Release characteristics of ascorbic acid encapsulated chitosan treated alginate microspheres.

A Dissertation submitted in partial fulfillment of the requirements for the award of degree of

Masters of Science
in
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Submitted by:

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Certificate

This is to certify that the dissertation entitled "**Release characteristics of ascorbic acid encapsulated chitosan treated alginate microspheres"** submitted by Randeep Kaur in partial fulfillment of the requirement for the award of degree of Master of Science in Biotechnolgy to Thapar University, Patiala, is a record of students 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 "**Release characteristics of ascorbic acid encapsulated chitosan treated alginate microspheres**" in partial fulfillment of the requirement for the award of degree of Masters of Science in Biotechnology is an authentic record of my own work during a period of six months January 2015 to June 2015, under the guidance of Mrs. M. Vasundhara, Assistant professor, Department of Biotechnology, 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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Abstract

Novel drug delivery systems have several advantages over conventional multi dose therapy. Much research effort in developing novel drug delivery system has been focused on controlled release and sustained release dosage forms. Now considerable efforts are being made to deliver the drug in such a manner so as to get optimum benefits. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs.

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. Chitosan and sodium alginate as a natural biodegradable polymer provides good barrier to the core material from the surrounding environment as well as very effective in controlled drug delivery system. It also helped in retaining L-ascorbic acid for longer duration in human body.

Ionotropic gelation method is an effective method where harsh and harmful chemicals are not used and hence safer for human consumption. The control of various manufacturing parameters plays a very important role in obtaining microspheres of good sphericity, high yield and high drug encapsulation.

L-ascorbic acid microspheres were prepared by dropping the drug containing solution of sodium alginate. The droplets instantaneously formed gelled spheres by the ionotropic gelation method. 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 B2 and B9 were the best having concentration of sodium alginate 2% at 100 rpm for 30 minutes. Entrapment efficiency and in vitro release was better observed in chitosan coated microspheres as shown in batch no. 9 and batch no. 4 with equal concentration but batch no. 9 was chitosan coated and batch no. 4 was sodium alginate microspheres.

Chapter 1

Introduction

Introduction

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.

Natural or synthetic substance which (when taken into a living body) affects its functioning or structure, and is used in the diagnosis, mitigation treatment, or prevention of a disease or relief of discomfort also called as legal drug or medicine. A medicinal drug (such as amphetamines), however, can be harmful and addictive if misused.

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology. (Aruna Rastogi et al, 2010)
Novel drug delivery systems have several advantages over conventional multi dose therapy. Much research effort in developing novel drug delivery system has been focused on controlled release and sustained release dosage forms. Now considerable efforts are being made to deliver the drug in such a manner so as to get optimum benefits.

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 system as shown in figure 1.

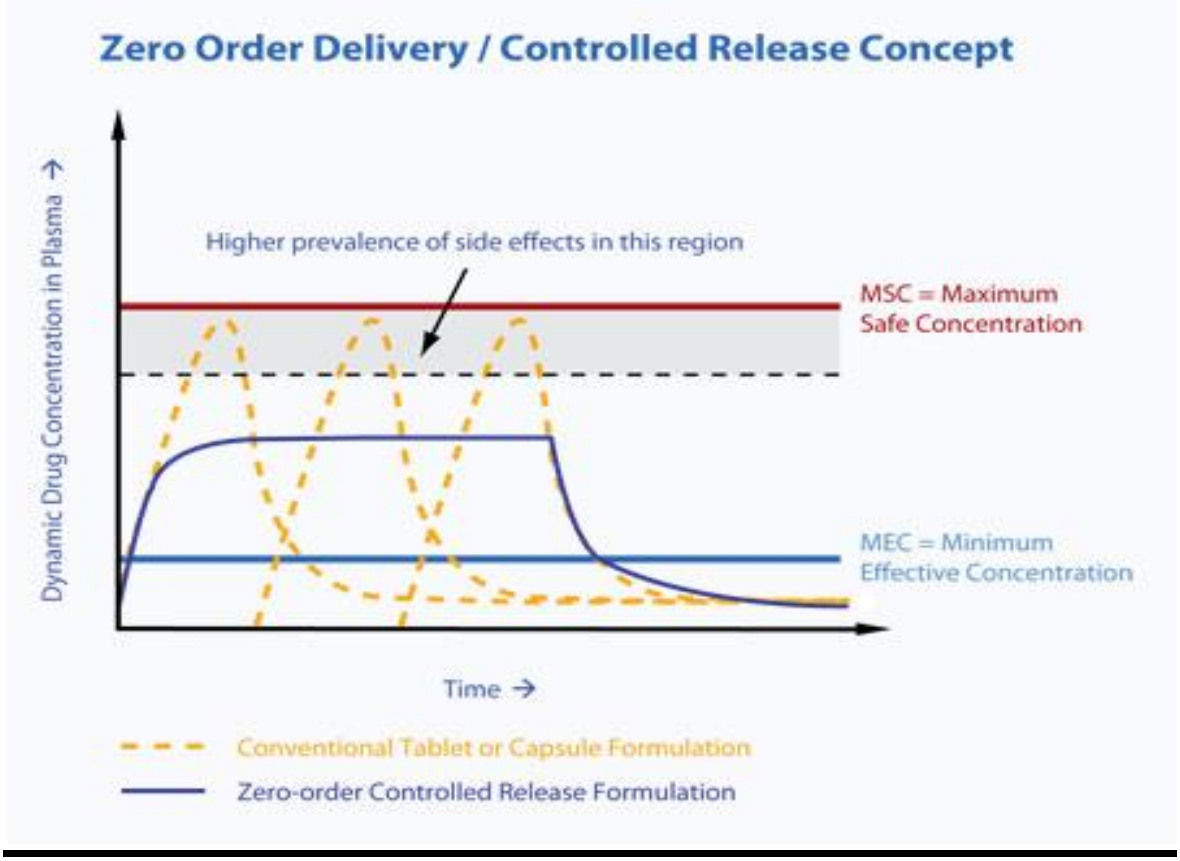


Figure 1: Drug level in the blood with

(a) Traditional drug dosing

(b) Controlled-delivery dosing

According to P.Venkatesan *et al* (2009) , advantages of controlled drug delivery system 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 some drugs like steroids, narcotics, anti-cancerous.

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).

There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs.

Microspheres

Microspheres are characteristically free flowing spherical particles consisting of drug within the polymer matrix, which are biodegradable in nature and ideally having a particle size range of 1 to 2000 μm. Microspheres can encapsulate many types of drugs including small molecules, proteins, and nucleic acids and are easily administered through a syringe needle. They are generally biocompatible, can provide high bioavailability, and are capable of sustained release for long periods of time. These carriers received much attention not only for prolonged release but also for the targeting anti-cancer drugs to the tumour. (Kataria Sahil *et al*, 2011)

Advantages of microspheres

According to Sahil Kataria et al (2011)

1. Microspheres provide constant and prolonged therapeutic effect.
2. Reduces the dosing frequency and thereby improve the patient compliance.
3. They could be injected into the body due to the spherical shape and smaller size.
4. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
5. Microsphere morphology allows a controllable variability in degradation and drug release.
6. They facilitate accurate delivery of small quantities of potent drug and reduce concentration of drug at site other than the target organ or tissue.
7. They provide protection for unstable drug before and after administration, prior to their availability at the site of action.

8. They provide the ability to manipulate the *in vivo* action of the drug, pharmacokinetic profile, tissue distribution and cellular interaction of the drug.
9. They enable controlled release of drug.Examples: narcotic, anticancerous, steroid hormones.

Limitations of Microspheres

Some of the disadvantages were found to be as follows

1. The modified release from the formulations.
2. The release rate of the controlled release dosage form may vary from a variety of factors like food and the rate of transit though gut.
3. Differences in the release rate from one dose to another.
4. Controlled release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form may lead to potential toxicity.
5. Dosage forms of this kind should not be crushed or chewed.

Chapter 2

Literature Review

Literature Review

Sezer D. And Akbuga J., (1999) studied release characteristics of chitosan treated alginate beads. Ionotropic gelation method was used in preparation of beads and effect of various factors like alginate, chitosan, drug, calcium chloride were studied.

Mofidi N., Aghai-Moghadam M., Sarbolouki M N., (1999), investigated easy method for the mass preparation of the alginate microspheres of controlled size with desirable properties (nonsticky, heat stable, sterilizable and driable). Water content, average diameter, size distribution, average pore size, and pore population density of the gel microspheres are reported.

Takka S. And Acarturk F., (1999) studied about calcium alginate microparticles for oral administration. Its effect on sodium alginate type on drug release and drug entrapment efficiency. The natural polymers alginate and chitosan were used for the preparation of controlled release nicardipine HCl gel microparticles.

Ko J. A. *et al*, (2002) formulated and optimized chitosan microparticles with tripolyphosphate (TPP) by ionic crosslinking. The particle sizes of TPP chitosan microparticles were in range from 500 to 710 µm and encapsulation efficiencies of drug were more than 90%. The morphologies of TPP-chitosan microparticles were examined with scanning electron microscopy. The release of drug from TPP-chitosan microparticles decreased when cross-linking time increased. These results indicate that TPP-chitosan microparticles may become a potential delivery system to control the release of drug.

Naidu K. A. *et al*, (2003) studied importance of ascorbic acid and it is essential for collagen, carnitine and neurotransmitters biosynthesis. For health benefits routine drug allowance should be between 100-120 mg/day.

Sinha V. R. *et al*. (2004) reported that chitosan microspheres as a potential carrier for drugs as well as studied its biodegradable, properties and applications, encapsulation of drugs, factors affecting encapsulation efficiency and release kinetics.

Desai KG , Liu C, Park HJ., (2005) reported the properties of vitamin C encapsulated sodium alginate beads prepared by alternative encapsulation process. It mainly involves immobilization of vitamin C in hydrated zinc oxide layers and encapsulation of prepared immobilized particles in sodium alginate bead. Morphology was observed by scanning

electron microscopy (SEM) when vitamin C immobilized particles are encapsulated in sodium alginate bead.

Deshmukh V N., *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 concentrations 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.

Aysu Yurdasiper and Ferhan Sevgi, (2010) reviewed about different formulation types of microparticulate systems such as beads, microbeads, microspheres and microsponges using special attention to chitosan, alginate and eudragit RS100. They have excellent potential for pharmaceutical and biopharmaceutical applications This review also includes non steoridal anti-inflammatory drug (NSAID) microparticle formulations which have been prepared with these polymers to minimize side effects and to obtain controlled release drug delivery systems.

Hammad Umer *et al.* (2011) studied process of microencapsulation techniques, factors influencing encapsulation efficiency. Reviewed advantages and applications of microspheres and also developed a carrier system for site-specific DDS.

Kataria Sahil *et al* (2011) reported that microspheres are characteristically free flowing powders containing proteins or synthetic polymers with a particle size ranging from 1-1000 μm. It is a reliable means to deliver the drug to the target site with specificity and to maintain the desired concentration at the site of interest.

Yueling Zhang *et al* (2011) studied about preparation and evaluation of alginate–chitosan microspheres for oral delivery of insulin. The alginate–chitosan microspheres were prepared by membrane emulsification technique in combination with ion (Ca^{2+}) and polymer (chitosan) solidification. Therefore, the alginate–chitosan microspheres were found to be promising vectors showing a good efficiency in oral administration of protein or peptide drugs.

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.

Mullaicharam A. R., *et al*, (2013) prepared ascorbic acid loaded microspheres by phase separation coacervation technique. The aim of this work was to investigate the micromeritic properties for prepared microspheres and incorporate the results with the uniformity of size and shape of microspheres and also its flow property.

Nirmala Devi and Dilip Kumar Kakati (2013) studied about porous microparticles of different sizes by polyelectrolyte complexation of biopolymers gelatine A and sodium alginate for microencapsulation of food bioactives. The surface morphology and sizes of the microparticles were investigated by scanning electron microscope (SEM). Fourier transform infrared spectroscopy (FTIR) study indicated the formation of polyelectrolyte complex between gelatine and sodium alginate and successful encapsulation of ascorbic acid into the microparticles.

Thangaraj S *et al* (2014) developed microcapsules for vitamin C by extrusion process. The aim of this work was to encapsulate vitamin C with sodium alginate and calcium chloride and to find out encapsulation efficiency of microspheres formed. Encapsulation efficiency was noticed higher in 100mg of Vitamin C beads. The average efficiency rate is 74%.

Soni M L *et al* reported sodium alginate microspheres for extending drug release: formulation and *in vitro* evaluation of spherical microspheres of theophylline (TP) using sodium alginate as the hydrophilic carrier were prepared to prolong the release.

Aysu Yurdasiper *et al* (2010) reported that sulindac loaded alginate beads were prepared by ionotropic gelation method for a mucoprotective and controlled drug release. Sulindac beads were investigated in vitro for a possible sustained drug release and their use in vivo as a gastroprotective system. Sulindac loaded alginate beads led to a significant reduction of

macroscopic histological damage in the stomach and duodenum in mice. Also microscopic analyses of the mucosal damage demonstrated a significant muco protective effect of all beads formulation compared to the free drug.

Dewi Melani Hariyadi *et al* (2014) studied about effect of cross linking agent and polymer on the characteristics of Ovalbumin loaded alginate microspheres. Ovalbumin-loaded alginate microspheres were successfully produced by aerosolisation with maximum encapsulation efficiency and loadings of about 89%. Smooth and spherical microspheres were shown for both alginate microspheres produced using Ca^{2+} and Ba^{2+} of the aerosolisation method with average sizes from 12 to 30μm. Size of bigger microspheres was produced of around 1-3 mm. This result suggested that variation in cross linking agent and polymer concentration were important for sustained release characteristics of ovalbumin-loaded alginate microspheres.

Hire N.N , Dr. Derle D.V (2014) developed various types of microspheres such as bio-adhesive, magnetic, floating, radioactive and polymeric microspheres for various purposes. Microspheres occupied a central place in novel drug delivery. There are various departments of medicine like cancer, pulmonary, cardiology, radiology, gynaecology, and oncology etc where numerous drugs are used and they are delivered by various types of drug delivery system. The purpose of the review is to compile various types of microspheres, different methods to preparation, its applications and also various parameters to evaluate their efficiency.

Chapter 3

Objective of study

Objective of Study

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

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

Chapter 4

Research Envisaged

Research Envisaged

Drug delivery systems have many advantages over conventional multi dose therapy. Now considerable efforts are being made to deliver the drug in such a manner so as to get optimum benefits. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs, (P.Venkatesan et al. , 2009)

Microencapsulation is the creation of a barrier to avoid chemical reactions and to enable the controlled release of the ingredients (Vilstrup, 2001).

Microencapsulation system offers potential advantages over conventional drug delivery systems and also established as unique carrier systems for many pharmaceuticals (sustained drug delivery systems). Although significant advances have been made in the field of microencapsulation, still many challenges need to be rectified during the appropriate selection of core materials, coating materials and process techniques.

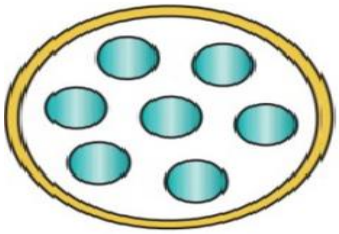
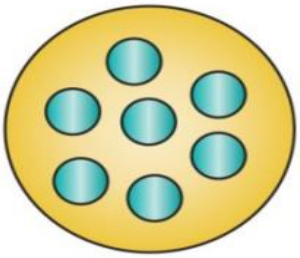
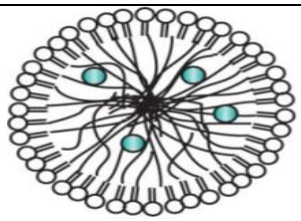
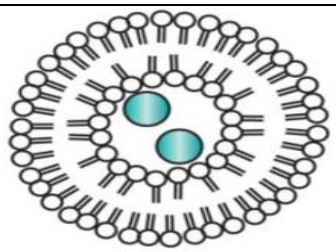
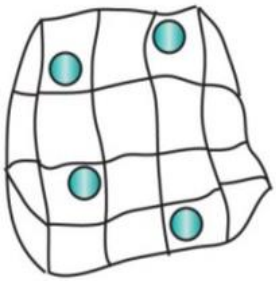
Generally multiparticulate drug delivery systems are intended for oral, parentral and topical formulations and approaches include formulations in the form of pellets, granules, beads, gelispheres, microcapsules, microspheres, lipospheres, microparticles and nanoparticles. In these systems, the dosage of the drug substances is divided on a plurality of subunit, typically consisting of thousands of spherical particles with selective diameter range.

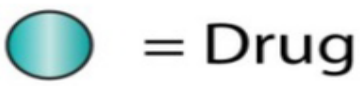
Microsphere is a spherical shell that is usually made up of a biodegradable or resorbable plastic polymer, that has a very small diameter usually in the micron or nanometer range, and that is often filled with a substance (as a drug or antibody) for release as the shell is degraded.

Drug loaded microspheres are drug delivery systems designed to provide a therapeutic agent in the needed amount, at the right time, to the proper location in the body, in a manner that optimizes efficacy, increases compliance and minimizes side effects. Such systems offer several potential advantages over traditional methods of administration (Kyekyoon “Kevin” Kim and Daniel W. Pack)

Biodegradable polymer microspheres are one of the most common types and hold several advantages. Microspheres can encapsulate many types of drugs including small molecules, proteins, and nucleic acids and are easily administered through a syringe needle. They are generally biocompatible, can provide high bioavailability, and are capable of sustained release for long periods of time.

Table: 1 Microencapsulation Classification

Terminology	Description	Size	Diagrams
Microcapsules	Products of coating liquid nuclei with solid walls.	µm	
Microspheres or Microparticles	The cores and walls are both solid. Often, there is no clear distinction between them: the thick solid wall functions as a porous matrix where active substances are embedded.	µm	
Micelles	an aggregate of surfactant molecules dispersed in a liquid colloid, with the hydrophilic "head" regions in contact with surrounding solvent	nm	
Liposomes	Lipid wall, often made of Phospholipids and cholesterol. Subtypes: unilamellar (one lipid layer) and multilamellar (several lipid layers).	µm to nm	
Hydrogels	A network of polymer chains that are hydrophilic, sometimes found as a colloidal gel in which water is the dispersion medium.	µm	



****** Jyothi Sri.S, A.Seethadevi, K.Suria Prabha, P.Muthuprasanna and ,P.Pavitra (2012)

Ascorbic acid (also called as vitamin C) is a naturally occurring organic compound with antioxidant properties. It is a white solid but impure samples can appear yellowish. The name is derived from *a-* (meaning "no") and *scorbutus* (scurvy), the disease caused by a deficiency of vitamin C. Being derived from glucose, many animals are able to produce it, but humans require it as a nutritional supplement**.** Ascorbic acid is one of the important water soluble vitamins. It is essential for collagen, carnitine and neurotransmitters biosynthesis. Most plants and animals synthesize ascorbic acid for their own requirement. However, apes and humans cannot synthesize ascorbic acid due to lack of an enzyme **gulonolactone oxidase**. Hence, ascorbic acid has to be supplemented mainly through fruits, vegetables and tablets. The current US recommended daily allowance (RDA) for ascorbic acid ranges between 100–120 mg/per day for adults. Many health benefits have been attributed to ascorbic acid such as antioxidant, anti atherogenic, anti-carcinogenic, immunomodulator and prevents cold etc. (Higdon J, 2006)

- The chemical formula - (R)-5-((S)-1,2-dihydroxyethyl)-3,4-dihydroxyfuran-2(5H)-one.
- Molar mass- 176.12 g mol-1
- Density 1.65 g/cm3
- Melting point 190-192 °C
- Solubility in water about 33 g/100 mL.

It is a water-soluble vitamin which can be found in many biological systems and foodstuffs (fresh vegetables and fruits, namely, citrus). Ascorbic acid plays an important role in collagen biosynthesis, iron absorption, and immune response activation and is involved in wound healing and osteogenesis. It also acts as a powerful antioxidant which fights against free-radical induced diseases.

Vitamin C is one of the most important antioxidants that may reduce the risk of cancer using various mechanisms (Esposito et al., 2002; Jacobs et al., 2001). However, environmental factors, such as temperature, pH value, oxygen, metal ion, UV and X-ray affect the stability of ascorbic acid (Alishahi et al., 2011; Kirby et al., 1991; Liao and Seib, 1988; Uddin et al., 2001).

Ascorbic acid is a mild reducing agent. For this reason, it degrades upon exposure to oxygen, especially in the presence of metal ions and light. It can be oxidized by one electron to a radical state or doubly oxidized to the stable form called dehydroascorbic acid.

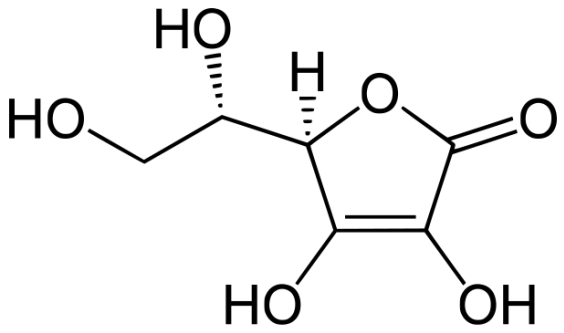


Figure 2: Structure of L-Ascorbic acid

Pharmacokinetics and Pharmacodynamics of L-ascorbic acid

Pharmacokinetics is what the body does to the drug. Pharmacodynamics is how the drug effects the body and its target organs, biological functions, chemical processes and/or microbes and parasites. Ascorbic acid Pharmacokinetics deals with how it is administered effectively in what form, amounts and frequencies. Ascorbic acid is in foods, more in certain sources. Ascorbic acid is supplemented by oral uptake in the gut, by injection and via IV infusions directly into the blood. Oral intake has many forms (ascorbic acid, sodium ascorbate, calcium ascorbate, magnesium ascorbate, and ascorbyl palmitate), the most effective gut-to-blood uptake is liposomal ascorbic acid where ~95% is transferred vs. less than 15% for water-soluble forms of ascorbic acid. Pharmacokinetics deals with rates of change in concentration: how & where & how much & how fast it is distributed, stored, converted, metabolized and eliminated.

Ascorbic acid pharmacodynamics deals with how it acts, where it acts, and when. It acts, taking an active part of the body's functioning biochemistry. It performs protective functions, blocks enzymes, disables a wide range of toxins, inhibits cortisone-related degeneration, restores immune system activity, and acts as an antibiotic in sufficient quantities. (Jorge Duconge., Jorge R. Miranda-Massari., 2008).

Pharmacokinetics of L-ascorbic acid

Absorption : Absorbed almost completely from distal small intestine.

Distribution: Distributed throughout water-soluble compartments. Adrenal cortex, leukocytes, platelets, and pituitary gland contain high concentrations.

Elimination: Excreted in the urine.

Functions of Vitamin C

- Metabolic reactions in human body require vitamin C as a cofactor, such as the synthesis of epinephrine from tyrosine.
- vitamin C involvement is suspected in the process of adrenal steroidogenesis.
- Biochemical roles of ascorbic acid are in thyroxine synthesis, amino acid metabolism and aiding in the absorption of iron.
- Vitamin C can quench aqueous reactive oxygen species, plays an important role in antioxidant defense system and immunocompetence, and in strengthening resistance to infection.
- In addition, vitamin C protects against deoxyribonucleic acid (DNA) mutations and, therefore, clinical value in the treatment of certain types of cancer and other diseases.
- Vitamin C is required for the synthesis of collagen, an important structural component of blood vessels, tendons, ligaments, and bone.
- Ascorbic acid also plays an important role in the synthesis of norepinephrine, a neurotransmitter critical to brain function.
- In addition, vitamin C is required for the synthesis of carnitine, a small molecule that is essential for the transport of fat to mitochondria, where it is oxidized and converted to ATP.
- Recent research also suggests that vitamin C is involved in the metabolism of cholesterol to bile acids, which may have implications for blood cholesterol levels and the incidence of gallstones.
- Ascorbic acid is also a highly effective antioxidant. Even in small amounts, vitamin C can protect indispensable molecules in the body, such as proteins, lipids, carbohydrates, and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species (ROS) that can be generated during normal metabolism as well as through exposure to toxins and pollutants (e.g. smoking).
- Vitamin C may also be able to regenerate other antioxidants such as vitamin E.
- Ascorbic acid quenches reactive oxygen species in both the extracellular and intracellular compartments.
- Vascular endothelium and helps to protect against bowel cancer by deactivating potentially damaging nitrosamines in the gut.

Sodium alginate

Alginates are refined from brown seaweeds. A wide variety of brown seaweeds of the phylum *Phaeophyceae* are harvested throughout the world to be converted into the raw material commonly known as sodium alginate. Sodium alginate has a wide use across a wide variety of industries including food, textile printing and pharmaceutical. Dental impression material utilizes alginate as its means of gelling.

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.

As early as 1938, sodium alginate powder had already been included in USP (United States Pharmacopeia). In 1963, alginate was accepted in British Pharmacopoeia. Alginate is insoluble in water but will swell when put in water. Sodium alginate consists of sodium salt of alginic acid.. The chemical formula of sodium alginate is (C6H7NaO6) n.

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.

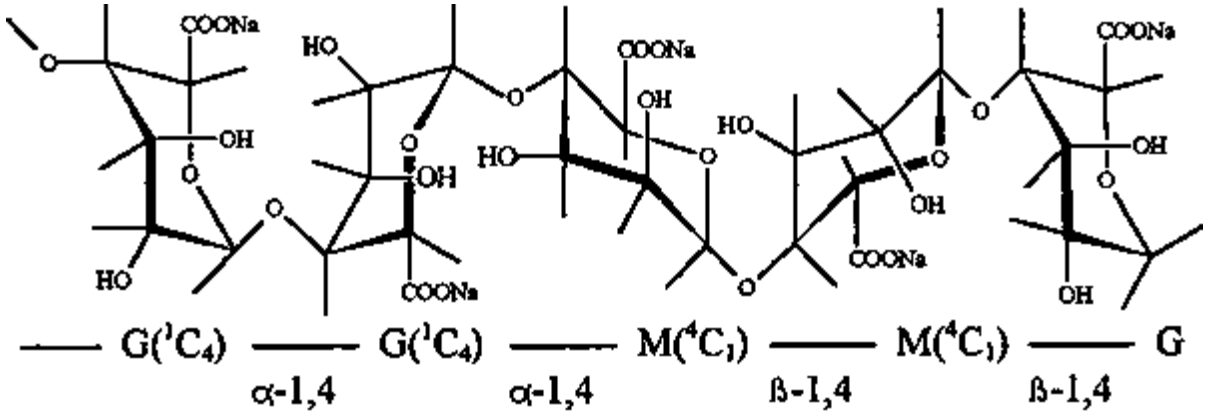


Figure 3: Structure of Sodium alginate

Calcium chloride

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 $CaCl_2$.

Addition of divalent ions (Ca^{2+}) and polyvalent ions to alginate solution cause gel formation due to cross linking.

Calcium chloride is a chemical compound made up of calcium and chlorine. It contains two atoms of chlorine and one atom of calcium as shown in figure 4

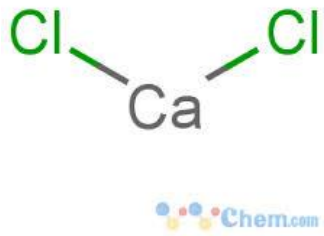


Figure 4: structure of Calcium chloride

Single layer chitosan–alginate beads released 70% of the drug within 4 h. Alginate is a suitable negatively charged agent which may interact with positively charged chitosan. Recently, alginate beads containing several substances have been prepared by the gelation of alginate with calcium cations.

According to Aysu Yurdasiper *et al* ., A process for preparing the gel beads of calcium alginate includes dispersing the aqueous solution of sodium alginate, which contains insoluble calcium salt, in the atoleine containing surfactant via inorganic membrane**,** reducing pH value to release Ca2+ ions and gelatinizing reaction. Its advantages are controllable granularity.

Chitosan

1. Chitosan is a biopolymer which could be used for the preparation of various polyelectrolyte complex products with natural polyanions such as xanthan, alginate, and carrangeenan .
2. Chitosan-polyanions complexes have been widely investigated for the applications like drug and protein delivery, cell transplantation, enzyme immobilization . Among these, complexes, chitosan-alginate complex may be the most important drug delivery microspheres.
3. The strong electrostatic interaction of amine groups of chitosan with the carboxyl groups of alginate lead to the formation of chitosan-alginate complex.
4. The chitosan-alginate gel beads with a chitosan core and a chitosan-alginate skin are prepared by dropping a solution of alginate into chitosan solution.
5. Due to the protonation of amino group on chitosan and the ionization of carboxylic acid group on alginate, the stability of chitosan influenced by the environmental parameters such as pH and ionic strength.
6. It was found that the macromolecular chitosan rapidly bind onto the surface of alginate droplet, but are limited to diffuse into the inner core . In order to increase the stability of chitosan-alginate complex, chitosan solution, consisting of calcium chloride was used for the gelation of alginate .
7. The presence of calcium ions in the chitosan solution during the incubation had a great effect on the ability of a gel bead to bind chitosan. As the concentration of calcium chloride increases, the rate and extent of chitosan binding process also proportionally increases.

Figure 5: Structure of Chitosan

Chapter 5

Experimental Work

Experimental Work

5.1 Experimental materials and equipments

Various chemicals and instruments used for the preparation and evaluation of L-Ascorbic acid microspheres are listed in table 2 as follows:

Table 2: Experimental materials and equipments

Experimental materials	Equipments
• Sodium alginate • Calcium chloride • Hydrochloric acid buffer (pH 1.2) • Phosphate buffer (pH 6.8) • Sodium hydroxide • Conc. hydrochloric acid • Chitosan (85% deacetylated, sigma Aldrich) • L-ascorbic acid (LOBA Chemie ltd) • Glacial acetic acid (1%) • Distilled water	• Analytical balance • pH meter • Lab stirrer • UV-Visible spectrophotometer • Incubator • Shaking incubator • Disposable syringes and needles • Glassware • Measuring cylinder • Micropipettes (T1000, T20)

5.2 Standard curve of ascorbic acid

Spectrophotometric method based on measurement of absorbance at 244nm 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 ascorbic acid.

5.2.1 Preparation of hydrochloric acid bufffer (pH 1.2)

50ml of 0.2M potassium chloride (KCl) was prepared. 85ml of 0.2M hydrochloric acid (HCl) was prepared. Both the solutions were mixed together and final volume was made upto 200ml by adding remaining volume of distilled water.

5.2.2 Preparation of phosphate buffer (pH 6.8)

50ml of 0.2M potassium dihydrogen phosphate solution was prepared. 22.4ml of 0.2M sodium hydroxide solution was prepared. Both the solutions were mixed together and final volume was made up to 200ml by adding remaining volume of distilled water.

5.2.3 Preparations of standard graph

Standard solutions were prepared by dissolving 100mg of L-Ascorbic acid in distilled water, hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 6.8) in a 100ml of volumetric flask. From the standard solution, different ascorbic acid dilutions were prepared by using the formulae:

$$M_1V_1 = M_2V_2$$

A series of dilutions containing 2, 4, 6, 8 and 10 μg/ml of drug were obtained. The absorbance of those diluted samples were measured in spectrophotometer at 244 nm using distilled water, hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 6.8) as a blank.

Standard curve data of L-ascorbic acid of distilled water, pH 1.2, pH 6.8 as shown in tables 3, 4, 5 respectively. Standard curves of L-ascorbic acid of distilled water, pH1.2, pH6.8 as shown in graphs 6, 7, 8.

Table 3: Standard curve data of ascorbic acid in distilled water

S. no.	Concentration(µg/ml)	Absorbance at 244nm
1	0	0
2	2	0.035
3	4	0.125
4	6	0.219
5	8	0.289
6	10	0.35

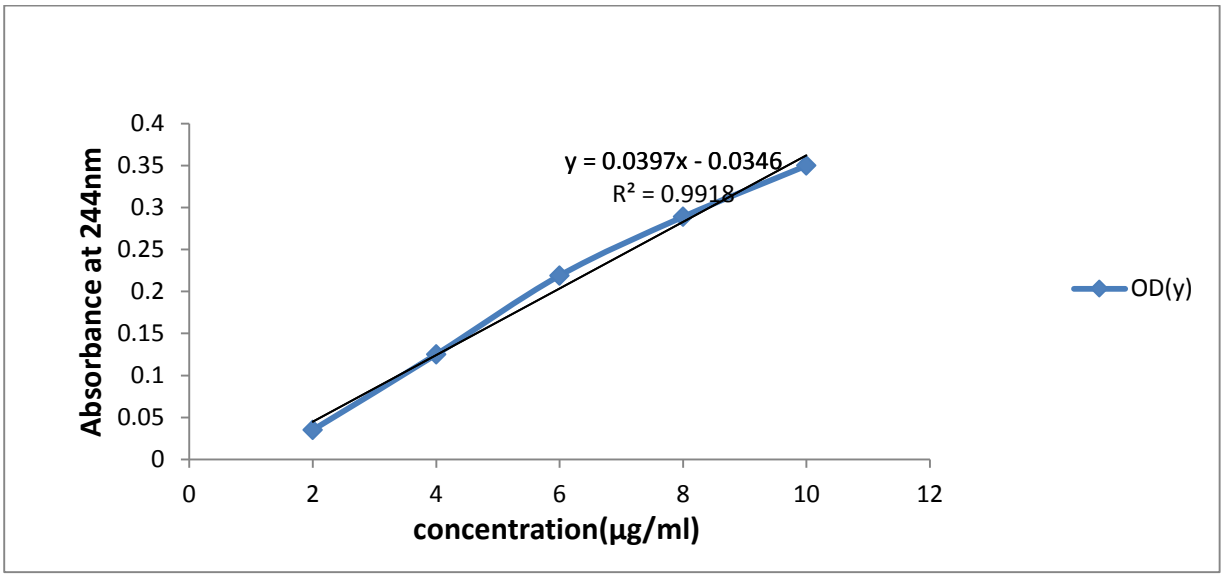


Figure 6**:** Standard curve of L-ascorbic acid in distilled water

Table 4: Standard curve data of L-ascorbic acid in hydrochloric acid buffer

S. no.	Concentration(µg/ml)	Absorbance at 244nm
1	0	0
2	2	0.117
3	4	0.276
4	6	0.434
5	8	0.58
6	10	0.758

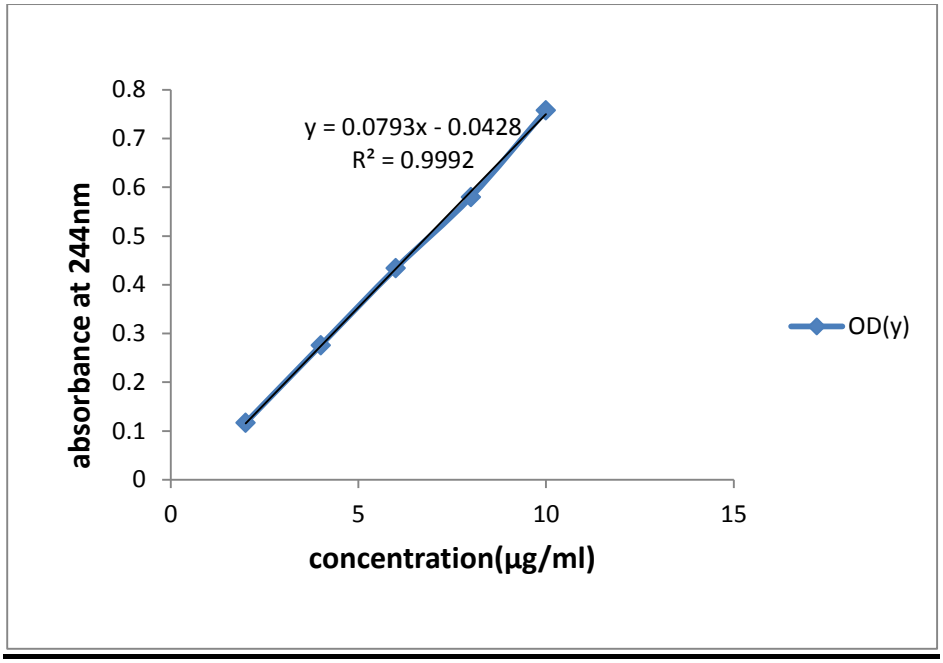


Figure 7: Standard curve of L-ascorbic acid in hydrochloric acid buffer

Table 5: Standard curve data of L-ascorbic acid in phosphate buffer (6.8)

S. no.	Concentration(µg/ml)	Absorbance at 244nm
1	0	0
2	2	0.075
3	4	0.139
4	6	0.209
5	8	0.275
6	10	0.342

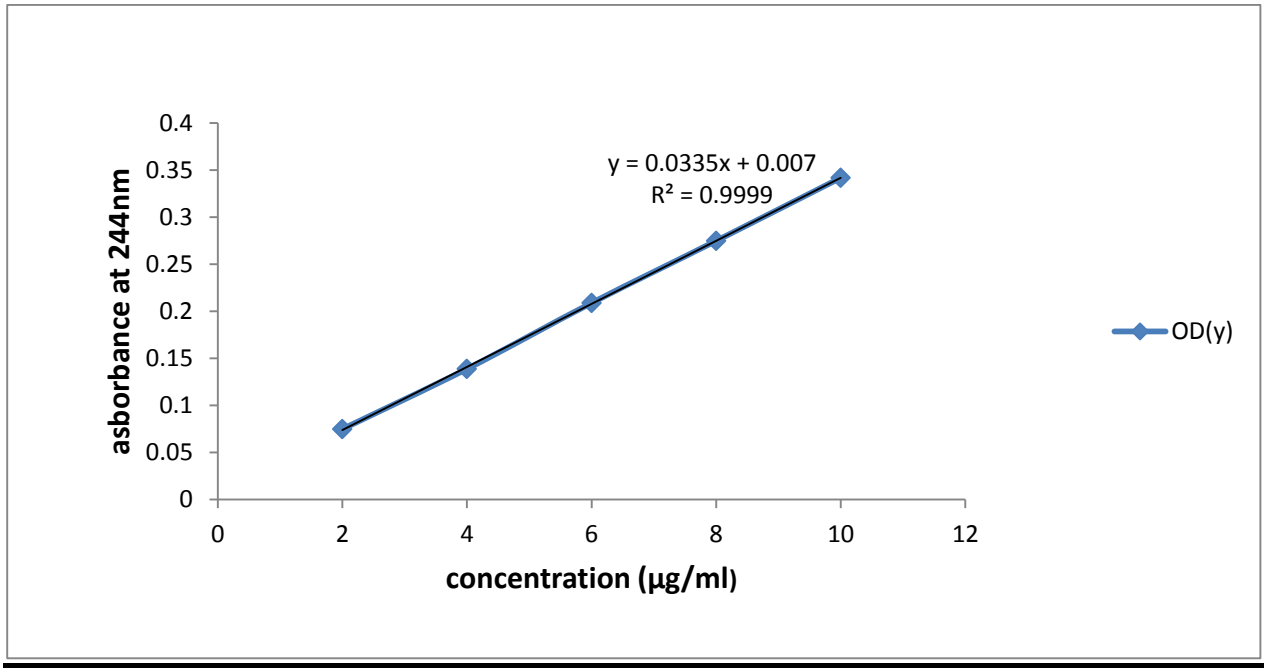
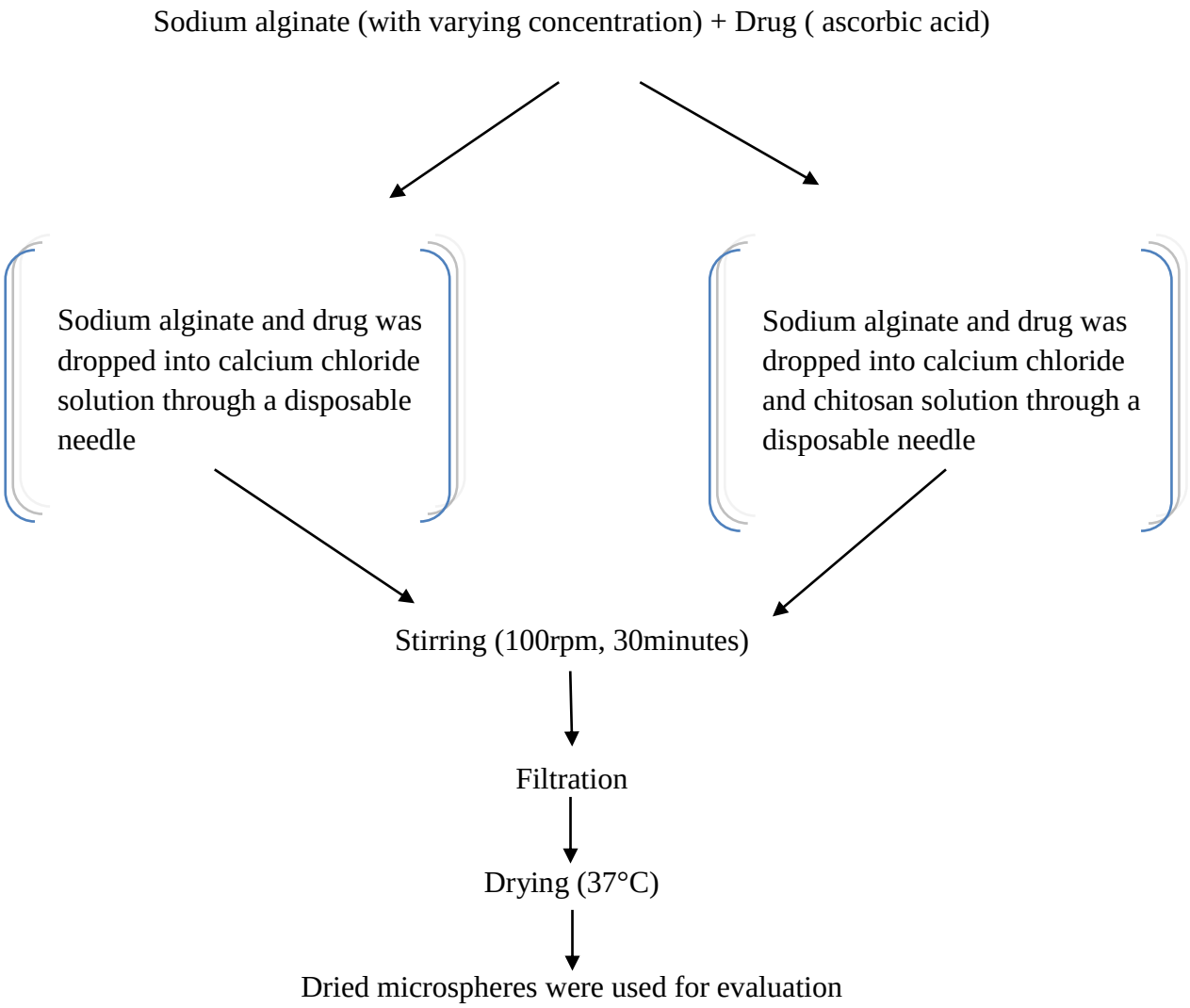


Figure 8: Standard curve of L-ascorbic acid in phosphate buffer (pH 6.8)

5.3 Preparation of L-ascorbic acid microspheres

Microspheres of L-ascorbic acid were prepared by ionotropic gelation technique. Two methods were followed for preparation of microspheres. First, varying concentration of sodium alginate and calcium chloride without chitosan and second with varying concentration of sodium alginate and calcium chloride with chitosan. 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 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 and chitosan with continuous stirring by magnetic stirrer.

Ionotropic gelation method for preparation of L-ascorbic acid microspheres



5.4 Formulation and process variables

Different batches of L-ascorbic acid microspheres were prepared by altering the following variables:

Concentration of drug (1%)

Concentration of polymer (1.5%-2%)

Concentration of cross-linking agent (1%-2%)

Stirring time (30minutes)

Stirring speed (80-100 rpm)

Volume of drug-polymer and cross linking agent

Table 6: Formulation of ascorbic acid microspheres prepared by varying polymer concentration without chitosan

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)
B1	1	1.5	1	30	100	10
B2	1	2	1	30	100	10

Table 7: Formulation of ascorbic acid microspheres prepared by varying $CaCl_2$ without chitosan

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)
B3	1	2	2	30	100	10
B4	1	2	1.5	30	100	10
B5	1	1.5	1.5	30	100	10

Table 8: Formulation of ascorbic acid microspheres prepared by varying polymer concentration with chitosan

Batch no.	Conc. of Drug (%w/v)	Conc. of polymer (%w/v)	Conc. of $CaCl_2$ (%w/v)	Stirring time (min)	Stirring speed (rpm)	Chitosan (%w/v)	Volume of Drug polymer (ml)
B6	**1**	**1.5**	**1**	**30**	**100**	**0.35**	**10**
B7	**1**	**2**	**1**	**30**	**100**	**0.35**	**10**

Table 9: Formulation of ascorbic acid microspheres prepared by varying $CaCl_2$ with chitosan

Batch no.	Conc. of Drug (%w/v)	Conc. of polymer (%w/v)	Conc. of $CaCl_2$ (%w/v)	Stirring time (min)	Stirring speed (rpm)	Chitosan (%w/v)	Volume of Drug polymer (ml)
B8	**1**	**2**	**2**	**30**	**100**	**0.35**	**10**
B9	**1**	**2**	**1.5**	**30**	**100**	**0.35**	**10**
B10	**1**	**1.5**	**1.5**	**30**	**100**	**0.35**	**10**

Drying time for all batches was 37ºC.

5.5 Evaluation of Ascorbic acid microspheres

All batches of Ascorbic acid 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. Each batch was repeated twice and mean of both the batches were taken. The percentage yield was calculated as:

$$Percentage\ Yeild = \frac{Wt\ of\ dried\ microspheres}{(wt\ of\ polymer + drug + crosslinker)} X100$$

Table 10: Percentage yield of different batches of ascorbic acid microspheres

Batch no.	Percentage yield
B1	28.37
B2	29.89
B3	25.3
B4	26.19
B5	33.49
B6	29.52
B7	34.22
B8	27.75
B9	27.11
B10	25.95

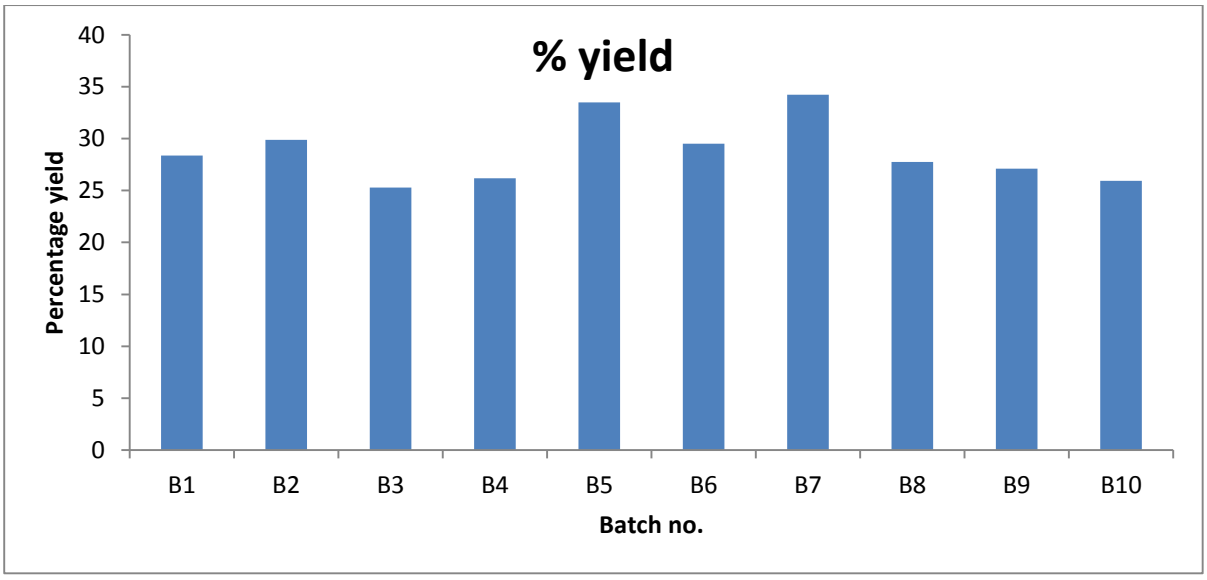


Figure 9: Histogram showing effect of various formulation parameters on percentage yield

5.5.2 Equilibrium swelling studies

The swelling ability of dried microspheres of chitosan was determined in distilled water, pH 1.2 buffer solutions and pH 6.8 buffer solution. 20mg of dried microspheres were immersed in 5ml distilled water, pH 1.2 buffer, pH 6.8 buffer in different glass vials respectively. These immersed microspheres were kept at 37°C for 24 hours. Swollen microspheres were filtered, blotted and weighed immediately on an electronic balance. The percentage swelling index of microspheres at equilibrium was calculated by using the following formula:

$$\%Esw = \frac{We - Wo}{We} X100$$

Wo= Initial weight of microspheres
We= Weight of microspheres at equilibrium

Table 11: Percent swelling of different batches of ascorbic acid microspheres

Batch No.	Distilled water (%)	pH 1.2 (%)	pH 6.8 (%)
B1	36.2	69.4	77.6
B2	24.6	63.4	277
B3	26	71.2	98.4
B4	36.8	65	112
B5	40.4	61.8	70.6
B6	32	26	21.2
B7	37.6	51	126
B8	67.8	74	88
B9	31.6	65	260
B10	50	38	76

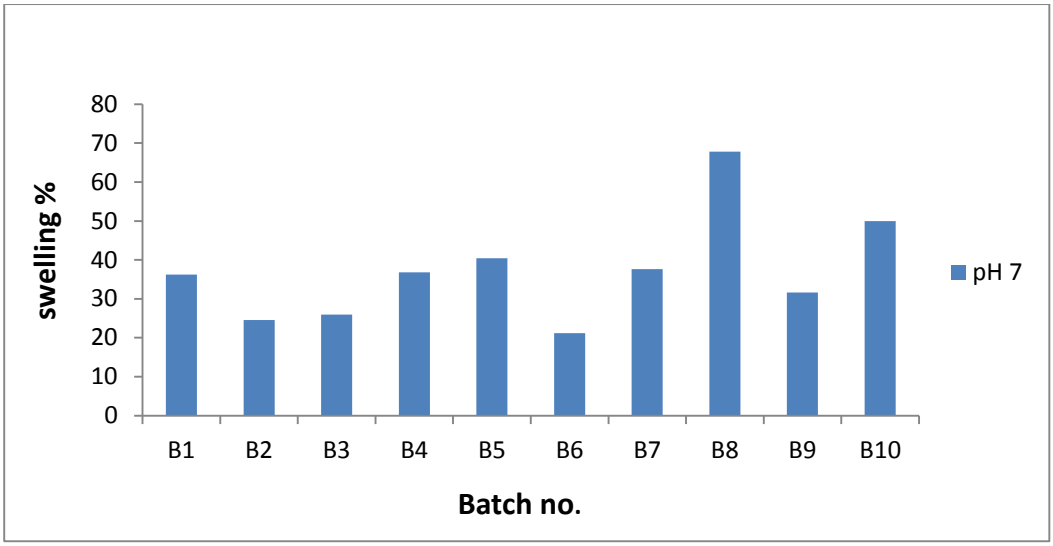


Figure 10: Swelling ratio of L-ascorbic acid microspheres at pH 7

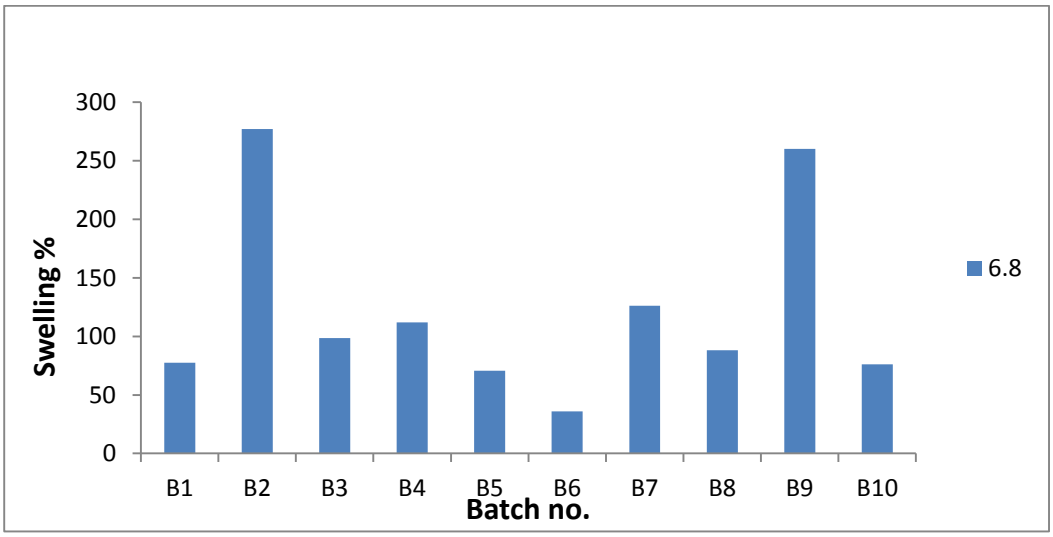


Figure 11: Swelling ratio of L-ascorbic acid microspheres at pH 6.8

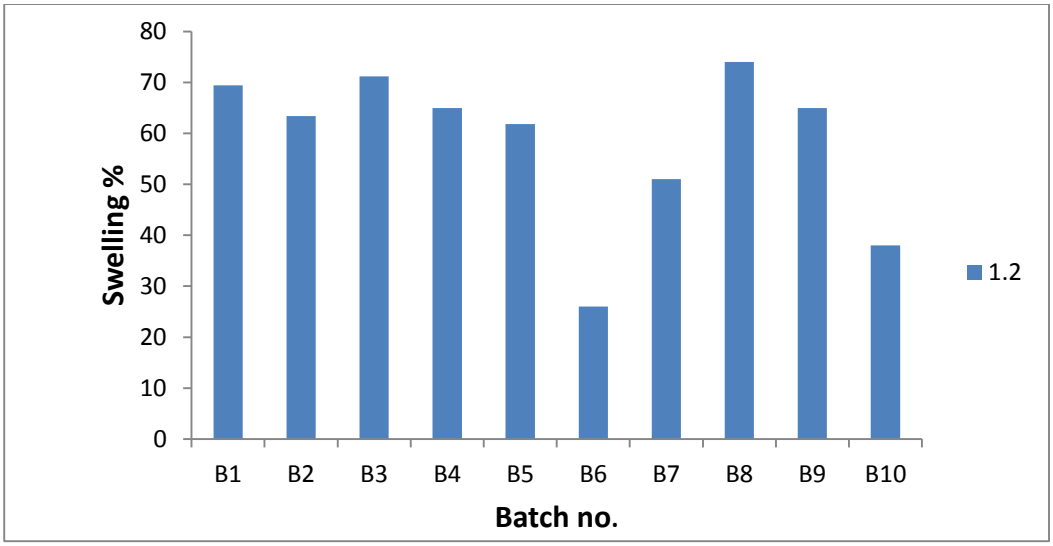


Figure 12: Swelling ratio of L-ascorbic acid microspheres at pH 1.2

5.5.4 Determination of encapsulation efficiency

Drug entrapment efficiency of ascorbic acid microspheres was performed by accurately weighing 50mg of microspheres and crushing them properly using pestle and mortar into fine powder. These powdered microspheres were then suspended into 10ml of pH 1.2 buffer. Then after suitable dilution vitamin C content in the filterate was analyzed spectrophotometrically at 244nm using spectrophotometer.

$$\%\ Entrapment\ efficiency = \frac{\%\ Drug\ loading}{(\%\ theoretical\ loading)} X100$$

Table 12: Encapsulation efficiency of different batches of Ascorbic acid

Batch No.	Entrapment efficiency (%)
B1	50
B2	73.6
B3	20.8
B4	40.7
B5	19.9
B6	72.79
B7	46.4
B8	13.07
B9	79.1
B10	35.73

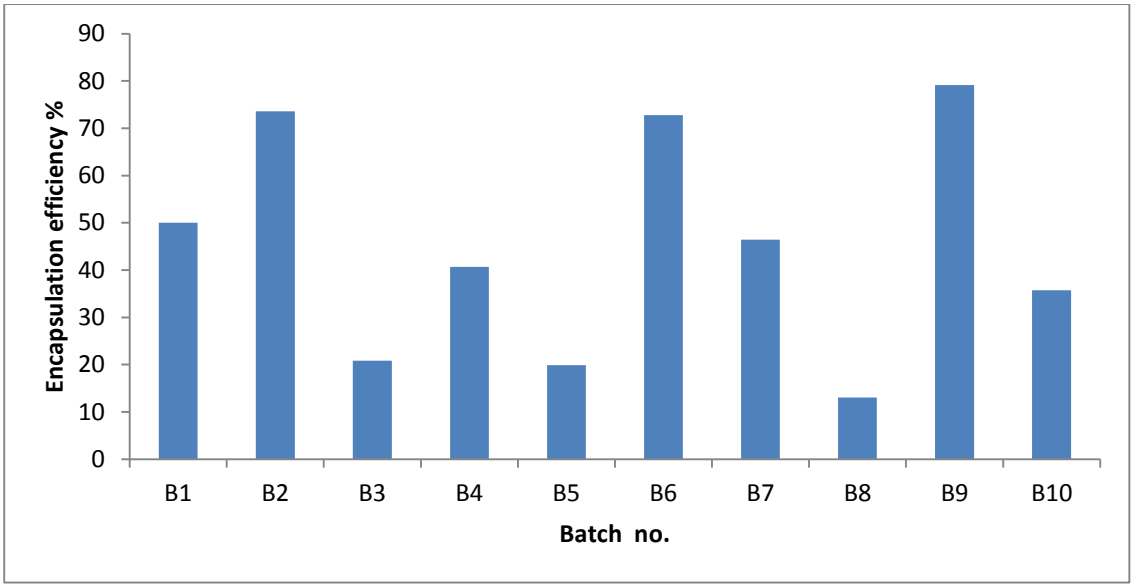


Figure 13 Histogram showing effect of various formulation parameters on encapsulation efficiency of L-Ascorbic acid microspheres.

5.5.5 Particle size determination

Microspheres of ascorbic acid were evaluated with respect to their size using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle size of microspheres with different concentration was determined . Scanning electron microscopy was done to determine size and morphological features.

Table 13: Average size of different batches of L-Ascorbic acid microspheres

Batch No.	Particle size(µm)
B1	905
B2	851
B3	915
B4	937
B5	953
B6	942
B7	966
B8	921
B9	1000
B10	1101

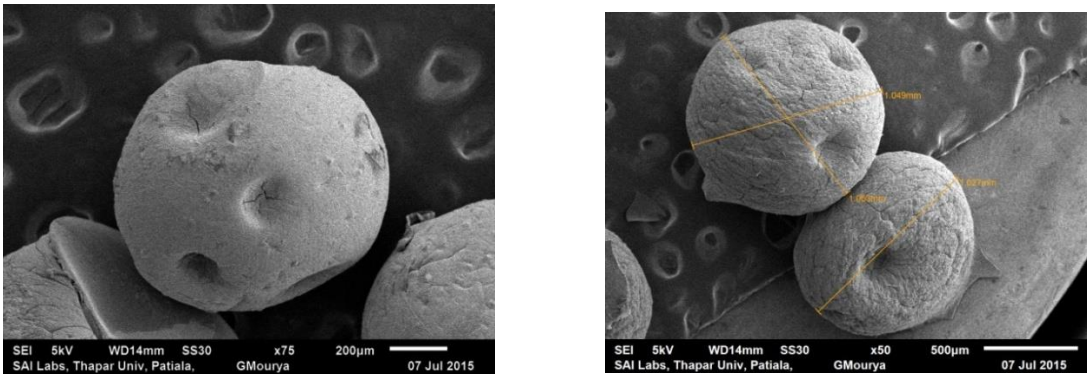


Figure 14: Scanning electron micrographs of sodium alginate chitosan coated microspheres

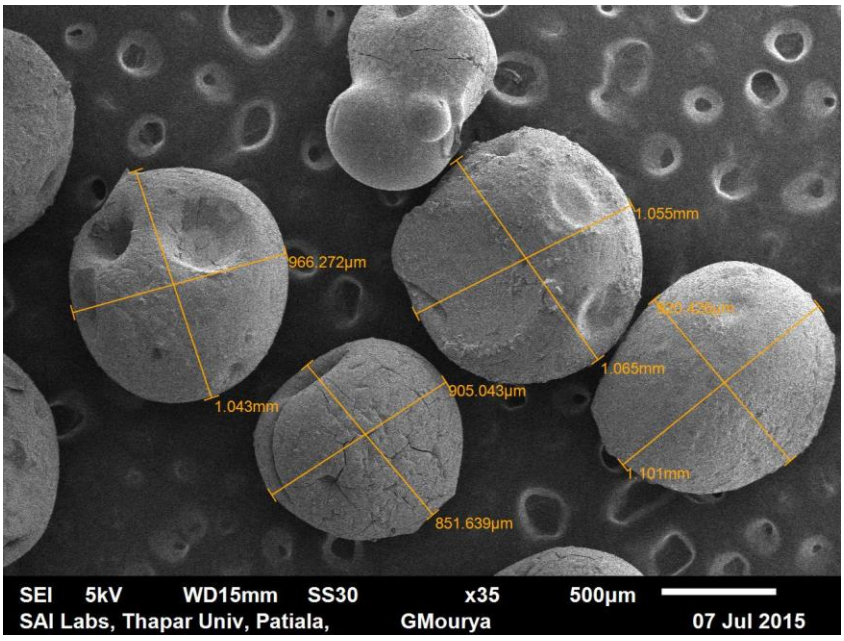


Figure 15: Scanning electron micrographs of sodium alginate microspheres

5.5.6 Evaluation of *in-vitro* drug release

50 mg of ascorbic acid microspheres were incubated in 50 ml buffer (pH1.2) in a 150 ml conical flask kept in a shaking incubator at 37ºC and at 100 rpm. After 4 hours microspheres were filtered and transferred into 50 ml buffer (pH 6.8) and incubated at 37ºC and at 100 rpm. Starting from time 0 hour and at desired intervals of time, 5ml sample was withdrawn and replaced with same amount of fresh medium. Drug released into the buffer medium at different intervals of time was analyzed by measuring the absorbance at 244nm using a UV spectrophotometer. Released concentration of vitamin C released from encapsulated chitosan microspheres of various batches was studied.

Table 14: Drug release data of ascorbic acid microspheres by varying concentration of $CaCl_2$ with chitosan.

S.no.	Time(hrs)	Cumulative percentage release(%)		
		B8	B9	B7
1	0	0	0	0
2	0.5	36.5	67	44.7
3	1	37.3	70	46.5
4	1.5	39.7	70.7	47.8
5	2	41.2	71.9	52.1
6	2.5	44.1	72.3	54
7	3	44	72.9	55.6
8	4	43	72.2	56.3
9	4.5	44	72.7	57
10	5	43.1	71.9	57.6
11	6	44	71	56.6
12	7	43.5	70.1	56.5
13	24	42.9	70.4	53.8

The drug release curve of L-ascorbic acid microspheres by varying polymer Concentration.

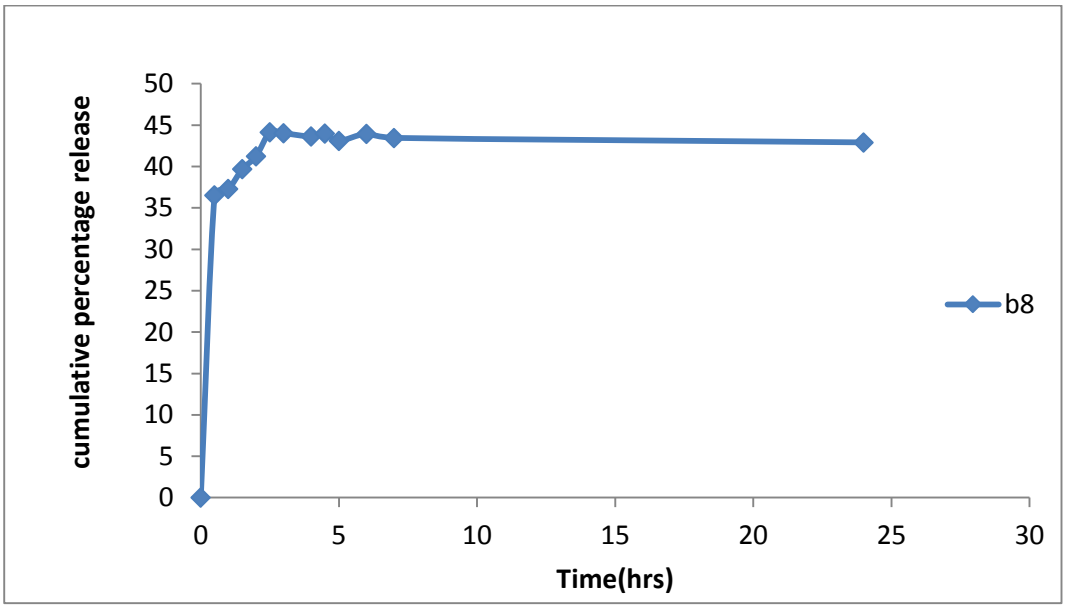


Figure 16 : The drug release curve of B8

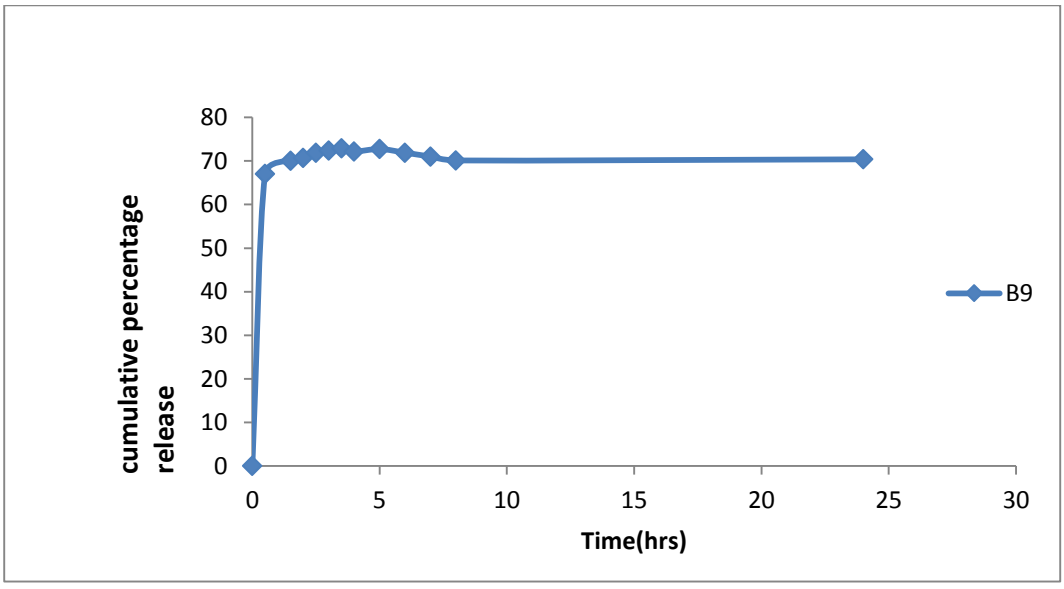


Figure 17: The drug release curve of B9

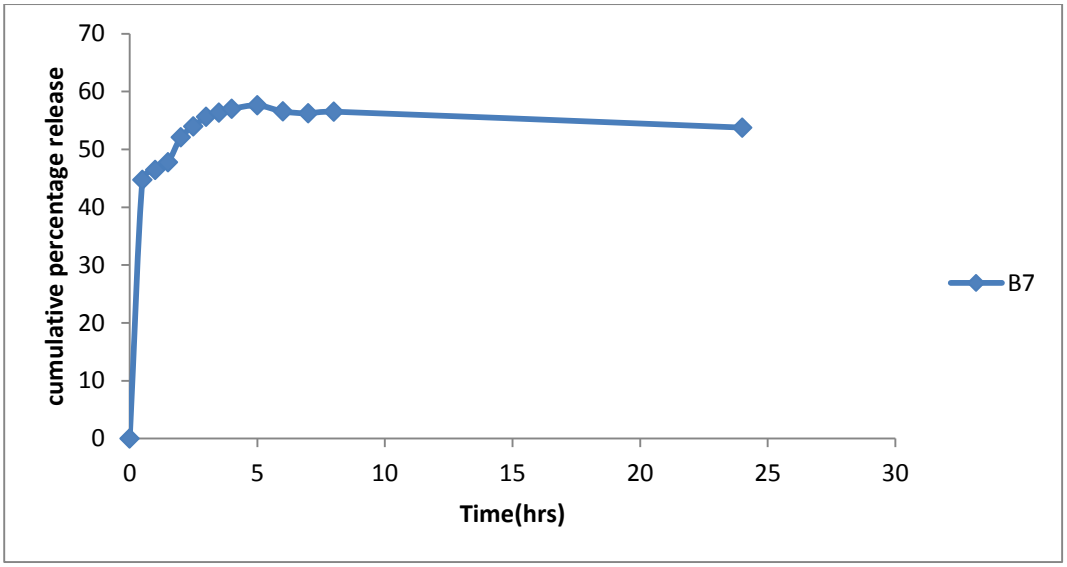


Figure 18: The drug release curve of B7

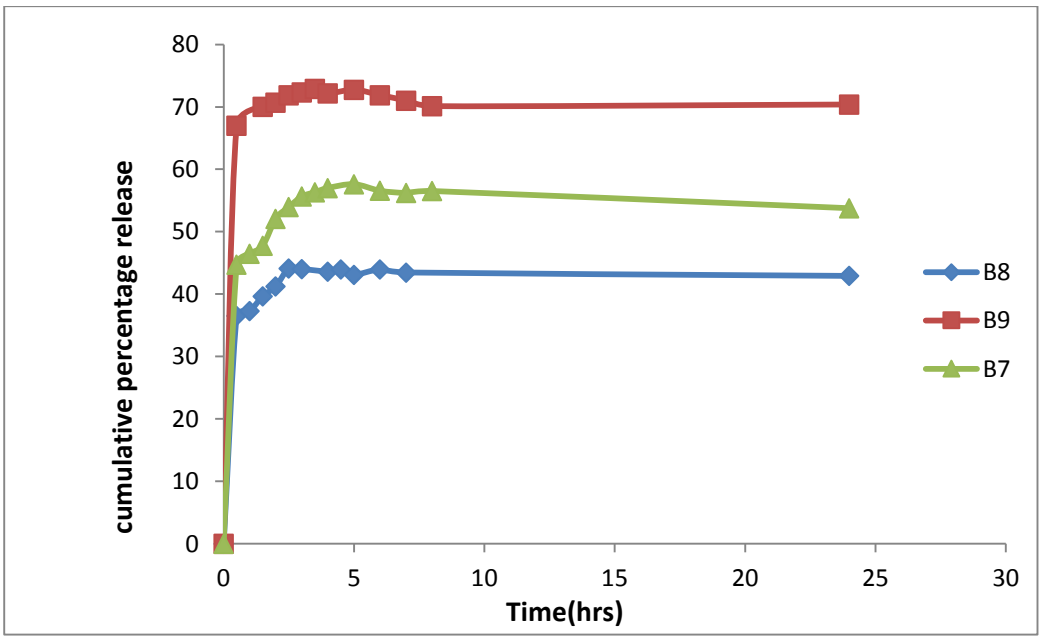


Figure 19: The Comparative drug release curves of B8-B9-B7

Table 15: Drug release data of chitosan coated ascorbic acid microspheres by varying concentration of polymer.

S. No.	Time(hrs)	Cumulative percentage release(%)	
		B10	B6
1	0	0	0
2	0.5	44.8	38.5
3	1	47.3	39.7
4	2	49.3	41.2
5	2.5	49.1	41.8
6	3	48.9	44.4
7	3.5	49.8	46.7
8	4	50.2	47.9
9	5	49.1	47.9
10	6	49.4	47.3
11	7	49.1	47.3
12	8	49.4	47.0
13	24	50.8	45.5

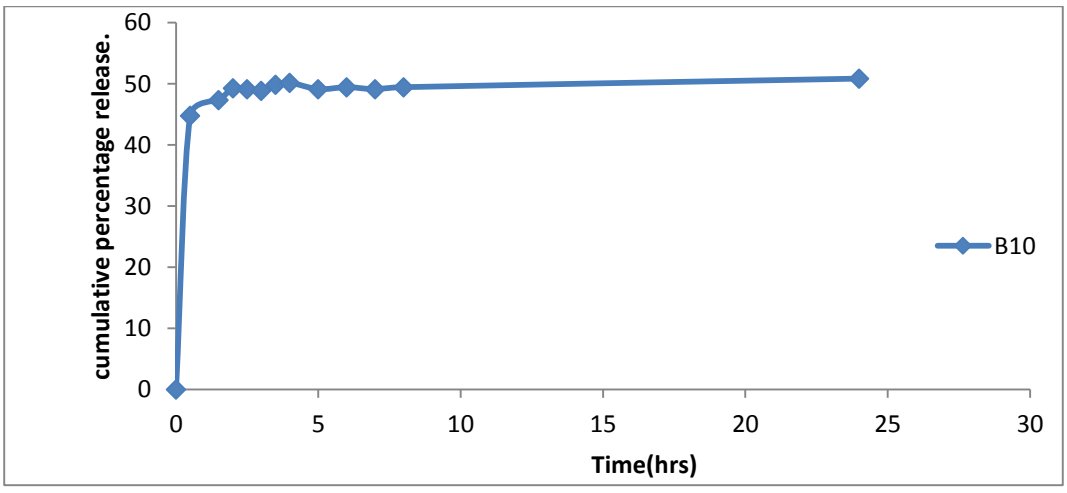


Figure 20: The drug release curve of B10

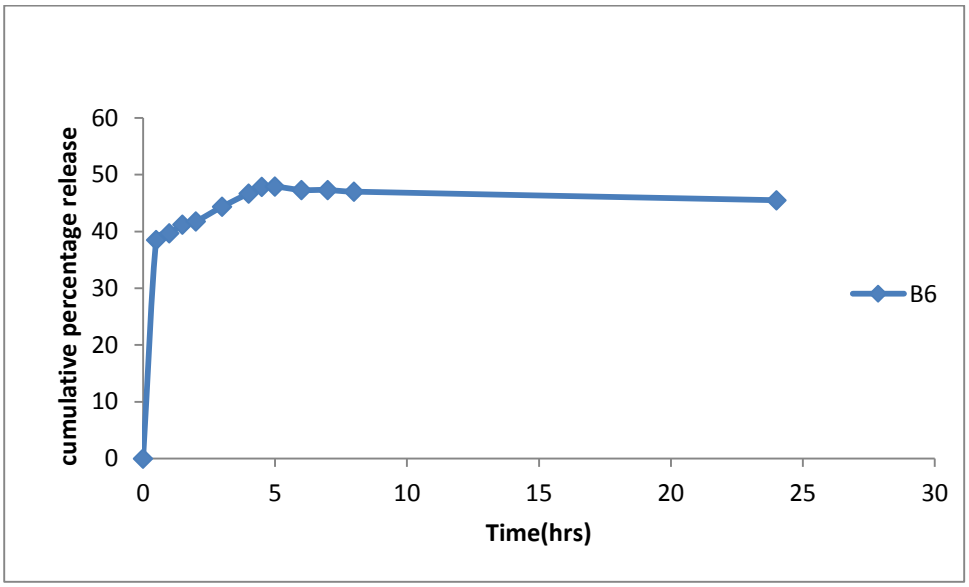


Figure 21: The drug release curve of B6

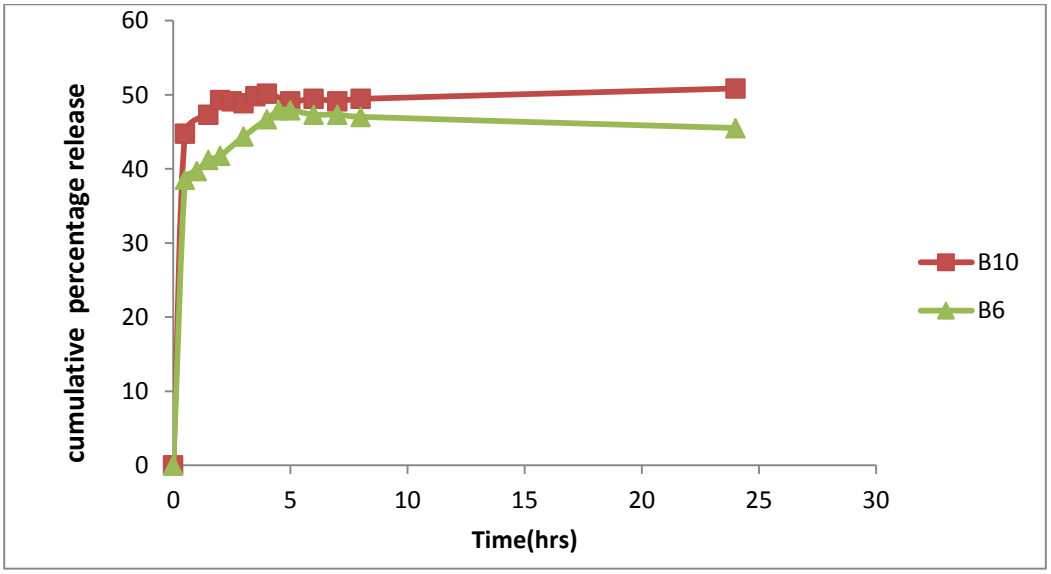


Figure 22: The Comparative drug release curves of B10-B6

Table 16: Drug release data of ascorbic acid microspheres by varying $CaCl_2$ without chitosan.

S.no.	Time(hrs)	Cumulative percentage release(%)		
		B1	B3	B4
1	0	0	0	0
2	0.5	39.2	21.3	45.5
3	1	40.6	22.1	48.0
4	1.5	41.3	22.5	49.4
5	2	44.1	23.3	50.9
6	3	47.7	24.9	52
7	4	48.0	25.0	53.8
8	4.5	46.9	27.4	55.4
9	5	46.9	27.6	56.7
10	6	47.3	27.1	57.7
11	7	49	27.5	57.6
12	8	48.3	27.2	56.0
13	24	46.9	27.0	55.1

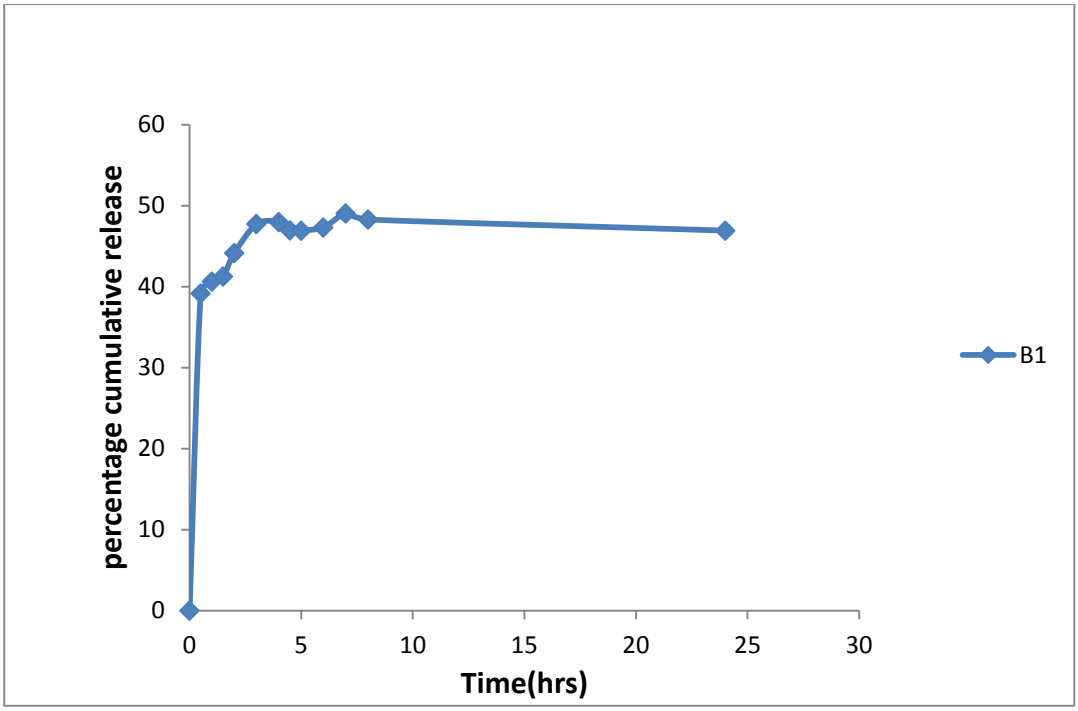


Figure 23: The drug release curve of B1

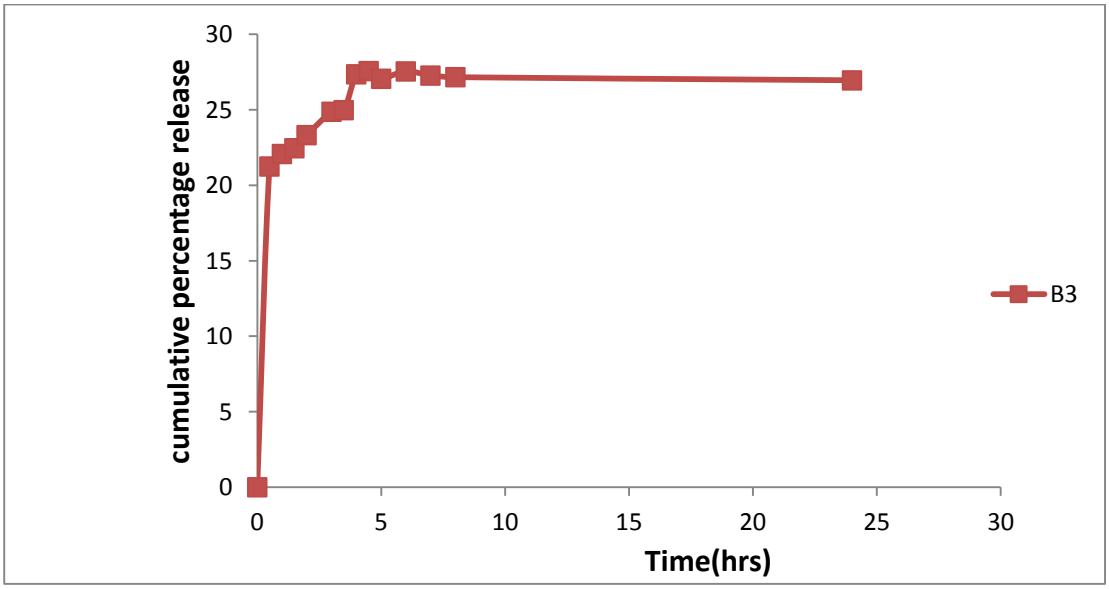


Figure 24: The drug release curve of B3

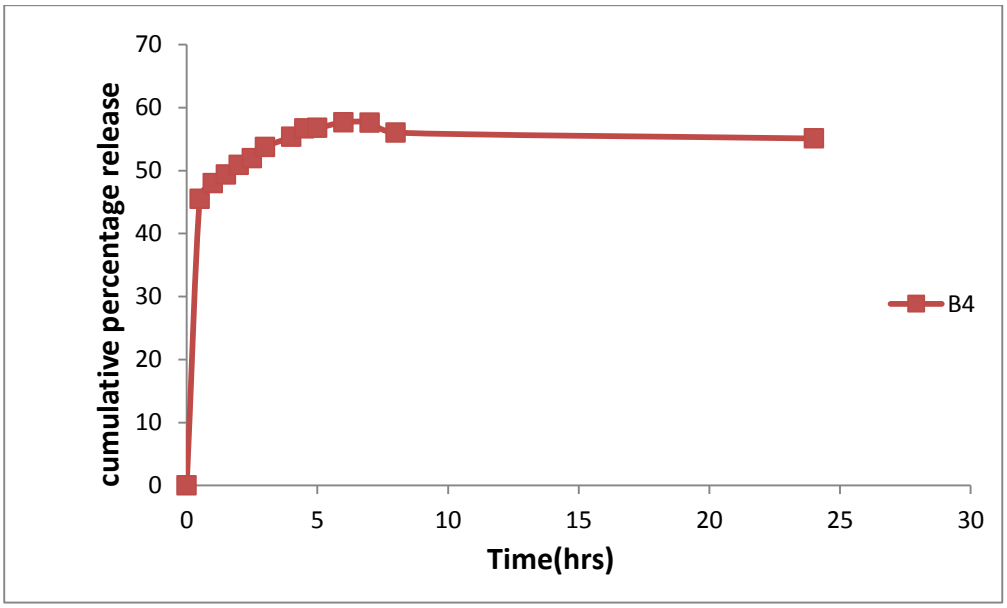


Figure 25: The drug release curve of B4

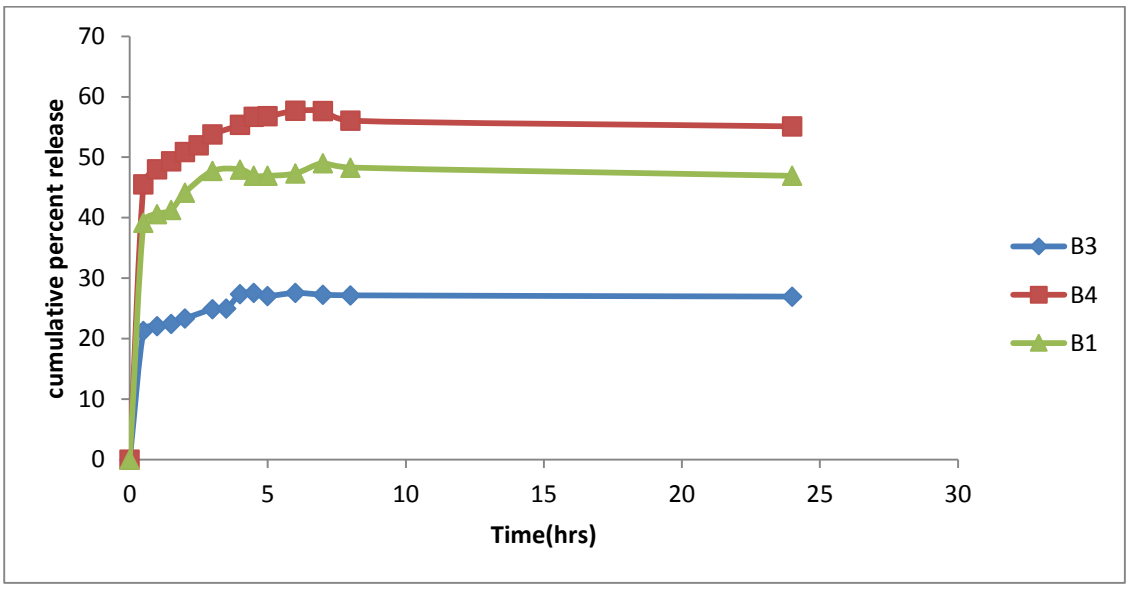


Figure 26: The Comparative drug release curves of B1-B3-B4

Table 17: : Drug release data of chitosan coated ascorbic acid microspheres by varying concentration of polymer

S. No.	Time(hrs)	Cumulative percentage release(%)	
		B2	B5
1	0	0	0
2	0.5	70.4	20.9
3	1	75.6	21.7
4	2	80.7	21.8
5	3	81.3	22.0
6	4	81.6	22.0
7	4.5	80.4	22.3
8	5	80.4	22.3
9	6	81.0	22.3
10	7	81.3	22.5
11	8	80.1	22.8
12	24	79.8	23.1

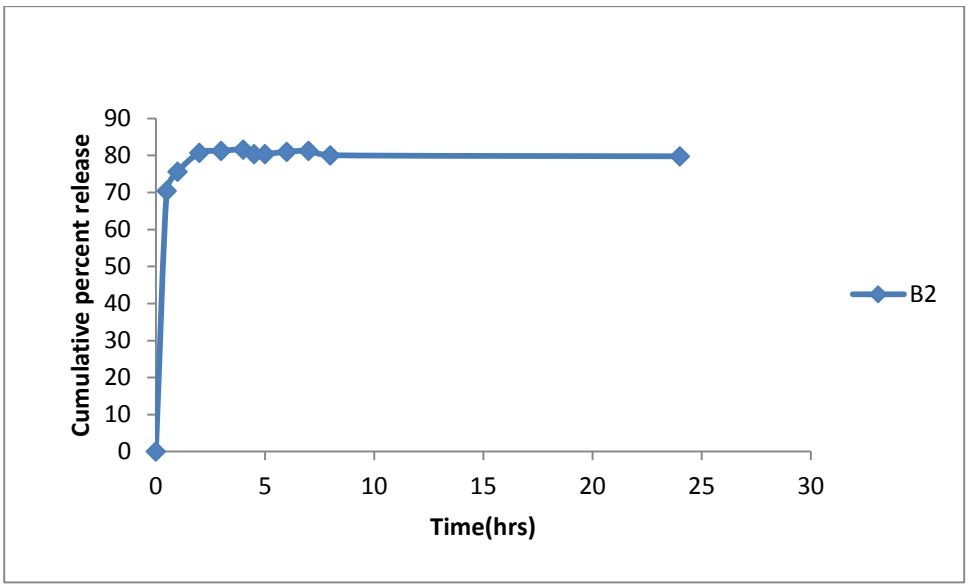


Figure 27: The drug release curve of B2

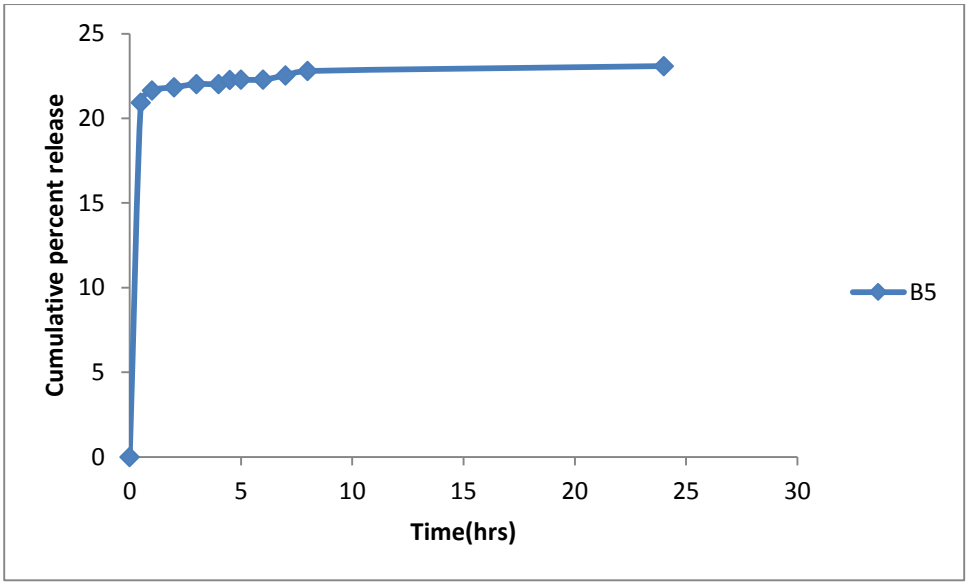


Figure 28: The drug release curve of B5

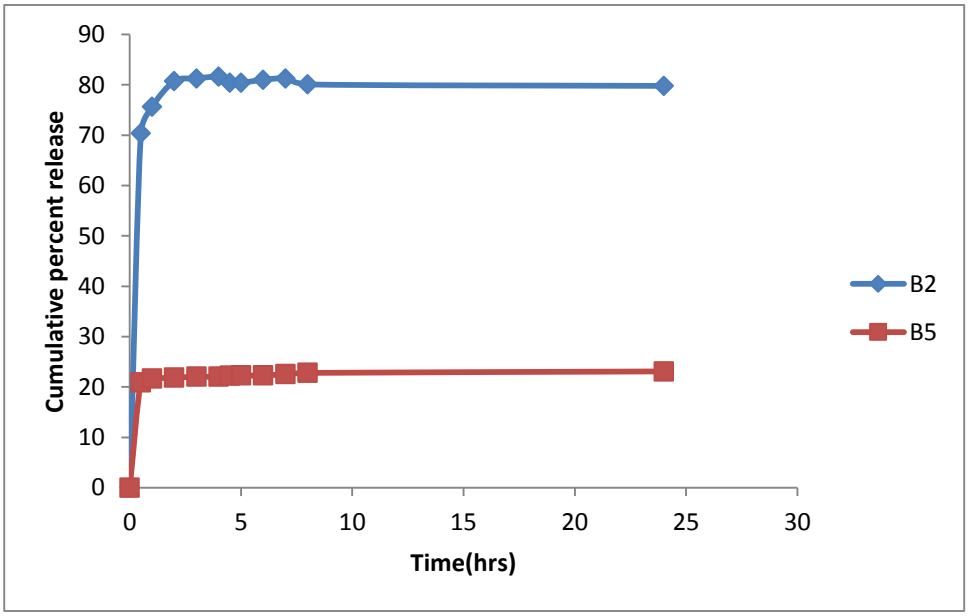


Figure 29: The Comparative drug release curves of B2-B5

Chapter 6

Results and Discussion

Results and Discussion

Microspheres constitute an important part of controlled drug delivery systems by virtue of their small size and efficient carrier capacity. Microspheres with different concentrations were prepared by ionotropic gelation method. Sodium alginate and chitosan were used as polymers. Two methods were used for preparation of microspheres. First was with chitosan and second method was without chitosan.

It was observed that with decrease in amount of polymer, a decrease in the size of the microparticles was observed. Besides this, the dispersive force of the stirrer became less efficient in presence of higher amount of polymer and as a result larger microparticles formed. (Nirmala Devi, Dilip Kumar Kakati, 2013)

Without proper crosslinking there was always chance of aggregation of the soft microparticles to produce polymer lump. With the increasing of the amount of crosslinker, the microparticles progressively developed proper shape from aggregated to relatively less aggregated and to free-flowing spherical microparticles but percentage yield decreases with increase in polymer concentration and drug concentration. Percentage yield also decreases with increase in $CaCl_2$ concentration (crosslinker) as shown in batch no. B3 and B8 whereas in batch no. B2 and B7 yield was high as shown in table no10 and figure: 9.

Similarly, stirring speed also affected the size and morphological features of microspheres. At low stirrer speed (100 rpm), microspheres formed were uniform in size as compared to those of particles produced at higher stirrer speed (300 rpm). Improper stirring and inaccurate time were responsible for clump formation instead of proper microspheres. The morphology of ascorbic acid microspheres was determined under light microscope. The microspheres were found to be spherical in shape. Scanning electron micrographs were also done of chitosan coated and sodium alginate microspheres as shown in figure 14, 15,mean particle sizes of all the batches were shown in table 13.

Swelling ratio was high in batch no. B2, B4, B7, B9 at pH 6.8. Swelling ratio was lower in pH 7 and pH 1.2 as compared to pH 6.8 as shown in table no. 11 and figures 10, 11, 12. 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. Microparticles with higher crosslinking showed lesser water, buffer uptake than the microparticles with low crosslinking. This was due to the formation of more compact wall (Agnihotri and Aminabhavi, 2004) caused by crosslinking. These swelling studies were done in hydrochloric acid buffer (pH 1.2). Ascorbic acid 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 ascorbic acid in simulated intestinal fluids.

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 and this was due to the more diffusion of ascorbic acid into the microparticles. Again, higher the amount of crosslinker in the microparticles, the lower was the loading efficiency. The decrease in loading efficiency might be attributed to the formation of more compact wall due to crosslinking that led to decrease in diffusion rate as shown in table no. 12 and figure no. 13.

The *in vitro* release studies were performed in buffer (pH 1.2) and subsequently in buffer (pH 6.8) close to the physiological gastrointestinal conditions. The dissolution behavior of the L-ascorbic acid microspheres was dependent on pH. The microspheres swelled in buffer (pH 1.2) while they disintegrated in buffer (pH 6.8).

The *in vitro* drug release rates of different batches were listed in Table 14, 15, 16, 17 and comparative studies in figures 19, 22, 26, 29. It was observed that 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. Charge density of beads, which is an important factor in electrostatic interaction also explained the pH responsive release and depends on solution pH.

Chitosan coated sodium alginate ascorbic acid microspheres (batch no. 9) showed better entrapment efficiency and *in vitro* drug release profile as compared with sodium alginate microspheres (batch no.4).

Conclusion

L-ascorbic acid microspheres can be prepared by ionotropic gelation method using sodium alginate and calcium chloride. Sodium alginate is a natural polymer which is biodegradable and biocompatible in nature and considered as safe material. Secondly, sodium alginate is a good drug delivery carriers that is ideal for broad category of drugs. Calcium chloride is used as crosslinking agent for uniform preparation of microspheres.

Several formulation parameters such as type and concentration of cross linking agents, concentration of polymer resulted significant influence on the size, morphology, yield, encapsulation efficency of L-ascorbic acid loading and release characteristics.

It was observed that Batch no. 2 and Batch no. 9 were best of all the batches that were studied on the basis of percentage yield, particle size, swelling studies, encapsulation efficiency and *in vitro* drug release. Batch no. 2 was prepared by with 2% sodium alginate, 1% calcium chloride, 1% drug without chitosan with 100 rpm, 30minutes stirring time. In Batch no. 9, 2% sodium alginate, 1.5% calcium chloride, 1% drug, chitosan coated at 100 rpm, 30 minutes stirring time.

Yield of both the batches is almost same i.e. 27.11% and 29.89%, B9 and B2 respectively. Swelling ratios are 260% and 277%, in vitro drug release studies are 79% and 70% of Batch no. 9 and 2 respectively. Morphology of both the batches were also good as compared to other batches.

Chitosan as a natural biodegradable polymer provides good barrier to the core material from the surrounding environment as well as very effective in controlled drug delivery system. It also helped in retaining L-ascorbic acid for longer duration in human body.
Ionotropic Gelation method is an effective method where harsh and harmful chemicals are not used and hence safer for human consumption.

Chapter 8

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