DEVELOPMENT AND EVALUATION OF MULTIPARTICULATE DRUG DELIVERY SYSTEM

Submitted by

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CERTIFICATE OF APPROVAL

This is to certify that **MOUMOYEE CHAKRABORTY** has carried out her M. Pharm thesis on the topic "**DEVELOPMENT AND EVALUATION OF MULTIPARTICULATE DRUG DELIVERY SYSTEM**" (Examination Roll No.:**M4PHP23032**) under my direct supervision in the division of **Pharmaceutics** at the **Department of Pharmaceutical Technology, Jadavpur University.** She has incorporated her finding into the thesis submitted by her in partial fulfilment of requirements for the award of the degree of **MASTER OF PHARMACY**. I am satisfied that she has carried out this work with proper care attention to my entire satisfaction.

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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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CONTENTS

ABBREVIATIONS

SA	Sodium Alginate
CCS	Calcium Chloride Solution
SAS	Sodium Alginate Solution
DDS	Drug Delivery System
EE	Entrapment Efficiency
SI	Swelling Index
GC	Gelatin Concentration
HCHO	Formaldehyde
PCM	Paracetamol
Fig	Figure

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Dedicated to

My Beloved Family

CHAPTER 1

INTRODUCTION

INTRODUCTION

Over the past few decades there are several approaches employed in order to treat some serious health disorders and to achieve the desired pharmacological action over a sustained period of time. The majority of multi-particulate drug delivery methods are oral dosage forms made up of numerous tiny discrete units, each of which possesses a variety of desired properties. These methods divide the medication dosage into a number of subunits, which are typically made up of thousands of spherical particles with a diameter of 0.05 to 2.00 mm. As a result, pharmaceutical formulations known as multiparticulate dosage forms contain the active ingredient in the form of numerous tiny, independent subunits. These subunits are combined or compressed into a tablet, sachet, or capsule to produce the recommended total dose. A multipleunit system is made up of distinct particles called multiparticulates. Due to their tiny size, they offer various benefits over single-unit systems. Comparing multiparticulate systems to conventional (monolithic) formulations, the former exhibit more consistent pharmacokinetic behaviour. Coating multiparticulates is one method of changing the medication release profile in those materials. Multiparticulates are coated for a variety of reasons, including to create functional coatings, increase chemical stability, boost patient acceptance, and improve physical properties. Aqueous polymer dispersions, polymer solutions, molten polymers, and dry powders are the main types of polymeric coating materials used to create coats. Functions including sustained release (SR), targeted release, delayed release, and pulsatile release can be accomplished depending on the type of coating material utilised [1,2].

For biomedical purposes, the field of naturally occurring polysaccharides of marine origin is already substantial and growing. Alginate is one of the seaweed-derived marine polysaccharides that is extremely prevalent in marine brown algae. Bacteria are another source of alginate. The resulting alginic acid can subsequently be transformed into a salt, such as sodium alginate, which is the primary form used at the moment. Alginates are widely used in the food and pharmaceutical industries as tablet disintegrants, thickeners, and suspending agents due to their advantageous properties like biocompatibility, non-toxicity, and ease of gelation. . Hence there was an attempt to formulate the macroscopic Alginate beads to enable its sustained action for a long time for the treatment of a group of serious health disorders [3].

Multiparticulate Sustained Release Drug Delivery System

A recurring dose of different medications must be delivered as part of the chronic disease therapy. Additionally, medications with a relatively short half-life are administered multiple times during the day. To overcome this, a sustained release dosage form must be created, which aids in maintaining the drug's therapeutic effective concentration for a prolonged length of time. The sustained release dosage form is a type of drug delivery system created to deliver medication continuously over an extended period of time following the administration of a single dose of a drug or tablet in order to prolong the therapeutic effect [4].

The ultimate goal of the treatment is to maintain a constant state tissue level that is therapeutically useful and non-toxic for a long time. An essential element in obtaining, providing, and completing this goal is the creation of the appropriate dosage form. By striving for and achieving a maximum rate and extent of drug absorption, the highest drug bioavailability can be achieved. The optimum method for maximizing drug distribution, which is the most important factor in achieving a measure of control over the therapeutic impact and reducing in vivo variability, is thought to be sustained release dosage forms. The main goals of sustained release dosage forms are to increase the time that a drug remains active, reduce fluctuations in plasma level, improve drug utilization, and reduce side effects. They also aim to reduce frequency of dosing and provide uniform drug delivery. By altering the pharmacokinetics and pharmacodynamics of pharmacologically active drug molecules using novel drug delivery systems or by altering the molecular structure and physiological parameters inherent in a chosen route of drug administration, sustained release delayed drug delivery systems are able to demonstrate their well-defined action. The sustained drug delivery process takes place when a drug or active agent is combined with a polymer in a way that the release from the bulk material is pre-designed and its release is also predetermined by the use of film forming polymer and enteric depending on its use, respectively. Both controlled release and sustained release are employed in a uniform and perplexing manner. Both are used to denote various drug administration methods. As any dose form that delivers medication over a lengthy period of time qualifies as sustained release, it demonstrates the system's ability to provide some real therapeutic control, which may be of a temporal, spatial, or both natures. While sustained release systems typically fail to achieve zero order type release, they do their best to imitate it by dispensing drugs in a sluggish first order [5,6]

Method of Formulation of Sustained Release Drug Delivery System

There are two basic components of Sustained Release Dosage Form: A Loading Dose and A Maintenance Dose. All drug delivery methods that provide a slow release of the medicine over an extended period of time are considered sustained release dosage forms. In essence, a sustained release oral dosage form is made to quickly release a predetermined portion of the total dose (the loading dose) into the digestive tract, producing the desired pharmacological response as soon as possible, and then slowly release the remaining portion of the total dose (the maintenance dose) to maintain the steady state. Desired controlled release of the Drug Product is constructed so that the release rate for the maintenance dose is equal to the elimination rate. Controlled release systems can be used to achieve constant blood levels, and the dosage form's prolonged release reduces plasma fluctuation by slowing down its absorption rate, allowing for both the achievement and maintenance of the drug's slower release rate. Hence the maintenance dose, also known as the slowly released part, releases the medication gradually and keeps the blood level of the drug at the therapeutic range for a long time. The immediate-access part of the loading dose aids in achieving the therapeutic level rapidly after administration [7].

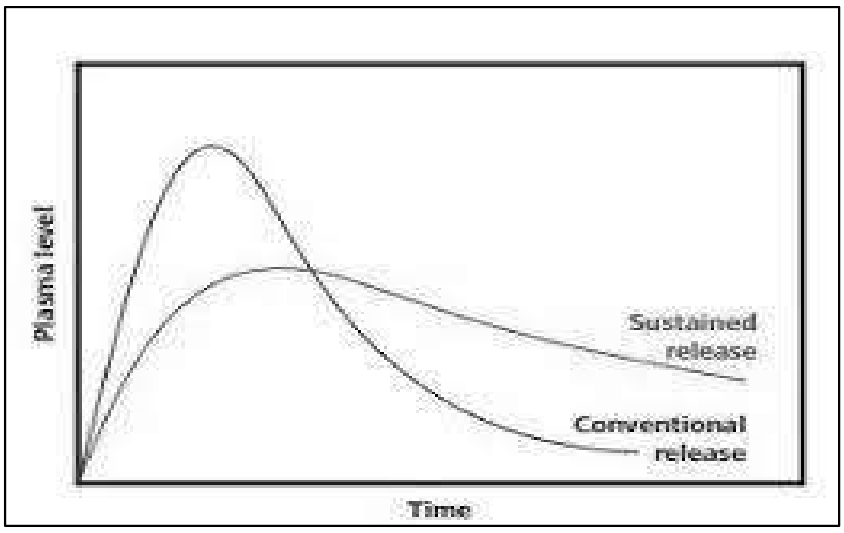


Fig 1: Plasma level-Time Curve for Sustained Release Drug Delivery System

The rate of drug release from the maintenance dose should follow zero order rate if the drug concentration at the absorption site is to remain constant. Zero order denotes that the drug release is unaffected by the original drug concentration or dosage. The drug should release from the loading dose according to first order rate release, which is dependent on the drug's initial concentration [8].

Benefits of Sustained Release Drug Delivery System

- The main benefit of the sustained release dosage form is that it constantly releases the medication, resulting in long-lasting therapeutic effects.
- It prolongs the effects of the medications and minimizes frequent dosage.
- A sustained release dose form can be used to control medication therapy.
- Utilizing a sustained release dosage form allows for the modification of the scope and rate of medication absorption.
- Another significant advantage of a sustained release dose form is patient compliance.
- It is simple to handle and transport, and it does not require particular storage conditions.
- Coating or granulating active medicinal substances might cover up their disagreeable taste and odor.
- Additionally stable in terms of their physical, chemical, and microbiological characteristics are these dosage forms.
- This technology delivers the API directly to the target site and offers the advantage of a single dose for the length of treatment.
- By lowering the overall dosage of the medicine, it lessens the local negative effects.
- To reduce the number of dosages the patient must take, several medications and excipients or components might be combined.
- Maximize the availability of minimum dose.
- Increases the safety margin of high potency drug.

Hence as a whole Sustained release dosage form outweighs many risks offered by the conventional dosage formulations and it has several benefits over the conventional therapies.

Characteristics of Sodium Alginate Beads

Many studies on the protection and controlled release of bioactive substances utilized in the pharmaceutical, cosmetic, and food industries have been done as a result of extensive study in the field of encapsulation [9, 10]. The usage of hydrogel beads that included encapsulated drugs has generated a lot of attention, making the quest for suitable excipients that provide stability and controlled release a significant problem. Due to their functional and sensory qualities, as well as their properties linked to wall protection and distribution, a number of hydrocolloids and biopolymers are used for this purpose. Due to its encapsulating/gelling capabilities when cross-linked with divalent ions like calcium [11], sodium alginate (derived from brown algae) is one of the most popular excipients utilized in the formulation of hydrogel beads.

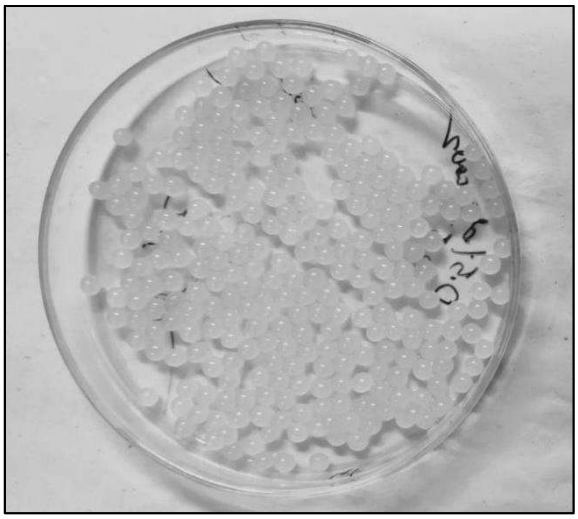


Fig 2: Sodium Alginate Beads prepared by crosslinking with CCS

Advantages

- a quick and inexpensive preparation technique that involves adding an aqueous solution (or emulsion) of the biopolymer and the bioactive substance drop by drop into CaCl2.
- The biocompatible characteristics improves it release and modifies the wall properties.
- Since the inside of it is believed to be chemically inert, it is versatile, permitting the encapsulation of a variety of bioactive compounds, which are extremely susceptible to external conditions.
- It is environmentally friendly and biodegradable.

Brief Insight

Because of its qualities including natural breakdown, creation of gels, biological compatibility, and non-toxicity, alginates are especially well recognized for their uses in the pharmaceutical sector for controlled drug delivery, wound repair, skincare, and scaffolds. Alginates, a type of natural gum, are more advantageous than synthetic polymers since they may be easily obtained, produce hydrogels, and are economical. A variety of pharmaceutical uses are made possible by the relatively simple oral administration of alginate gels into the human body. Alginate gels represent exciting biomaterials for tissue engineering and transplantation of cells that can be used to replace missing or failing organs and tissues in sufferers.

Mechanisms of drug release from Multiparticulate sustained release DDS

There are few mechanisms based on which drug is released from Mutiparticulate sustained release dosage forms.

1. Diffusion: Water dissipates into the inside of the particle when it comes into touch with water-based fluids in the GIT. Drug solutions may dissolve and diffuse through the release membrane to the outside.

2. Erosion: There are Certain coats can be made to progressively wear away over time, releasing the medication that is trapped inside the particle.

3. Osmosis: If the correct conditions are met and water is allowed to enter, an osmotic pressure may develop inside the particle. By the protective coating, the medication was compelled from the particle and onto the surface [12].

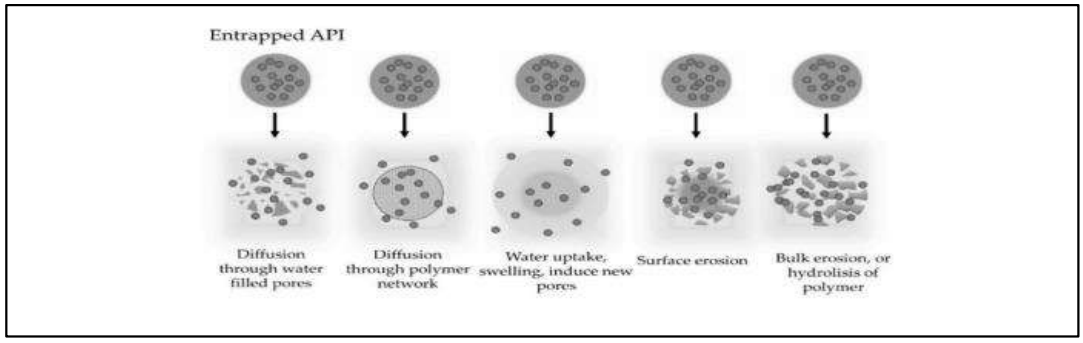


Fig 3: Mechanism of Drug release from beads [37]

Methods of Preparation of Sodium Alginate Beads

There are different methods employed to formulate sodium alginate beads by optimum crosslinking and drug loading at the same time.

Ionotropic Gelation Method

At first SAS is prepared by dissolving certain amount of Sodium Alginate in double distilled water to make an aqeous solution and then allowed to drop it from a syringe into CaCl2 solution in an appropriate concentration. After an hour of continuous stirring, the calcium alginate beads ought to be collected by filtration, cleaned with distilled water, and dried by air overnight. The medications are loaded using both the simultaneous and sequential methods.
A) In the sequential method Calcium alginate beads were made according to the instructions in the preceding paragraph. After being submerged and agitated in a solution containing an active medication for 1 hour, the wet beads were filtered and rinsed with distilled water. Calcium alginate beads with loaded drug are finally dried.
B) In the simultaneous technique, the drug loading into the beads and the calcium ion-induced gelation of the beads happened simultaneously. Dropwise additions of SAS were made to CaCl2 solutions containing the drug. These beads were taken out of the counter ion solution after 1 hour [13].

Fig 4 : Drug loading into beads by Ionotropic gelation method

Emulsion-Gelation Method

The emulsion gelation procedure was used to produce the calcium alginate beads with oil trapped within them. At a speed of 100 rpm, the polymer became dispersed in the water. The polymer solution was supplemented with chosen oils. It had 50 mg of the medication put to it. The homogenised or non-homogenized mixture was gently stirred at 37°C 0.5°C at room temperature before being extruded into a calcium chloride solution. The produced beads are left in the solution for 5 min before being decanted, filtered, and then cured at room temperature for a whole night [14].

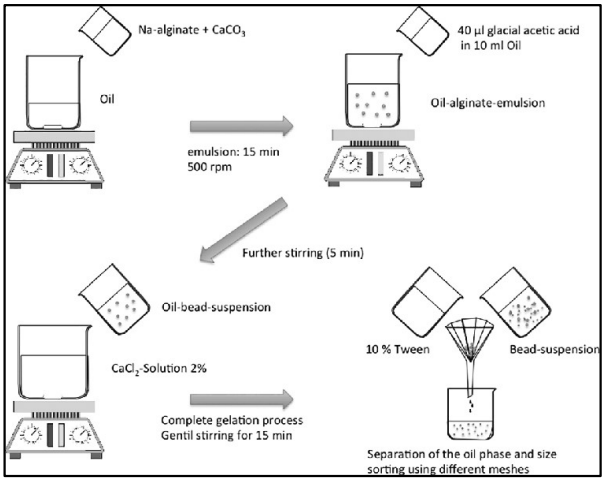


Fig 6: Emulsion-Gelation Technique [36]

Emulsion Gelation Crosslinking Technique

This sort of gelatine solution, which had previously been heated at 400C for 1 hour, included the drug dissolved in it. Without creating an emulsion, the solution was put in droplets to liquid paraffin while it was still at 350°C. The mixture was then stirred at 1500 rpm for 10 minutes. Optional stirring should be done for 10 minutes at 150C. The spherically formed beads were cleaned three times with acetone and isopropyl alcohol, respectively, and air-dried and placed in 5 mL of an aqueous glutaraldehyde saturated toluene solution at 280C for three hours. Then, 100 mL of a 10 mm glycine solution with 0.1 percent w/v between 80 and 370C is kept for 10 minutes to lump unreacted glutaraldehyde into the produced beads [15]

Influence of Co-Polymer Gelatin on Sodium Alginate Beads

Gelatin, a denatured protein generated from collagen, is abundant in nature and contains significant quantities of glycine, proline, and hydroxyproline. With respect to its excellent biocompatibility and ease of gelation, gelatin—the main component of skin, bones, and connective tissue—has been employed in the medical field, for example as a biopharmaceutical material for drug release [16-18]. Gelatin is appropriate for use in food, photography, and medicinal applications [19-21] because it can create thermoreversible gels at melting temperatures ranging from 30 to 40 °C [22].

In a well-known study, the unique ability of gelatin was employed to keep the beads' spherical shape when the gelatin was poured into the alginate solution. The difficulty of pelletizing and drug release during the preparation stage could be solved with this straightforward one-step extrusion procedure, and the shell regulated the outward difusion of the pharmaceuticals. To be able to adjust the inner core's erosion and release qualities, the beads' outer shell must be more stable. This study used a one-step extrusion technique to create sodium alginate/gelatin core-shell beads. Cross-linked gelatin was used as the core and SA as the outer layer of the drug delivery system [23].

CHAPTER 2

LITERATURE REVIEW

LITERATURE REVIEW

1. Charde M.S.et al studied about Sustained Release Sodium Alginate beads withtheir methods of preparation such as ionic gelation and emulsion gelation, different characterization techniques such as FTIR, swelling properties, buoyancy and other morphological features. It also included the In-Vitro release characteristics. The study reveal that One benefit of using sodium alginate beads as drug delivery systems is the increased adaptability and flexibility of dose formulations. The ionotropic gelation method was used to create alginate beads with ease and success. It has been noted that a significant restriction on drug leakage via the holes during the gelation process is caused by the loose network of beads. The extra Hpmc k100m polymer delays medication release, which can last for up to 12 hours. An increase in the concentration of cross-linking agents like aluminium chloride led to a more spherical form for the particle drug delivery system. Particulate spheres can address the issue of curcumin medicines' limited bioavailability because they are hydrophobic and less soluble in aqueous solutions.

2. Sharma D.et al conducted a study on Sustained Release Drug Delivery System with the role of natural polymers. The methods of formulation of Sustained release Drug Delivery system, the classification and mechanism of acton of the same were also studied. They concluded that Matrix tablets are useful in overcoming issues with adherence by patients and dosage form effectiveness in evoking desired therapeutic response that corresponds to conventional dose forms. The advantages include a once-daily dose and cost effectiveness, among others. Therefore, the dosage form design for sustained-release matrix tablets tends to be optimized.

3. Treenate, P.et al performed a study about the in vitro drug release profiles of pH-sensitive hydroxyethylacryl chitosan/sodium alginate hydrogels using paracetamol as a soluble model drug. They formulated the beads with hydroxyethylacryl derivative of chitosan and evaluated the outcome suggesting that HC and SA together could postpone the hydrogels' rate of deterioration. Compared to copper or zinc crosslinking systems, the calcium crosslinking

system demonstrated greater stability. The in vitro drug release characteristics of paracetamol, a soluble model medication, were investigated. In simulated gastric fluid (SGF), only about 20% of paracetamol was released. By increasing the HC content and/or using crosslinker, the burst release of paracetamol in simulated intestinal fluid (SIF) was suppressed.

4. Aslani, P.et al did a study on effect of gelation conditions and dissolution media on the release of paracetamol from alginate gel beads. They conducted 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 released 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.

5. Guo, T., Zhang, N., Huang, J. et al carried out a study under the title of A facile fabrication of core–shell sodium alginate/gelatin beads for drug delivery systems, where gelatin is used in order to formulate sodium alginate beads and their entrapment efficiency and drug release is being evaluated.

6. Sagar Kishore Savale conducted a study on Formulation and Evaluation of Metformin HCl microbeads by ionotropic gelation method. He also evaluated the drug encapsulation efficiency, drug content, particle size distribution and its release pattern. Better sustained release activity is seen when CMC (Carboxy Methyl Cellulose) and Sodium Alginate are combined. The values for Drug Content and% Encapsulation Efficiency are within the pharmacopeia's permitted range. The maximum percentage of drug release, which was 71.15%, was shown by in vitro dissolution experiments to occur within 4 hours.

7. Kubo, W.et al published a work on oral sustained delivery of paracetamol from in situ-gelling gellan and sodium alginate formulations and studied about its in vivo pharmacokinetics, encapsulation efficiecny of the formulations. They confirmed that creation of gel depots in the stomachs of rabbits and rats was a result of 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. This was due to the calcium ions being released in the acidic environment. Diffusion-controlled release of paracetamol from the gels over a 6-hour period was shown by in vitro tests. 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.

8. Navnath B.et al studied Sustained Release behavior of Metformin Hydrochloride through incorporation of stomach and intestine specific sodium alginate beads. The study includes study of swelling properties, beads size characterization, in-vitro release patterns and surface morphological characteristics.

9. Badwan, A. A.et al studied about a sustained release drug delivery system using calcium alginate beads. There was a study conducted to analyze the morphological characteristics as well as the release pattern of the formulations and the conclusion drawn was that the sulphamethoxazole 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 looked 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.

10. Amit Kumar Nayak and Dilip Kumar Pal studied about Ionotropically gelled mucoadhesive beads for oral metformin HCl delivery and its formulation, optimization followed by evaluation. In the wash-off test, the optimised mucoadhesive beads demonstrated 94.86 3.92% drug encapsulation efficiency, good mucoadhesivity with the biological membrane, and sustained drug release

profile beyond 10 h. With a super case-II transport mechanism, the in vitro drug release from these beads followed a controlled-release (zero-order) pattern (R2 = 0.9873 to 0.9980). These findings unambiguously showed that the created mucoadhesive beads were effective in managing non-insulin dependent diabetes mellitus while maintaining blood glucose levels due to sustained drug release and mucoadhesive properties after oral administration.

11. Kulkarni, A. R.et al carried out a study on Controlled release of diclofenac sodium from sodium alginate beads crosslinked with glutaraldehyde and evaluated changes in different parameters and the final results were It was discovered that the percentage entrapment efficiency varied between 30 and 71 according to the circumstances of their preparations. Reduced entrapment efficiency but longer DS release from the beads produced at elevated temperatures and greater exposure duration to the crosslinking agent. The molecular level dispersion of the medicines in the beads was revealed by the scanning electron microscopy examinations, which revealed nonporous smooth surfaces.

12. Patil, V. B.et al did a research on Preparation and evaluation of sustained release nimesulide microspheres prepared from sodium alginate and they described about the microsphere characteristics in detail.

13. Menon, T. V.et al formulated and evaluated sustained release sodium alginate microbeads of carvedilol and characterized its morphological along with its entrapment efficiency and drug release parameters at different pH.Carvedilol microbeads' in vitro drug release profile was studied in phosphate buffer pH 7.4 for the final two hours after the first two hours in pH 1.2 N hydrochloric acid. The sodium alginate micro beads appeared to have good mucoadhesive qualities based on the in vitro wash-off test. The specifically designed beads demonstrated greater drug loading, entrapment efficiency, small particle size, and moisture content. Their formulation had better mucoadhesion and released carvedilol over a longer period of time (24 hours).

14. Malviya, V. R.et al Prepared and evaluated sustained release beads of zolmitriptan hydrochloride. They formulated calcium Alginate beads by crosslinking methods, evaluated its in vitro release parametres. They revealed that the oral controlled release systems exhibit a characteristic pattern of drug release where the drug concentration is kept in the therapeutic window for an extended period of time (sustained release), hence ensuring sustained therapeutic activity. They are employed as single-dose forms. In the current study, Sodium alginate, a natural polymer, Hydroxyl Propyl Methyl Cellulose K15, and Chitosan Hydrochloride are used to prepare and analyse sustained release beads of Zolmitriptan Hydrochloride. Ionotropic gelation is the method used to prepare the drug's beads.

CHAPTER 3

AIMS AND OBJECTIVES

AIM AND OBJECTIVES

For years there has been tremendous efforts to formulate oral sustained release drug delivery systems with the incorporation of different drugs intended for the purpose of prolonged action.

Everyday there are modern approaches employed to expand the application of pharmaceutical formulations. Extensive research is going on towards the development of multiparticulate sustained release DDS with various modifications and usage of different additives and other polymers.

The main aim of this research work is development and evaluation of multi-particulate drug delivery system based on sodium alginate for sustained release. Here, paracetamol will be used as a model drug. Any other drug having similar solubility profile may be substituted in the developed formulations.

The objectives of this research work to achieve the projected aim, the extensive studies of sodium alginate, gelatin, calcium chloride and formaldehyde as base polymer, additive polymer and crosslinking agents are to be carried out respectively.

The objectives are

- Optimization of SA concentration alone for bead formation.

The beads are to be prepared by ionotropic gelation method and it is aimed to optimize certain parameters such as SAS preparation time, crosslinking time between SAS and CCS and curing time of the beads. The primary aim is to find out the minimum concentration of SA capable of forming beads and the highest concentration which can form beads distinctly and can be extruded through the syringe.

- Optimization of Calcium Chloride concentration for bead formation.

Along with finding the optimum concentration capable of forming beads, at the same time ideal concentration of Calcium Chloride is also determined.

- Optimization of Gelatin concentration in presence of alginate for bead formation.

The next plan is to determine the maximum amount of gelatin which can be added to individual concentration of SA thereby keeping the morphological and fluidity in mind.

- Preparation and Characterization of beads.

The beads are to be prepared by Ionotropic Gelation method and further characterization studies like drug entrapment efficiency, particle size analysis, drug release etc to be conducted.

CHAPTER 4

DRUG AND POLYMER PROFILE

DRUG AND POLYMER PROFILE

Acetaminophen(Paracetamol)

In the 4-aminophenol class of phenols, which includes paracetamol, one of the hydrogens linked to the amino group has been swapped out for an acetyl group. It performs the functions of a cyclooxygenase 2 inhibitor, cyclooxygenase 1 inhibitor, non-narcotic analgesic, antipyretic, non-steroidal anti-inflammatory drug, cyclooxygenase 3 inhibitor, xenobiotic, environmental contaminant, human blood serum metabolite, hepatotoxic agent, ferroptosis inducer, and geroprotector. It belongs to the acetamide family and the phenol family. It is connected to a 4-aminophenol functionally [24].

IUPACname:

N-(4-hydroxyphenyl)acetamide

Structure:

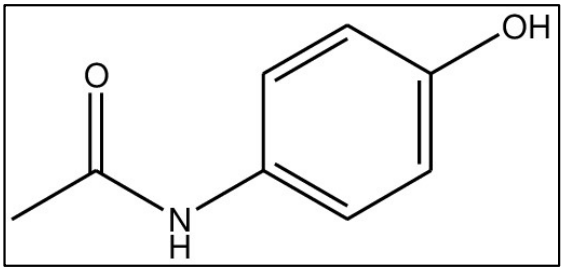


Fig 5: Structure of Acetaminophen

Formula	$C_8H_9NO_2$
Molarmass	151.16 g/mol
Meltingpoint	168-172°C(A)(lit.)
Boilingpoint	420 °C
Density	1.26 g/cm^3
Refractiveindex	1.5810
Storagetemp.	Room Temperature
Color	White [24]

Description

The World Health Organisation (WHO) recommends acetaminophen (paracetamol), generally referred to as Tylenol, as the first line of treatment for pain disorders. It is the most widely used painkiller in the world. It is also employed for its antipyretic properties, which lower fever. This medication was first authorised by the U.S. FDA in 1951 and is offered in a number of dosage forms, including syrup, injection, suppository, normal tablets, effervescent tablets, and other dosage forms. More than 600 over-the-counter (OTC) products, including allergy treatments, cold medications, sleep aids, pain relievers, and other items, frequently contain paracetamol in combination with other pharmaceuticals.

The fact that this medication is available in a variety of formulations, strengths, and dose recommendations for kids of various ages may contribute to confusion regarding how much to take. It is crucial to adhere to the most recent manufacturer and government dosing recommendations while taking or being given paracetamol due to the risk of lethal overdose and liver failure brought on by improper use [24].

Clinical pharmacology

Mechanism of Action: Although the precise mechanism of action of paracetamol has not been fully identified, according to FDA labeling, it is frequently grouped with NSAIDs (nonsteroidal anti-inflammatory medications) because of its capacity to inhibit the cyclooxygenase (COX) pathways. It is believed to exert central effects that ultimately result in the relief of pain symptoms. According to one idea, paracetamol raises the pain threshold by blocking the production of prostaglandin (PG) by two isoforms of cyclooxygenase, COX-1 and COX-2. Pain feelings are brought on by prostaglandins. Acetaminophen has no peripheral anti-inflammatory benefits because it does not inhibit cyclooxygenase in peripheral tissues. Additionally, studies indicate that paracetamol specifically inhibits a COX variant distinct from the recognised COX-1 and COX-2 versions [24].

Pharmacodynamics: Acetaminophen has been found to have both antipyretic and analgesic effects, according to both animal and human research. There is evidence that this medication has no anti-inflammatory properties. If used at the recommended doses, paracetamol does not disturb tubular secretion of uric acid, unlike the salicylate medication class, and it has no effect on acid-base balance. Acetaminophen does not interfere with homeostasis and does not inhibit platelet aggregation. Rarely, using paracetamol can cause allergic responses [24].

Pharmacokinetics: Following oral treatment, paracetamol is quickly and virtually entirely absorbed from the GI tract. The stable state oral bioavailability of 1.3-g doses of acetaminophen given once every eight hours for an aggregate of 7 doses in healthy men was the same as that of 1-g doses of acetaminophen given at intervals of six hours for a total of 7 doses in traditional tablets. Food may slightly slow down the absorption of acetaminophen extended-release pills. Peak plasma concentrations are reached about 10–60 or 60–120 minutes after oral administration of immediate- or extended-release paracetamol formulations, respectively. An average plasma acetaminophen concentration of 2.1 or 1.8 ug/mL occurs at 6 or 8 hours after oral administration of a single 500-mg conventional tablet or a single 650-mg extended-release tablet, respectively.

Metabolites of paracetamol are primarily eliminated in the urine. At least 90% of the amount injected is eliminated within 24 hours, with less than 5% of the dose excreted in the urine as free (unconjugated) acetaminophen. About 0.9L/kg makes up the distribution volume. Red blood cells hold between 10 and 20 percent of the medication. With the exception of fat, acetaminophen seems to be broadly disseminated across the majority of bodily tissues. Clearance for Adults is nearly 0.27 L/h/kg after an intravenous (IV) dosage of 15 mg/kg and for Children0.34 L/h/kg after an intravenous (IV) dosage of 15 mg/kg [24].

Adult Dosage: For pain or fever:

For oral and rectal dosage forms (capsules, granules, powders, solution, suppositories, suspension, or tablets):

- Adults and teenagers—650 to 1000 milligrams (mg) every 4 to 6 hours as needed. Dose is based on form and strength. Carefully follow the label instructions for the maximum dose per day.
- Children—Dose is based on weight or age. Carefully follow the label instructions for the maximum dose per day.
- Children 11 to 12 years of age: 320 to 480 mg every 4 to 6 hours as needed.
- Children 9 to 11 years of age: 320 to 400 mg every 4 to 6 hours as needed.
- Children 6 to 9 years of age: 320 mg every 4 to 6 hours as needed.
- Children 4 to 6 years of age: 240 mg every 4 to 6 hours as needed.
- Children 2 to 4 years of age: 160 mg every 4 to 6 hours as needed.
- Children under 2 years of age: Use and dose must be determined by your doctor [24].

Indications: Acetaminophen is a fever reducer and pain reliever.

When used with opiates, paracetamol is used to treat moderate to severe pain as well as mild to moderate discomfort and fever. Headache, muscle aches, arthritis, backaches, toothaches, sore throats, colds, flu, and fevers are among the most prevalent ailments treated [24].

Dosage and administration

Acetaminophen comes in many different forms such as capsules, liquid, chewable or disintegrating tablets, and dissolving powders or granules. Acetaminophen made for infants is available in two different dose concentrations, and each concentration comes with its own medicine dropper or oral syringe. These dosing devices are not equal between the different concentrations. To use the acetaminophen effervescent granules, dissolve one packet of the granules in at least 4 ounces of water. The oral powder should be placed directly on the tongue and swallowed [24].

Dosage forms and strengths

oral capsule (325 mg; 500 mg), oral granule, effervescent (650 mg), oral liquid (160 mg/5 mL; 325 mg/10.15 mL; 500 mg/15 mL; 650 mg/20.3 mL), oral powder (500 mg), oral suspension (160 mg/5 mL; 650 mg/20.3 mL), oral tablet (325 mg; 500 mg), oral tablet, chewable (160 mg; 80 mg), oral tablet, disintegrating (160 mg; 325 mg; 80 mg), oral tablet, extended release (650 mg) [24].

Warnings and precautions

Acetaminophen overdose may result in significant (and even deadly) **liver damage.** Acetaminophen dosage for adults shouldn't exceed 4 grammes (4,000 milligrammes) per day. Acetaminophen dosage should be reduced for young children and those with liver issues [24].

Adverse reactions

After ingesting toxic dosages of the medication, nausea, vomiting, and stomach discomfort typically start to appear 1-2 hours later. CNS stimulation, excitation, and delirium may first manifest in cases of severe poisoning. CNS depression, stupor, hypothermia, significant prostration, quick shallow breathing, rapid weak irregular pulse, low blood pressure, and circulatory failure may occur after this. Even if there are no obvious side effects, a person who has consumed a toxic dose of paracetamol should be hospitalised for several days of observation. This is because maximum liver damage and/or cardiotoxic effects typically do not manifest until 2-4 days after the drug has been consumed [24].

- **Hepatotoxicity**

A fraction of patients receiving chronic acetaminophen therapy at doses of 4 grammes per day saw temporary increases in serum aminotransferase levels, usually beginning after 3 to 7 days and reaching peak values exceeding 3-fold increased in 39% of cases. These elevations are typically asymptomatic and go away quickly when the therapy is stopped or the dosage is decreased. In some cases, they go away even when the treatment is continued at the full dose. While new findings imply that acetaminophen's routine use can cause severe hypersensitivity reactions such Stevens Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), acetaminophen has few negative effects when used in therapeutic amounts. Both of these diseases may be followed by signs of liver damage and both are potentially fatal [24].

- **Uses in specific conditions**

One of the most popular medications for pain and fever that pregnant women use is paracetamol. Typically, the substance, sold under the trade name Tylenol, is regarded as safe during pregnancy [24].

Drug Interactions

Because of the greater transformation of the medication to hepatotoxic metabolites, anticonvulsants (such as phenytoin, barbiturates, and carbamazepine) that promote hepatic microsomal enzymes may enhance acetaminophen-induced liver toxicity. Patients who consume more acetaminophen than is advised while taking anticonvulsants are at much higher risk of developing acetaminophen-induced liver damage.

The specific mechanism of this interaction is unknown, although concurrent administration of isoniazid and paracetamol may raise the risk of hepatotoxicity. When patients take more paracetamol than is advised while taking isoniazid, the risk of liver toxicity is significantly enhanced. As a result, while taking isoniazid, patients should avoid self-medicating with paracetamol.

Acetaminophen causes dose-dependent decrease in concentration of hepatic glutathione. Agents such as diethyl maleate, which depletes hepatic glutathione, potentiate /hepatic & renal tubular/ necrosis. Conversely, administration of cysteine, glutathione precursor, protects against damage [24].

Overdosage

The initial symptoms of an acetaminophen overdose are weakness or disorientation, loss of appetite, nausea, vomiting, and discomfort in the stomach. Later signs and symptoms could include upper stomach pain, black urine, and yellowing of the skin or eye whites.Acetaminophen overdose may be manifested by renal tubular necrosis, hypoglycemic coma, and thrombocytopenia. Sometimes, liver necrosis can occur as well as liver failure [24].

Sodium Alginate USP-NF 91%

Synonym

Sodium Salt of Alginic Acid, Algiline.

Structure

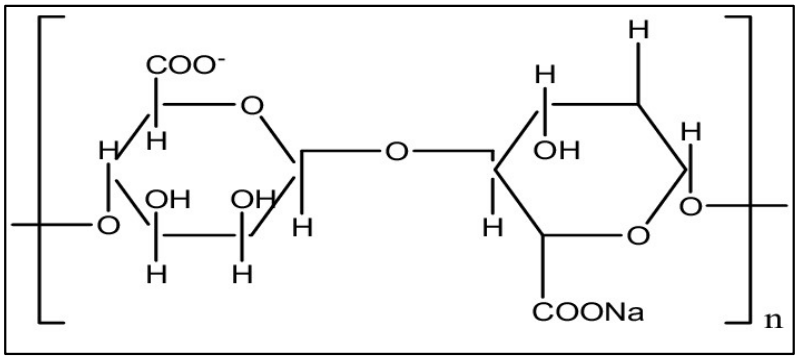


Fig 6: Chemical Structure of Sodium Alginate

Chemical name

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

Empirical formula

$(NaC_6H_7O_6)_n$

Molecular weight

Average Molecular Weight 216.1212

Functional category

Stabiliser, emulsifier, thickener, formulation aid.

Description

Natural polysaccharide sodium alginate has a structure that is linear, and it is biodegradable, biocompatible, and is harmless for the human body. It gives tissues strength and flexibility, and it can be employed in industries due of its gelling, viscous, stabilising, and water-retentive qualities. Sodium alginate is considered to be a polyanionic copolymer which structurally is the sodium salt of alginic acid, an acid consisting of several successive groups of the two uronic acids: β-D-manuronic acids (M) and α-L-glucuronic (G), linearly linked to each other by 1–4 glycosidic bonds.

Properties

- **Gelling properties**

It has been thoroughly investigated how to make carriers for controlled or sustained administration of medicinal medicines by treating aqueous alginate solutions with divalent ions (Ca2+, Sr2+, and Ba2+) or trivalent ions (Fe3+ and Al3+). This is a result of intramolecular bonding and ionic interactions that take place between the cations and carboxylic acid groups on the polymer matrix [25,26].

- **Biocompatibility**

Alginate's biological compatibility has been thoroughly examined in-vivo and in-vitro at various purity levels. According to certain studies, alginates with rich M monomers are more immunogenic and 10 times more effective at stimulating cytokine generation than those with G monomers [27], whereas other studies found either a very weak or nonexistent immune response to alginate implants [28]. Variable reactions may be brought on by contaminants in the alginates, such as heavy metals, endotoxins, proteins, and polyphenolic chemicals, at the injection or implantation sites. However, there were not many severe inflammatory effects associated with commercially available, certified, or alginates purchased from reputable businesses [29].

- **Bioadhesion**

Bioadhesion is the adhesion or contact of two surfaces, one of which is a biological substrate. The mucosal layer is employed, for instance, in mucoadhesion [30]. Alginates contain a mucoadhesive anionic polymeric layer represented by the carboxyl group. In comparison to polycation or non-ionic polymers, polyanion polymers are said to be more effective bioadhesives [31]. In comparison to polymers like Polystyrene, Chitosan, Carboxymethyl cellulose, and Poly (lactic acid), Alginate has a stronger mucoadhesive strength. Alginate's bioadhesive qualities would be useful as a mucosal drug delivery vehicle to the GI tract and nasopharynx because they increase the effectiveness of the drugs by prolonging their residence duration at the site of action.

- **Effect of pH on Alginates**

The viscosity of alginates is uninfluenced above pH > 5, whereas solution having pH 5 causes the COO- group present in alginates to get protonated to -COOH, and the electrostatic repulsion between chains reduces, allowing them to shift closer together to create hydrogen bonds, whereby the viscosity of alginates increases.

Applications

- Alginates have been utilised in a range of foods over the years because of its special capabilities, including thickening, gelling, emulsification, stabilisation, and texture functioning. Examples include ice cream toppings, fruit jams, jelly, milk products, food packaging, instant noodles, beer, etc.
- In the current medical profession, a large number of medications or therapies given to patients are resulting in a large number of negative effects. As a result, there is a huge market for drug loading carriers that lengthen the duration a medication remains in the body, either in vitro or in vivo. These carriers should also be safe and non-toxic for use around the body, notably in the gastrointestinal system.
- Alginates gels often have a porous structure with a size of around 5 nm, which enables small molecular weight medications to fill this gap by either physical or chemical bonding. The drug release is regulated when a drug that is embedded or loaded with a drug comes into contact with an aqueous medium. Crosslinking the alginates with bivalent or trivalent cations will improve the durability of the gels or films because the drug-loaded carriers are water-soluble and may degrade in an aqueous medium. These characteristics facilitate our investigation of the drug release kinetics.
- Applying ointments like antiseptic, antimicrobial, anti-inflammatory, antipruritic, pain-relieving gels, or anti-mycotic with fungal action may cause skin irritation or other side effects and may prolong the healing time for any injury, burns, torn muscles, cuts, or burns that occur on the human body. Alginates are therefore widely employed in wound healing due to their commendable features and due to the fact that they are non-toxic to load the necessary medications in alginate gels, which enhances the retention duration of the drug, allowing the drug to release in small doses on the precise site.

Gelatin

A by-product of the hydrolysis of collagen in animal bones and skin is gelatin. Transparent, tasteless, solid, and water-soluble protein is known as gelatin. According to research, gelatin contains roughly 19 amino acids (AAs) as well as a variety of proteins and peptides. Gelatin is one of the fibrous proteins found in the skin, bones, and cartilage of animals [33,34]. The characteristics of gelatin are greatly influenced by its age and source. Gelatin develops an incredibly viscous character in an aquatic environment, which cools the gel. Similar to collagen, the gelatin component can produce a homogenous gel that can support an endless 3-D gel network. Gelatin films are adversely affected by its chemical and physical qualities because of its mechanical properties. Fatty acids, certain polysaccharides, and soy protein are examples of other polymers that can be combined with gelatin to enhance its physical qualities. Gelatin is therefore frequently utilised as an emulsifier, a colloid stabiliser, and a biodegradable packaging material in a variety of food industries as well as cosmetics, food decoration, and a wide range of biomedical applications.

Properties

In the presence of diluted acids, collagen found in animal skin and bones is thermally denaturated to create gelatin, a biopolymer. It is a byproduct of collagen, a fibrillar protein, degrading. Gelatin has a colourless or faintly yellow look and is almost tasteless and odourless. It might appear as sheets, flakes, or powder and is translucent and fragile. Gelatin can be dissolved in polar solvents like hot water, glycerol, and acetic acid, but it is insoluble in organic solvents like alcohol. Gelatin has a relative density of 1.3–1.4 and 8–13% moisture content. In cold water, it hydrates into distinct, inflated particles.

Applications

The desirable characteristics of gelatin particles as drug carriers have been noticed. According to the objectives of the research, the degree of gelatin crosslinking makes it simple to regulate the release of drugs from gelatin particles. The gelatin particles that can release drugs are useful in drug screening and wound healing models.

CHAPTER 5

EXPERIMENTAL

EXPERIMENTAL

Preformulation studies

Materials used

Drug: Acetaminophen.

Gelling agent: Sodium Alginate USP NF 91% (Loba Chemicals Pvt. Ltd., Gelatin.

Cross Linking Agent: Calcium Chloride, Formaldehyde

Solvent: Doubled distilled water, 0.1 N HCl pH 1.2, Phosphate Buffer pH 6.8.

Other Chemicals: Potassium Dihydrogen Phosphate, Dipotassium Hydrogen Phosphate, NaCl.

Instruments used

1) UV-Vis Spectrophotometer (Model No. 3200, Shimazdu, Japan, LabIndia)
2) Dissolution Apparatus USP Type-II (Electrolab)
3) Magnetic stirrer (Model No. 1MLH, Remi and Techno makes, Mumbai)
4) Micropipette (0.5-10µl) (Accupipet V19347)
5) Balance (Metler Toledo)
6) Brookfield Viscometer (Toki Sangyo Company Ltd)
7) Magnetic Stirrer (REMI)
8) Test tubes, Pipettes, Beakers, Volumetric Flasks, Conical Flasks
9) Syringe 22G
10) Digital pH meter

UV Scanning for λ_{max} detection of Paracetamol

To determine the wavelength of maximum absorbance (λ_{max}) of Paracetamol for developing the analytical procedure for its spectrophotometric determinations, a solution of suitable concentration of the drug in 0.1N HCl and phophate buffer pH 6.8 was prepared and scanned from a wavelength of 190-400 nm in a UV-Visible Spectrophotometer using appropriate blank. The λ_{max} was found to be 243 nm, which was used for spectrophotometric determination of Paracetamol in different formulations.

Standard Curve Preparation of Paracetamol:

For quick and accurate analysis of Paracetamol by spectrophotometric method at the determined λ_{max}, an operating calibration curve or standard curve was prepared. A series of standard samples were prepared by using 0.1N HCl and Phosphate Buffer pH 6.8. The different concentration of drug taken and corresponding absorbances are plotted. The corresponding standard curve generated by linear regression analysis along with the mathematical equation representing the curve is mentioned here.

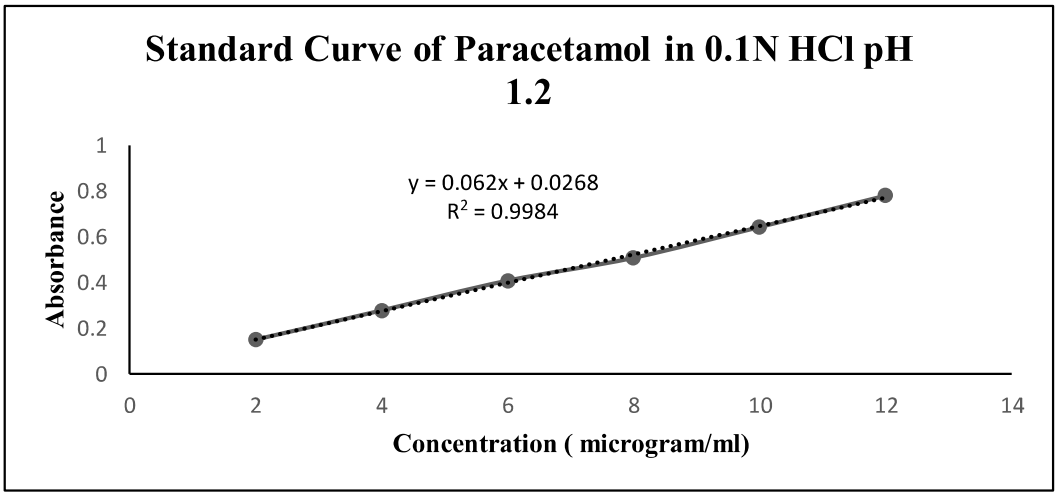


Fig 7: Standard Curve of Paracetamol in 0.1N HCl pH 1.2

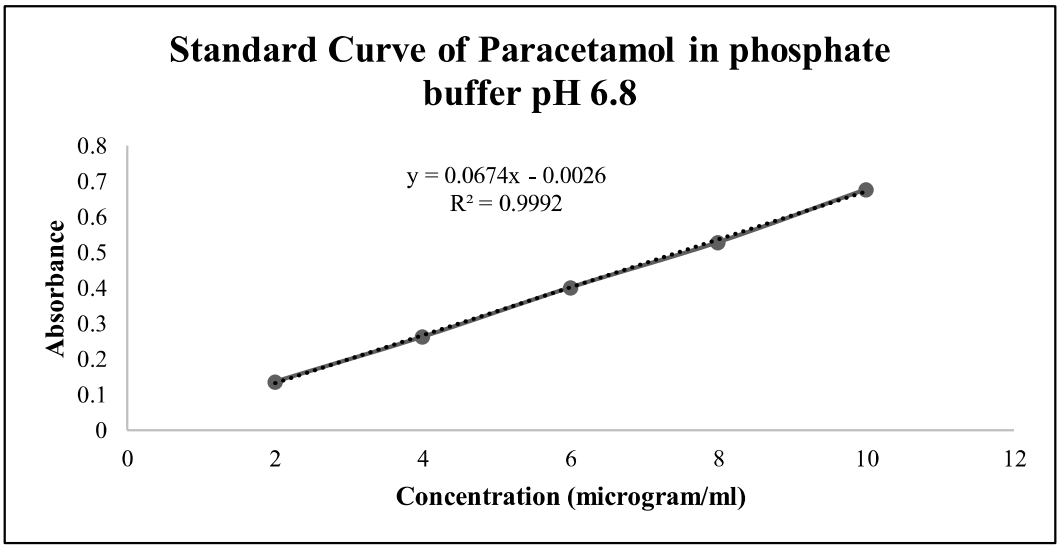


Fig 8 : Standard Curve of Paracetamol in phosphate buffer pH 6.8

Preparation of polymer solutions

The Sodium Alginate polymer solution was prepared by dissolving specific amount of Sodium Alginate in double distilled water and stirred for an hour and the volume of the solution was kept fixed at 20ml. After the SAS is prepared, definite amount of Gelatin is added to the SAS and 20-30°C of heat is provided and stirred for another half and hour. After both the polymer is dissolved then a certain amount of drug is added accordingly.

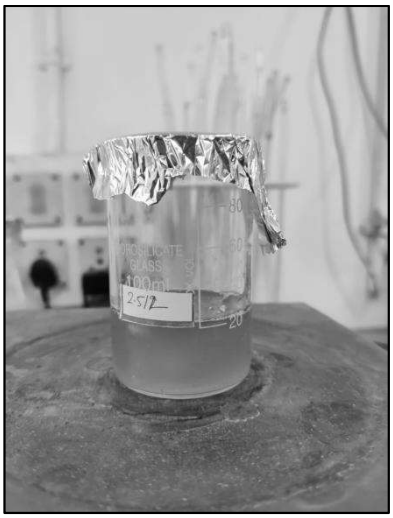


Fig 9: Preparation of Sodium Alginate Polymer Solution

Optimization of Ideal Concentration of Sodium Alginate capable of forming Beads

The work started as per the principal that SA when allowed to extrude through a Syringe into CCS, **a cross linking** takes place and there is formation of spherical beads with certain characteristics. So the experiment began with variation of sodium alginate concentration and followed by variation of Cacl2. So the concentration of Alginate was being varied from **1-3.5%,** but when **1%** alginate solution when dropped from the syringe into Cacl2 solution it showed **irregular transparent structure** with the presence of tip which couldn't retain its shape and no vesicular structures were formed. From **1.5%** of the alginate solution spherical beads were observed and it continued till **3%** up to which the beads were spherical. But when the alginate solution concentration was extended to **3.5**% although the beads were formed, but the solution itself becomes extremely viscous, difficult to extrude from the syringe and also renders irregular structure of the beads, hence the batch was discarded.

Optimization of Ideal Calcium Chloride Concentration for bead formation

Now at the same time our concern is to optimize ideal concentration of CCS. Taking two concentrations such as 2% and 2.5% of SA fixed and varying the CaCl2 concentration from **0.5-5%,** using Paracetamol as a model drug, when the supernatant fluid obtained after bead formation was analyzed by UV-Spectrophotometer**,** it could be concluded that till 1.5% of CaCl2 concentration, the beads were somewhat irregular in shape and from **2%** onwards CaCl2 concentration, the beads were more uniform structured with no irregularity. However the fact that increase in concentration from **2-5%** did not make significant change in the **entrapment efficiency** of the beads, so 2% of CaCl2 concentration was fixed.

Table 1: Optimization of $CaCl_2$ Concentration based on Morphology of the prepared beads.

Concentration of Sodium alginate (%)	Concentration of $CaCl_2$ (%)	Observation
1	0.5, 1, 1.5, 2	Irregular structure, no vesicle formed, couldn't retain its shape.
1.5	0.5, 1, 1.5	Spherical structure with very minute tips present, Vesicle is present, translucent pale color
1.5	2	Spherical beads obtained with no tip.
2	2	Spherical beads produced
2.5	2	Spherical beads produced
3	2	Spherical beads produced but the solution starts becoming viscous

Table 2: Optimization of $CaCl_2$ Concentration based on Entrapment Efficiency of the prepared beads.

Sodium alginate Concentration (%)	Calcium Chloride Concentration (%)	Entrapment Efficiency(%)
2	1.5	77.87
2	2	71.35
2	2.5	71.35
2	3	69.98
2	3.5	73.54

2	4	70.13
2	4.5	68.56
2	5	69.59
/2.5	1.5	72.89
2.5	2	79.61
2.5	2.5	79.47
2.5	3	80.63
2.5	3.5	79.47
2.5	4	79.61
2.5	4.5	79.47
2.5	5	81.7

Optimization of Ideal Concentration of Gelatin

Now after the determination of ideal concentration of SA and CCS when additive polymer Gelatin was added with individual concentration of SAS initially beads were formed with presence of irregularity in their structure but from a certain concentration spherical beads were obtained. However, after a certain limit the addition of Gelatin when total polymer concentration reaches above 4% becomes difficult to be extruded from the syringe. Therefore, the amount of gelatin permitted to be added with the optimized alginate concentration 1.5 to 3% was varied keeping total polymer concentration not exceeding 4%.

Table 3: Effect of Addition of Gelatin in Increment on structure of beads

Concentration of Sodium alginate (%)	Concentration of $CaCl_2$(%)	Concentration of Gelatin(%)	Observation
1.5	2	1 2	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	2	1 2	Circular beads were formed, and no observable tip was observed.

2.5	2	1 2	Circular beads were formed, and no observable tip was observed.
3	2	1	Circular beads were formed, and no observable tip was observed. Further increase in gelatin concentration reduces fluidity

Optimization of Ideal Concentration of Formaldehyde

Keeping the safety health limit in mind 0.05ml of 38% (w/v) Formaldehyde solution was added to 50ml of $CaCl_2$ solution [32].

Fourier Transform Infra Red Spectroscopy Studies

Since a crosslinking takes place during the formation of beads, it is expected that thedrug and the polymer interact with each other. To evaluate the interaction of the drug and the polymers, the developed sample formulations were reduced to powder fom and analyzed. FTIR Studies are conducted in FTIR Spectrophotometer.

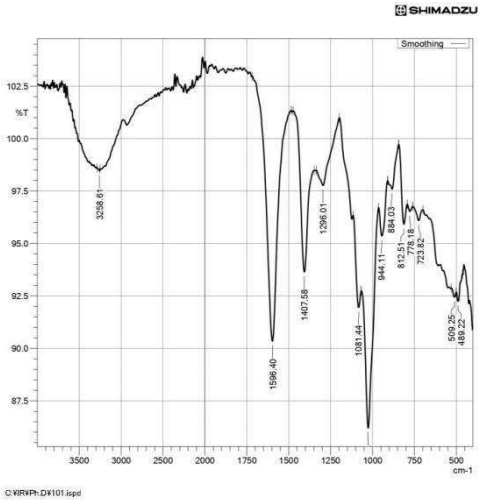
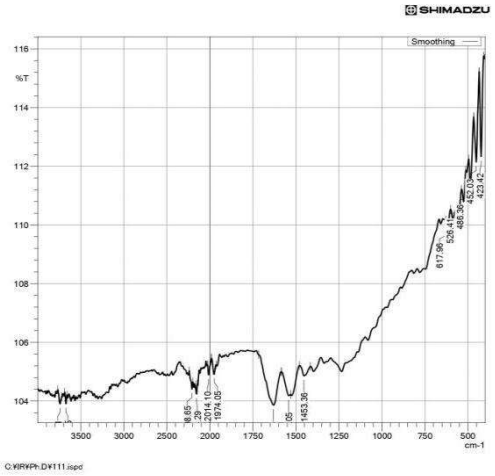


Table 4 : FTIR Spectrum of Sodium Alginate		
SERIAL NO.	**FUNCTIONAL GROUP**	**RANGE**
1.	O-H Stretch of intermolecular hydrogen bond	3258.61 cm^{-1}
2.	O-C=O Asymmetric stretching	1596.40 cm^{-1}
3.	O-C=O Asymmetric stretching	1407.58 cm^{-1}
4.	N-O Symmetric stretching of nitro compound	1296.01 cm^{-1}

Table 5: FTIR Spectrum of Gelatin



SERIAL 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}

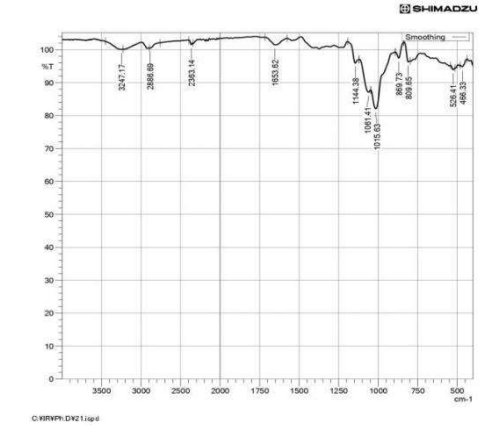


Table 6: FTIR Spectrum of Paracetamol		
SERIAL NO.	**FUNCTIONAL GROUP**	**RANGE**
1.	O-H Stretch of hydrogen bond of Alcohol	3247.17 cm^{-1}
2.	C=O Stretch of Amide	1653.62 cm^{-1}
3.	O-H Stretch of Carboxylic acid	2886.69 cm^{-1}
4.	CH_3 Stretching	2363.14 cm^{-1}

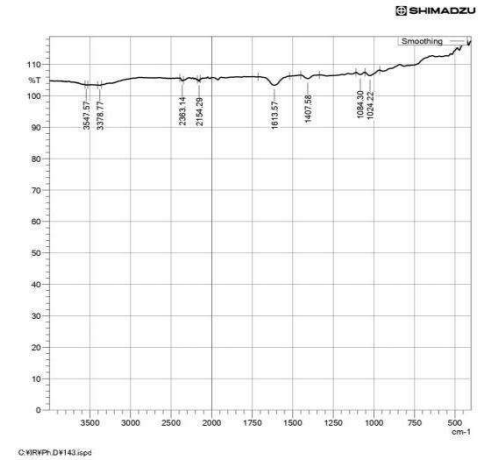


Table 7 : FTIR Spectrum of SA-$CaCl_2$		
SERIAL NO.	**FUNCTIONAL GROUP**	**RANGE**
1.	O-H Stretch enlargement of region due to $CaCl_2$	3547.57 cm^{-1} & 3378.77 cm^{-1}
2.	C-H Stretching region enlargement	2363.14 cm^{-1} & 2154.29 cm^{-1}
3.	O-C-O Stretch free carboxyl group region enlargement	1613.57 cm^{-1} & 1407.58 cm^{-1}

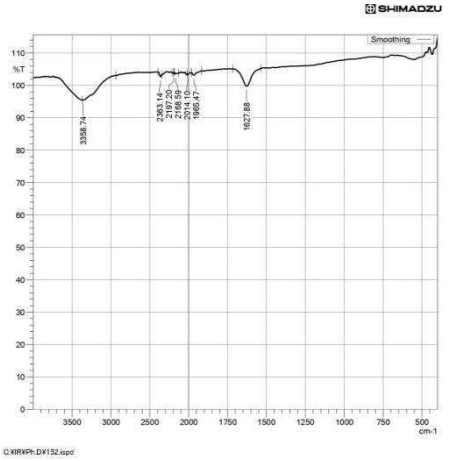


Table 8: FTIR Spectrum of Gelatin-$CaCl_2$		
SERIAL NO.	**FUNCTIONAL GROUP**	**RANGE**
1.	O-H Stretch enlargement of region due to $CaCl_2$	3547.57 cm^{-1} & 3378.77 cm^{-1}
2.	C-H Stretching region enlargement	2363.14 cm^{-1} & 2154.29 cm^{-1}
3.	O-C-O Stretch free carboxyl group region enlargement	1613.57 cm^{-1} & 1407.58 cm^{-1}

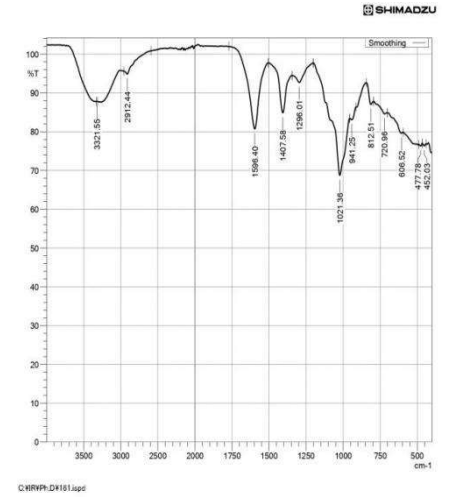


Table 9: FTIR Spectrum of SA-HCHO		
SERIAL NO.	**FUNCTIONAL GROUP**	**RANGE**
1.	O-H Stretch of hydrogen bond	3321.55 cm^{-1}
2.	C-H Stretch	2912.44 cm^{-1}
3.	C-OH deformation vibration with O-C-O symmetric stretch of carboxyl group	1407.58 cm^{-1}
4.	C-O & C-C Stretch of pyranose ring	1021.36 cm^{-1}

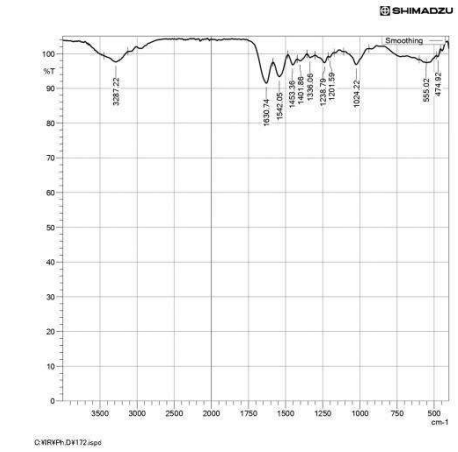


Table 10: FTIR Spectrum of Gelatin-HCHO		
SERIAL NO.	**FUNCTIONAL GROUP**	**RANGE**
1.	O-H Stretch of hydrogen bonding	3287.22 cm^{-1}
2.	C=O Stretch vibration along with C-N Stretch	1630.74 cm^{-1}
3.	N-H Bending & C-N Stretching	1542.05 cm^{-1}
4.	C-N & N-H vibration in plane of bound amide	1453.36 cm^{-1}

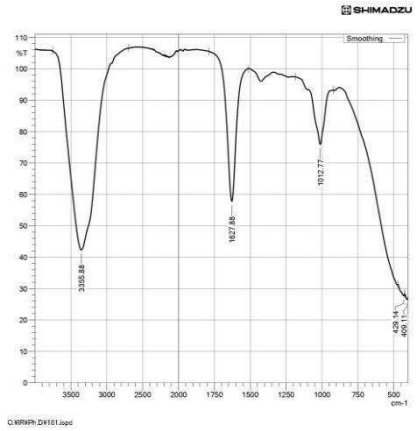


Table 11 : FTIR Spectrum of SA-HCHO-$CaCl_2$		
SERIAL NO.	**FUNCTIONAL GROUP**	**RANGE**
1.	O-H Stretch of hydrogen bond	3355.88 cm^{-1}
2.	O-C-O Asymmetric stretch of carboxyl	1627.88 cm^{-1}
3.	C-O & C-C Stretch	1012.77 cm^{-1}

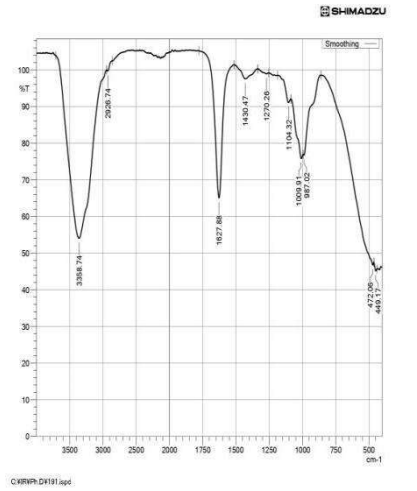


Table 12: FTIR Spectrum of Gelatin-HCHO-$CaCl_2$		
SERIAL NO.	**FUNCTIONAL GROUP**	**RANGE**
1.	O-H Stretch of hydrogen bonding	3358.74 cm^{-1}
2.	C=O Stretch with COO	1627.88 cm^{-1}
3.	C-H Stretching	2926.74 cm^{-1}
4.	C-N & N-H vibration in plane of bound amide	1430.47 cm^{-1}

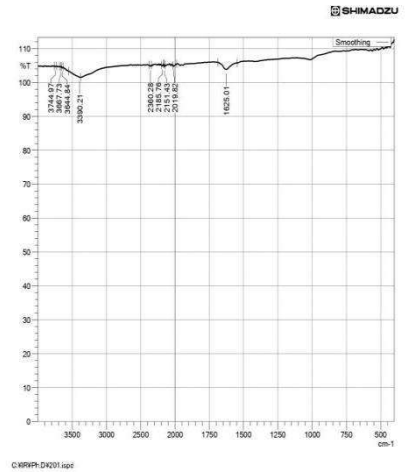


Table 13: FTIR Spectrum of SA-Gelatin-HCHO		
SERIAL NO.	**FUNCTIONAL GROUP**	**RANGE**
1.	O-H Stretch of free hydroxyl group (gelatin)	3744.97 cm^{-1}, 3667.73 cm^{-1} & 3644.84 cm^{-1}
2.	O-H Stretch of hydrogen bonding	3390.21 cm^{-1}
3.	C=O Stretch associated with NH_2, Alginate could be associated with gelatin	1625.01 cm^{-1}
4.	C≡C Stretch of alkyne group (alginate)	2185.76 cm^{-1} & 2151.43 cm^{-1}

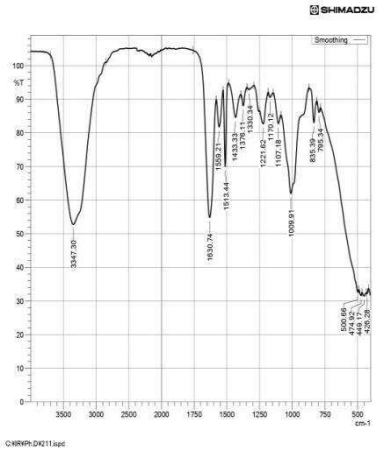


Table 14:FTIR Spectrum of PCM-SA-HCHO-$CaCl_2$		
SERIAL NO.	**FUNCTIONAL GROUP**	**RANGE**
1.	O-H Stretch of hydrogen bonding	3347.30 cm^{-1}
2.	C=O Stretch associated with NH_2, Alginate could be associated with gelatin	1630.74 cm^{-1}
3.	N-H Bending & C-N Stretching (gelatin)	1559.21 cm^{-1}

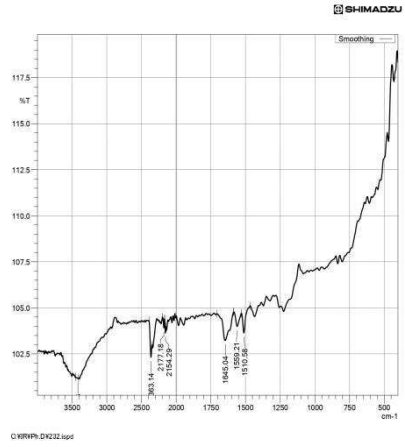


Table 15:FTIR Spectrum of PCM-SA-$CaCl_2$		
SERIAL NO.	**FUNCTIONAL GROUP**	**RANGE**
1.	O-H Stretch of hydrogen bonding	3400 cm^{-1} – 3330 cm^{-1}
2.	C=O Stretch associated with NH_2, Alginate could be associated with gelatin	1645.04 cm^{-1}
3.	CH_3 Stretching of paracetamol	2363.14 cm^{-1}
4.	C≡C 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}

From the FTIR Spectrum it was found that there was interaction between alginate molecules with calcium ions. It was also found that Gelatin Molecules were crosslinked by Formalin. No interaction was there between drug and other composite materials.

Optimization of Beads Preparation Time and Curing Time

The initial step to prepare the beads were to make a solution of SA by dissolving a definite amount of SA into double distilled water maintained at 50-60°C with continuous stirring until a homogeneous solution was obtained. The solutions were kept overnight for hydration. Gelatin solution was also prepared the same way by dissolving appropriate amount of Gelatin into Double distilled water and then mix both the solutions together as per the percentage of polymer required. Finally after the mixing the model drug was added to the solution and stirred. The prepared solution was extruded through 22G syringe into the CCS. And kept for five minutes. It was visually observed that five minutes curing time was ideal for the formation of uniform vesicular structure. The the beads were filtered, washed, collected and subjected to air drying.

Formulation Development and Evaluation

Preparation of Beads

Beads were prepared by dropping the polymer solutions in $CaCl_2$ solution through 22G Syringe as per the composition stated in table 4. For the preparation of Formaldehyde treated beads, 0.1 % of Formaldehyde was added in $CaCl_2$ solution before dropping the polymer solutions. The beads were allowed to stay in the $Cacl_2$ solution for five minutes for the formation of uniform structure. Then the beads were filtered, washed and subjected for air drying. The Composition of the formulations for 20ml each is mentioned in table 4.

Table 16: Composition of Beads

Formulation	Sodium Alginate (%)	Gelatin (%)	Volume of CCS (ml)	Amount of Drug (mg)	Formalin treated formulation
F1	1.50	0	50	20	FF1
F2	1.50	1	50	20	FF2
F3	1.50	2	50	20	FF2
F4	2	0	50	20	FF4
F5	2	1	50	20	FF5
F6	2	2	50	20	FF6
F7	2.50	0	50	20	FF7
F8	2.50	1	50	20	FF8
F9	2.50	2	50	20	FF9
F10	3.00	0	50	20	FF10
F11	3.00	1	50	20	FF11

Rheological Evaluation

The formulations are prepared on the basis of the optimized concentration of SA alone or with the additive polymer Gelatin. The instrument Brookfield Viscometer was chosen for the purpose of evaluation of Viscosity of the polymer composite of the formulations. The spindle M2 and M3 were used during the experiment.

Determination of pH

30 ml of each of the formulations are developed and the pH of the prepared formulations were analyzed by Digital pH meter and Noted.

Determination of Entrapment Efficiency

After the preparation of beads, the beads are filtered and the supernatant fluid is collected and the volume of supernatant was noted. Next 0.1 ml of the sample is diluted to 10ml(100 times) with 0.1N HCl pH 1.2 buffer and phosphate buffer pH 6.8 and scanned at 243 nm wavelength in UV-3200 Double beam spectrophotometer and the amount of drug present in the supernatant fluid was determined from the standard curve prepared. So the rest amount is entrapped in the beads. Now the entrapment efficiency is calculated as per the formula,

$$\%\ Entrapment\ Efficiency = \frac{(Theoritical\ amount\ of\ drug - amount\ of\ drug\ in\ supernatent)}{Theoritical\ amount\ of\ drug} \times 100$$

Particle Size Analysis

The particle size analysis is carried out by Sieving Method where the sieves are arranged in the order starting from sieve number 8 to sieve number 120 and the sieves are shaken to determine the amount of beads retained on the specific sieves.

Swelling Study

To evaluate the swelling pattern of the drug entrapped beads, the initial weight of the beads are noted, the beads are suspended in 100 ml of 0.1N HCl pH 1.2 for a period until a constant weight is achieved. Based on the weights achieved the swelling index of the beads are calculated.

$$\%\ Swelling = \frac{(Final\ weight\ at\ time\ t - Initial\ weight)}{Initial\ weight} \times 100,\ t = 1, 2, 3, \ldots..$$

In-Vitro Drug Release Study

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

CHAPTER 6

RESULTS AND DISCUSSIONS

Results and Discussions

Table 17: Characterization of formulation composites and developed formulations

Formulation	pH of Composite	Viscosity (cP) of Composite	Entrapment efficiency (%)	Swelling Index (%)	Particle Size (mm)
F1	7.4	266.4	63.645	89.91	1.38
F2	6.9	340.1	56.927	90.14	1.39
F3	6.55	439.1	69.175	90.86	1.50
F4	7.41	656.6	62.854	90.2	1.49
F5	6.89	774.8	62.46	91.44	1.55
F6	6.47	889.9	68.78	91.9	1.58
F7	7.43	1226	60.6	91.6	1.54
F8	6.74	1301	61.66	92.54	1.56
F9	6.52	1651	68.38	93.1	1.59
F10	7.45	2360	56.137	92.9	1.56
F11	7.25	3037	64.04	93.24	1.58

Table 18: Characterization of formulation composites and developed formulation with Formalin

Formalin treated formulation	Entrapment efficiency (%)	Swelling Index (%)	Particle Size (mm)
FF1	71.548	81.88	1.16
FF2	69.03	82.46	1.19
FF2	69.2	83.2	1.26
FF4	72.5	82.9	1.20
FF5	81	83.98	1.25
FF6	69.03	84.4	1.28
FF7	71	83.8	1.25
FF8	63.61	84.43	1.27
FF9	76	85.12	1.28
FF10	74.06	84.87	1.28
FF11	65.55	85.23	1.29

Drug Entrapment Efficiency

The Entrapment Efficiency (%) of all the formulations, with or without formaldehyde are mentioned in the table 4. From the table it was observed that the entrapment efficiency was decreased from about 64 to 56% when Sodium alginate concentration was increased from 1.5-

3%. This happens due to the fact that at lower concentration alginate solution when dropped into $cacl_2$, instant crosslinking of the sodium alginate molecules occur at the surface and all the molecules due to the pulling by the $CaCl_2$ molecules approaches towards the outer surface faced to $CaCl_2$ Solution. The drug containing water component present in the drop mixture was encapsulated by the formed crosslinked alginate gel. The drug diffusion from that gel layer was comparatively lower since the total polymer embedded in the outer film increases and the viscosity of that outer film also increases and the aqueous drug remains within the vesicle. Whereas in case when the polymer concentration rises, the crosslinking was restricted, the pulling action exerted by the $CaCl_2$ molecules are less and the outer film formation is somewhat lesser. So the drug containing water component present in the drop mixture was less encapsulated by the formed crosslinked alginate gel. The aqueous drug becomes dispersed than remaining within the crosslinked layer, the drug diffusion from that gel layer is more because the total polymer embedded in the outer film decreases and the viscosity of that outer film also less and the drug mixture was obstructed from staying in the vesicle. Whereas when the additive polymer Gelatin was added to the batches, the %EE had increased gradually to a certain concentration. When **the total polymer concentration that is Sodium alginate along with Gelatin lies within 3.5-4%, the %EE becomes maximum**. This is due to the increase viscosity of the polymer composite and the surface of the formed beads. When same formulations are treated by Formaldehyde, increase in Drug entrapment efficiency was observed compared to the same formulations without the treatment of Formaldehyde. This is because of synergistic effect of crosslinking between Gelatin molecules by formaldehyde in addition to the crosslinking of alginate molecules by calcium ions.

Study of pH and Rheological Evaluation

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

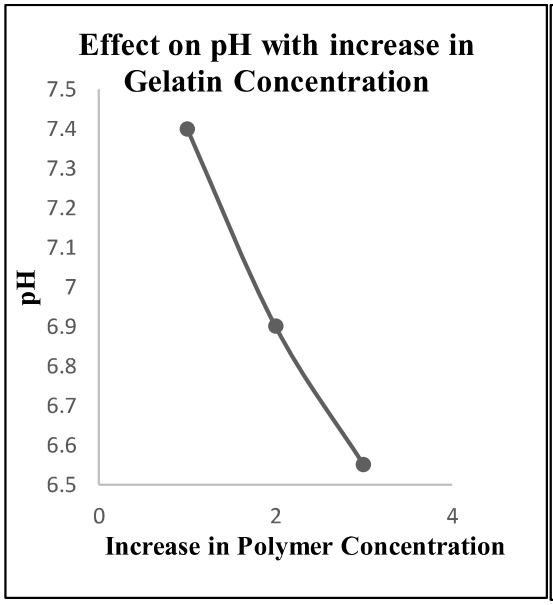


Fig 10: Effect on pH due to rise GC.

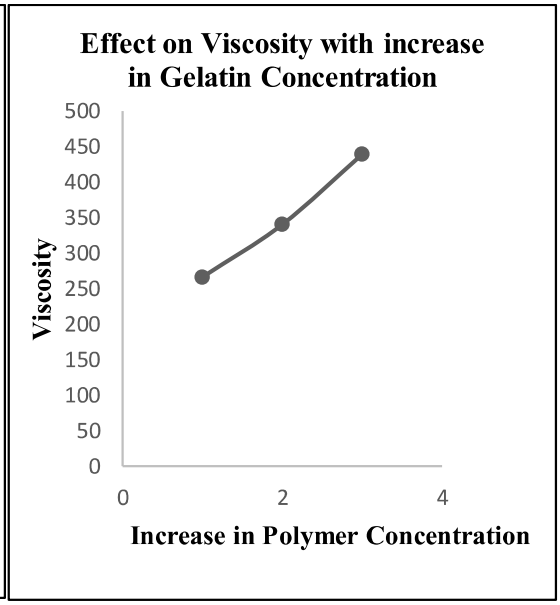


Fig 11: Effect on Viscosity due to rise in GC

Swelling Index

From the table 4 and 5 it was observed that the swelling index of beads were increased with increase in sodium alginate concentration due to high content of sodium alginate. At fixed sodium alginate concentration when gelatin was added the SI of the beads was also increased in increment of gelatin concentration. This is due to the increase total polymer content, increase in viscosity. When swelling behaviour of formalin treated beads were compared with the ones without the formalin treatment, it was found that the swelling index was less in the former case than the later. This is because of synergistic effect of crosslinking between Gelatin molecules by formaldehyde in addition to the crosslinking of alginate molecules by calcium ions.

Particle Size Analysis

The aperture size of the sieves are noted in millimeters. It was noted that none of the beads were able to pass through sieve number 24 (0.707 mm) and all the particle passes through Sieve number 12 (1.68 mm).

In-Vitro Drug Release Profiles

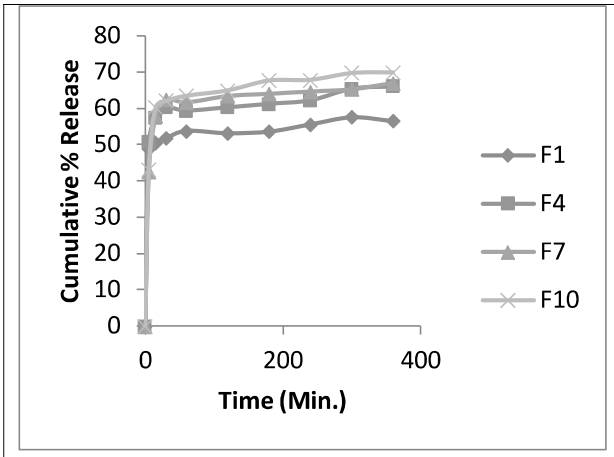


Figure 12: Comparison of release profiles of F1, F4, F7 and F10.

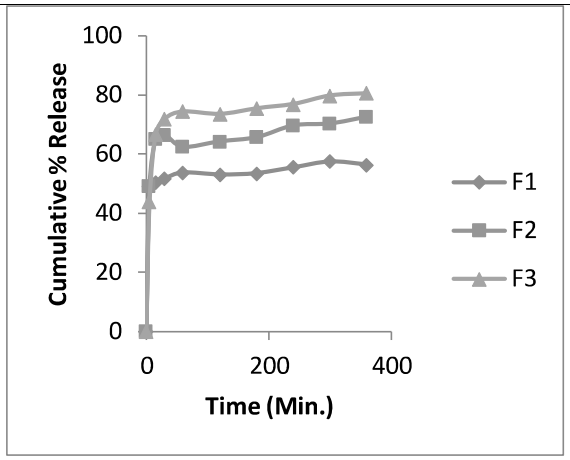


Figure 13: Comparison of release profiles of F1-F3.

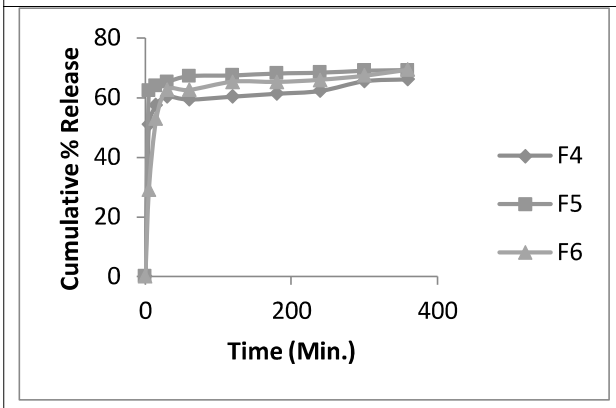


Figure 14 : Comparison of release profiles of F4-F6.

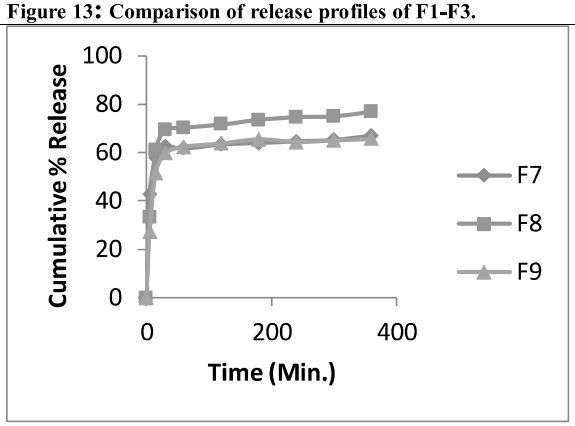


Figure 15 : Comparison of release profiles of F7-F9.

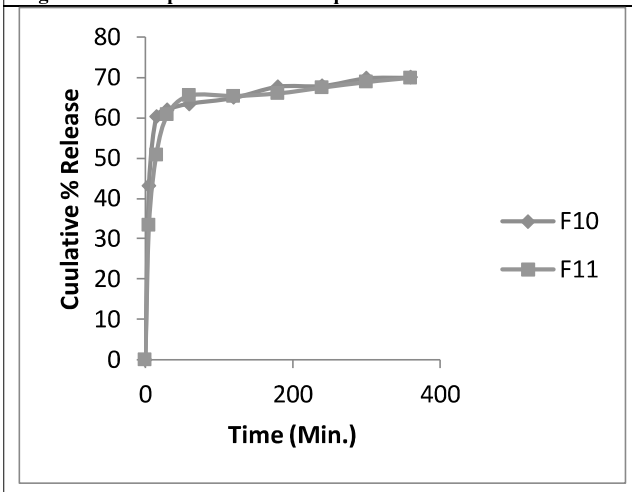


Figure 16: Comparison of release profiles of F10-F11.

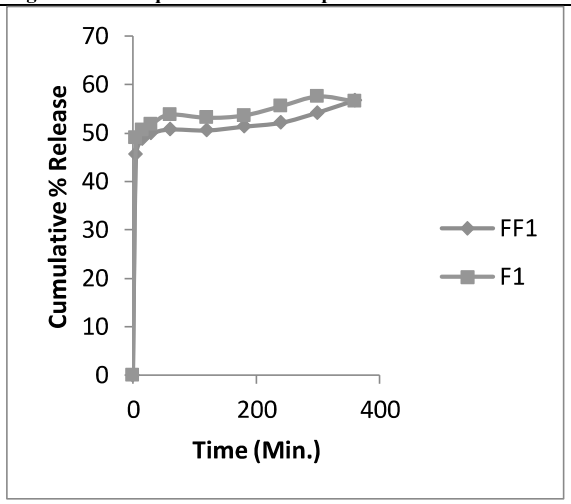


Figure 17 : Comparison of release profiles of F1-FF1.

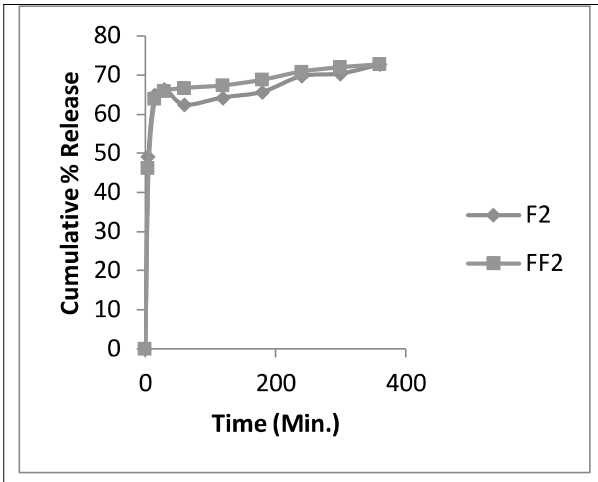


Figure 18 : Comparison of release profiles of F2-FF2

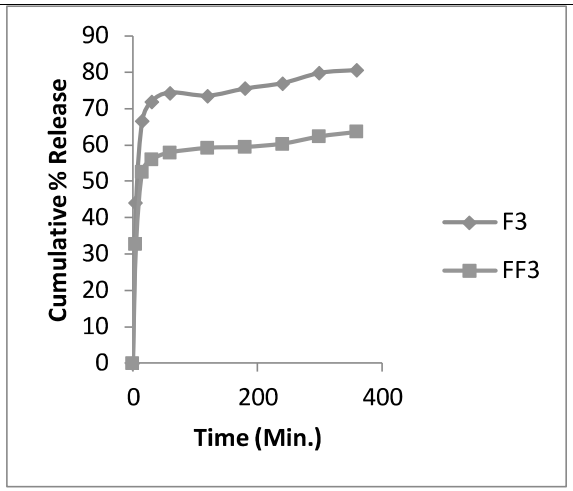


Figure 19 : Comparison of release profiles of F3-FF3

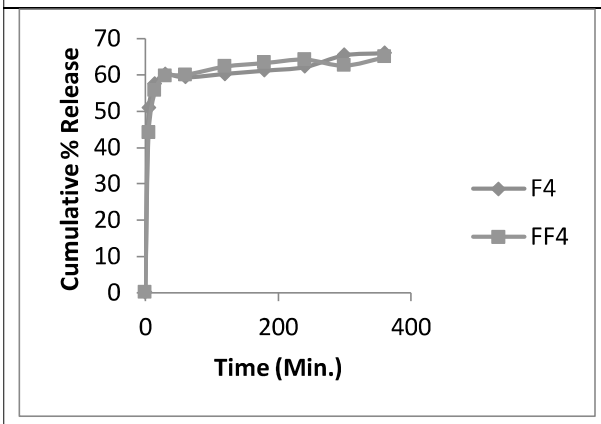


Figure 20 : Comparison of release profiles of F4-FF4

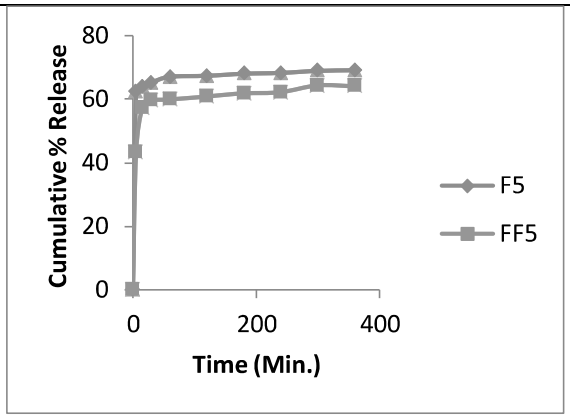


Figure 21 : Comparison of release profiles of F5-FF5

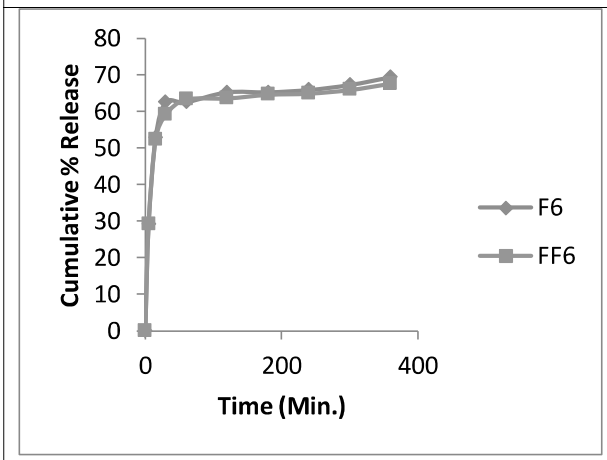


Figure 22 : Comparison of release profiles of F6-FF6

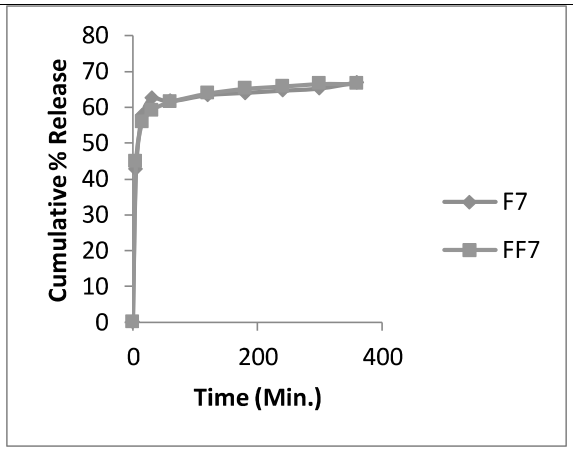


Figure 23 : Comparison of release profiles of F7-FF7

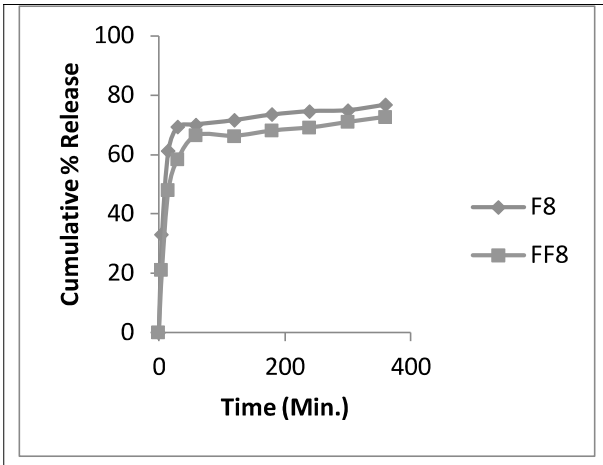


Figure 24 : Comparison of release profiles of F8-FF8

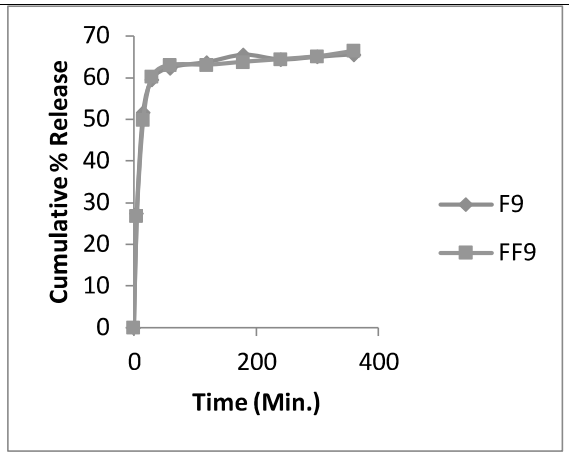


Figure 25 : Comparison of release profiles of F9-FF9

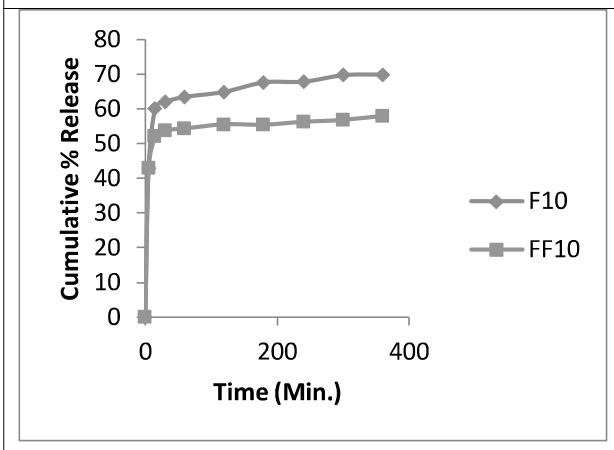


Figure 26 : Comparison of release profiles of F10-FF10

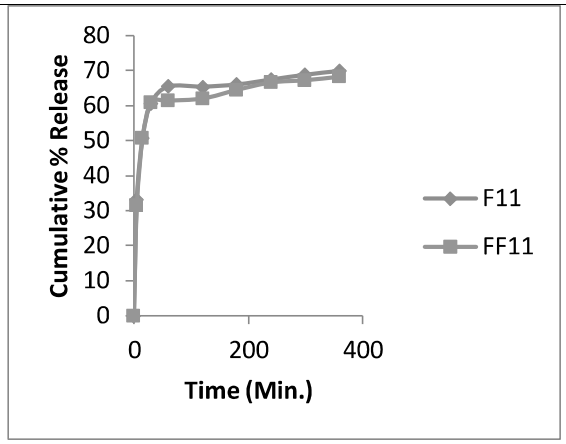


Figure 27 : Comparison of release profiles of F11-FF11

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 12-27). From Fig. 12 it was found that drug release was increased with increase in alginate concentration in spite of two different pH. This may be because of lesser degree of crosslinking at the surface of the bead, restriction of crosslinking in the core once surface was crosslinked and retard subsequent crosslinking of the core material. The Figure 13,14,15,16 revealed that the increased concentration of gelatin in a fixed concentration of alginate the drug release also increases. It may be due to formation of gel structure at the surface of beads which turn to nearly to be 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 corresponding formulations without formalin treatment depicted in Figure 17-27. It was observed that very little deviations in drug release

profile was observed when Alginate concentration was low but at a higher concentration of alginate with formalin treatment showed a significant retardation in of drug release profile. When Formalin is added, Gelatin molecules crosslinked and made a strong network additionally with the sodium alginate network, as a synergistic effect drug release was significantly retarded nearly 5-10%.

In- vitro Release Kinetics

All the in-vitro dissolution data were subjected for zero order, first order and higuchi equation to interpret the release pattern from the developed beads. From the table 19 it was observed that the drug release was best explained by Higuchi's kinetics upto 2 hours in 0.1N HCl followed by zero order in phosphate buffer, as the plot showed highest linearity. All the kinetic data were fitted in the Korsmeyer Peppas equation. The acceptable linearity was observed shown in table 19 and the release exponent 'n' varied from 0.026 to 0.352 in 0.1N HCl upto two hours and 0.02-0.113 in Phosphate buffer, which indicated that drug release followed by diffusion only.

Table 19 : Drug Release Kinetics

Formulation	Zero Order		First Order		Higuchi's		Korsmeyer-Peppas			
	r^2		r^2		r^2		r^2	n	r^2	n
	0-2h	2-6h	0-2h	2-6h	0-2h	2-6h	0-2h	0-2h	2-6h	2-6h
F1	0.604	0.807	0.605	0.801	0.773	0.824	0.901	0.028	0.832	0.071
F2	0.17	0.959	0.155	0.962	0.292	0.961	0.503	0.07	0.951	0.113
F3	0.3876	0.9791	0.4345	0.9777	0.5659	0.9776	0.7528	0.156	0.9662	0.0859
F4	0.3946	0.9371	0.4051	0.9327	0.5637	0.9155	0.7634	0.0512	0.8857	0.0878
F5	0.7636	0.9755	0.7736	0.9768	0.9006	0.9831	0.9694	0.0263	0.9788	0.0239
F6	0.4586	0.8583	0.5189	0.8502	0.6338	0.7991	0.7854	0.2424	0.7363	0.051
F7	0.4018	0.9358	0.4318	0.928	0.5714	0.9002	0.7572	0.1173	0.8612	0.0449
F8	0.4107	0.9538	0.4755	0.9556	0.5859	0.961	0.7516	0.2307	0.9605	0.0587
F9	0.471	0.4287	0.5359	0.4263	0.6492	0.4429	0.7923	0.2561	0.4599	0.0188
F10	0.4176	0.8937	0.4602	0.9024	0.5833	0.9237	0.7569	0.1198	0.9422	0.0672
F11	0.5368	0.988	0.5907	0.9863	0.7198	0.9712	0.8641	0.2156	0.9437	0.0624
FF1	0.476	0.932	0.483	0.923	0.661	0.888	0.854	0.032	0.845	0.099
FF2	0.359	0.9696	0.3893	0.9749	0.5246	0.984	0.7168	0.11	0.985	0.0727
FF3	0.4315	0.9344	0.4767	0.9305	0.6026	0.8933	0.7655	0.1744	0.8444	0.0662
FF4	0.5074	0.4041	0.5502	0.4067	0.6757	0.4095	0.6757	0.1037	0.4095	0.0253
FF5	0.3885	0.8942	0.4146	0.8904	0.5567	0.8932	0.7452	0.1005	0.8826	0.0516

FF6	0.4726	0.9422	0.5338	0.9352	0.6525	0.9117	0.7989	0.2389	0.8778	0.0498
FF7	0.6034	0.8852	0.659	0.8901	0.7673	0.9294	0.8994	0.1075	0.9612	0.0386
FF8	0.5328	0.9931	0.6106	0.9896	0.7144	0.9817	0.8255	0.3522	0.9636	0.0809
FF9	0.4656	0.9762	0.517	0.9717	0.6489	0.9516	0.8007	0.2656	0.9204	0.0436
FF10	0.4646	0.8992	0.4891	0.8966	0.6327	0.8502	0.8077	0.0751	0.7934	0.0379
FF11	0.4458	0.9386	0.4829	0.9487	0.6276	0.9692	0.7975	0.2111	0.9862	0.0875

CHAPTER 7

CONCLUSION

CONCLUSION

From the research it could be concluded that optimized concentration of sodium alginate, gelatin, calcium chloride and formaldehyde was successfully determined. Through the results we could reach the conclusion that increase in sodium alginate concentration as a base polymer from 1.5-3% resulted in insignificant variation of entrapment efficiency (54-63%). The ideal concentration of Calcium Chloride for cross linking was found to be 2% since no significant variation in entrapment efficiency was observed with varying concentration of $CaCl_2$ with a fixed concentration of sodium alginate. Increase in additive polymer concentration gelatin, modified the entrapment efficiency by 6-7% and increased the rate of drug release from 5-10%. Now the presence of Formaldehyde seemed to improve the entrapment efficiency by 7-18% and retarded the drug release in presence of gelatin by 5-10%. Finally is was concluded that the above research may be a modified approach for the development of multiparticulate drug delivery system in an alternative way.

CHAPTER 8

FUTURE WORK

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 behaviour.
- Extensive study with other drugs
- Extensive In-Vitro release study
- In-Vivo Study
- In-Vitro In-Vivo Correlation
- Statistical Analysis

CHAPTER 9

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