FORMULATION AND EVALUATION OF METFORMIN HYDROCHLORIDE MICROSPHERES BY IONOTROPIC GELATION TECHNIQUE

A Dissertation

Submitted in partial fulfillment of the requirement

For the award of degree of

Masters of Science in Biotechnology

Submitted by:

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CERTIFICATE

This is to certify that the dissertation entitled **"Formulation and evaluation of Metformin hydrochloride microspheres by ionotropic gelation technique"** submitted by Kamalpreet Kaur in partial fulfillment of the requirement for the award of the Degree of Master of Science in Biotechnology to Thapar University, Patiala, is a record of student's own work carried out by her under my supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other University.

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DECLARATION

I hereby declare that the work presented in the dissertation entitled **"Formulation and evaluation of Metformin hydrochloride microspheres by ionotropic gelation technique"** in partial fulfillment of the requirement for the award of the degree of Master of Sciences in Biotechnology, is an authentic record of my own work during a period of six months from January 2013 to June 2013, under the guidance of Mrs. M. Vasundhara, Assistant professor, Department of Biotechnology and Environmental Sciences, Thapar University, Patiala. The report has not been submitted for the award of any other degree or certificate in this or any other University.

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301101016

Place: Patiala

Date: 15 July, 2013

ACKNOWLEDGEMENT

It's said that life is a carnival of experience and a journey with various goals. So in my journey where I experienced this project I want to thank the supreme almighty for his presence in my soul and in my mind.

I take this opportunity to thank my guide **Mrs. M. Vasundhara**, Assistant professor, Department of Biotechnology and Environmental Sciences without whole presence the whole project just would have been a dream. I am extremely indebted to her for the scientific attitude and utmost patience she has installed in me which definitely stand in all future endeavours and it was because of her that I was able to learn so much in this short period.

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ABSTRACT

Controlled drug delivery technology is concerned with systematic release of a pharmaceutical agent to maintain a therapeutic level of the drug in the body for a sustained period of time. Various different approaches are used to develop controlled drug delivery systems. One such approach is using microspheres as carriers for drugs. Due to its small size they are widely distributed throughout the gastrointestinal tract which improves drug absorption and reduces side effects due to localized build-up of irritating drugs against the gastrointestinal mucosa. There are many methods for preparation of microspheres. Among them, ionotropic gelation method is one.

The aim of the present work was formulation and evaluation of microspheres by ionotropic gelation method using sodium alginate as polymer and $Cacl_2$ as cross-linking agent. Sodium alginate is biodegradable natural polymer with a great potential for pharmaceutical applications due to its good biocompatibility and non-toxicity. $Cacl_2$ is also called a common salt which is made up of calcium and chlorine. Metformin hydrochloride was selected as a model drug.

Metformin hydrochloride microspheres were prepared by dropping the drug containing solution sodium alginate. The droplets instantaneously formed gelled spheres by the ionotropic gelation technique. The microspheres were characterized by their percentage yield, morphology, particle size, swelling studies, encapsulation efficiency and in vitro drug release rate. Release studies were done in buffer (pH 1.2) and subsequently in buffer (pH 6.8). The release of drug from microspheres was greatly affected by drug concentration, polymer concentration, $Cacl_2$ concentration, stirring time and stirring speed. After studying various parameters it was examined that B10 was best having 2% drug, 2% polymer and 2.5% $Cacl_2$ at 100 rpm for 30 minutes.

Metformin hydrochloride microspheres have good properties. High drug incorporation in microspheres could be achieved by ionotropic gelation method without using any toxic chemicals which causes undesirable effects. Hence, this method is of interest in the pharmaceutical field. Also the ionotropic gelation can be carried out under very mild conditions using simple equipments. The control of various manufacturing parameters plays a very important role in obtaining microspheres of good sphericity, high yield and high drug encapsulation.

CHAPTER 1

INTRODUCTION

INTRODUCTION

A drug is defined as a chemical substance used in the treatment, cure, prevention, or diagnosis of disease or used to otherwise enhance physical or mental well-being. Drugs may be prescribed for a limited duration, or on a regular basis for chronic disorders. Drugs are usually distinguished from endogenous biochemical by being introduced from outside the organism.

Drugs are rarely administered as pure chemical substance alone and are almost always given as formulated preparations or medicines (i.e. drug delivery systems or dosage forms). These can vary from relatively simple solutions to complex drug delivery systems through the use of appropriate additives or excipients in the formulations. The method by which a drug is delivered (i.e. called drug delivery system) can have significant effect on its efficacy.

According to Jorge *et al*., (2010), the main purpose of drug delivery system is not only to deliver a biologically active compound in a controlled manner (time period and releasing rate) but also to maintain drug level in the body within therapeutic window.

Controlled drug delivery (CDD) occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the active agent is released from the material in a predesigned manner. The release of active agent may be constant over a long period, it may be cyclic over a long period, or it may be triggered by the environment or other external events. The goal of many of the original controlled release systems was to achieve a delivery profile that would yield a high blood level of the drug over a long period of time. With traditional tablets or injections, the drug level in the blood follows the profile shown in figure 1(a), in which the level rises after each administration of the drug and then decrease until the next administration. The key point with traditional drug administration is that the blood level of the agent should remain between a maximum value, which may represent a toxic level, and a minimum value, below which the drug is no longer effective. In controlled drug delivery systems designed for long term administration, the drug level in the blood follows the profile shown in figure 1(b), remaining constant, between the desired maximum and minimum, for an extended period of time. (Rafi Shaik *et al*., 2012).

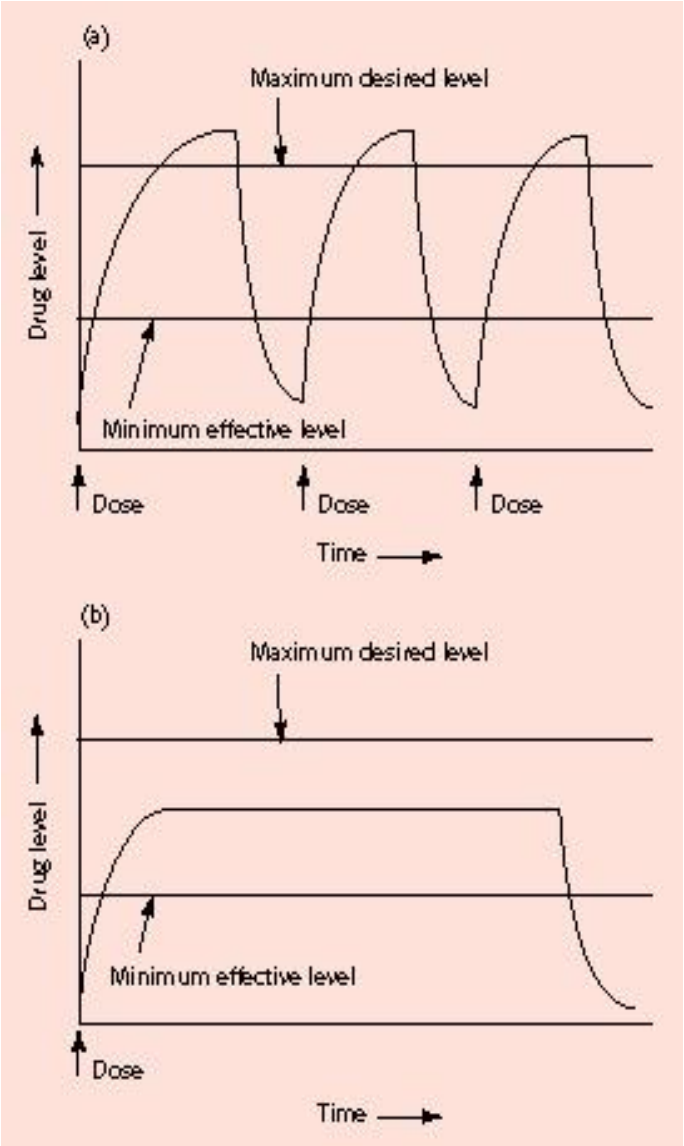


Figure 1: Drug level in the blood with

(a) Traditional drug dosing and (b) Controlled-delivery dosing

According to Shivadas Wani, (2008), advantages of controlled drug delivery systems include

I. Patient compliance due to reduction in the frequency of designing.
II. Employ minimum drug.
III. Minimize or eliminate local and systemic side effects.
IV. Minimize drug accumulation with chronic dosing.
V. Improves efficacy in treatment.
VI. Cure or control confirm more promptly.
VII. Improve control of condition i.e. reduce fluctuation in drug level.
VIII. Improve bioavailability of same drugs.

Disadvantages of controlled drug delivery system are

I. They are costly.
II. Unpredictable and often poor in-vitro in-vivo correlations, dose dumping, reduced potential for dosage adjustment and increased potential first pass clearance.
III. Poor systemic availability in general.
IV. Effective drug release period is influenced and limited by GI residence time.

According to Rajgor, (2011), the controlled drug delivery systems are based on three basic delivery mechanisms

- Swelling control
- Osmotic pumping
- Diffusion

The ideal drug delivery system should be inert, biocompatible, mechanically strong, comfortable for the patient, capable of achieving high drug loading, safe from accidental release, simple to administer and remove, and easy to fabricate and sterilize. (Raghanaveen, 2009).

Most biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer chains into biologically acceptable, and progressively smaller, compounds. In some cases for example, polylactides, polyglycolides, and their copolymers- the polymers will eventually break down to lactic acid and glycolic acid, enter the Krebs's cycle, and be further broken down into carbon dioxide and water and excreted through normal process. Degradation may take place through hydrolysis, in which the polymer degrades in a fairly uniform manner throughout the matrix.

Various different types approaches to developing controlled drug delivery systems. One such approach is using microspheres as carriers for drugs. Microspheres are made up of proteins or polymers. Due to its small size they are widely distributed throughout the gastrointestinal tract which improves drug absorption and reduces side effects due to localized build-up of irritating drugs against the gastrointestinal mucosa. (Ketul *et al*., 2012). There are many methods for preparation of microspheres. Among them, ionotropic gelation method is one.

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Majeti N. V. Ravi Kumar, (2000) reported that microparticles have potential applications for the administration of therapeutic molecules and in research areas covers novel properties that have been developed increased efficiency of drug delivery, improved release profiles and drug targeting.

P K Choudhury and Mousumi Kar, (2005) used emulsion gelation method to prepare gel beads for a highly water-soluble drug Metformin hydrochloride using sodium alginate as the polymer. They used oil with sodium alginate for preparation of microspheres. The oil entrapped calcium alginate gel beads showed good sustained release and scanning electron photomicrographs demonstrated minute oil globules on the beads and also through the inner surface of the beads.

KM Manjunatha *et al*., (2007) prepared beads based on dispersing drug in solutions of ionic polysaccharide such as chitosan and sodium alginate. This is ionotropic gelation technique. The beads were characterized by scanning electron microscopy and X- ray diffraction studies.

M. K. Das and P. C. Senapati, (2008) prepared Furosemide- loaded alginate microspheres by ionic cross- linking technique using $Cacl_2$, $Al_2(SO_4)$ and $Bacl_2$. The effect of sodium alginate concentration, cross- linking agent, drying conditions was evaluated with respect to entrapment efficiency, particle size, surface characteristics, and in vitro release behavior. Infrared spectroscopic study confirmed the absence of any drug-polymer interaction and scanning electron microscopy study analyze that the drug was molecularly dispersed in the alginate microspheres matrices showing rough surface.

VN Deshmukh *et al*., (2009) prepared microspheres by ionic- cross linking technique. Chemical reaction between sodium alginate and calcium chloride to form calcium alginate was utilized for microspheres. For slowing the rate of release from microspheres the hydrophilic polymer locus bean gum and xanthan gum and their combinations was added in different concentration so that drug will be released constantly for 12hrs. Stability studies revealed that polymers used were stable and compatible with the drug and there is no significant effect on physical characteristics, drug content and dissolution profile of the microspheres.

MD Dhanaraju *et al*., (2009) fabricated sustained release polymeric beads containing diclofenac sodium with hydrophilic polymers, sodium carboxymethyl cellulose and sodium alginate by ionotropic gelation method using calcium chloride as cross-linking agent. Prepared beads were evaluated for their different properties. The drug entrapment efficiency varied between 73% and 92% in different formulations and the release of drug was observed to be 82% and 91% respectively at the end of 10 hours.

Dr. K. P. Namdeo *et al*., (2010) prepared spherical microspheres of theophylline (TP) using sodium alginate as the hydrophilic carrier. They observed that with increase in concentration of polymer, mean particle size of microspheres increases and percent drug release decreases. Optimized alginate microspheres were found to possess good spherical shape, size and adequate entrapment efficiency. TP- loaded alginate microspheres showed extended in vitro drug release thus use of microspheres potentially offers sustained release profile along with improved delivery of TP.

K. M. Manjanna *et al*., (2010) developed a microparticulate oral sustained release dosage form, to reduce dosing frequency, to eliminate the dose related adverse effects and to ultimately improve compliance in the pharmacotherapy of arthritis. The microbeads were prepared by an ionotropic external gelation technique, by using sodium alginate as the hydrophilic carrier and $Cacl_2$ as cross-linking agent. The shape and surface characteristics, size distribution and physical state of drug were determined. While increasing the concentration of sodium alginate dispersion increased flow properties, mean particle size, swelling ratio and drug entrapment efficiency.

Shailendra Shukla *et al*., (2010) developed cyst-targeted alginates microspheres of diloxanide furoate (DF) for the effective treatment of amoebiasis. Alginate microspheres were produced by the emulsification method using calcium chloride. Formulations were characterized for particle size and shape, surface morphology, entrapment efficiency, and in vitro drug release in simulated gastrointestinal fluids. XRD and differential scanning calorimetry were used to confirm successful entrapment of DF into the alginates microspheres. Calcium alginate retarded the release of DF at low pH (1.2 and 4.5) and released microspheres slowly at pH 7.4 in the colon without colonic enzymes.

Sanchita Mandal *et al*., (2010) developed a sustained release dosage form of trimetazidine-dihydrochloride (TMZ) using a natural polymeric carrier prepared in a completely aqueous environment. The drug was incorporated either into preformed calcium alginate beads

(sequential method) or incorporated simultaneously during the gelation stage (simultaneous method). Drug entrapment in the sequential method was higher with increased calcium chloride and polymer concentration but lower with increased drug concentration. In the simultaneous method, drug entrapment was higher when polymer and drug concentration were increased and also rose to a certain extent with increase in calcium chloride concentration, where further increase resulted in lower drug loading. FTIR studies revealed that there is no interaction between drug and calcium chloride and XDR studies showed that the crystalline drug changed to an amorphous state after formulation.

Prasanth V V *et al*., (2011) prepared salbutamol sulphate (SS) loaded alginate microspheres by ionotropic gelation method with calcium chloride, aluminium sulphate, magnesium aluminium silicate. The microspheres were dried in hot air oven and dried at room temperature. The particle size and drug content of hot air oven dried microspheres were less than air dried microspheres. During and at the end of the accelerated stability study, the tested microspheres showed almost similar drug content and drug release as observed at the beginning of the study.

Anupama Singh *et al*., (2011) formulated and optimized alginate microspheres by employing 3^2 factorial designs for treatment of chronic ulceritis using famotidine drug, sodium alginate as polymer and calcium chloride: calcium carbonate as cross- linking agent. The prepared microspheres were characterized in terms of drug- excipient compatibility study, percentage yield, micromeritics study, swelling index, particle size analysis, shape analysis, drug content, drug encapsulation efficiency, and in vitro drug release study. Good surface and size of microspheres was found to be responsible for slow release of drug from matrix through erosion.

Akhilesh V Singh, (2011) reported that biopolymers are choice of research as excipients in pharmaceutical drug delivery system because of its low toxicity, biodegrability, stability and renewable nature.

Swati Aggarwal *et al*., (2011) reported that polymers have been main tool to control the drug release rate from the formulations and widely used in biomedical applications because of their known biocompatibility and biodegradability.

Kataria Sahil *et al*., (2011) reported that microspheres are the reliable means to deliver the drug to the target site with specificity and in future by combining various strategies,

microspheres will find the central place in novel drug delivery, particularly in diseased cell sorting, diagnostics, safe, targeted and effective in vivo delivery and supplements as miniature version of diseased organ and tissues in the body.

Amrinder Singh *et al*., (2011) prepared polymeric gel beads of cefixime for controlled release. They used calcium ions as cross linking agents in formulations of alginate and alginate pectin beads by ionotropic gelation method. In results, they showed that, as the concentration of alginate was increased in the formulation, spherical shape of the beads was maintained and also more sustained action was observed. But when pectin was used along with sodium alginate the shape of beads turned irregular or disc like and the sustained action was reduced.

Jakir Ahmed Chowdhury *et al*., (2011) prepared sustained release polymeric beads containing diclofenac sodium with sodium alginate by ionotropic gelation method using calcium chloride and aluminium sulphate. In aluminium sulphate solution the entrapment efficiency of beads was highest than in calcium chloride solution. In vitro dissolution data showed that, with increasing drug, polymer and electrolyte amount diclofenac release percentage also decreased.

Raja Chakraverty, (2012) prepared and evaluated of sustained release of microspheres of norfloxacin employing sodium alginate as natural polymer by ionotropic gelation technique.

Ramteke K. H, (2012) incorporated Metformin hydrochloride into beads giving sustained release of Metformin and optimized best cross linking agent amongst calcium and aluminium. Formulations were prepared by ionotropic gelation technique. Calcium cross-linked beads showed sustained release of drug for about 8 hr while aluminium cross-linked beads shown sustained release of drug about 10 hrs. The entrapment efficiency was also lesser in case of calcium cross-linked beads. Aluminium was found to be better cross- linking agent than calcium.

Sreenivasulu *et al*., (2012) prepared and evaluated sodium alginate microspheres loaded with Metformin hydrochloride by ionotropic gelation technique. The prepared microspheres were evaluated for swelling studies, drug loading, drug entrapment efficiency, SEM and in vitro drug release. FTIR studies revealed that there was no interaction between drug and polymer and in vitro release profile of all formulations showed slow controlled release up to 8 hrs.

Christina. E *et al*., (2012) formulated different microsphere formulations of diclofenac sodium by ionotropic gelation technique using sodium alginate as carrier. Microspheres were evaluated for flow properties, drug entrapment efficiency and drug release from entrapment. Microspheres prepared using drug polymer ratio of 1: 2 and were found to sustain the release of active substrate for 12 hours when examined in pH 7.5 phosphate buffer. They demonstrated the suitability of the prepared microspheres of diclofenac sodium as sustained release dosage form.

Ruma Maji *et al*., (2012) investigated the effect of various formulation variables like drug-polymer ratio, stirring speed and surfactant (Span 80) concentration on the properties of ethyl cellulose microparticles containing Metformin hydrochloride. The drug entrapment efficiency, particle size, and drug release behavior of these microparticles was influenced by these formulation variables. The sustained release characteristic of microparticles was more prominent in pH 6.8 and pH 1.2. The drug release from ethyl cellulose microparticles was found to follow the Fickian (diffusion-controlled) release mechanism. The drug polymer interaction and surface topology of these microparticles was analyzed by FTIR spectroscopy and SEM, respectively.

Mahammad Rafi Shaik *et al*., (2012) stated that biodegradable materials are used in packing, agriculture, medicines and other areas. Two classes of biodegradable polymers are synthetic or natural polymers. Polymers produced from feedstock derived either from petroleum resources or from biological resources, but natural polymers offer fewer advantages than synthetic polymers and these polymers have many applications in controlled drug delivery systems.

Pandya Ketul *et al*., (2012) reported that microsphere drug delivery system has wide range of applications as it covers targeting the drug to particular site to imaging and helping the diagnostic features. It has advantage over liposome as it is physiochemically more stable.

Mohamed Aly Kassem *et al*., (2012) reported that microspheres constitute an important part in drug delivery systems, by the virtue of their small and uniform size and efficient carrier characteristics. Their study shows that optimum polymer concentration, crosslinker concentration, drug concentration, curing time and stirring speed are 3%, 7.5%, 1.5%, 15 minutes and 400 rpm respectively. Different mathematical models of drug release were obtained for the microspheres indicating that the drug release from the microspheres is controlled by first order kinetics.

CHAPTER 3

OBJECTIVE OF STUDY

OBJECTIVE OF STUDY

The objectives of the present study were to optimize the variables influencing the preparation of the Metformin hydrochloride microspheres. The study involved:

- Preparation of Metformin hydrochloride microspheres by altering the process variables.
- Characteristics of Metformin hydrochloride microspheres such as weight variation, percentage yield, swelling study, particle size and encapsulation amount of drug.
- Evaluation of the drug release behavior of the prepared Metformin hydrochloride microspheres.

CHAPTER 4

RESEARCH ENVISAGED

RESEARCH ENVISAGED

The use of microsphere based therapy allows drug release to be carefully tailored to the specific treatment site through the choice and formulation of various drug- polymer combinations. The total dose of medication and the kinetics of release are variables, which are manipulated to achieve the results. Using innovative microencapsulation technologies, and by varying the copolymer ratio, molecular weight of polymer etc., microspheres can be developed into an optimal drug delivery system which provide the desired release profile. Microsphere based system may increase the lifespan of active agents and control the release of constituents. Being small in size, microspheres have large surface to volume ratio and can be used for controlled release of insoluble drugs. (Sinha *et al*., 2004).

It is desirable that the duration of drug action becomes more a design property of a rate controlled dosage form, and less, or not at all, a property of the drug molecules inherent kinetic properties. Thus, optimal design of controlled release systems necessitates a thorough understanding of the pharmacokinetics and pharmcodynamics of drug. The successful designing of a drug delivery system also involves a basic understanding of the properties and characteristics of polymer and a thorough knowledge of the nature of polymer.

4.1 Selection of drug

The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. Design of controlled release product is normally a very difficult task because of the interplay of physiochemical and biological properties of the drug, the patient's disease state and the technological limitation in fabrication of the final dosage form. The selection of drug for preparation in controlled release dosage forms involves consideration of a number of properties.

4.1.1 Properties of drug

4.1.1.1 Physico-chemical properties

- The therapeutic dose of the drug should be low.
- Extremes in aqueous solubility of a drug are undesirable.
- Extremes in partition coefficient of a drug are also undesirable.

- Drug used for sustained drug delivery should be stable over the entire length of the gastrointestinal tract.
- Large molecules will show small diffusion coefficient and may be unsuitable for a sustained release system.

4.1.1.2 Biological properties

- A drug with a short half life requires dosing and this makes it a desirable candidate for sustained release formulation.
- Drugs that are slowly absorbed or absorbed with variable absorption rate are poor candidates for sustained release formulation.
- Drugs with high apparent volumes of distribution are poor candidates.
- Drugs with a narrow therapeutic index require precise control over the blood levels of drug placing a constraint on controlled release dosage form.

Metformin hydrochloride was chosen as a model drug for the preparation of microspheres.

4.2 Metformin hydrochloride

4.2.1 Description

Metformin hydrochloride is an oral antihyperglycemic drug used in the management of type 2 diabetes. Metformin hydrochloride (N, N-diethylimidodicarbonimidic diamide hydrochloride) is not chemically or pharmacologically related to any other classes of oral antihyperglycemic agents. Metformin hydrochloride is a white to off-white crystalline compound with a molecular formula of $C_4H_{11}N_5$.HCL and a molecular weight of 165.63. Metformin hydrochloride is a freely soluble in water and is practically insoluble in acetone, ether and chloroform. The pK_a of Metformin is 12.4. The pH of a 1%aqueous solution of Metformin hydrochloride is 6.68. The chemical structure of Metformin hydrochloride is as follows (Indian pharmacopoeia, 1996).

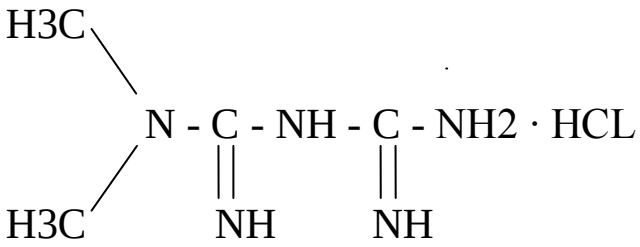


Figure 2: Chemical structure of Metformin hydrochloride

4.2.3 Mechanism of action

Metformin is an antihyperglycemic agent which improves glucose tolerance in patients with type 2 diabetes, lowering both basal postprandlal plasma glucose. Its pharmacologic mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects and does not cause hyperinsulinemia. With Metformin therapy, insulin secretion remains unchanged while fasting insulin levels and day-long plasma insulin response may actually decreases. The glucose lowering effects of Metformin are mainly a consequence of reduced hepatic glucose output (primarily through inhibition of gluconeogenesis and, to a lesser extent, glycogenolysis) and increased insulin-stimulated glucose uptake in skeletal muscle and adipocytes (Bailey C J *et al*., 1996; Hundal R S *et al*., 2000).

4.2.4 Pharmacokinetics

4.2.4.1 Absorption and bioavailability

The absolute bioavailability of a Metformin hydrochloride 500 mg tablet given under fasting conditions is approximately 50-60%. Studies using single oral doses of Metformin tablets of 500 mg and 1500 mg, and 850 mg, and 2550 mg, indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination.

4.2.4.2 Distribution

The apparent volume of distribution (V/F) of Metformin following single oral doses of 850 mg averaged 654 L. Metformin is negligibly bound to plasma proteins. At usual clinical

doses and dosing schedules of Metformin hydrochloride tablets, steady state plasma concentration of Metformin are reached within to 24-48 hours and are generally <1µg/ml. During controlled clinical trials, maximum Metformin plasma levels did not exceed 5µg/ml, even at maximum doses.

4.2.4.3 Metabolism and elimination

Intravenous single dose studies in normal subjects demonstrate that Metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) nor biliary excretion. Renal clearance is approximately 3.5 times greater than creatinine clearance which indicates that tubular secretion in the major route of elimination. Following oral administration, approximately 90%of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination half life of approximately 6.2 hours. In blood the elimination half life is approximately 17.6 hours, suggesting that the erythrocytes mass may be compartment of distribution.

4.2.5 Uses

Metformin is primarily used for type 2 diabetes, but is increasingly being used in polycystic ovary syndrome (PCOS), non alcoholic fatty liver disease (NAFLD) and premature puberty, three other disease that feature insulin resistance; these indications are still considered experimental. The benefit of Metformin in NAFLD has not been extensively studied and may be only temporary; although some randomized controlled trials have found significant improvement with its use, the evidence is still insufficient.

Women may also take the Metformin to treat polycystic ovary syndrome, as it helps stimulate the ovaries to release an egg.

4.2.6 Adverse effects

The most common side effect of Metformin is gastrointestinal upset, including diarrhea, cramps, nausea, vomiting and increased flatulence. The most seriously potential side effect is Metformin use is lactic acidosis which has fewer occurrences in usual peoples but risk is high in those with impaired liver or kidney function (Khurana et al., 2010). Feeling very weak, tired, unusual muscle pain and stomach discomfort, trouble breathing, feeling cold and feeling dizzy are some common signs of lactic acidosis. Metformin has also been reported to

reduce the blood levels of thyroid-stimulating hormone in patients with hypothyroidism, in men, luteinizing hormone and testosterone, The clinical significance of these changes is still unknown (Vigersky R A, 2006; Shegem N S *et al*., 2002).

4.2.7 Precautions

- Drinking alcohol while taking Metformin may cause an unhealthy decrease in blood sugar levels and increases the risk of developing lactic acidosis.
- Individuals with kidney disease or congestive heart failure should not take Metformin.
- Metformin is not recommended for use in pregnancy and for nursing mothers.
- Metformin therapy should be temporarily suspended for any surgical procedure and should not be restarted until the patient's oral intake has resumed and renal function has been evaluated as normal.

4.3 Selection of polymer

Polymers first developed in search for biodegradable materials have been proven to be useful for long term drug delivery applications. Biodegradable polymers are highly desirable in these situations because they degrade in the body to biologically inert and compatible molecules. By incorporating drugs in biodegradable polymers, dosage forms that release the drug over a prolong length of time can be prepared in variety of shapes and sizes. As a result biodegradable polymers offer a novel approach for developing sustained release drug delivery system that are simple and convenient to patient.

4.3.1 Advantages of biodegradable polymers as drug carriers

A polymer is a large molecule composed of smaller units called monomers that are bonded together. In addition to eliminating the necessity of removal, the five key advantages that polymeric delivery products can offers are: localized delivery of drug, sustained delivery of drug, stabilization of the drug, release rate which is less dependent of drug properties and steadier release rate with time.

4.3.2 Factors affecting selection of polymer

If an application requires rapid development and commercialization, then the polymer selection will most likely be made from those polyesters that already received regulatory

approval. Another factor to be taken into account is choice, whether to use homopolymers or copolymers containing multiple monomer species. If copolymers are employed then the relative ratio of different monomers may be manipulated to change polymeric properties i.e. morphology, structure and extent of drug polymer interactions. Ultimately all these properties will influence the performance of the drug delivery systems via changes to relative rates of mass transport and degradation rate of both, the polymer and the device. (Kotwal and Saifee, 2007).

4.3.3 Development of polymers

The development of appropriate carriers for drug delivery is the challenge for biomedical scientists. Peptides, proteins, oligonucleotides, and genes are unstable compounds that need to be protected from degradation in the biological environment. Moreover, their efficacy is highly limited by their ability to cross biological barriers and reach the target site. As such, the future of these molecules as therapeutic agents clearly depends on the design of an appropriate carrier for their delivery to the body. Several studies have been reported so far in the development of these carriers, among which the design of biodegradable nanoparticles has drawn considerable interest. In particular, nanoparticles made of hydrophobic or amphiphilic polymers such as poly (lactide-co-glycolide) (PLGA) and poly (lactic) acid (PLA)-poly (ethylene) glycol (PEG) offer great promise (Blanco and Alonso1997; Tobio *et al*., 1998; Tobio *et al*., 2000). But these nanoparticles have a limitation in their preparation procedure, which requires the use of organic solvents and surfactants as well as sonication. A challenging alternative to these hydrophobic particles are colloidal carriers made of hydrophilic polymer for the association and delivery of labile macromolecular compounds (M. Prabaharan., 2005). Among water-soluble polymers available, sodium alginate is one of the most extensively studied.

4.4 Sodium alginate

Sodium alginate is the purified carbohydrate product extracted from brown seaweeds by the use of dilute alkali. Sodium alginate consists of sodium salt of alginic acid. The chemical formula of sodium alginate is $(C_6H_7NaO_6)_n$.

4.4.1 Origin

The sodium alginate was studied for the first time in 1881 by English chemist ECC Stanford. He had at the time extracted a viscous liquid from brown seaweed of the *Laminaria* species, with an alkaline solution. He called this product "Algin", a term still commonly used to describe sodium alginate.

Different species are therefore harvested according to the purpose for which they are intended and the two most popular are the *Macrocystis pyrifera* of California and the *Ascophyllum nodosum*, grown in the North Atlantic.

Sodium alginate is a salt extracted from the viscous liquid from the cell wall of brown algae. Its natural function is to increase the flexibility of the algae. Thus, algae developing in troubled waters generally have large alginate content than those growing in calm water.

4.4.2 Structure

The number and sequence of the mannuronate and glucuronate residues shown in figure 3 vary in the naturally occurring alginate. The water molecules associated with the alginate molecule are not shown in the structural formula.

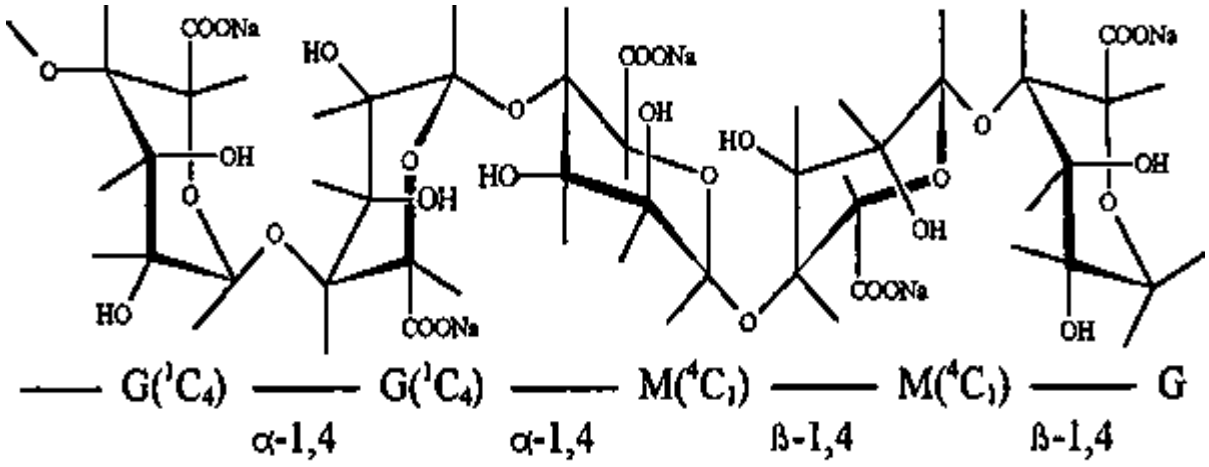


Figure 3: Chemical structure of sodium alginate

4.4.3 Physical properties

1. Molecular weight: 10,000-600,000.
2. Form: Sodium alginate is white or light yellow powder almost odorless and tasteless.

3. Solubility: Sodium alginate soluble in water and insoluble in alcohol, ether, chloroform and other organic solvents. When dissolved in water it will form a sticky liquid. The pH of 1% of this solution is 6-8 and stickiness will be stable when pH becomes 6-9 and will decrease when is heated to 80℃.
4. Toxicity: Sodium alginate toxic, LD50>5000mg/kg.
5. Gelation: Chelating agent on the properties of sodium alginate solution, chelating agents can be complex systems of divalent ions, making the alginate to stabilize in the system

4.4.4 Applications

- In food industry: Sodium alginate use in food industry is based on its thickening, gelling and in general colloidal properties. Thickening is useful in sauces, syrup, and topping for ice cream, pie filling, cake mixes, and canned meat and vegetables. Gel formation lead to uses in milk desserts and jellies, bakery filling cream, fruit pie, animal foods and reformed fruit. General colloidal properties are difficult to define but are illustrated by results obtained by adding sodium alginate to ice cream and water ices, or propylene glycol alginate to stabilize beer foam or suspended solids in fruit drinks.
- In textile industry: Sodium alginate is used as thickener for the paste containing dye. These pastes may be applied to the fabrics by either screen or roller printing equipment.
- Immobilization: Sodium alginate has been used for cell or enzyme immobilization, on laboratory and large scale. Examples are: production of ethanol from starch, production of citric acid, continuous yoghurt production etc.
- In paper industry: The main use of sodium alginate in paper industry is in surface sizing. It is also used in starch adhesives for making corrugated boards because it stabilizes the viscosity of the adhesive and allows control of its rate of penetration.
- Sodium alginate is also used in the welding roads, in pharmaceutical industry, in cosmetic industry, in water treatment and in dental industry.

4.5 Calcium chloride (Cross-linking agent)

Calcium chloride is a chemical compound made up of calcium and chlorine. It contains two atoms of chlorine and one atom of calcium. Thus its chemical formula is $Cacl_2$.

4.5.1 Preparation

Calcium chloride can be prepared by various methods. When calcium carbonate or calcium oxide is dissolved in hydrochloric acid, calcium chloride is produced. Calcium chloride is obtained on a large scale as a byproduct of the Solvay process or the ammonia-soda process. In this process, when calcium carbonate reacts with sodium chloride, sodium carbonate and calcium chloride are formed.

4.5.2 Physical properties of calcium chloride

- Physical state: Calcium chloride can be found in solid state at room temperature, and is available as flakes, granules and powdered form.
- Taste: Calcium chloride is salty to taste. Hence, it is added to many food products like pickles etc.
- Color: In solution form, calcium chloride is white in color, white in liquidated form, it is colorless.
- Electrical conductivity: Usually in a molten state, it is a good conductor of electricity.
- Thermal conductivity: Calcium chloride is a bad conductor of heat.
- Boiling point: Its boiling point is as high as 1600ºC.
- Solubility: It is soluble both in inorganic solvents like water, as well as organic solvents like ethanol.
- Hygroscopy: It is hygroscopic in nature and absorbs moisture from air. If exposed to open air, it tends to become liquid. That is why; it is often called a deliquescent substance.
- Melting point: It has a low melting point, which means it can be dissolved at a very low temperature.

4.5.3 Chemical properties of calcium chloride

- Exothermic: Calcium chloride is exothermic in nature, that is, it releases heat during any chemical reaction.
- Reaction with water: When calcium chloride is exposed to water, a large amount of heat is liberated, which can cause sputtering and boiling. The end product of this reaction is calcium hydroxide and chlorine gas.
- Reaction with sulfuric acid: When it comes in contact with sulfuric acid, hydrochloric acid is formed, which is highly caustic in nature.
- Reactions with metals: Calcium chloride is a non inflammable substance. However, when it comes in contact with metals like zinc or sodium, it produces hydrogen, which is highly inflammable.

4.5.4 Uses

- Calcium chloride has the ability of lowering the freezing point of water. It is often used to de-ice roads in region where icy roads are problem and also applied in working mines to minimize the levels of dust.
- Calcium chloride is used as a preservative in food because it is able to prevent food from spoiling.
- It is also used as an additive in the pasteurization of milk and in cheese making.
- Calcium chloride is commonly used as drying agent because it is capable of absorbing moisture. The property is used to remove moisture from the air, to dry wet products like kelp.
- Calcium chloride is also used in medicinal industry. It is used for hypocalcaemia, magnesium intoxication and hyperkalemia.
- It is commonly used in swimming pool water with low calcium content. This can reduce damage to the materials used around the pool.
- It is also added to many sports drinks because it is an electrolyte as well as being a good source of calcium.

4.5.5 Toxicology

It can cause skin irritation and must be handled carefully.

CHAPTER 5

EXPERIMENTAL WORK

EXPERIMENTAL WORK

5.1 Experimental materials and equipments

Various chemicals and instruments used for the preparation and evaluation of Metformin hydrochloride microspheres are listed in table as follows

Table 1: Experimental materials and equipments

Experimental materials	Equipments
• Metformin hydrochloride • Sodium alginate • Calcium chloride • Hydrochloric acid buffer (pH 1.2) • Phosphate buffer (pH 6.8) • Sodium hydroxide • Conc. hydrochloric acid.	• Analytical balance • pH meter • Lab stirrer • Microscope • UV-Visible spectrophotometer • Incubator • Shaking incubator • Disposable syringes and needles • Glassware • Measuring cylinder • Micropipettes

5.2 Standard curve of Metformin hydrochloride

Spectrophotometric method based on measurement of absorbance at 232nm of UV region in different media like distilled water, hydrochloric acid (pH 1.2), and phosphate buffer (pH 6.8) were used for estimation of Metformin hydrochloride.

5.2.1 Preparation of phosphate buffer (pH 6.8)

Phosphate buffer was prepared by placing 50 ml of 0.2M potassium dihydogen orthophosphate solution and 39.1 ml of 0.2N sodium hydroxide in a 200 ml volumetric flask and then distilled water was added to volume. The pH was found to be 6.8.

5.2.2 Preparation of hydrochloric acid (pH 1.2)

Hydrochloric acid was prepared by using 50 ml of potassium chloride and 95 ml of 0.2N hydrochloric acid in 200 ml volumetric flask and then distilled water was added to volume. The pH was found to be 1.2.

5.2.3 Preparation of standard graph

Standard solution was prepared by dissolving 10mg of Metformin hydrochloride in distilled water/ hydrochloric acid buffer (pH 1.2)/ phosphate buffer (pH 6.8) in a 100ml of volumetric flask. From the standard solution, a series of dilutions containing 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 µg/ml of drug were obtained. The absorbance of those diluted samples were measured in spectrophotometer at 232 nm using distilled water hydrochloric acid (pH 1.2) and phosphate buffer (pH 6.8) as a blank. (Sreenivasulu *et al*., 2012).

Table 2: Standard curve data of Metformin hydrochloride in distilled water

S.No.	Concentration (µg/ml)	Absorbance at 232 nm
1.	1	0.071
2.	2	0.133
3.	3	0.223
4.	4	0.303
5.	5	0.342
6.	6	0.416
7.	7	0.491
8.	8	0.577
9.	9	0.649
10.	10	0.717

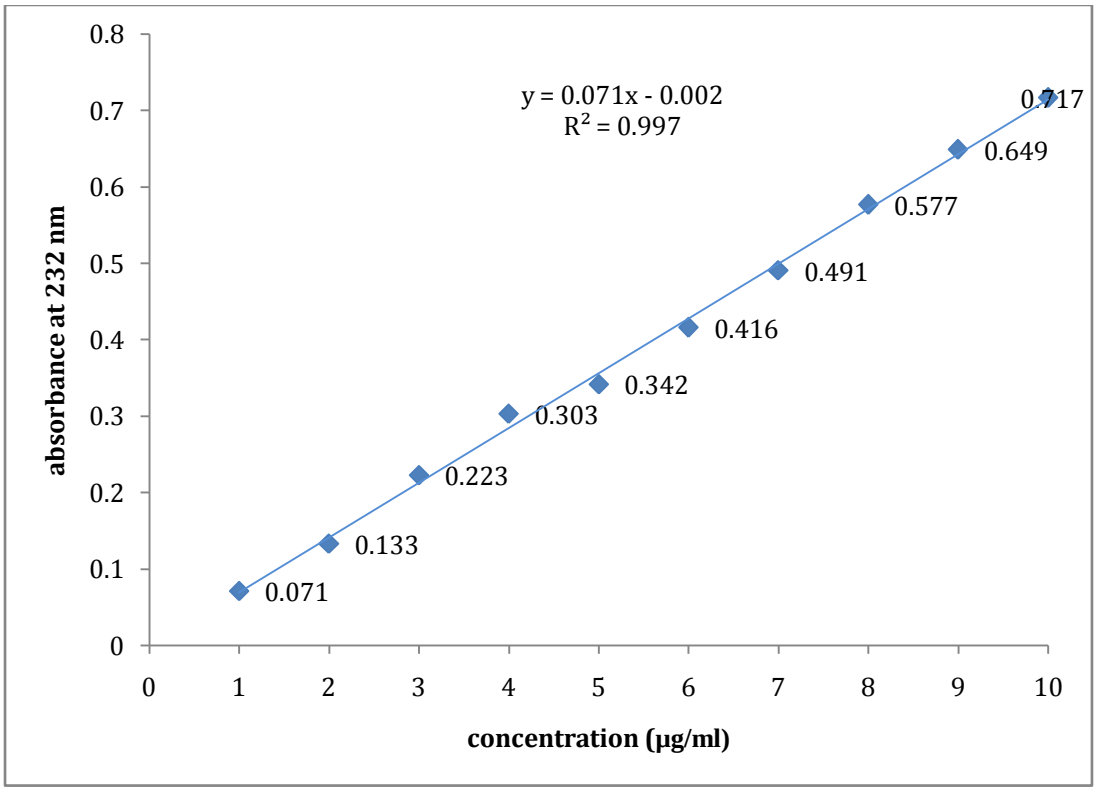


Figure 4: Standard curve of Metformin hydrochloride in distilled water

Table 3: Standard curve data of Metformin hydrochloride in hydrochloric acid buffer

S.No.	Concentration (µg/ml)	Absorbance (at 232 nm)
1.	1	0.052
2.	2	0.079
3.	3	0.110
4.	4	0.131
5.	5	0.179
6.	6	0.213
7.	7	0.229
8.	8	0.262
9.	9	0.308
10.	10	0.333

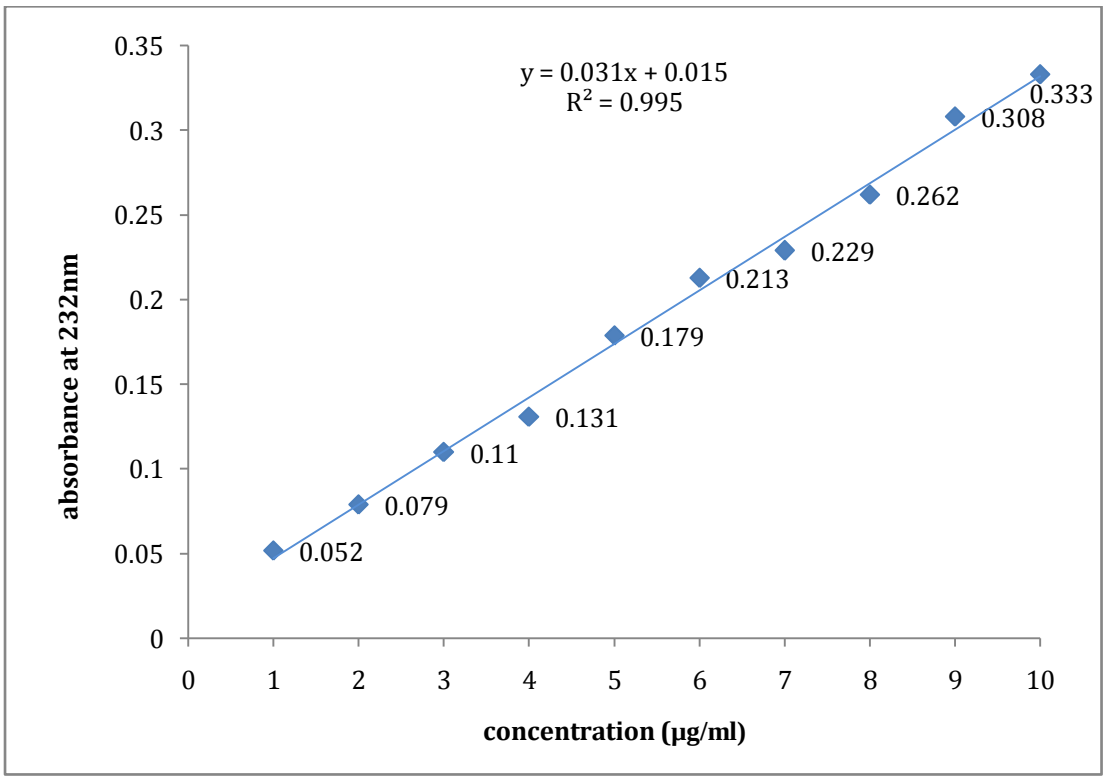


Figure 5: Standard curve of Metformin hydrochloride in hydrochloric acid buffer

Table 4: Standard curve data of Metformin hydrochloride in phosphate buffer

S. No.	Concentration (µg/ml)	Absorbance (at 232 nm)
1.	1	0.223
2.	2	0.327
3.	3	0.435
4.	4	0.545
5.	5	0.698
6.	6	0.776
7.	7	0.885
8.	8	0.996
9.	9	1.143
10.	10	1.278

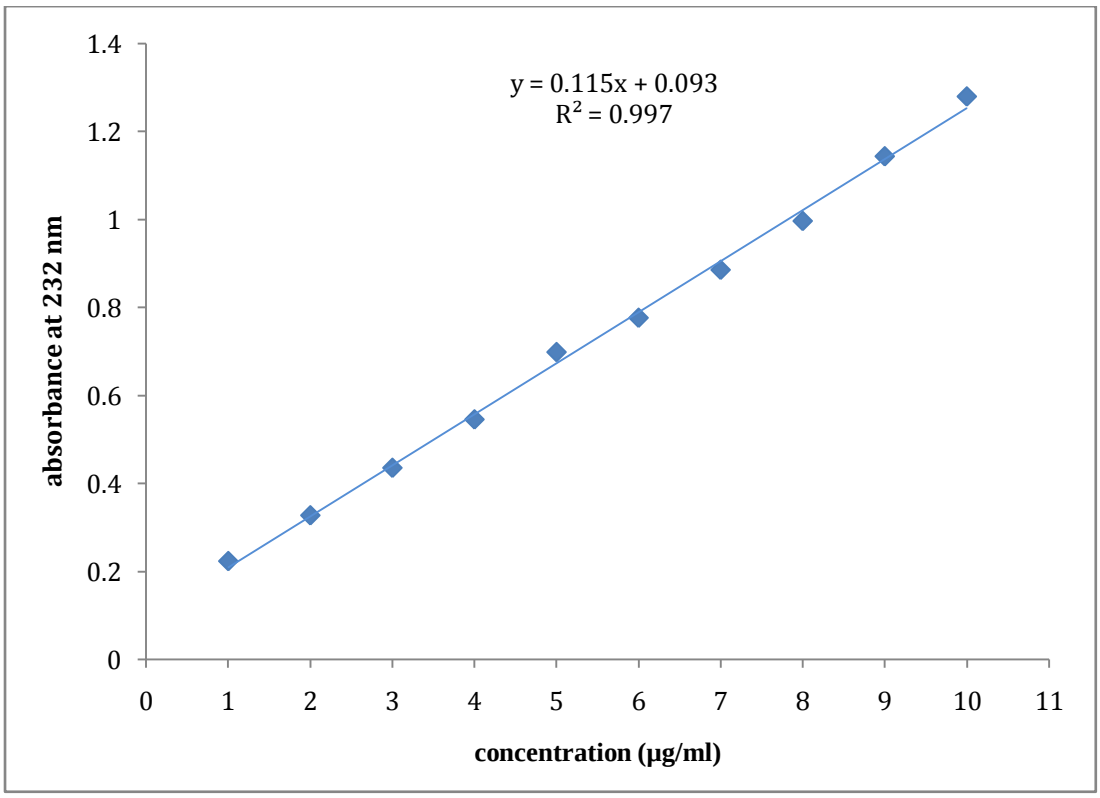
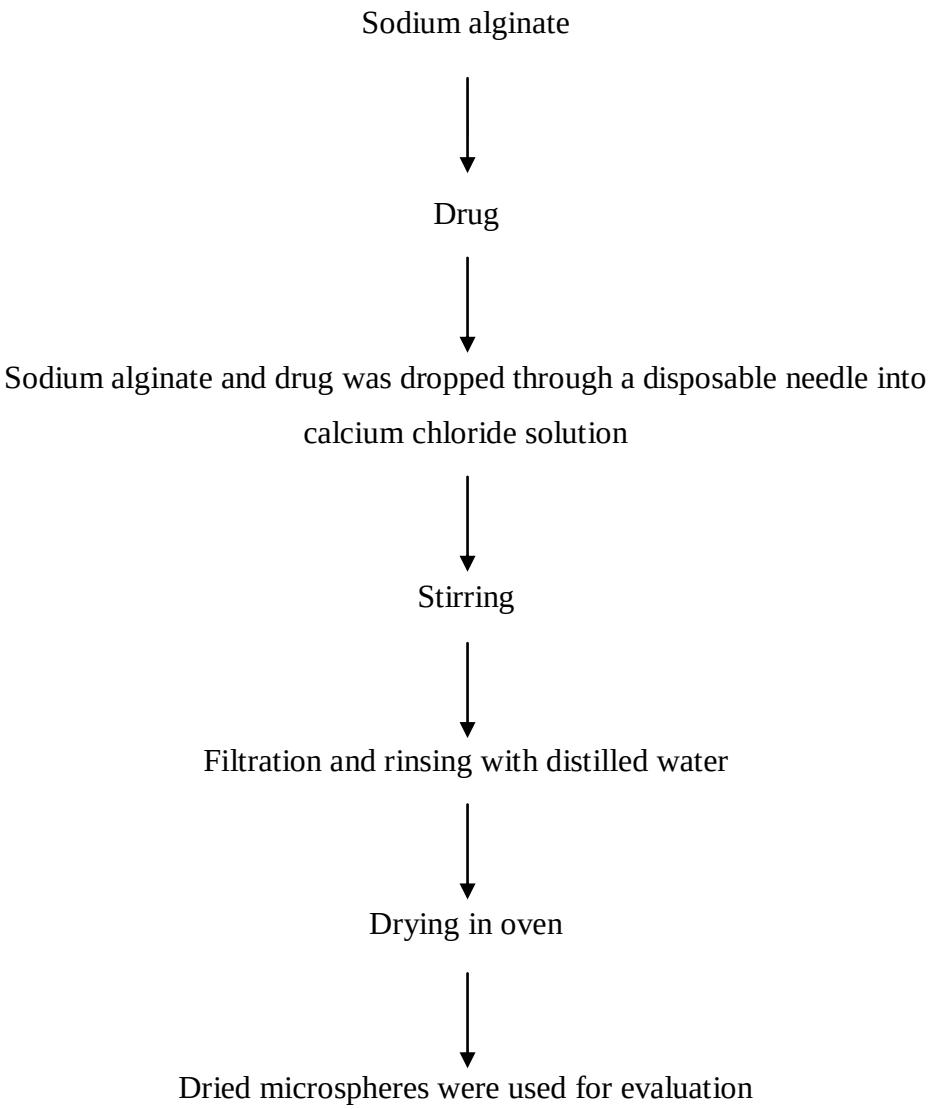


Figure 6: Standard curve of Metformin hydrochloride in phosphate buffer

5.3 Preparation of Metformin hydrochloride microspheres

Microspheres of Metformin hydrochloride were prepared by ionotropic gelation technique. In beaker, a suitable amount of sodium alginate was taken and mixed properly and kept for overnight. Accurately weighed quantity of drug was dissolved in sufficient quantity of distilled water. The drug solution was added to sodium alginate solution with proper mixing. After complete mixing, the polymer-drug solution was added drop wise by using a disposable syringe from a height of about 5 cm into a beaker containing calcium chloride with continuous stirring by magnetic stirrer. Then the solution was filtered. The microspheres were washed two-three times with distilled water and dried at 37ºC.



5.4 Formulation and process variables

Different batches of Metformin hydrochloride microspheres were prepared by altering the following variables:

- Concentration of drug (0.5% - 2%)
- Concentration of polymer (2% - 2.5%)
- Concentration of cross-linking agent (2% - 4%)
- Stirring time (30–45 minutes)
- Stirring speed (100-200 rpm)
- Volume of drug-polymer and cross linking agent

Table 5: Formulation of Metformin hydrochloride microspheres prepared by varying polymer concentration

Batch No.	Conc. of drug (%w/v)	Conc. of polymer (%w/v)	Conc. of $Cacl_2$ (%w/v)	Stirring time (min)	Stirring speed (rpm)	Volume of drug-polymer (ml)
1	2	2	2	30	100	10
2	2	2.5	2	30	100	10

Table 6: Formulation of Metformin hydrochloride microspheres prepared by varying drug concentration with 4% $Cacl_2$

Batch No.	Conc. of drug (%w/v)	Conc. of polymer (%w/v)	Conc. of $Cacl_2$ (%w/v)	Stirring time (min)	Stirring speed (rpm)	Volume of drug-polymer (ml)
3	0.5	2	4	45	200	10
4	1	2	4	45	200	10
5	1.5	2	4	45	200	10
6	2	2	4	45	200	10

Table 7: Formulation of Metformin hydrochloride microspheres prepared by varying drug concentration with 2.5% $Cacl_2$

Batch No.	Conc. of drug (%w/v)	Conc. of polymer (%w/v)	Conc. of $Cacl_2$ (%w/v)	Stirring time (min)	Stirring speed (rpm)	Volume of drug-polymer (ml)
7	0.5	2	2.5	30	100	10
8	1	2	2.5	30	100	10
9	1.5	2	2.5	30	100	10
10	2	2	2.5	30	100	10

- Drying time for all batches was 37ºC.

5.5 Evaluation of Metformin hydrochloride microspheres

All batches of Metformin hydrochloride microspheres were evaluated for the following properties:

1. Percentage yield
2. Equilibrium swelling studies
3. Average particle size
4. Encapsulation efficiency
5. In vitro release properties

5.5.1 Percentage yield

The prepared microspheres were collected, dried and weighed. The percentage yield was calculated as

$$\text{Percentage yield (w/w)} = \frac{\text{weight of dried microspheres recovered}}{\text{Weight of sodium alginate + weight of calcium chloride}} \times 100$$

Table 8: Percentage yield of different batches of Metformin hydrochloride microspheres

Batch No.	Yield (gm)	Percentage yield
1	0.320	22.8
2	0.268	18.4
3	0.356	15.8
4	0.335	14.5
5	0.333	14.1
6	0.331	13.7
7	0.384	25.6
8	0.402	25.9
9	0.389	24.3
10	0.393	23.8

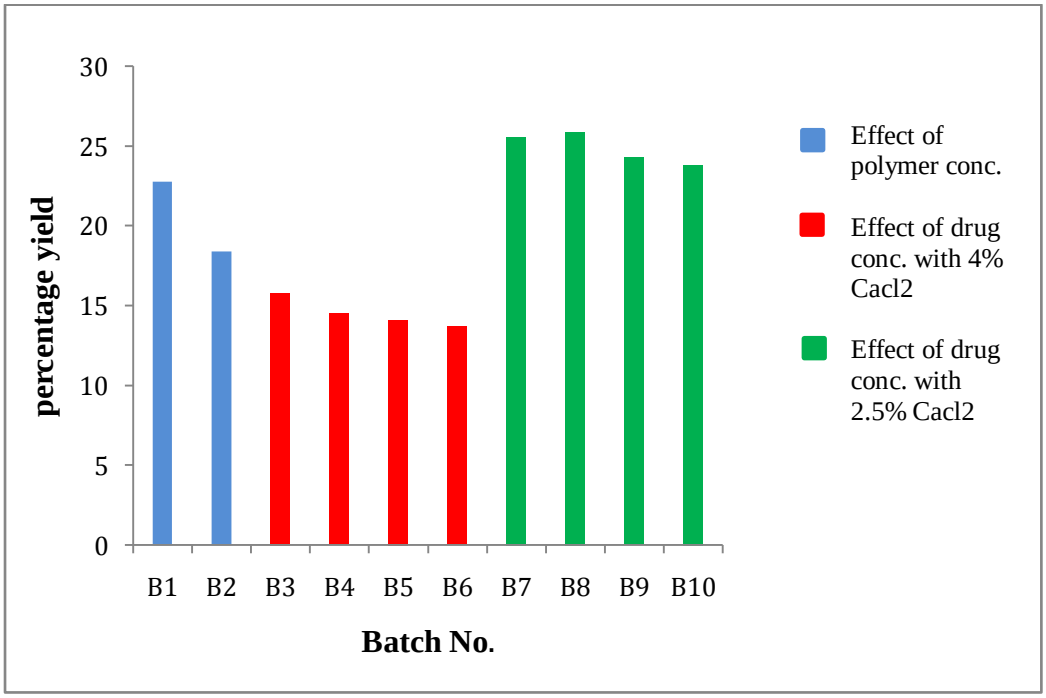
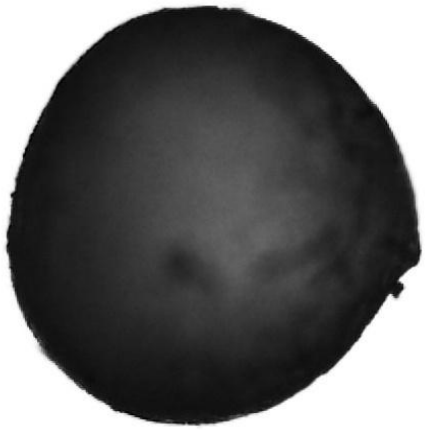


Figure 7: Histogram showing effect of various formulation parameters on Percentage yield of Metformin hydrochloride microspheres

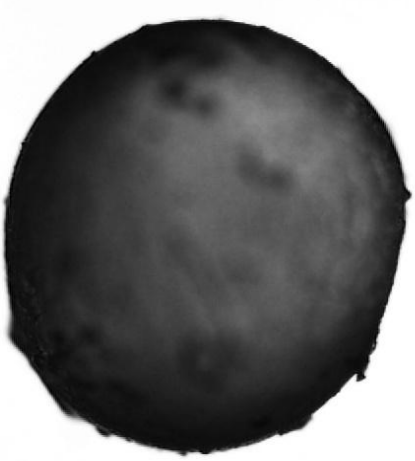
5.5.2 Particle size determination

All the microspheres were evaluated with respect to their size using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle size of more than 50 microspheres was measured randomly by optical microscope. The average particle size of microspheres was determined by the total size of the microspheres divided by the number of microspheres

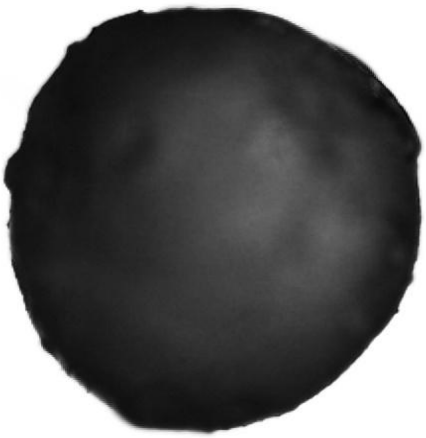
The morphological characteristics of Metformin hydrochloride microspheres were also observed under light microscope.



Unloaded microsphere

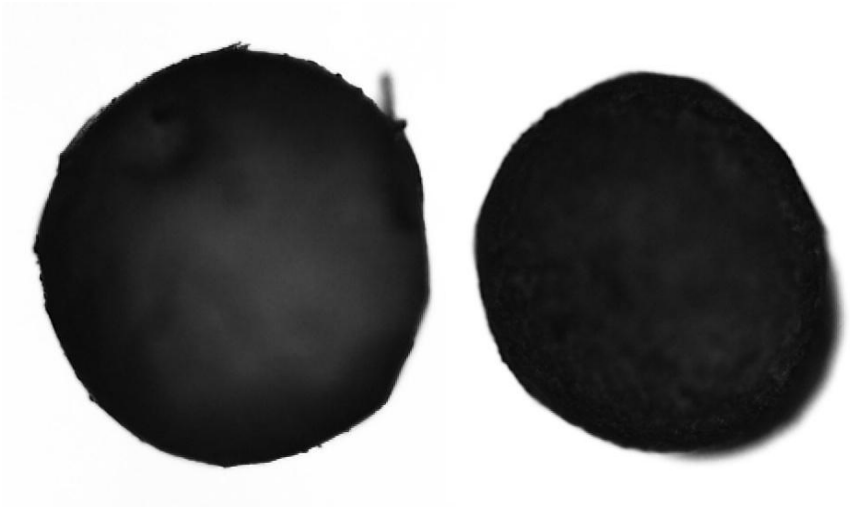


B1



B5

B6



B9 **B10**

Figure 8: Morphology of unloaded microsphere and loaded microspheres (B1, B5, B6, B9, and B10)

Table 9: Average size of different batches of Metformin hydrochloride microspheres

Batch No.	**Particle size (µm)**
1	961
2	980
3	953
4	942
5	933
6	916
7	937
8	909
9	841
10	812

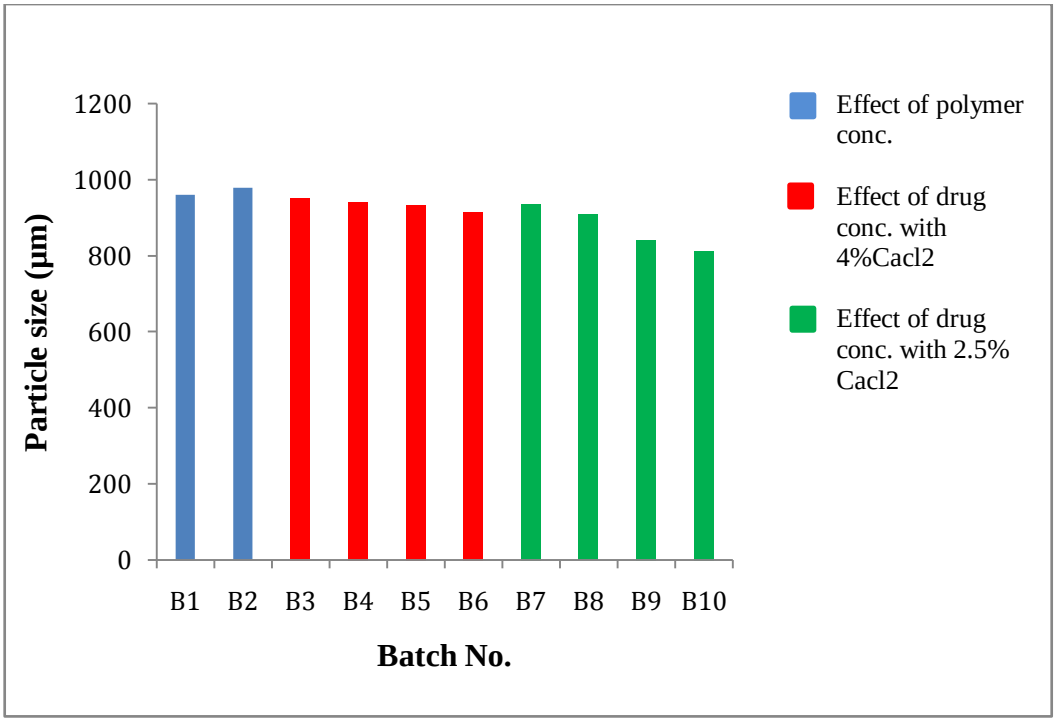


Figure 9: Histogram showing effect of various formulation parameters on average particle size of Metformin hydrochloride microspheres.

5.5.3 Equilibrium swelling studies

The swelling ability of the dried microspheres of Metformin hydrochloride was determined in hydrochloric acid buffer (pH 1.2). 5 mg of dried microspheres were immersed in 5 ml of hydrochloric acid buffer (pH 1.2) in a glass vial. These microspheres were kept at 37ºC for 24 hours. Swollen microspheres were filtered, blotted, and weighed immediately on an electronic balance. The percentage swelling of microspheres at equilibrium was calculated from formula given below:

$$\% E_{SW} = \frac{W_e - W_o}{W_o} \times 100$$

Where, E_{SW} is percent swelling of microspheres at equilibrium, W_o is initial weight of microspheres, and W_e is weight of microspheres at equilibrium.

Table 10: Percent swelling of different batches of Metformin hydrochloride microspheres

Batch No.	Swelling ratio (%)
1	66.4
2	76.6
3	67.4
4	77.4
5	106.8
6	108.8
7	116.2
8	133.1
9	137.4
10	145.2

Figure 10: Histogram showing effect of various formulation parameters on swelling ratio of Metformin hydrochloride microspheres

5.5.4 Determination of encapsulation efficiency

Drug entrapment efficiency of Metformin hydrochloride microspheres was performed by accurately weighing 50 mg of microspheres and crushing them properly in a glass mortar and pestle. These weighed microspheres were suspended in 50 ml of hydrochloric acid buffer (pH 1.2) and it was kept aside for 24 hours. Then, after suitable dilution, Metformin content in the filtrate was analyzed spectrophotometrically at 232 nm using U.V spectrophotometer.

$$\text{Percentage encapsulation efficiency} = \frac{\text{Estimated drug content}}{\text{Theoretical drug content}} \times 100$$

Table 11: Encapsulation efficiency of different batches of Metformin hydrochloride

Batch No.	**Drug encapsulation efficiency (%)**
1	11.4
2	9.7
3	21.3
4	30.1
5	21.7
6	33.4
7	22.4
8	25.3
9	32.2
10	48.9

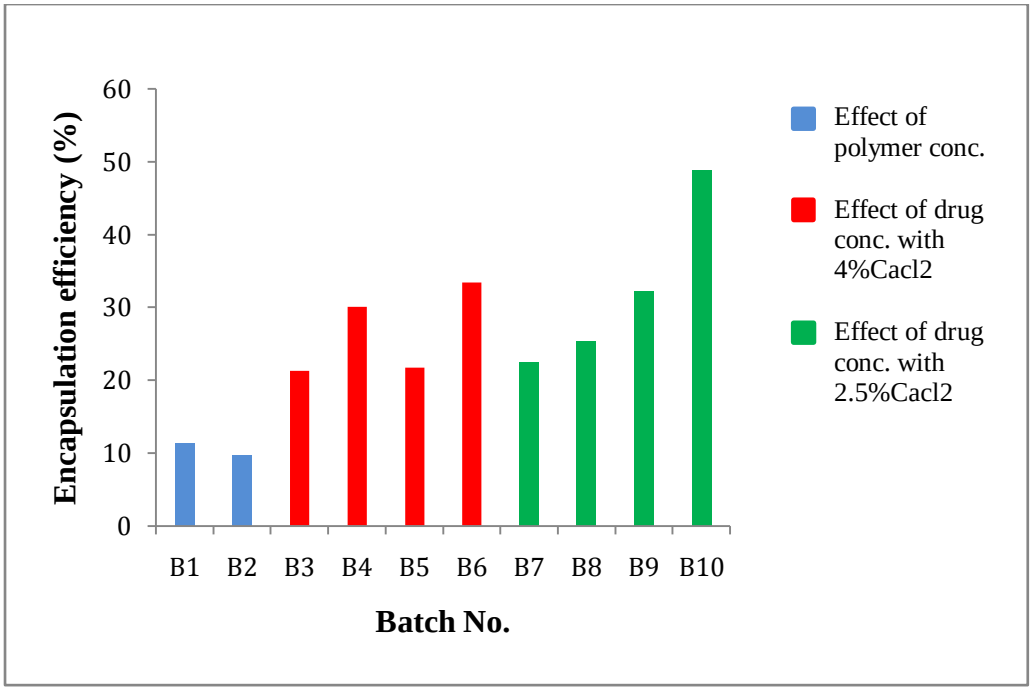


Figure 11: Histogram showing effect of various formulation parameters on encapsulation efficiency of Metformin hydrochloride microspheres.

5.5.5 Evaluation of in-vitro drug release

50 mg of Metformin hydrochloride microspheres were incubated in 50 ml buffer (pH1.2) in a 150 ml conical flask kept in a shaking incubator at 37ºC and at 50 rpm. After 4 hours microspheres were filtered and transferred into 50 ml buffer (pH 6.8) and incubated at 37ºC and at 50 rpm. Starting from time 0 hour and at desired intervals of time, 50ml sample was withdrawn and replaced with same amount of fresh medium. Drug in the release medium was measured spectrophotometerically. Release studies and release profile of Metformin hydrochloride microspheres of various batches are given below:

Table 12: Drug release data of Metformin hydrochloride microspheres by varying polymer concentration

S. No.	Time (hrs)	Cumulative percentage release	
		B1	**B2**
1	1	5.6	6.2
2	2	5.7	7.5
3	3	7.9	8
4	4	9.7	11.3
5	5	12.4	13.3
6	6	12.8	13.3
7	7	12.9	13.6
8	8	12.9	13.6
9	24	13.1	13.7

The drug release curves of Metformin hydrochloride microspheres by varying polymer concentration.

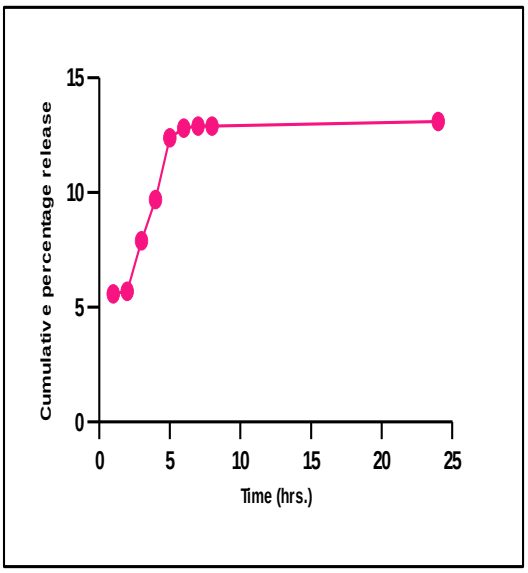


Figure 12: The drug release curve of B1

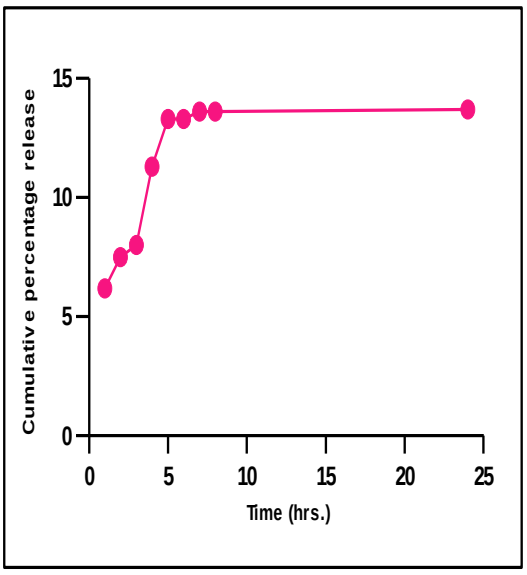


Figure 13: The drug release curve of B2

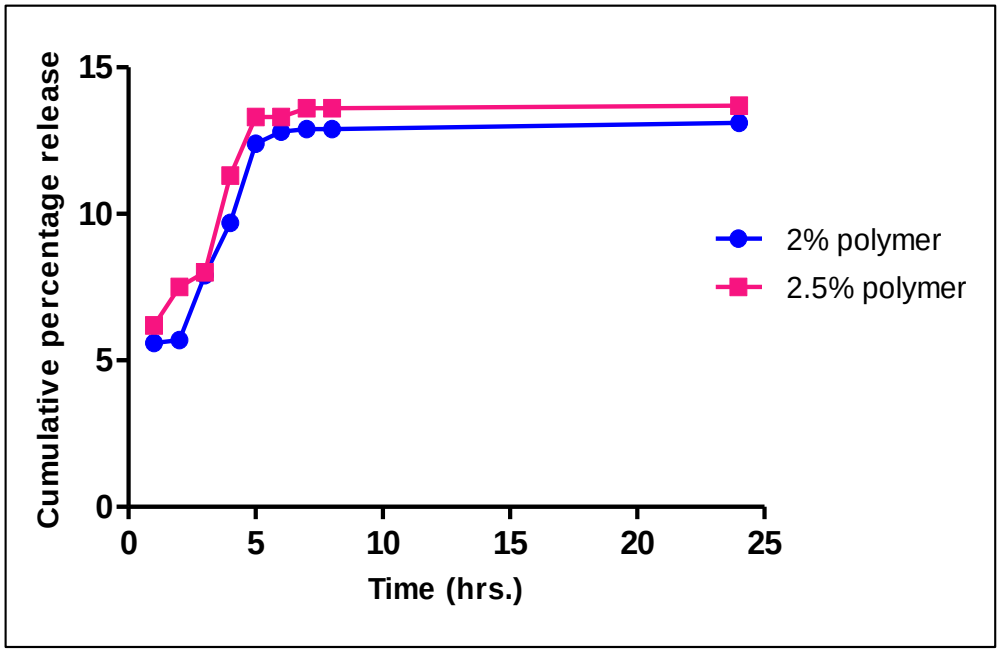


Figure 14: The Comparative drug release curves of B1-B2

Table 13: Drug release data of Metformin hydrochloride microspheres by varying drug concentration with 4%$Cacl_2$

S. No.	Time (hrs.)	Cumulative percentage release			
		B3	B4	B5	B6
1	1	10.2	28.8	31.1	26.7
2	2	20.3	34.4	34.6	27.7
3	3	46.1	34.5	36.7	29.7
4	4	46.2	37.7	40.0	29.9
5	5	47.7	72.1	71.3	56.6
6	6	47.8	73.2	75.7	56.6
7	7	47.8	73.2	75.8	56.7
8	8	47.8	73.4	75.8	56.9
9	24	47.9	73.5	75.9	56.9

The drug release curves of Metformin hydrochloride microspheres by varying drug concentration with 4% $Cacl_2$.

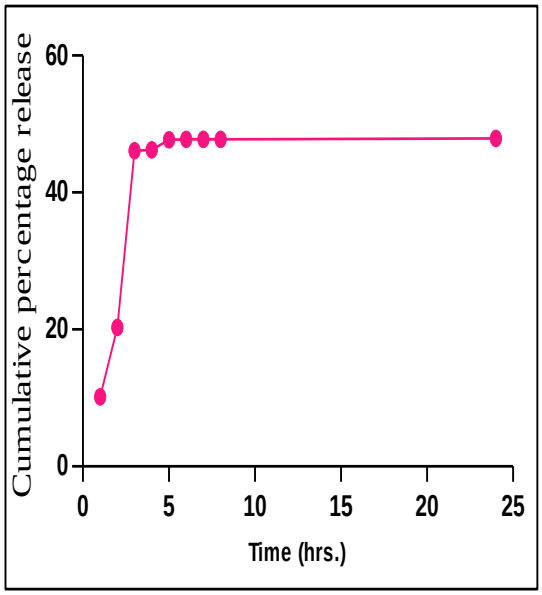


Figure 15: The drug release curve of B3

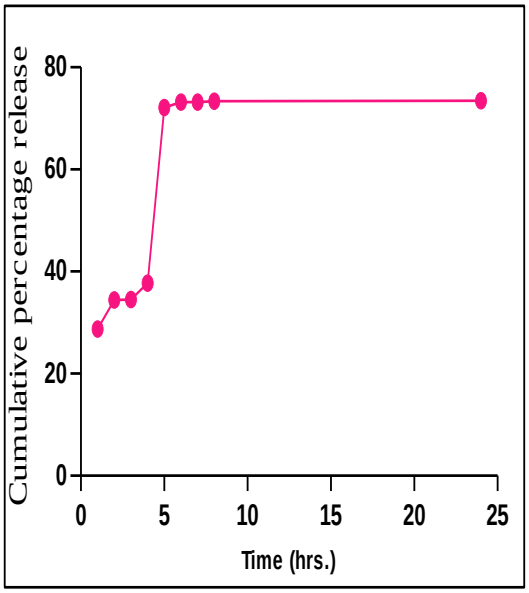


Figure 16: The drug release curve of B4

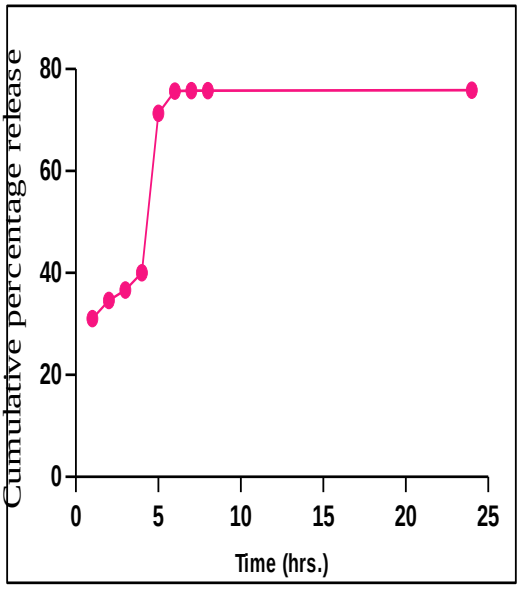


Figure 17: The drug release curve of B5

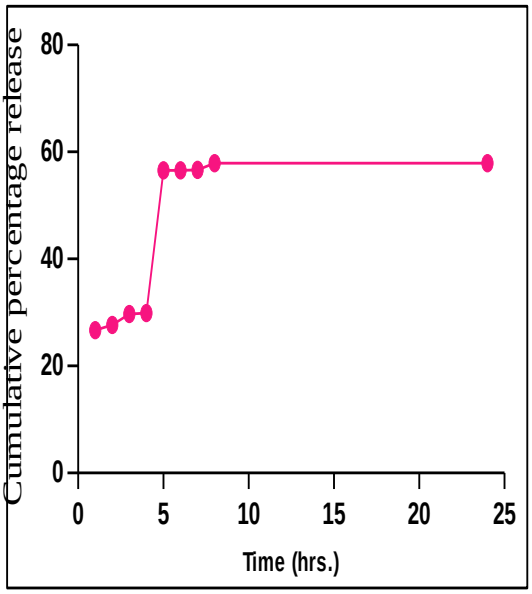


Figure 18: The drug release curve of B6

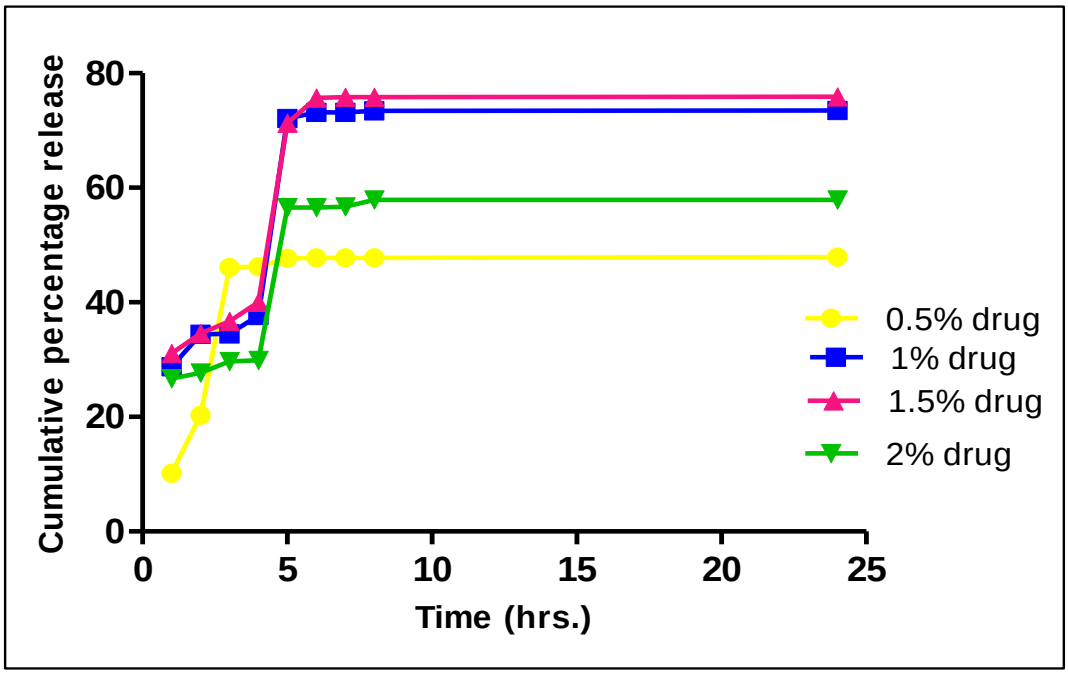


Figure 19: The comparative drug release curves of B3-B6

Table 14: Drug release of Metformin hydrochloride microspheres by varying drug concentration with 2.5%$Cacl_2$

S. No.	Time (hrs.)	Cumulative percentage release			
		B7	B8	B9	B10
1	1	18.8	14.2	16.8	20.8
2	2	22.9	15.1	28.0	28.7
3	3	29.0	24.4	28.1	39.1
4	4	33.6	27.3	29.1	42.3
5	5	44.7	39.5	39.3	72.4
6	6	45.5	39.6	39.4	72.7
7	7	45.5	39.6	39.8	77.9
8	8	45.6	39.9	40.2	78.2
9	24	46	40	40.4	78.2

The drug release curves of Metformin hydrochloride microspheres by varying drug concentration with 2.5% $Cacl_2$.

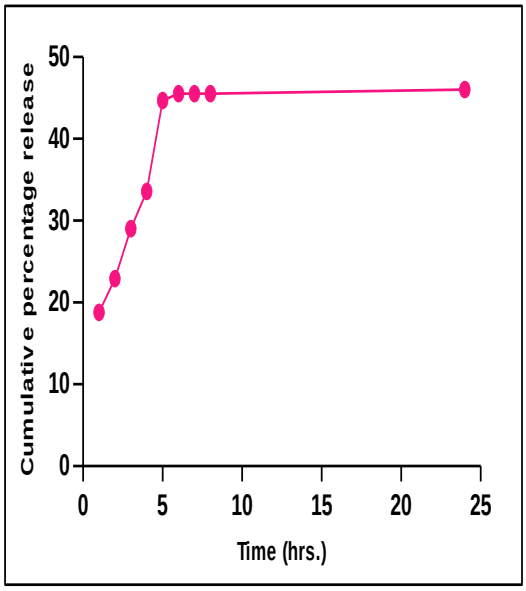


Figure 20: The drug release curve of B7

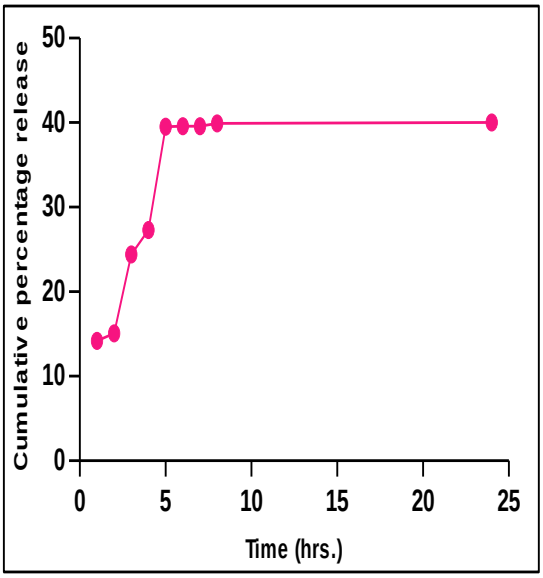


Figure 21: The drug release curve of B8

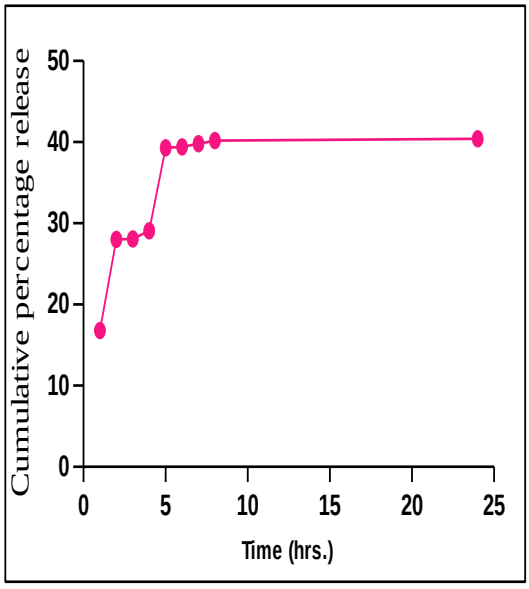


Figure 22: The drug release curve of B9

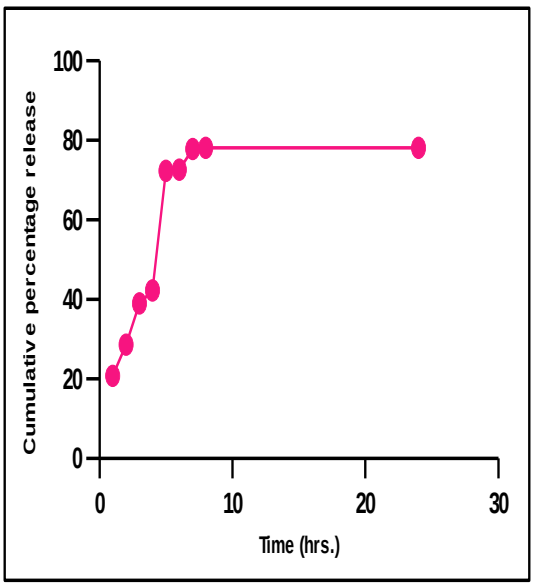


Figure 23: The drug release curve of B10

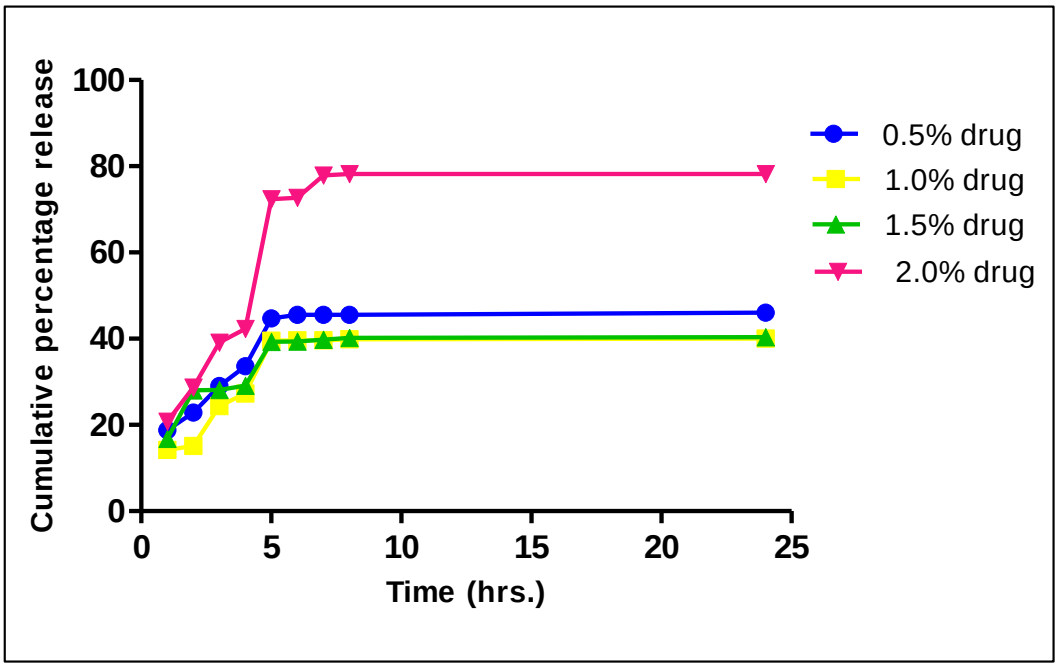


Figure 24: The comparative drug release curves of B7-B10

CHAPTER 6

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

There are many methods reported in literature for the preparation of microspheres for achieving controlled drug delivery. Some of the important methods are emulsion cross-linking, chemical cross-linking, coacervation, spray drying, precipitation and ionotropic gelation. Some of these methods use organic solvents and chemicals, whose residual amount in the microspheres may cause undesirable effects like irritation to mucosal membranes. The ionotropic gelation method is found to be suitable for the preparation of Metformin hydrochloride microspheres of sodium alginate. By this method, the microspheres could be easily prepared using simple instruments and the drug could be entrapped within microspheres in a completely aqueous environment.

The objective of this study was to investigate the effect of parameters- drug concentration, polymer concentration, cross linking agent concentration, stirring time and stirring speed. The Metformin hydrochloride microspheres thus prepared by varying the formulation parameters were studied for percentage yield, average particle size, swelling studies, drug encapsulation efficiency and in vitro drug release rates.

Table 5-7 indicates batches that were prepared by dropping sodium alginate-drug solution into $Cacl_2$ solution by varying polymer concentration, drug concentration with 4% $Cacl_2$ and 2.5% $Cacl_2$ respectively.

Percentage yield of different batches of Metformin hydrochloride microspheres are listed in table 8. It varies from 13.7% to 25.9%. The comparative study of percentage yield is shown by histogram in figure 7. Maximum percentage yield was obtained from batch 8 having 1% drug, 2% polymer and 2.5% $Cacl_2$ prepared at 100 rpm for 30 minutes. Percentage yield decreases with increase in polymer concentration and drug concentration. Percentage yield increases with decrease in $Cacl_2$ concentration.

The average particle size of the various batches of Metformin hydrochloride microspheres is listed in table 9. It varies from 812µm-980µm. The comparative study of the average particle size is shown by histogram in figure 8. Minimum size was obtained from batch 10 having 2% polymer, 2% drug and 2.5% $Cacl_2$ prepared at 100 rpm for 30 minutes. Particle size increases with increase in the polymer concentration due to increase in viscosity of the polymer solution which increases the droplets size. When $Cacl_2$ concentration, stirring speed and

stirring time is increased, the particle size also increases. Particle size decreases with increase in the concentration of drug.

The morphology of Metformin hydrochloride microspheres is determined under light microscope. The microspheres are found to be spherical in shape. The outer dark area is sodium alginate which encapsulates the Metformin hydrochloride drug in the centre of the microsphere. The photos of unloaded and after loading drug microspheres is shown in fig. 8.

The swelling study data of the various batches of Metformin hydrochloride microspheres is listed in table 10. The swelling ratio varies from 66.4% to 145.2%. The comparative study of the swelling ratio is shown by histogram in figure 9. Maximum swelling ratio was obtained from batch 10 having 2% polymer, 2% drug and 2.5% $Cacl_2$ prepared at 100 rpm for 30 minutes. Swelling ratio increases with increase in the polymer concentration and drug concentration. Swelling ratio decreases with increase in the concentration of $Cacl_2$, stirring time and stirring speed. These swelling studies were done in hydrochloric acid buffer (pH 1.2). Metformin hydrochloride microspheres disintegrated in phosphate buffer (pH 6.8) after 30 minutes, these results suggest that sodium alginate microspheres do not disintegrate in the stomach, and thus result in delayed release of Metformin hydrochloride in simulated intestinal fluids.

The encapsulation efficiency of drug is expressed as the amount of total available drug that is actually entrapped in the microspheres. The encapsulation efficiency of the various batches of Metformin hydrochloride microspheres is listed in table 11. The encapsulation efficiency of drug obtained from Metformin hydrochloride microspheres is from 9.7% to 48.9%. The comparative study of the encapsulation efficiency is shown by histogram in figure 10. Maximum encapsulation efficiency was obtained from batch 10 having 2% drug, 2% polymer and 2.5% $Cacl_2$ prepared at 100 rpm for 30 minutes. Encapsulation efficiency increases with increase in the drug concentration. The encapsulation efficiency decreases with increase in $Cacl_2$ concentration, stirring time and stirring speed. The reason for low entrapment efficiency may be high solubility of drug. The drug encapsulation efficiency also decreases with increase in the polymer concentration due to its higher viscosity which affects the diffusion coefficient of drug.

The in vitro release studies were performed in buffer (pH 1.2) and subsequently in buffer (pH 6.8) close to the physiologically gastrointestinal conditions. The dissolution behavior of the Metformin hydrochloride microspheres was dependent on pH. The microspheres swelled in

buffer (pH 1.2) while they disintegrated in buffer (pH 6.8). An initial burst release of drug was observed from all batches that can be attributed to two reasons: the leaching of drug on the bead outer surfaces and faster ingress of dissolution medium and subsequent diffusion of drug. However on changing pH from lower to higher level, drug release slowed. The pH responsive release can be explained based on the charge density of beads, which is an important factor in electrostatic interaction and depends on solution pH. The in-vitro drug release of different batches of Metformin hydrochloride microspheres are listed in table 12, 13, and 14. The comparative study of drug release curves are shown in figure 22, 23 and 24. Maximum drug release profile 78.2% is obtained from batch 10 having 2% polymer, 2% drug and 2.5% $Cacl_2$ prepared at 100 rpm for 30 minutes. With increase in the concentration of sodium alginate and drug, the drug release rate also increases. In vitro drug release rate is also dependent upon the swelling study and encapsulation efficiency of drug; if swelling ratio and encapsulation efficiency of drug is high then drug release rate is also high.

Metformin hydrochloride microspheres with more than 2.5% sodium alginate concentration could not be prepared as it became difficult to drop sodium alginate solution through syringe needle due to increase in the viscosity of the solution.

Metformin hydrochloride microspheres were also prepared with 2% and 3% $Cacl_2$ concentration, 15 minutes stirring time and 300 rpm stirring speed but their encapsulation efficiency was very less.

CHAPTER 7

CONCLUSION

CONCLUSION

Metformin hydrochloride microspheres have shown to be prospective drug delivery carriers as they offer many advantages. First, sodium alginate is considered as a safe material as it is natural polymer that possesses biocompatible and biodegradable properties. Second, it is a water-soluble polymer which has an ideal property as a drug carrier; therefore, simple and mild preparation methods can be applied. This renders sodium alginate microspheres as promising drug delivery carriers that are suitable for a broad category of drugs including macromolecules and labile drugs.

Based on percentage yield, particle size, swelling studies, encapsulation efficiency and in vitro drug release, it was observed that batch 10 prepared at 2% drug, 2% polymer, 2.5% $Cacl_2$ concentration , stirring time of 30 minutes and stirring speed of 100 rpm is the best of all the batches that were studied. It has 23.8% percentage yield, 812 µm particle size, 145.2% swelling ratio, 48.9% encapsulation efficiency and cumulative percentage drug release of 78.2%. The morphology of batch 10 is also good as compared to other batches.

It may be concluded at the end that ionotropic gelation method may be widely used to encapsulate a broad range of drugs to achieve controlled delivery of drugs. It has the advantage that it is a mild, effective method where harsh chemicals are not utilized.

CHAPTER 8

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