence of alginate for bead production**: To calculate the maximum amount of gelatin that can be added to each specific concentration of sodium alginate while maintaining morphology and fluidity in mind.
- **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.

CHAPTER 4

DRUG-EXCIPIENT PROFILE

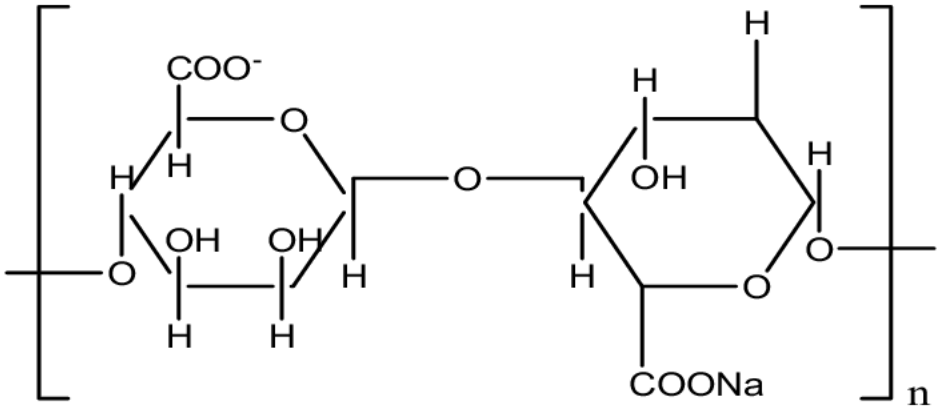
4. DRUG-EXCIPIENT PROFIE.

4.1. Sodium Alginate.

Chemical name:

Sodium-3,4,5,6-tetrahydroxyoxane-2-carboxylate

Chemical structure:



Synonym:

Sodium alginate, D-Galacturonic acid sodium salt, Alginic acid monosodium salt, Sodium-3,4,5,6-tetrahydroxyoxane-2-carboxylate.

Chemical formula:

$NaC_6H_7NaO_6$

Molecular Weight:

216.121 g/mol

CAS Number:

9005-38-3

Computed properties:

Hydrogen bond doner count	:	4
Hydrogen bond acceptor count	:	7
Rotatable bond count	:	1
Exact mass	:	216.025 g/mol
Monoisotopic mass	:	216.025 g/mol
Topological polar surface area	:	130 A^2
Heavy atom count	:	14
Formal change	:	0
Complexity	:	210
Isotope atom count	:	0
Defined atom stereocenter count	:	0
Undefined atom stereocenter count	:	5
Defined bond stereocenter count	:	0
Undefined bond stereocenter count	:	0
Covalently bonded unit count	:	0
Compound is canonicalized	:	Yes

Physical Description:

Nearly odorless, white to yellowish fibrous or granular powder.

Pharmacology:

Sodium alginate is the sodium salt form of alginic acid and gum mainly extracted from the cell walls of brown algae, with chelating activity. Upon oral administration, sodium alginate binds to and blocks the intestinal absorption of various radioactive isotopes, such as radium 226 (Ra-226) and Strontium (Sr-90).

Uses:

Food additives, Emulsifiers, Gelling agents, stabilizer, thickners.

4.2. Gelatin [53].

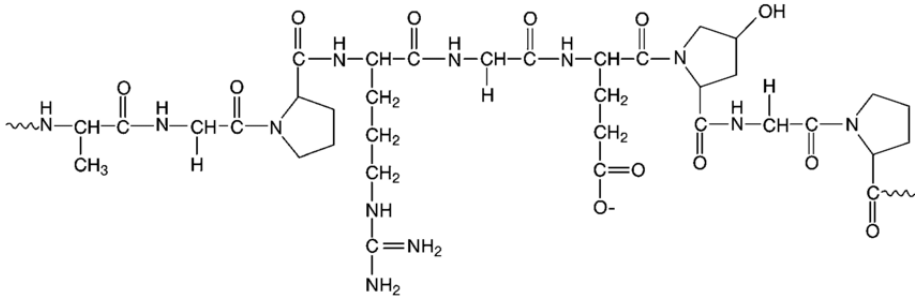
Synonyms:

Gelatine, jelly, gel ,aspic, Gelfoam, Teleostean Gelatin, Pharmagela.

Chemical formula:

C102H151N31O39

Chemical structure:



CAS Number:

9000-70-8

Molecular Weight:

4.10^5 to 5.10^7 g/mol

Toxicity:

LD50 orally in Rabbit: > 5000 mg/kg.

Physical Description:

Gelatin, a solid that is transparent, colorless, brittle, and almost tasteless, is made from the collagen found in animal skin and bones.

Uses:

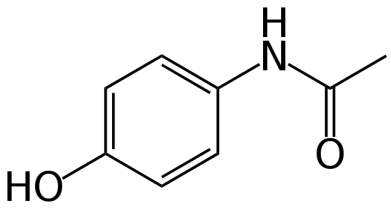
In the production of food, pharmaceuticals, photography, and cosmetics, it is frequently employed as a gelling agent. Collagen has been irreversibly hydrolyzed to create gelatin. It can be found in various gummy sweets as well as in marshmallows, gelatin desserts, and some low-fat yoghurt.

4.3. Paracetamol [54].

Synonym:

Acetaminophen

Chemical Structure:



Pharmacological Action:

Paracetamol is simply termed as analgesic and an antipyretic. Despite enduring assertions that it acts by inhibition of Cyclooxygenase (COX) mediated production of prostaglandins, unlike non-selective anti- inflammatory drugs (NSAIDs), paracetamol has been demonstrated not to reduce tissue inflammation.

There is very little anti-inflammatory activity of paracetamol. In the brain, COX is more active than it is in peripheral tissues, where it is a poorer inhibitor of PG (prostaglandin) production. Its inability to inhibit COX in the presence of peroxides, which are produced at sites of inflammation but are absent in the brain, has been suggested as one explanation for the disparity between its analgesic-antipyretic and anti-inflammatory activities. It's also possible that paracetamol's analgesic-antipyretic effects are due to its capacity to inhibit COX-3, an isoenzyme that has been discovered to be present in dog brain.

Pharmacokinetics:

Orally taken paracetamol is readily absorbed; only about $1/4^{th}$of it is protein bound in plasma, and it is evenly distributed throughout the body. The majority of metabolism involves the conjugation of glucuronic acid and sulphate; conjugates are quickly eliminated in the urine.Plasma $t_{1/2}$ is 2–3 hours. An oral dose has effects that linger for 3-5 hours.

Adverse effects:

Small children with poor hepatic glucuronide conjugating capacity are more susceptible to it. Serious toxicity may develop if a high dose (> 150 mg/kg or > 10 g in an adult) is consumed. More than 250 mg/kg frequently results in death.

Early symptoms consist only of nausea, vomiting, stomach discomfort, and tenderness in the liver; there is no impairment of consciousness. Centrilobular hepatic necrosis develops after 12 to 18 hours; this may be followed by renal tubular necrosis, hypoglycemia, and coma. Two days later, jaundice appears. Continuation of the course depends on the dosage. If the plasma levels are above the line connecting 200 g/ml at 4 hours and 30 g/ml at 15 hours, fulminating hepatic failure and death are likely. If the levels are lower, recovery with supportive treatment is the rule.

Treatment:

If the patient is brought in early, gastric lavage or vomiting should be induced. To stop further absorption, activated charcoal is administered orally or through a tube. As necessary, additional supportive actions should be implemented. *Specific:* 150 mg/kg of N-acetylcysteine (MUCOMIX, ANTIFEN 200 mg/ml inj in 2, 5 ml amps) should be administered intravenously (IV) over 15 minutes, then the same amount intravenously over the following 20 hours. A different option is to administer 75 mg/kg orally every 4-6 hours for 2-3 days. It replaces the liver's glutathione reserves and stops the harmful metabolite from attaching to other cellular components.

Uses:

One of the most popular 'over-the-counter' analgesics for headache, mild migraine, musculoskeletal pain, dysmenorrhea, etc., paracetamol is comparatively ineffectual when inflammation is high, as in rheumatoid arthritis. Many professional bodies advise using paracetamol as the first-line analgesic for osteoarthritis pain. With no danger of Reye's syndrome, it is one of the best medications to use as an antipyretic, especially in children.

Doses of Marketed products:

- Dose: 325–650 mg (children 10–15 mg/kg) 3–5 times a day.
- CROCIN 0.5, 1.0 g tabs;
- METACIN, PARACIN 500 mg tab, 125 mg/5 ml syrup, 150 mg/ml paed. drops,
- ULTRAGIN, PYRIGESIC, CALPOL 500 mg tab, 125 mg/5 ml syrup,
- NEOMOL, FEVASTIN, FEBRINIL300mg/2mlinj.,
- CROCIN PAIN RELIEF: 650 mg + Caffeine 50 mg tab.
- JUNIMOL-RDS 80, 170, 250 mg suppository (for children), PARACETAMOL RECTAL SUPPOSITORY-80, 170 mg.

CHAPTER 5

MATERIALS AND METHODS USED

5. MATERIALS AND METODS USED.

5.1. Chemicals Used.

The following chemicals are used during this project work:

S. No.	Chemicals Used	Manufacturer/ Source
1.	Sodium Alginate (91%)	LobaChemie Pvt. Ltd., Mumbai-400005
2.	Gelatin Powder 240 Bloom	S. D Fine-Chem Limited (SDFCL), Mumbai- 400030
3.	Calcium Chloride Dihydrate	Merck Life Science Pvt. Ltd., Mumbai-400079
4.	Hydrochloric Acid	Merck Life Science Pvt. Ltd., Mumbai-400079
5.	Formaldehyde	Merck Life Science Pvt. Ltd., Mumbai-400079
6.	Potassium Dihydrogen Phosphate GR	Merck Specialities Pvt. Ltd., Mumbai-400018
7.	Di-potassium Hydrogen Phosphate anhydrous GR	Merck Specialities Pvt. Ltd., Mumbai-400018
8.	Sodium Chloride	Merck Life Science Pvt. Ltd., Mumbai-400079
9.	Paracetamol	East India Pharmaceutical Works Ltd., Kolkata- 700071 Gift sample

5.2. Instruments used.

The following instruments are used during my project work:

S. No.	Instrument	Model no.	Manufacturer
1.	Electronic Balance	ML204/A01	Mettler Toledo, Switzerland
2.	Digital pH Meter	CL110	Chemi Line, Kolkata
3.	Sieves	18- 100 mesh size	Excel Enterprise, Kolkata
4.	Magnetic Stirrer	2ML & 2MLH	REMI Sales & Engineering Ltd., Mumbai
5.	UV- Visible FTIR Spectroscopy	IR- Prestige- 21	Shimadzu, Japan.
6.	UV- Visible Spectrophotometer	UV- 3200	Labindia Analytical Instrument Pvt. Ltd., Kolkata.
7.	USP Dissolution Apparatus II	TDT- 08L	Electrolab, Mumbai
8.	Brookfield Viscometer	TV- 10	Toki Sangyo Co. Ltd.

5.3. PREFORMULATION STUTY.

5.3.1. Determination of λmax for paracetamol in 0.1N HCL (pH 1.2) and Phosphate buffer (pH 6.8):

Accurately weighted paracetamol was dissolved in separately in 0.1N HCL (pH 1.2) and Phosphate buffer (pH 6.8). Suitable concentrated solutions for both preparations were prepared and scanned under UV-Vis spectrophotometer between 190 to 400 nm against corresponding blank buffer. The λmax of paracetamol in 0.1N HCL (pH 1.2) and Phosphate buffer (pH 6.8) is found to be 243 nm for both the buffer. The λmax 243 nm was used for determination of unknown concentration of paracetamol.

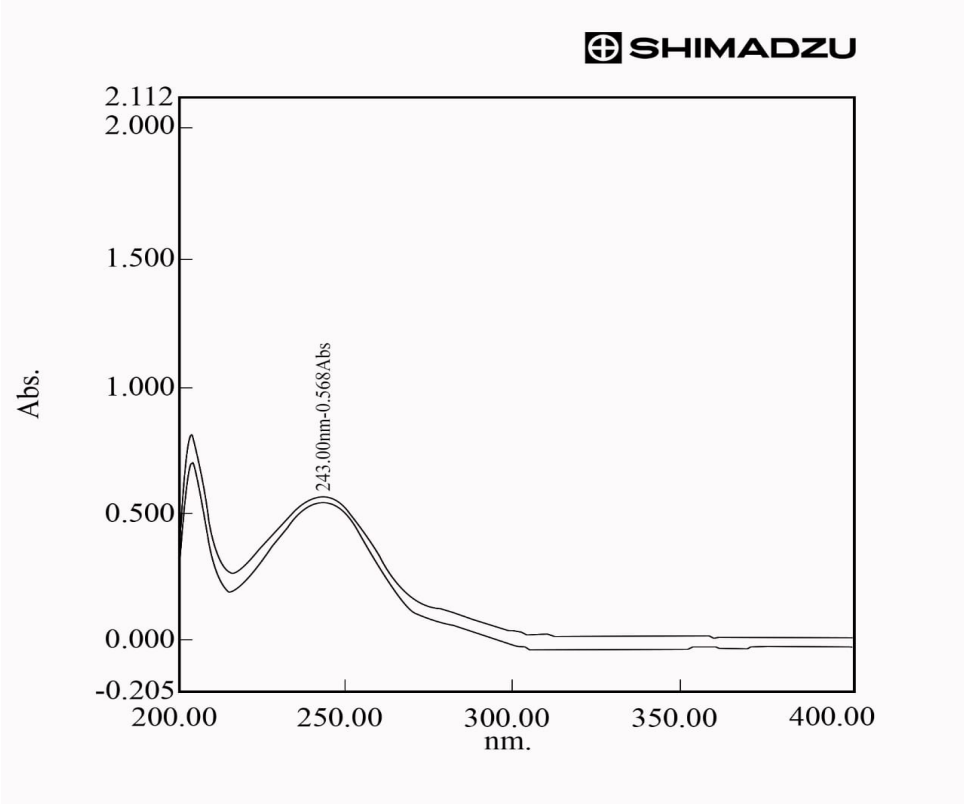


Fig. 5.1: λmax for paracetamol in 0.1N HCL (pH 1.2) and Phosphate buffer (pH 6.8).

5.3.1.1. Preparation of Standard Calibration Curves of Paracetamol in 0.1N HCL (pH 1.2) and Phosphate buffer (pH 6.8):

The calibration curves of paracetamol were prepared in 0.1N HCL (pH 1.2) and in phosphate buffer solution (pH 6.8). Accurately weighted paracetamol was dissolved separately in 0.1N HCL and phosphate buffer. From the stock paracetamol solution, different solutions with known paracetamol concentrations were prepared for both 0.1N HCl and phosphate buffer. All the

paracetamol solutions were subjected to UV-Vis spectrophotometric 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. The calibration curves were prepared by linear regression analysis, and the mathematical equations were drawn.

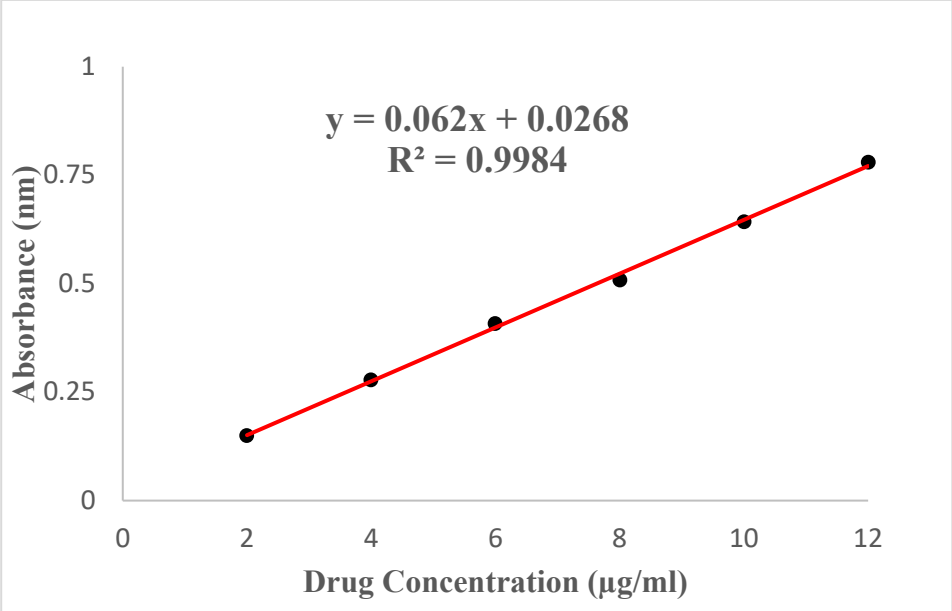


Fig. 5.2: Standard Curve of Paracetamol in 0.1N HCl pH 1.2.

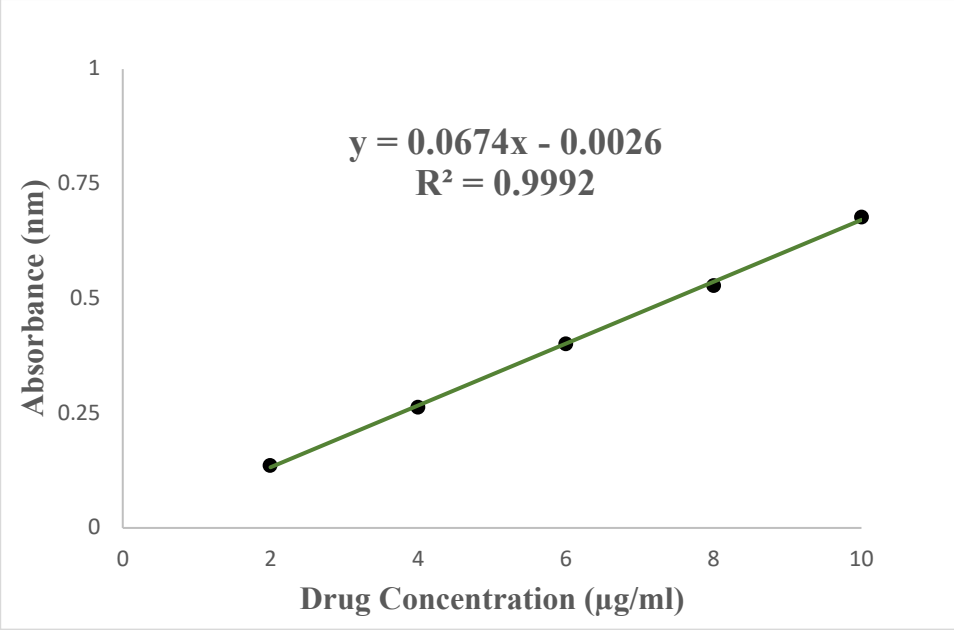


Fig. 5.3: Standard Curve of Paracetamol in Phosphate buffer pH 6.8.

Table 5.1: Concentration and corresponding absorbance of paracetamol in 0.1N HCL (pH 1.2).

Drug concentration (µg/ml)	Absorbance (nm)	Equation
2	0.150	y= 0.062x + 0.026 R^2= 0.9980
4	0.278	
6	0.408	
8	0.508	
10	0.642	
12	0.780	

Table 5.2: Concentration and corresponding absorbance of paracetamol in Phosphate Buffer (pH 6.8).

Drug concentration (µg/ml)	Absorbance (nm)	Equation
2	0.137	y= 0.0674x - 0.0026 R^2 = 0.9992
4	0.263	
6	0.402	
8	0.529	
10	0.678	

5.3.2. Optimization for an ideal concentration of Sodium alginate beads.

The observation started with the observation that when sodium alginate solution is allowed to pass through a syringe into a fixed concentration of calcium chloride solution (2%), cross-linking takes place and there is the formation of spherical beads with various morphological characteristics. So, the experiment started with variations in sodium alginate concentration and was followed by variations in calcium chloride solution. So, the concentration of sodium alginate was being experimented with from 1-3.5%, but when 1% sodium alginate solution dropped from the syringe into $CaCl_2$ solution, it showed an irregular transparent structure with the presence of a tip that couldn't retain its shape and no vesicular structures were formed. From 1.5% of the sodium alginate solution, spherical beads were observed, and they continued until 3%, at which point the beads were spherical. But when the alginate solution concentration exceeded 3.5%, the beads were formed, but the solution itself became too viscous, which made it difficult to syringe out and also rendered the beads irregular, hence the batch was discarded.

5.3.3. Optimization for ideal concentration of Calcium Chloride solution for Alginate beads formation.

As we have determined the ideal concentration of sodium alginate, it is also essential to optimise the concentration of calcium chloride solution. Now, by considering one or two ideal concentrations of sodium alginate (2% and 2.5%) fixed and by varying the $CaCl_2$ concentrations

from 0.5 to 5%, it was seen that till 1.5% of $CaCl_2$ concentration, the beads were irregular in shape, and from 2% onwards, the beads were more uniformly structured with no irregularity. However, it was seen that with an increase in concentration from 2-5%, it didn't show any significant change in the shape or the entrapment efficiency when the alginate beads were treated with the model drug paracetamol, so 2% of the $CaCl_2$ concentration was fixed.

Table 5.3: Optimization of $CaCl_2$ concentration on the basis of morphology of the beads.

Alginate concentration (%)	$CaCl_2$ Concentration (%)	Observation
1.0	0.5, 1.0, 1.5, 2.0	Irregular structure, no vesicle formed, couldn't retain its shape.
1.5	0.5, 1.0, 1.5	Spherical structure with very minute tips present, Vesicle is present, translucent pale color.
1.5	2.0	Spherical beads obtained with no tip.
2.0	2.0	Spherical beads produced.
2.5	2.0	Spherical beads produced.
3.0	2.0	Spherical beads produced but the solution starts becoming viscous.

Table 5.4: Experimental data of determination of optimization of $CaCl_2$ solution based on % Entrapment efficiency.

Alginate concentration (%)	$CaCl_2$ Concentration (%)	% Entrapment Efficiency
2.0	1.5	77.87
2.0	2.0	71.35
2.0	2.5	71.35
2.0	3.0	69.98
2.0	3.5	73.54
2.0	4.0	70.13
2.0	4.5	68.56
2.0	5.0	69.59
2.5	1.5	72.89
2.5	2.0	79.61
2.5	2.5	79.47
2.5	3.0	80.63
2.5	3.5	79.47
2.5	4.0	79.61
2.5	4.5	79.47
2.5	5.0	81.70

5.3.4. Optimization for ideal Gelatin concentration.

After the determination of ideal concentrations of sodium alginate and $CaCl_2$ solution, it is also required to find out the ideal gelatin concentration. When additive polymer gelatin was added with individual concentrations of alginate solution, initially beads were formed with irregularities in

their structure, but at certain concentrations, spherical beads were obtained. However, after a certain limit, further addition of gelatin made the solution viscous and difficult to extrude from the syringe, so the amount of gelatin that could be added to 1.5% of alginate solution was (0.5, 1, 1.5, 2, 2.5%), to 2% of alginate solution was (0.5, 1, 1.5, 2, 2.5%), to 2.5% alginate solution was (0.5, 1, 1.5, 2%), and to 3% alginate solution was (0.5, 1, 1.5%).

Table 5.5: Effect of addition of gelatin on bead structure.

Alginate Concentration (%)	CaCl2 Concentration (%)	Gelatin Concentration (%)	Observations
1.5	2.0	0.5, 1.0, 1.5, 2.0, 2.5	There was formation of beads as the gelatin concentration kept on increasing there was more transparency in the color of beads and the beads were light weight, easily broken my minute pressure.
2.0	2.0	0.5, 1.0, 1.5, 2.0, 2.5	Circular beads were formed, and no observable tip was observed.
2.5	2.0	0.5, 1.0, 1.5, 2.0	Circular beads were formed, and no observable tip was observed.
3.0	2.0	0.5, 1.0, 1.5	Circular beads were formed, and no observable tip was observed. Further increase in gelatin concentration reduces fluidity

5.3.5. Optimization of formaldehyde concentration.

In a 20 ml composite solution, 0.05 ml of 38% w/v formaldehyde was used for health safety as per the literature [55].

5.3.6. Optimization of curing time of beads.

The first step to preparing the sodium alginate beads and making the proper amount of sodium alginate is to dissolve them in double-distilled water by constantly stirring them in a magnetic stirrer at 300–400 rpm. The solutions are kept for 2-4 hours at a magnetic stirrer for hydration. Gelatin solution is also prepared the same way by dissolving an appropriate amount of gelatin into hydrated alginate solution and then mixing both solutions together with heat (20–40 °C). Then, at last, the model drug is mixed into the above-made alginate-gelatin solution, and at the same time, a $CaCl_2$ solution is prepared. The prepared solution is extruded through a 22-gauge syringe into the $CaCl_2$ solution; the beads are kept in solution for 5 minutes and then filtered, washed, and collected.

5.3.7. Fourier Transform Infra-Red Spectroscopy Studies (FTIR).

Because crosslinking occurs during the formation of beads, it was hypothesized that the drug and polymer may interact. The individual excipients, drug and their mixtures were subjected to FTIR study for determination of interaction if any between the composite materials and drug. An FTIR spectrophotometer was used for FTIR investigations.

The IR analysis of pure drug, polymer and drug loaded beads prepared by both the methods were analyzed with FTIR spectrophotometer. All the samples were crushed with potassium bromide to get pellets at 600 kg/cm^2. Spectral scanning was done in the range of 400-4000 cm^{-1}.

The FTIR analysis of drug and the formulation was done to observe the change in functional group in the formulation from its parent drug. Comparison of the reports with the additive polymer will give us the proper idea about the changes that has taken place in the formulation. We have studied FTIR study by using Prestige-21, Shimadzu, Japan, from Jadavpur University, Kolkata, W.B.

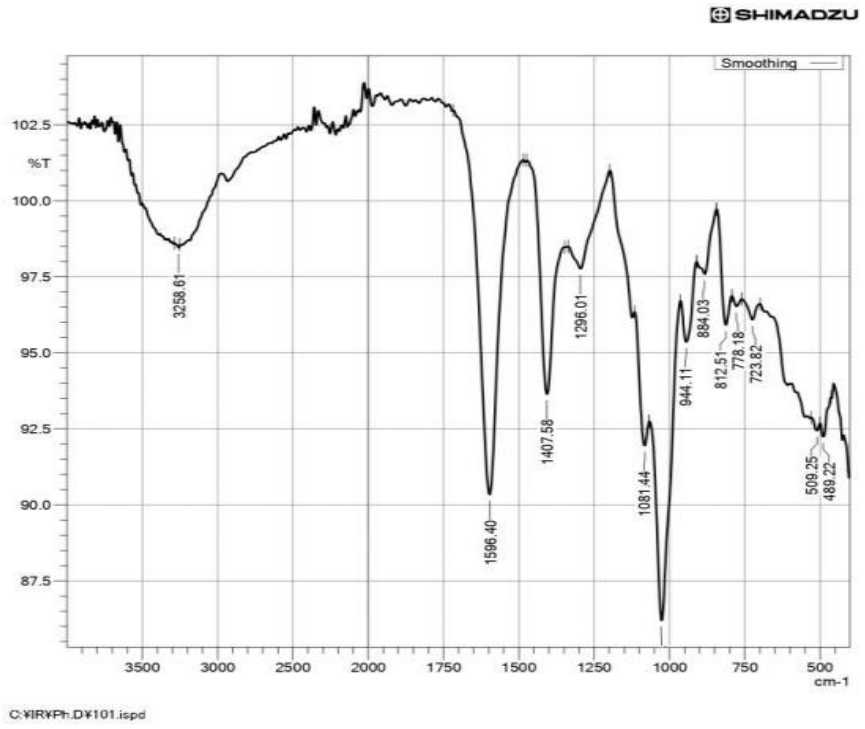


Fig. 5.4: FTIR Spectrum of sodium alginate

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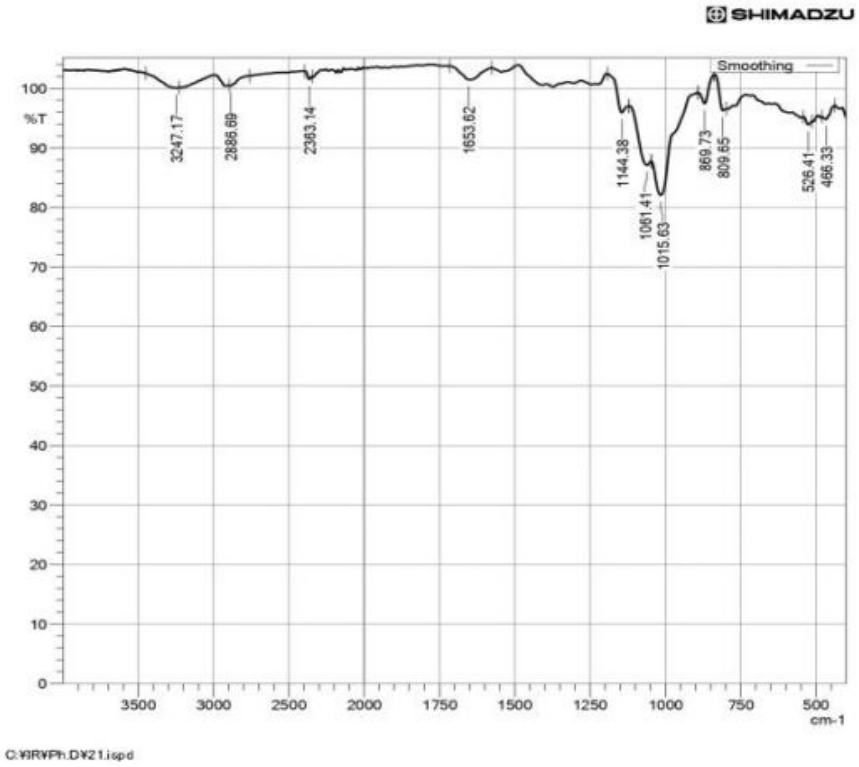

Fig. 5.5: FTIR Spectrum of Paracetamol.

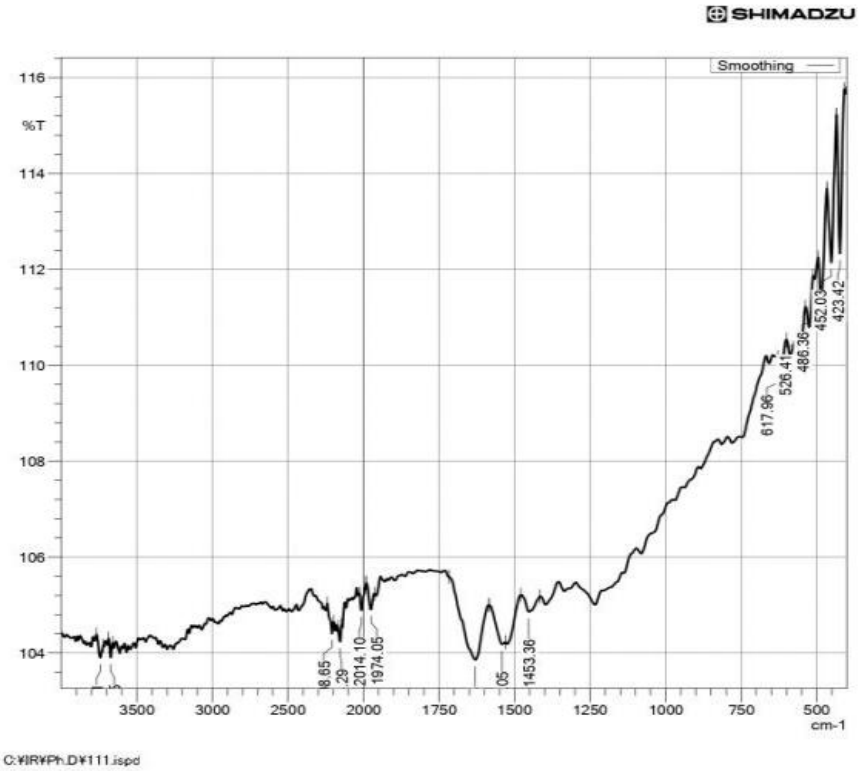

Fig. 5.6: FTIR Spectrum of Gelatin.

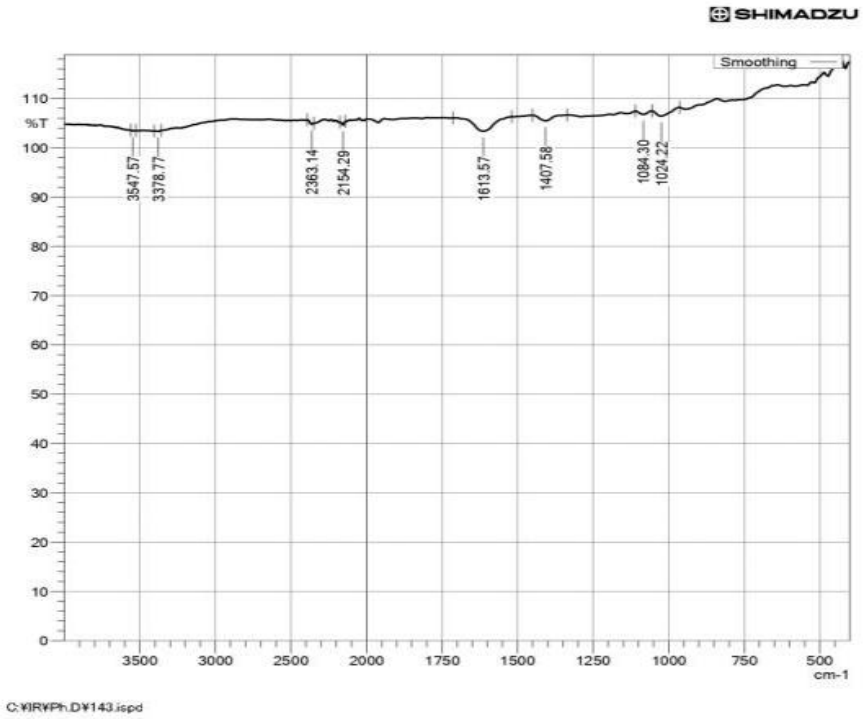

Fig. 5.7: FTIR Spectrum of sodium alginate and $CaCl_2$ mixture.

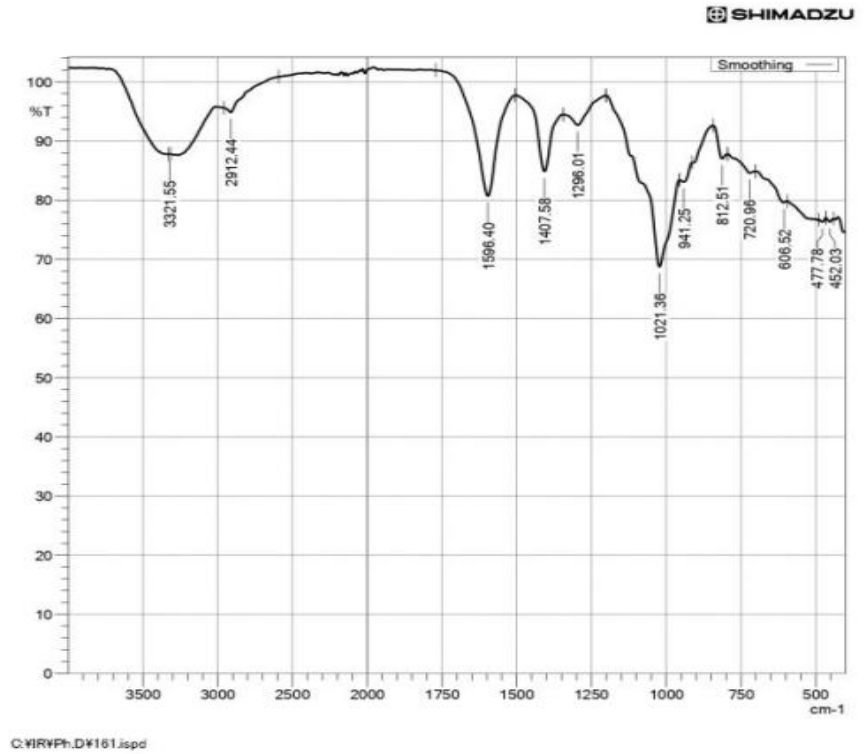

Fig. 5.8: FTIR Spectrum of Gelatin-Formaldehyde.

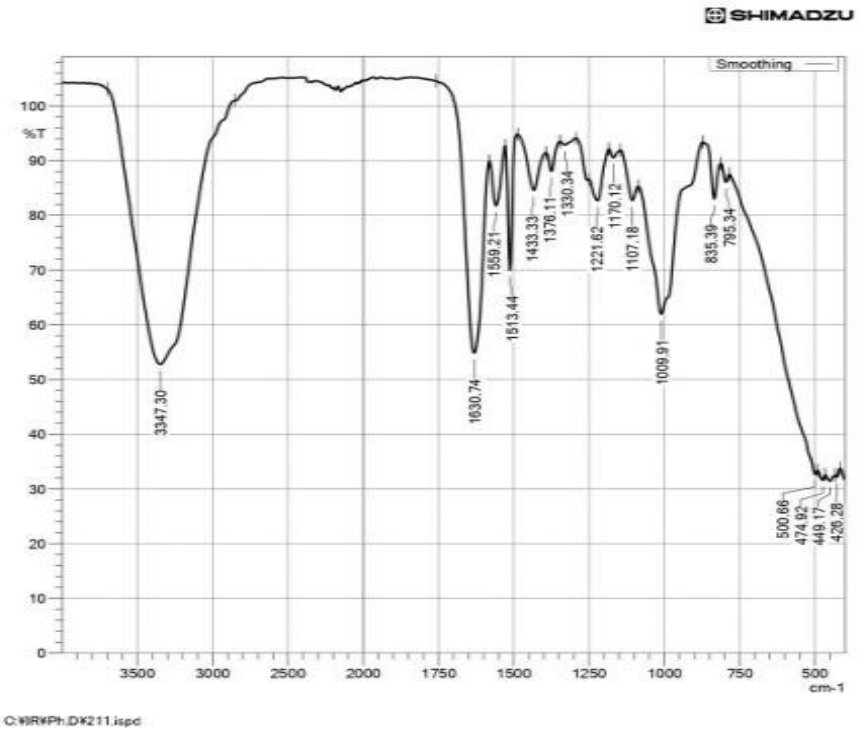

Fig. 5.9: FTIR Spectrum of crushed bead (Paracetamol-Sodium alginate-Formaldehyde-$CaCl_2$).

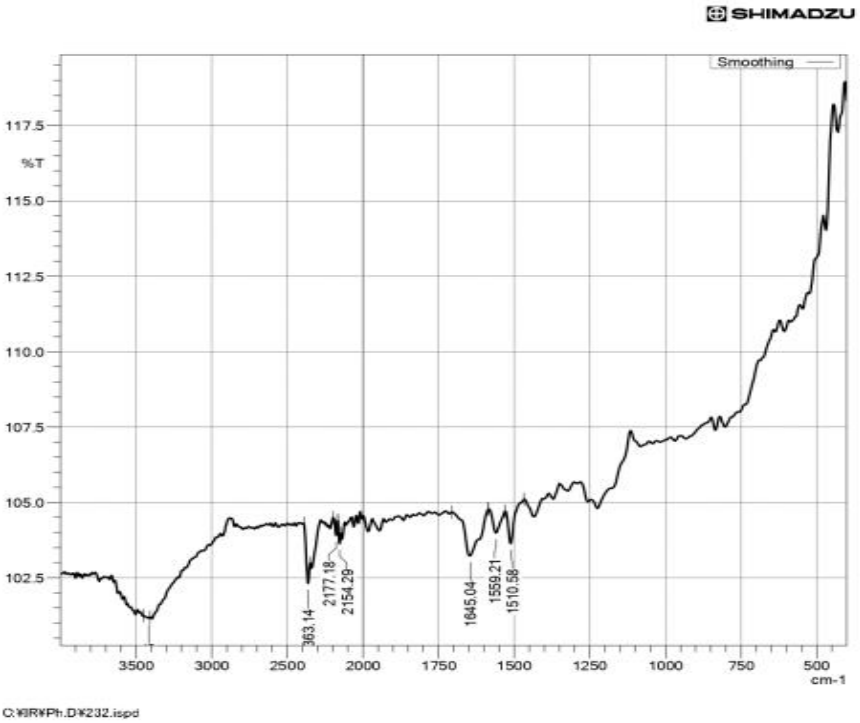

Fig. 5.10: FTIR Spectrum of crushed bead (Paracetamol-Sodium alginate-$CaCl_2$).

Table 5.6: FTIR Spectrum of Sodium alginate.

S.NO	FUNCTIONAL GROUP	RANGE
1.	O-H Stretch of intermolecular hydrogen bond	3258.61 cm^{-1}
2.	O-C=O Asymmetric stretching	1596.40 cm^{-1}
3.	O-C=O Asymmetric stretching	1407.58 cm^{-1}
4.	N-O Symmetric stretching of nitro compound	1296.01 cm^{-1}

Table 5.7: FTIR Spectrum of Paracetamol.

S. NO.	FUNCTIONAL GROUP	RANGE
1.	O-H Stretch of hydrogen bond of Alcohol	3247.17 cm^{-1}
2.	C=O Stretch of Amide	1653.62 cm^{-1}
3.	O-H Stretch of Carboxylic acid	2886.69 cm^{-1}
4.	CH_3 Stretching	2363.14 cm^{-1}

Table 5.8: FTIR Spectrum of Gelatin.

S. NO.	FUNCTIONAL GROUP	RANGE
1.	C=O Stretch hydrogen bond couple with COO	1680 cm^{-1} – 1630 cm^{-1}
2.	N-H Bending & C-N Stretching	1605 cm^{-1}
3.	C-N & N-H vibration in plane of bound amide	1453.36 cm^{-1}
4.	O-H Stretch of free hydroxyl group	3720 cm^{-1} – 3600 cm^{-1}
5.	O-H Stretch of hydrogen bond	3400 cm^{-1} – 3330 cm^{-1}

Table 5.9: FTIR Spectrum of Sodium alginate- $CaCl_2$ mixture.

S. NO.	FUNCTIONAL GROUP	RANGE
1.	O-H Stretch enlargement of region due to $CaCl_2$	3547.57 cm^{-1} & 3378.77 cm^{-1}
2.	C-H Stretching region enlargement	2363.14 cm^{-1} & 2154.29 cm^{-1}
3.	O-C-O Stretch free carboxyl group region enlargement	1613.57 cm^{-1} & 1407.58 cm^{-1}

Table 5.10: FTIR Spectrum of Gelatin- Formaldehyde.

S. NO.	FUNCTIONAL GROUP	RANGE
1.	O-H Stretch of hydrogen bonding	3287.22 cm^{-1}
2.	C=O Stretch vibration along with C-N Stretch	1630.74 cm^{-1}
3.	N-H Bending & C-N Stretching	1542.05 cm^{-1}
4.	C-N & N-H vibration in plane of bound amide	1453.36 cm^{-1}

Table 5.11: FTIR Spectrum of crushed beads (Paracetamol-Sodium alginate-Formaldehyde-$CaCl_2$).

S. NO.	FUNCTIONAL GROUP	RANGE
1.	O-H Stretch of hydrogen bonding	3347.30 cm^{-1}
2.	C=O Stretch associated with NH_2, Alginate could be associated with gelatin	1630.74 cm^{-1}
3.	N-H Bending & C-N Stretching (gelatin)	1559.21 cm^{-1}

Table 5.12: FTIR Spectrum of crushed bead (Paracetamol-Sodium alginate-$CaCl_2$).

S. NO.	FUNCTIONAL GROUP	RANGE
1.	O-H Stretch of hydrogen bonding	3400 cm^{-1} – 3330 cm^{-1}
2.	C=O Stretch associated with NH_2, Alginate could be associated with gelatin	1645.04 cm^{-1}
3.	CH_3 Stretching of paracetamol	2363.14 cm^{-1}
4.	C≡C Stretch of alkyne group (alginate)	2177.18 cm^{-1}& 2154.29 cm^{-1}
5.	N-H Bending & C-N Stretching (gelatin)	1559.21 cm^{-1}

The FTIR spectrums were compared and observed that the interaction occurred between alginate molecules and calcium ions. Formalin was also found to be cross linked with gelatin molecules. There was no interaction between the drug paracetamol and the other excipients.

5.4. FORMULATION DEVELOPMENT.

5.4.1. Preparation of sodium alginate- Gelatin beads.

Procedure:

i) Dissolved appropriate amount of sodium alginate into 20 ml of double distilled water.
ii) Then stirred it constantly in magnetic stirrer with 300- 400 rpm for around 3- 4 h until it was hydrated.

Fig. 5.11: Hydration of sodium alginate solution.

iii) Then the desired amount of gelatin was added to hydrate alginate solution and stirred constantly with minute heating (20º- 40ºC). After this the solution was cool down to room temperature. Then the model drug paracetamol was weighed 20 mg and mixed into this solution and stirred properly until it was dissolved completely.

iv) Then, 2 g calcium chloride was weighed properly and mixed it into 100 ml of double distilled water. And then the solution was divided into two parts. In one part added 0.05 ml of formaldehyde and the other part was kept without formaldehyde.
v) Then drug polymer solution was dropped in the calcium chloride solution through 22G needle.
vi) Then left it for 5 min for curing.
vii) The beads after curing were separated by filtration and washed them properly with distilled water.
viii) The Calcium chloride supernatant solution was collected after filtering the beads and the volumed was measured for further entrapment efficiency was calculation.
ix) The beads were air dried for further evaluations.

Table 5.13: Composition of the developed formulations.

Formulation	Alginate (%)	Gelatin (%)	$CaCl_2$ Volume (ml)	Drug Amount (mg)	Formaldehyde (ml)
F1	1.5	0.0	50	20	0.05
F2	1.5	0.5	50	20	0.05
F3	1.5	1.5	50	20	0.05
F4	1.5	2.5	50	20	0.05
F5	2.0	0.0	50	20	0.05
F6	2.0	0.5	50	20	0.05
F7	2.0	1.5	50	20	0.05
F8	2.0	2.5	50	20	0.05
F9	2.5	0.0	50	20	0.05
F10	2.5	0.5	50	20	0.05
F11	2.5	1.5	50	20	0.05
F12	3.0	0.0	50	20	0.05
F13	3.0	0.5	50	20	0.05
F14	3.0	1.5	50	20	0.05

5.5. CHARACTERIZATION AND EVALUATIONS.

5.5.1. Rheological Evaluation.

The formulations were developed using a concentration optimum of sodium alginate solution, calcium chloride solution, and the additive polymer gelatin. The Brookfield viscometer (TV-10; Toki Sangyo Co. Ltd.) was used to determine the viscosity of the formulations. During the experiment, the spindles M2 and M3 were used.

Fig. 5.12: Brookfield Viscometer.

5.5.2. pH determination.

The pH of the developed formulations was determined by a digital pH metre (CL-110; Chemi Line) and noted.

5.5.3. Determination of Entrapment Efficiency.

After the preparation of beads, the beads were filtered, the supernatant fluid was collected, and the volume of supernatant was noted. Next, 0.1 ml of the filtrate sample was diluted up to 10 ml (100 times) with 0.1 N HCl and phosphate buffer at pH 6.8 separately. The diluted sample was analysed at 243 nm in a UV-3200 double-beam spectrophotometer. The amount of drug present in the supernatant fluid was determined from the standard curve prepared. So, the rest was entrapped in the beads. Now the entrapment efficiency was calculated as per the formula:

$$\%\, \boldsymbol{E.E} = \frac{(\boldsymbol{Theroetical\ amount\ of\ drug - Amount\ of\ drug\ in\ Supernatant})}{(\boldsymbol{Theoretical\ amount\ of\ the\ drug})} \times \mathbf{100}$$

5.5.4. Particle Size Analysis.

The sieving method was used for particle size analysis, in which the sieves were positioned in an order ranging from sieve number 12 to sieve number 25, and the sieves were shaken to determine the number of beads retained on the individual sieves.

5.5.5. Swelling Study.

The initial weight of the drug-entrapped beads was recorded in order to examine the swelling trend. For a length of time, the beads were suspended in 100 ml of 0.1N HCl pH 1.2, and the

weight was recorded every hour until a steady weight was attained. The swelling index of the beads was determined based on the weights obtained as follows:

$$\textbf{\textit{Swelling Index}} = \frac{\boldsymbol{Wt - Wo}}{\boldsymbol{Wo}} \times \mathbf{100}$$

Where, Wt= Mass of swollen beads at time t

Wo= Mass of dry beads at time= 0

Fig. 5.13: Swelling study of drug loaded alginate beads.

5.5.6. In-Vitro Drug Release Study.

The in vitro drug release study of all the developed formulations was conducted by the Dissolution Apparatus USP Type-II (Paddle Apparatus). The flasks were filled with 900 ml of dissolution media, the temperature of the apparatus was kept constant at 37 ± 0.5 °C, and the paddles were rotated at 100 RPM. The dissolution was carried out initially for 2 hours in 0.1N HCl (pH 1.2), followed by phosphate buffer at pH 6.8. In intervals starting from 5, 15, and 30 minutes, followed by 1-hour intervals, 5 ml of sample were withdrawn and replenished with a specific amount of the corresponding buffer. The collected samples are then properly diluted and analysed by a UV spectrophotometer at 243 nm.

CHAPTER 6

RESULTS AND DISCUSSIONS

6. RESULTS AND DISCUSSIONS.

Table 6.1: Characterization of the formulation composites and the developed formulations.

Formulation	pH	Viscosity (cP)	Entrapment Efficiency (%)	Entrapment Efficiency with Formaldehyde (%)	Swelling Inder at 6 hours
F1	7.4	266.4	63.645	71.55	66.95
F2	7.11	307.6	60.483	66.81	70.86
F3	6.68	379.1	67.483	67.90	61.62
F4	6.48	512.2	68.387	68.64	56.70
F5	7.41	656.6	62.854	72.50	73.88
F6	7.07	687.8	58.300	79.90	53.26
F7	6.54	811.4	66.515	68.26	55.05
F8	6.36	1071.2	62.903	77.87	62.01
F9	7.43	1226	60.600	71.00	34.31
F10	7.09	1289	57.911	73.90	39.81
F11	6.63	1357	68.640	67.87	29.52
F12	7.45	2360	56.137	74.06	38.75
F13	7.31	2856	66.568	69.40	39.09
F14	6.98	3858	63.600	66.71	36.54

6.1. Drug Entrapment Efficiency.

The entrapment efficiency (%) of all the formulations, with or without formaldehyde was mentioned in the ***Table 6.1.*** From the table, it was observed that the entrapment efficiency was increased when the sodium alginate concentration was increased alone from 1.5–3%. This happens due to the fact that at lower concentrations of alginate solution, when dropped into $CaCl_2$, instant cross-linking of the sodium alginate molecules occurs at the surface, and all the molecules, due to the pulling by the $CaCl_2$ molecules, approach the outer surface facing $CaCl_2$ solution. The drug-containing water component present in the drop mixture was encapsulated by the cross-linked alginate gel. The drug diffusion from that gel layer was comparatively lower since the total polymer embedded in the outer film increased, the viscosity of that outer film also increased, and the aqueous drug remained within the vesicle. The aqueous drug becomes dispersed rather than remaining within the cross-linked layer; the drug diffusion from that gel layer was slow. Whereas when the additive polymer gelatin was added to the batches, the entrapment efficiency was increased gradually to a certain concentration. When the total polymer concentration, i.e., sodium alginate along with gelatin, lies within 3.5–4%, the entrapment efficiency is at its maximum. This may be due to the increased viscosity of the polymer composite and the surface of the formed beads. When the same formulation was treated with formaldehyde solution, drug entrapment

efficiency was observed to be increased as compared to the same formulations without treatment. This may be a synergistic effect of the cross-linking of gelatin molecules by formaldehyde in addition to the cross-linking of alginate molecules by calcium ions.

6.2. Study of pH and Rheological Evaluation.

The pH of the polymer composites of formulations was analysed by a digital pH metre, and the viscosity of the same was determined by a Brookfield viscometer. It was observed that when the gelatin concentration is gradually increased with a fixed sodium alginate concentration, the pH of the mixture solution is gradually decreased. Similarly, the viscosity increased with an increase in gelatin concentration to a fixed alginate concentration. This was due to the increased concentration of gelatin, which behaves slightly acidic to a fixed concentration of alginate.

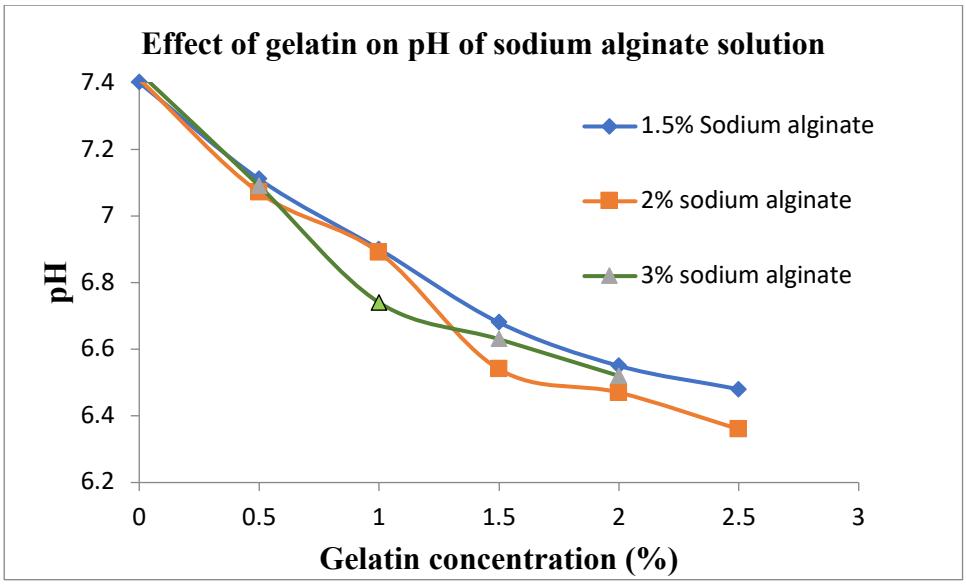

Fig. 6.1: Effect of Gelatin on pH of Sodium alginate solution.

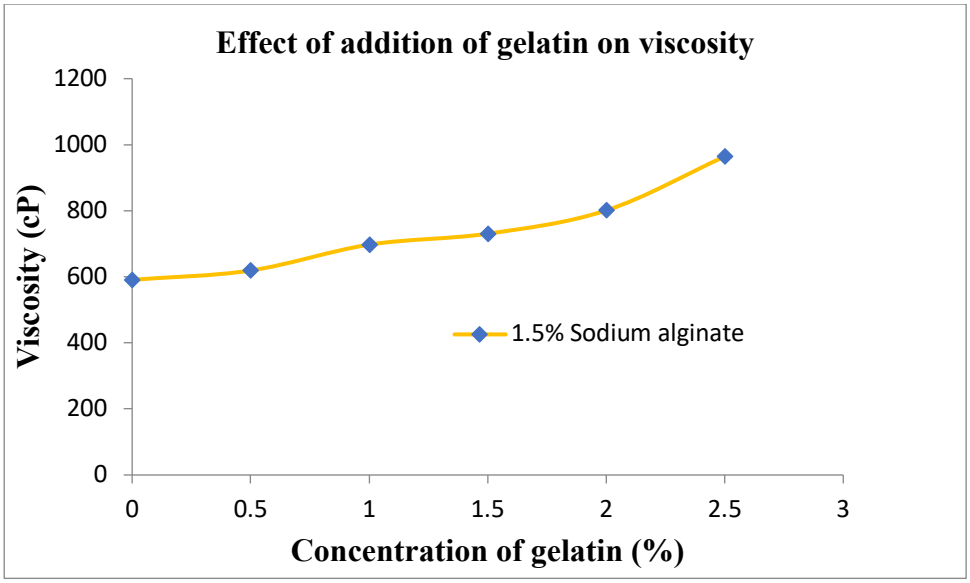

Fig. 6.2: Effect on viscosity on addition of gelatin in 1.5% Sodium alginate solution.

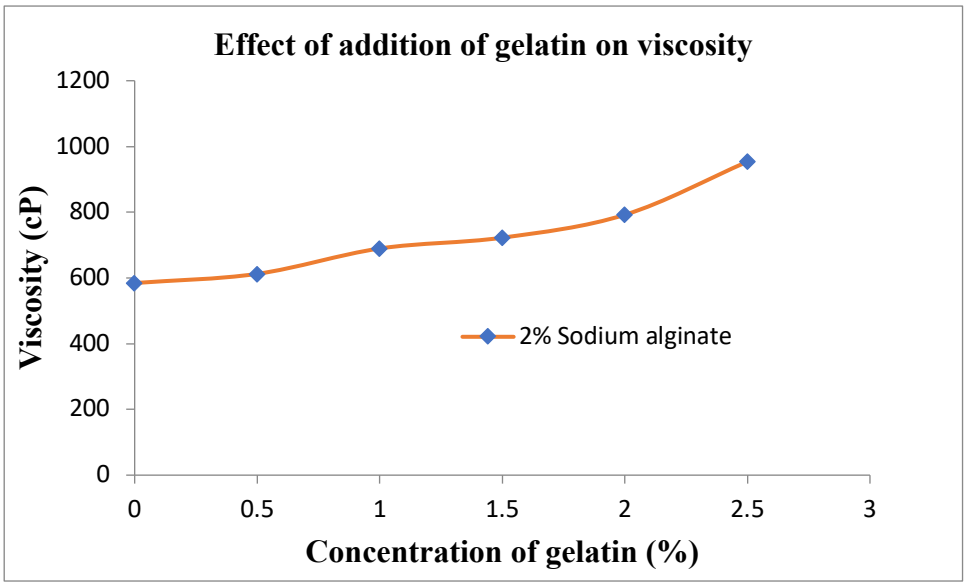

Fig. 6.3: Effect on viscosity on addition of gelatin in 2% Sodium alginate solution.

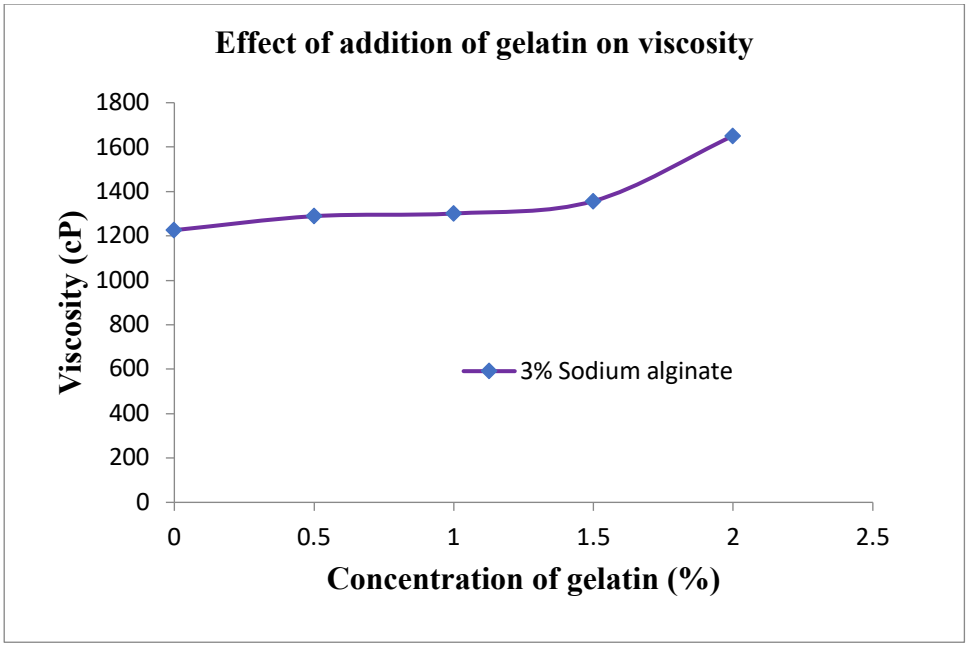

Fig. 6.4: Effect on viscosity on addition of gelatin in 3% Sodium alginate solution.

6.3. Swelling study.

From the ***Table 6.1.*** it was found that the swelling percentage increased with an increase in sodium alginate concentration from 1.5 to 2% due to the high content of sodium alginate. But above 2% sodium alginate, swelling percentages were found to decrease. It may be due to the high degree of cross-linking and retardation of water penetration. At a fixed sodium alginate concentration (up to 2%), when gelatin was added, the sweeling of the beads decreased with the increase in gelatin concentration. This is due to the increase in the degree of interaction and the increase in viscosity. But above 2% alginate concentration, the addition of gelatin does not significantly change the swelling property.

Table 6.2: Particle size analysis.

Formulation	Sieve No. 12 (1.7mm)	Sieve No. 16 (1.18mm)	Sieve No. 20 (0.85mm)	Sieve No. 25 (0.71mm)
	% Retention			
F1	0.726016	45.4624	48.40104	5.410545
F2	0	59.52381	35.71429	4.761905
F3	0	66.66667	27.77778	5.555556
F4	0.21575	79.5685	19.137	1.078749
F5	2.020202	48.48485	45.45455	4.040404
F6	3.030303	61.61616	32.32323	3.030303
F7	7.407407	64.81481	23.14815	4.62963
F8	13.76147	68.80734	17.43119	0

F9	12.71186	57.62712	27.11864	2.542373
F10	26.08696	52.17391	21.73913	0
F11	33.33333	45.83333	20.83333	0
F12	20	54.4	25.6	0
F13	32	48	20	0
F14	38.46154	42.30769	19.23077	0

6.4. Particle Size Analysis.

The aperture sizes of the sieves were noted in millimetres. It was noted that a maximum of 5% of the beads were able to pass through sieve number 25 (0.71 mm) for some developed formulations, but none of the particles passed when the total polymer concentration was more than 4% or 2.5% alginate alone. It was also observed that increases in alginate concentration result in a gradual increase in particle size, whereas the addition of gelatin to a fixed alginate also increases particle size.

6.5. In-vitro drug release profiles.

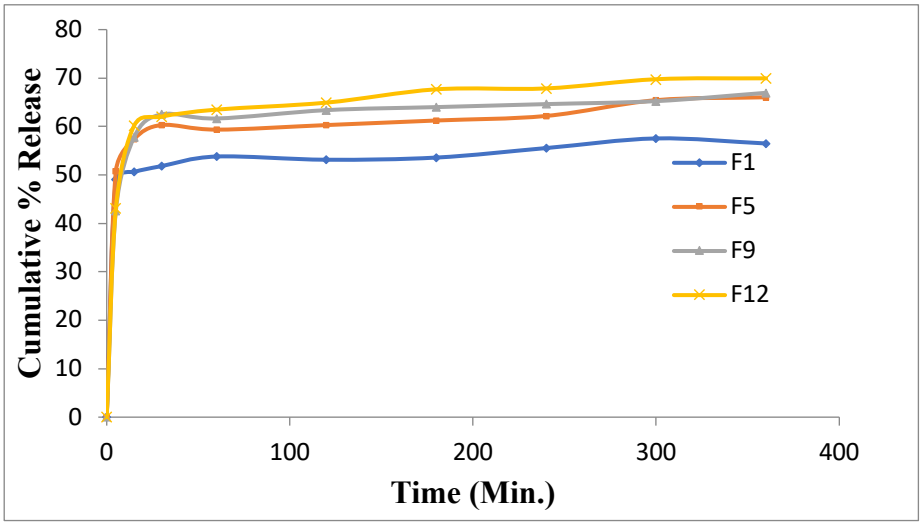

Fig. 6.5: Comparison of release profiles of F1, F5, F9 and F12.

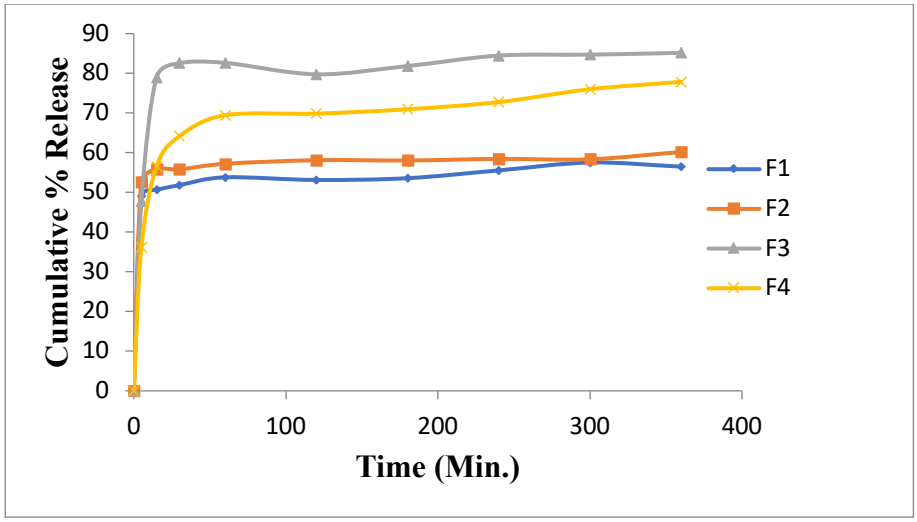

Fig. 6.6: Comparison of release profiles of F1, F1-F4.

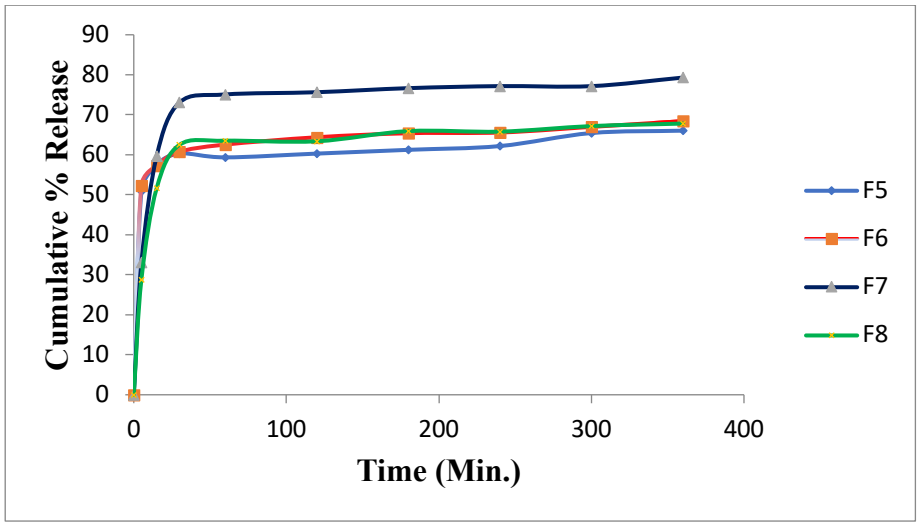

Fig.6.7: Comparison of release profiles of F5-F8.

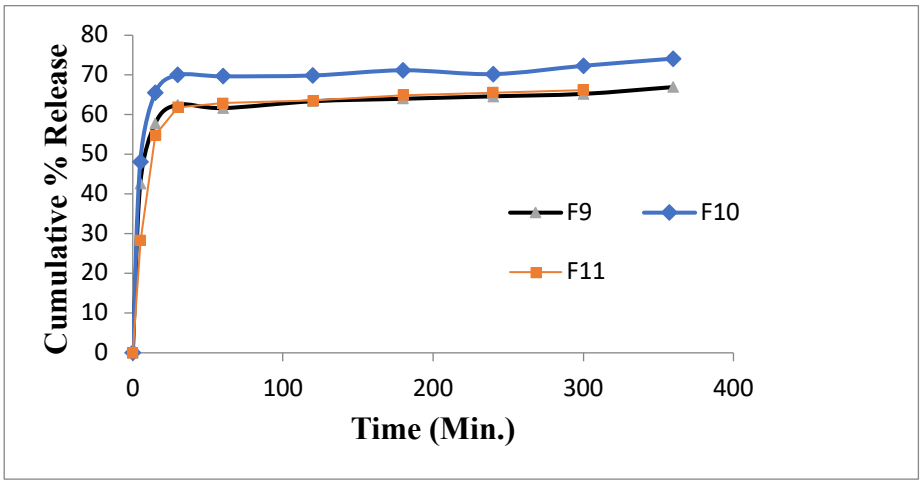

Fig. 6.8: Comparison of release profiles of F9-F11.

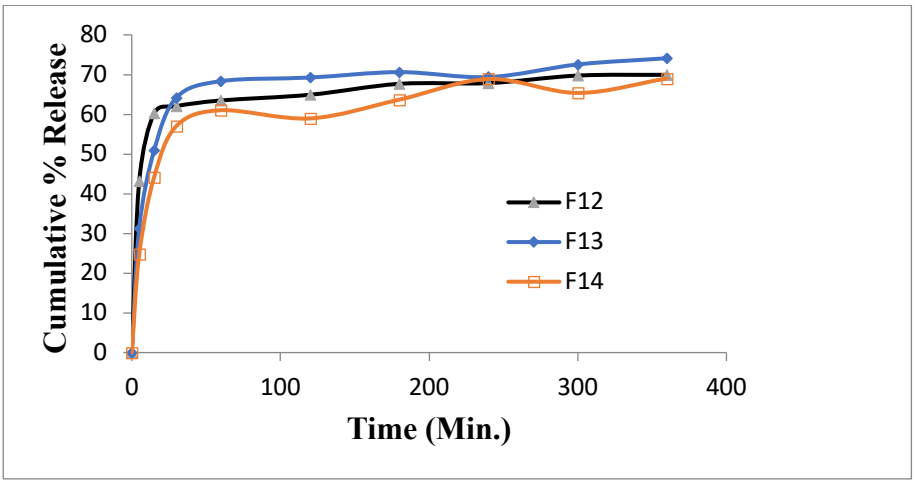

Fig.6.9: Comparison of release profiles of F12-F14.

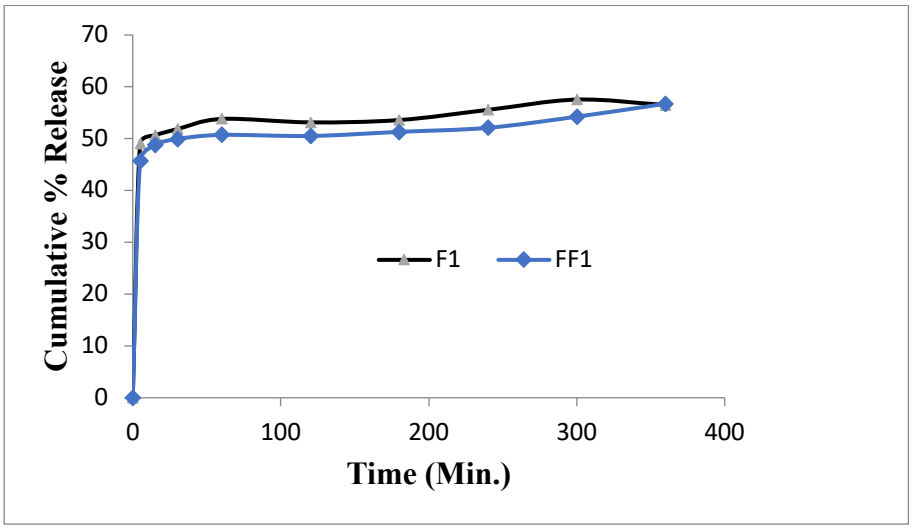

Fig. 6.10: Comparison of release profiles of F1 & FF1.

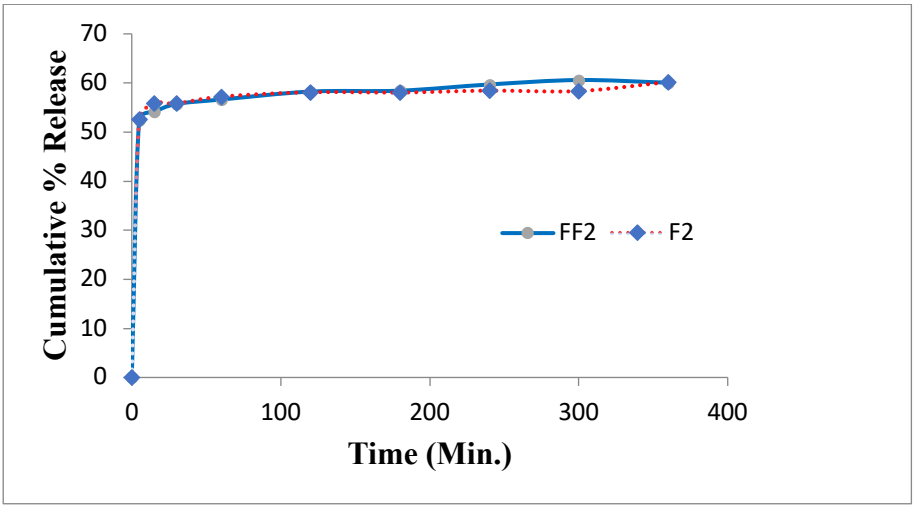

Fig. 6.11: Comparison of release profiles of F2 & FF2.

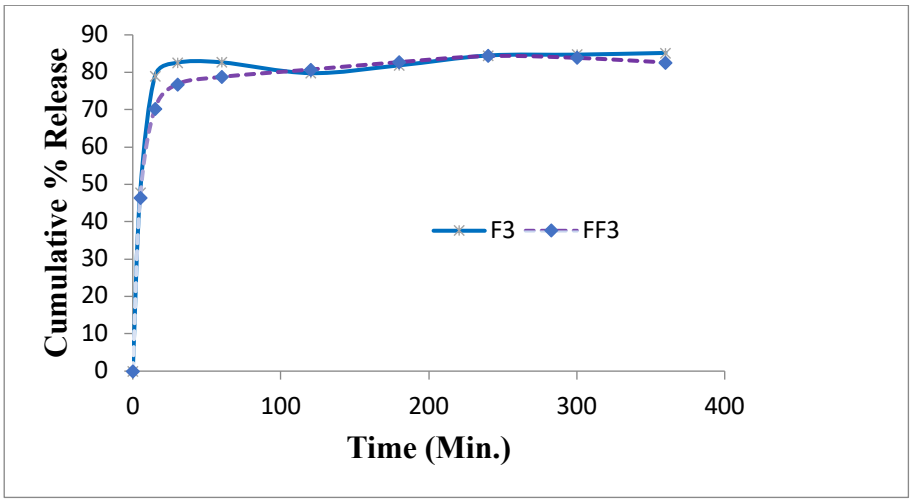

Fig. 6.12: Comparison of release profiles of F3 & FF3.

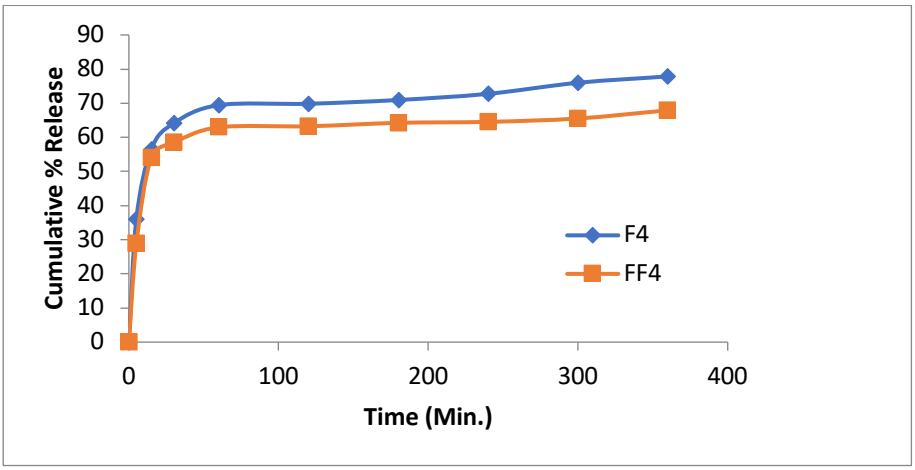

Fig. 6.13: Comparison of release profiles of F4 & FF4.

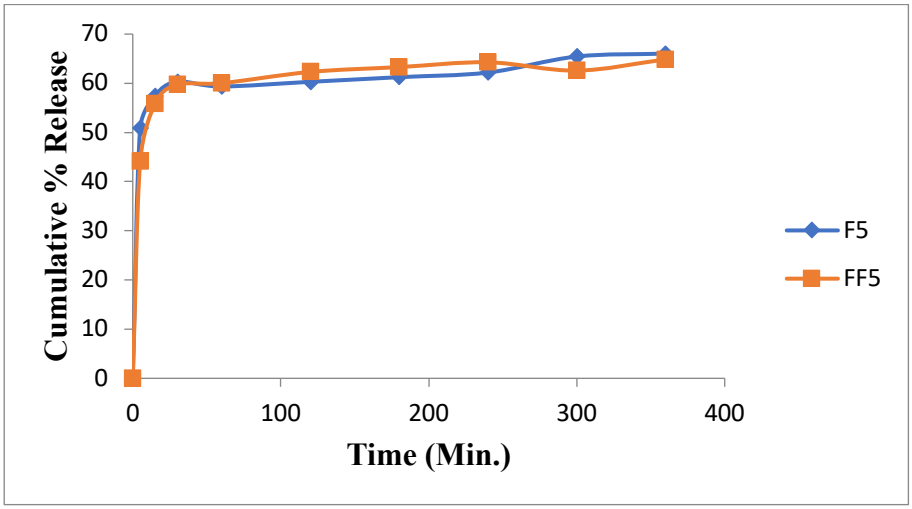

Fig. 6.14: Comparison of release profiles of F5 & FF5.

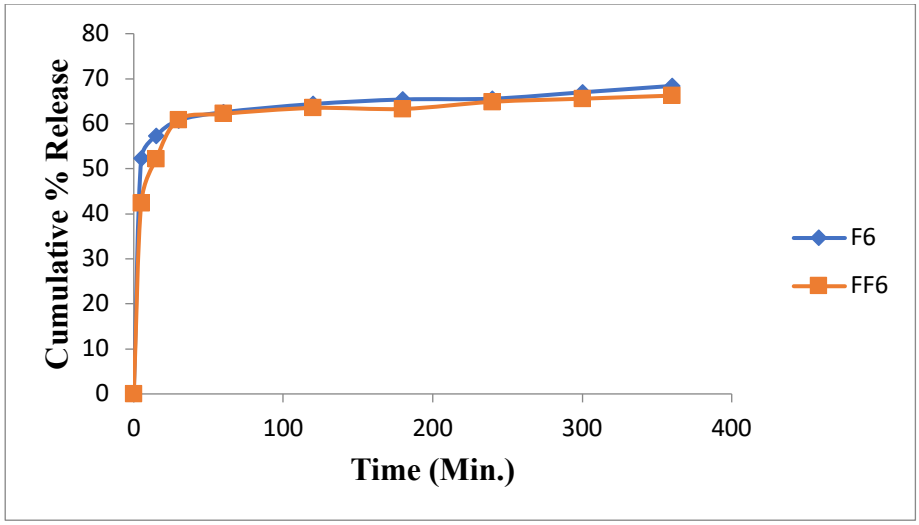

Fig. 6.15: Comparison of release profiles of F6 & FF6.

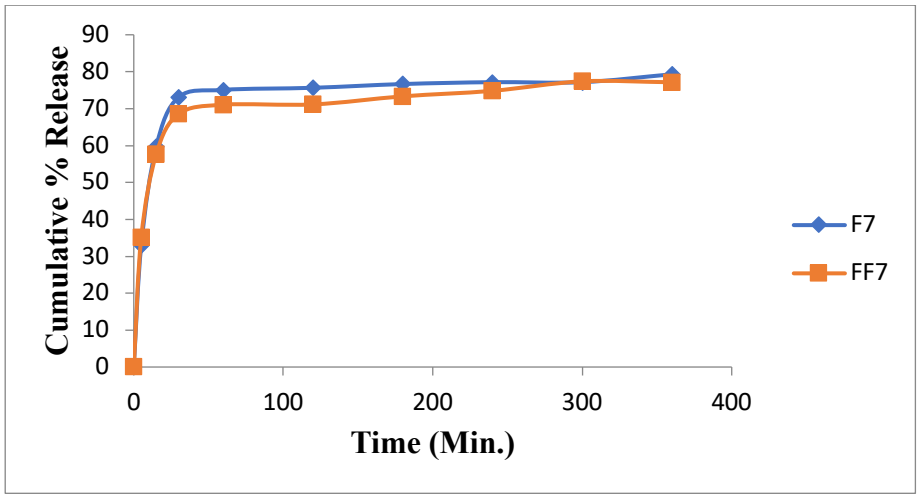

Fig. 6.16: Comparison of release profiles of F7 & FF7.

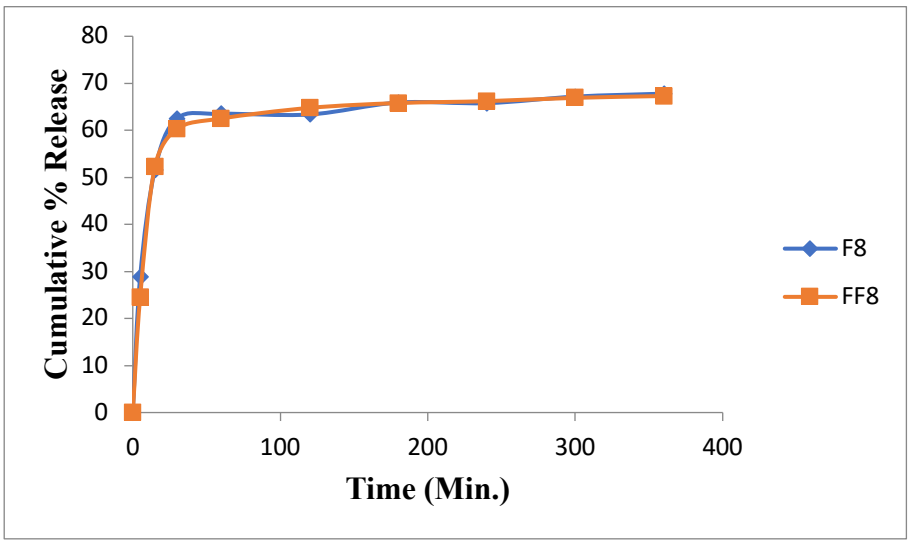

Fig. 6.17: Comparison of release profiles of F8 & FF8.

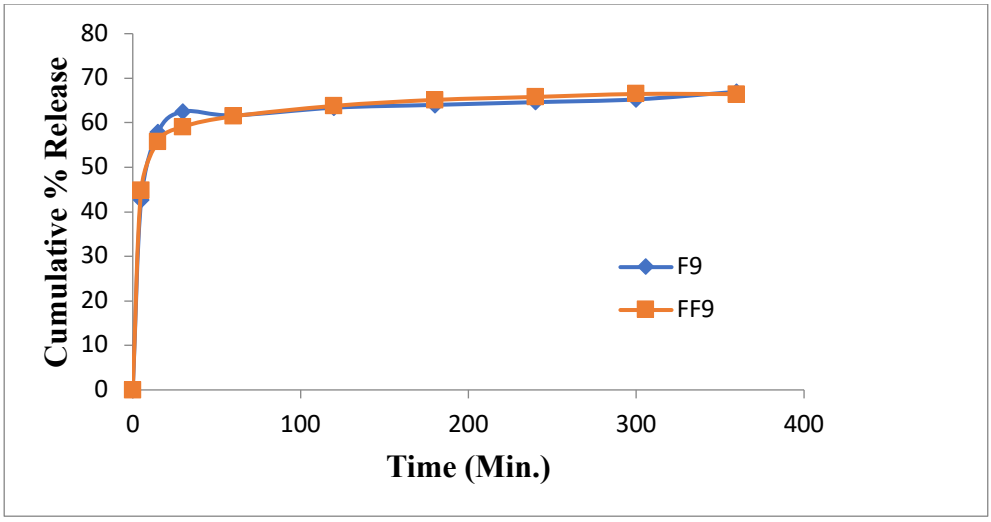

Fig. 6.18: Comparison of release profiles of F9 & FF9.

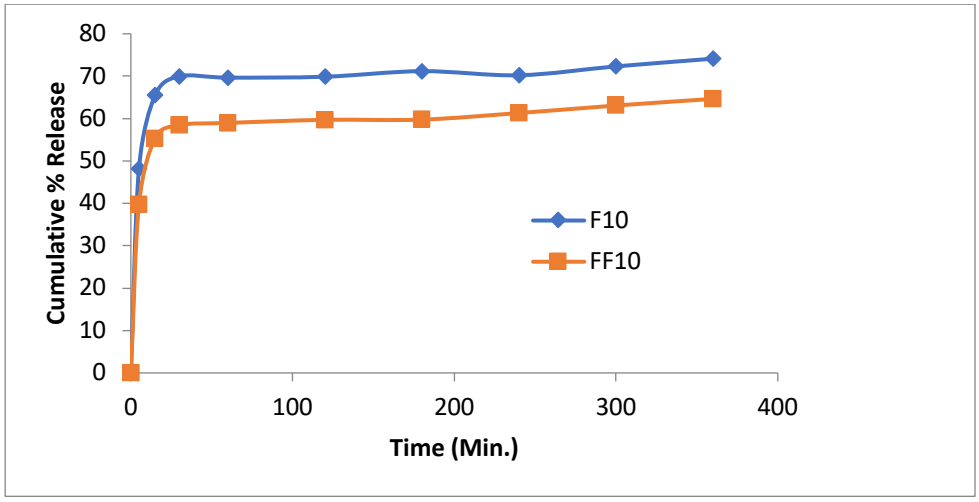

Fig. 6.19: Comparison of release profiles of F10 & FF10.

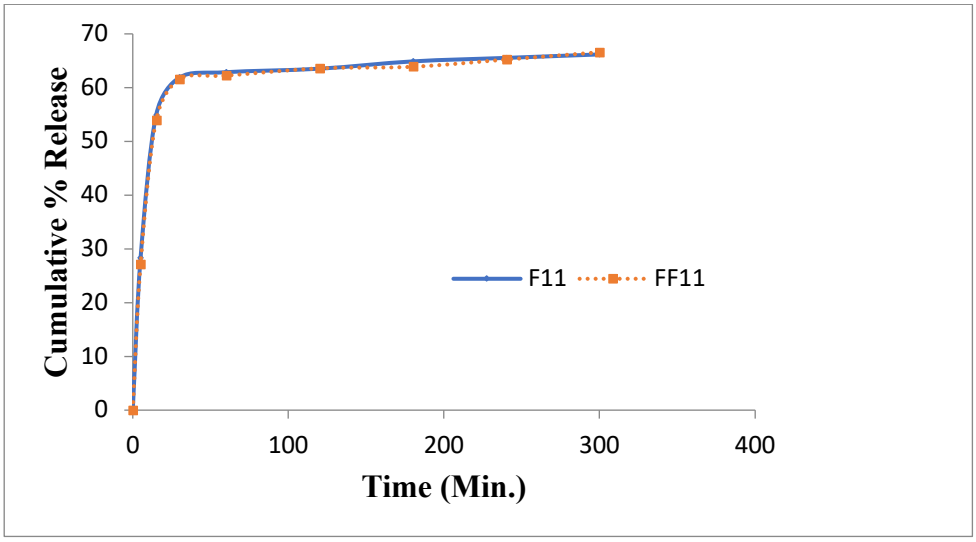

Fig. 6.20: Comparison of release profiles of F11 & FF11.

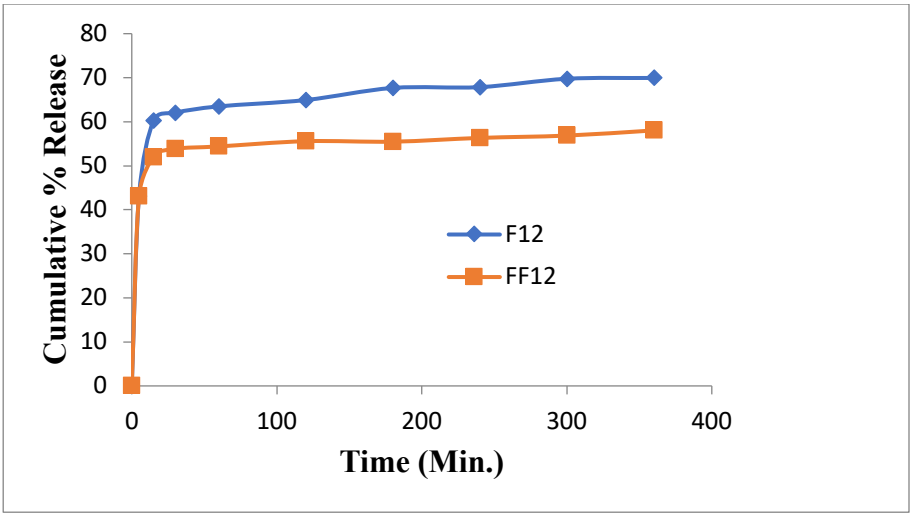

Fig. 6.21: Comparison of release profiles of F12 & FF12.

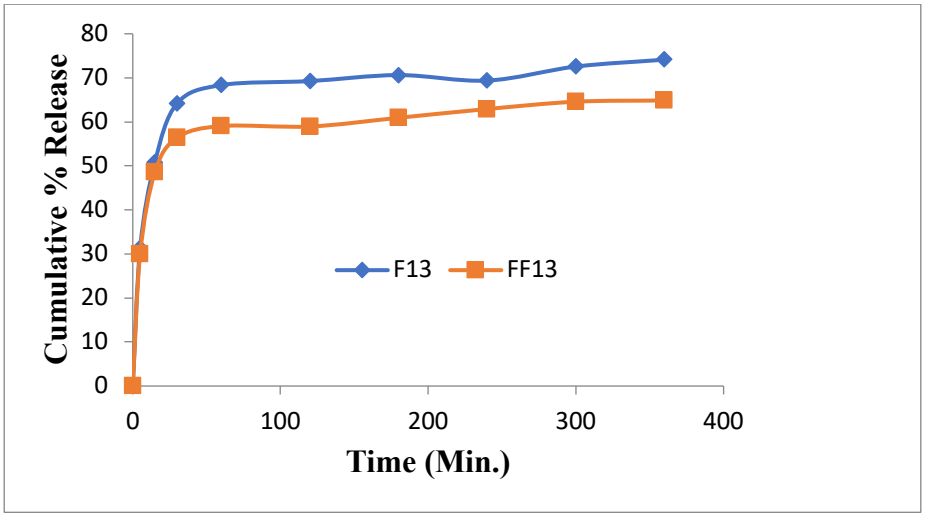

Fig. 6.22: Comparison of release profiles of F13 & FF13.

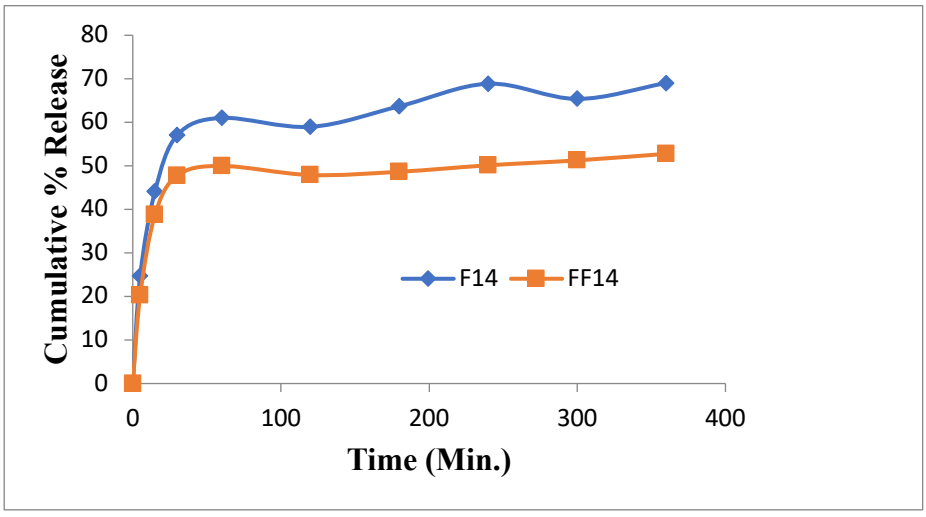

Fig. 6.23: Comparison of release profiles of F14 & FF14.

Drug release profiles in 0.1N HCl for two hours followed by in phosphate buffer pH 6.8 of all the formulations were represented graphically (***Fig.6.5***). From (***Fig. 6.6, 6.7, 6.8 & 6.9***) it was found that drug release increased with an increase in alginate concentration in spite of two different pH's. This may be because of the lesser degree of cross-linking at the surface of the bead, the restriction of cross-linking in the core once the surface was cross-linked, and the retardation of subsequent cross-linking of the core material. The figure revealed that with an increased concentration of gelatin in a fixed concentration of alginate, drug release also increases. It may be due to the formation of gel structures at the surface of beads, which turn to nearly a sol state at dissolution temperature, which is very close to gelatin sol temperature (above 40°C). The release profiles from all the formalin-treated beads were compared with the corresponding formulations without formalin treatment, as depicted in (***Fig. 6.10 to 6.23***). It was observed that very little deviation in the drug release profile was observed when alginate concentration was low, but at a higher concentration of alginate with formalin treatment, there was a significant retardation in the drug release profile. When formalin was added, gelatin molecules cross-linked and formed a strong network with the sodium alginate network; as a synergistic effect, drug release was significantly retarded.

Table 6.3: Drug Release Kinetics of the developed beads.

For mulation	Zero-Order		First Order		Higuchi		Korsmeyer-Peppas			
	R^2		R^2		R^2		R^2	n	R^2	n
	0-2h	2-6h	0-2h	2-4h	0-2h	2-4h	0-2h	2-4h	0-2h	2-4h
F1	0.604	0.807	0.605	O.801	0.773	0.824	0.901	0.832	0.028	0.071
F2	0.676	0.626	0.694	0.716	0.81	0.565	0.918	0.505	0.029	0.024
F3	0.232	0.873	0.217	0.511	0.389	0.915	0.613	0.945	0.151	0.063
F4	0.528	0.973	0.601	0.965	0.708	0.947	0.849	0.915	0.206	0.101
F5	0.395	0.937	0.405	0.933	0.564	0.915	0.763	0.886	0.051	0.088
F6	0.713	0.947	0.748	0.941	0.865	0.916	0.972	0.881	0.066	0.051
F7	0.459	0.857	0.530	0.846	0.642	0.833	0.799	0.806	0.256	0.036
F8	0.423	0.885	0.460	0.893	0.606	0.910	0.777	0.925	0.243	0.058
F9	0.402	0.936	0.432	0.928	0.571	0.900	0.757	0.861	0.117	0.045
F10	0.344	0.778	0.367	0.776	0.515	0.734	0.719	0.687	0.111	0.046
F11	0.391	0.960	0.436	0.965	0.566	0.981	0.736	0.993	0.242	0.044
F12	0.418	0.894	0.460	0.902	0.583	0.924	0.757	0.942	0.120	0.067
F13	0.547	0.763	0.613	0.767	0.728	0.719	0.864	0.668	0.253	0.056
F14	0.468	0.686	0.497	0.678	0.656	0.731	0.819	0.778	0.276	0.132

Table 6.4: Drug Release Kinetics of the developed beads treated with formaldehyde.

For Mulation	Zero-Order		First Order		Higuchi		Korsmeyer-Peppas			
	R^2		R^2		R^2		R^2	n	R^2	n
	0-2h	2-6h	0-2h	2-4h	0-2h	2-4h	0-2h	2-4h	0-2h	2-4h
FF1	0.476	0.932	0.483	0.923	0.661	0.888	0.854	0.845	0.032	0.099
FF2	0.859	0.792	0.873	0.79	0.96	0.81	0.990	0.935	0.011	0.007
FF3	0.459	0.311	0.562	0.294	0.633	0.384	0.794	0.463	0.167	0.027
FF4	0.447	0.895	0.508	0.884	0.623	0.855	0.772	0.815	0.235	0.056
FF5	0.507	0.404	0.550	0.407	0.676	0.410	0.676	0.410	0.104	0.025
FF6	0.563	0.914	0.599	0.917	0.740	0.890	0.891	0.850	0.131	0.042
FF7	0.464	0.930	0.523	0.931	0.648	0.951	0.810	0.961	0.220	0.080
FF8	0.460	0.972	0.534	0.975	0.636	0.987	0.772	0.991	0.290	0.034
FF9	0.603	0.885	0.659	0.890	0.767	0.929	0.899	0.961	0.107	0.039
FF10	0.385	0.943	0.412	0.940	0.556	0.904	0.744	0.856	0.121	0.074
FF11	0.406	0.940	0.456	0.937	0.581	0.906	0.744	0.867	0.254	0.050
FF12	0.465	0.899	0.489	0.897	0.633	0.850	0.808	0.793	0.075	0.038
FF13	0.465	0.953	0.505	0.957	0.648	0.975	0.809	0.985	0.210	0.095
FF14	0.401	0.990	0.418	0.988	0.588	0.971	0.768	0.945	0.269	0.087

6.6. In- vitro Release Kinetics.

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 6.3 & 6.4***) 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 and formalin treated beads. The kinetic data were subjected for fitment in the Korsmeyer Peppas equation. The acceptable linearity was observed shown in (***Table 6.3 & 6.4***) and the release exponent 'n' varied from 0.028 to 0.276 in 0.1N HCl up to 2 hours and 0.036-0.132 in phosphate buffer for beads without formalin treatment indicated that drug release followed by diffusion only. For formalin treated beads the 'n' varied from 0.011 to 0.29 in 0.1N HCl up to 2 hours and 0.007-0.099 in phosphate buffer indicated the drug release followed by diffusion also.

CHAPTER 7

CONCLUSIONS

7. CONCUSIONS.

The current study concluded that an optimal concentration of sodium alginate, gelatin, and calcium chloride was successfully determined. The conclusion may be inferred that increasing the concentration of sodium alginate as a base polymer from 1.5–3% resulted in a non-significant variation in entrapment efficiency (56–68%). The optimal calcium chloride concentration for cross-linking was found to be 2%. With different $CaCl_2$ concentrations and a fixed concentration of sodium alginate, there was no significant variation in entrapment efficiency. Increased additive polymer gelatin content in alginate improved entrapment efficiency by 4–10% and increased drug release significantly by 5-29%. Now, the introduction of formaldehyde appears to boost entrapment efficiency by 6-21% while slowing drug release. These research findings concluded that modified approaches for the development of hydrogel beads may be a better alternative for sustained-release drug delivery systems.

CHAPTER 8

FUTURE WORK

8. FUTURE WORK

The following work is to be done in future for the completion of this research work:

- Confirmatory study for polymer composite in entrapment and release behavior with other drugs.
- Extensive in-vitro release studies for other drugs.
- In-vivo studies.
- In-vitro - in-vivo correlation.
- Statistical analysis.

CHAPTER 9

REFERENCES

9. REFERENCES.

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