PREPARATION AND EVALUATION OF MICROSPHERES OF NATURAL GUMS CONTAINING AN ANTI VIRAL DRUG

By

Ms. JASTI HARIKA
B.Pharm.,

Reg.No:13PU080

A dissertation Submitted to the

Rajiv Gandhi University of health Sciences
Karnataka Bangalore

In partial fulfillment of the requirements for the

MASTER OF PHARMACY
IN
PHARMACEUTICS

Under the guidance of,
ABDUL NASIR KURNOOL
M.Pharm.,

DEPARTMENT OF PHARMACEUTICS
SAC COLLEGE OF PHARMACY
B.G. NAGARA, KARNATAKA-571448
2015

RAJIV GANDHI UNIVERSITY OF HEALTH SCIENCES, KARNATAKA, BANGALORE

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled PREPARATION AND EVALUATION OF MICROSPHERES OF NATURAL GUMS CONTAINING AN ANTI VIRAL DRUG is a bonafide and genuine research work carried out by me under guidance of ABDUL NASIR KURNOOL Assistant Professor, Department of Pharmaceutics, and SAC College of Pharmacy. B.G. Nagara.

Date:

Place: B.G. Nagara

Ms. JASTI HARIKA
B.Pharm

Department of Pharmaceutics,
SAC College of Pharmacy,
B.G. Nagara-571448
Karnataka

SRI ADICHUNCHANAGIRI COLLEGE OF PHARMACY
B.G. NAGARA-571448

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **PREPARATION AND EVALUATION OF MICROSPHERES OF NATURAL GUMS CONTAINING AN ANTI VIRAL DRUG** is bonafide research work done by **Ms. JASTI HARIKA** in partial fulfillment of the requirement for the Degree of MASTER OF PHARMACY IN PHARMACEUTICS. This work was carried out by his in the laboratory of SAC College of Pharmacy, B.G.Nagara, under my direct supervision and guidance.

Date:

Place: B.G.Nagara

ABDUL NASIR KURNOOL
M.Pharm.,
Assistant Professor
Dept. of Pharmaceutics
SAC College of Pharmacy
B.G.Nagara-571448
Karnataka

SRI ADICHUNCHANAGIRI COLLEGE OF PHARMACY
B.G. NAGARA-571448

ENDORSEMENT BY THE HEAD OF THE DEPARTMENT

This is to certify that the dissertation entitled "PREPARATION AND EVALUATION OF MICROSPHERES OF NATURAL GUMS CONTAINING AN ANTI VIRAL DRUG" is a bonafide research work carried out by **Ms. JASTI HARIKA** under the guidance of **ABDUL NASIR KURNOOL** Assistant Professor, Department of Pharmaceutics, SAC College of Pharmacy, B.G.Nagara.

Date:

Place: B.G. Nagara

Dr. MOHAMMED GULZAR AHMED
M.Pharm, Ph.D.,
Professor and Head,
Dept. of Pharmaceutics
SAC College of Pharmacy
B.G.Nagara-571448
Karnataka

SRI ADICHUNCHANAGIRI COLLEGE OF PHARMACY
B.G.NAGARA-571448

ENDORSEMENT BY THE PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"PREPARATION AND EVALUATION OF MICROSPHERES OF NATURAL GUMS CONTAINING AN ANTI VIRAL DRUG"** is a bonafide research work carried out by **Ms. JASTI HARIKA** under the guidance of **ABDUL NASIR KURNOOL** Assistant Professor, Department of Pharmaceutics, SAC College of Pharmacy, B.G.Nagara.

Date:

Place: B.G. Nagara

Dr. B. Ramesh
M.Pharm, Ph.D.,

Principal
SAC College of Pharmacy
B.G. Nagara-571448
Karnataka

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the **Rajiv Gandhi University of health Sciences, Karnataka** shall have the right to preserve, use and disseminate this dissertation in print or electronic format for academic / research purpose.

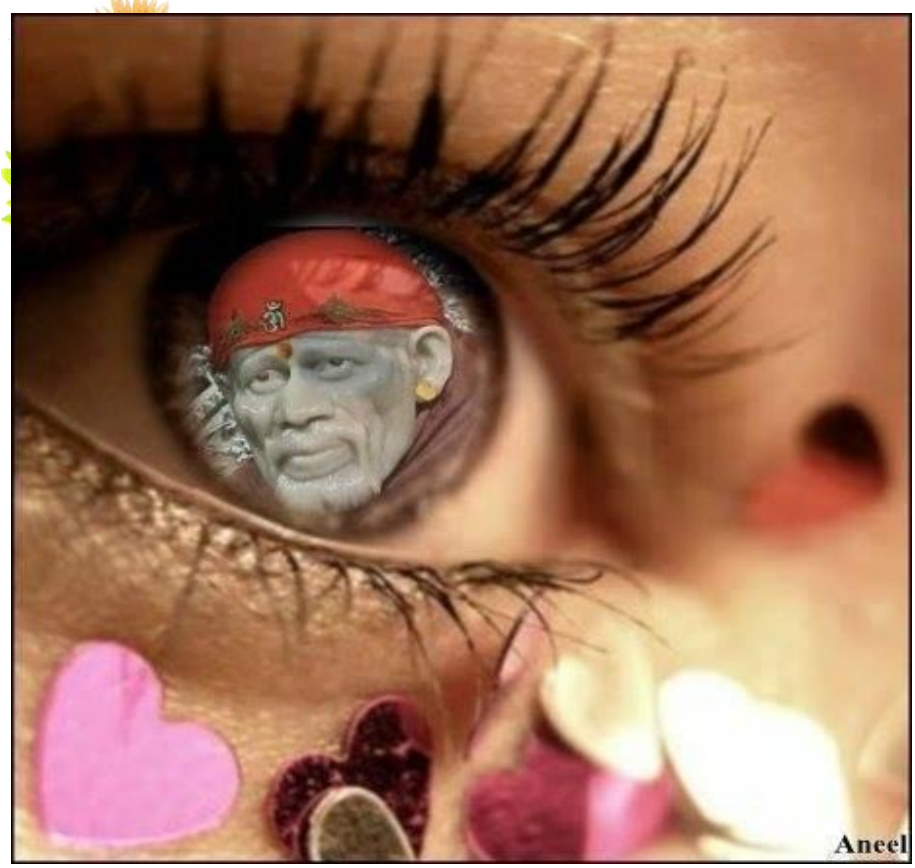
Date:

Palce: B.G. Nagara

Ms. JASTI HARIKA
B.Pharm

Department of Pharmaceutics
SAC College of Pharmacy
B.G. Nagara-571448
Karnataka

Dedicated to Beloved Parents, Friends & Guide



ACKNOWLEDGEMENT

"Gratitude makes sense of our past, brings peace for today and creates a vision for tomorrow"

First I thank The God Almighty ***Lord Saibaba, Lord Venkateswara*** *and* ***Lord Vinayaka*** *for giving me the patience, courage, and abundant blessings poured upon me for conducting the study and helping me in every walk of life with all that I have got.*

The completion of this dissertation is not only fulfillment of my dreams but also the dreams of my family who have taken lots of pain for me in completion of my higher studies.

I take this privilege and pleasure to acknowledge the contributions of many individuals who have been inspirational and supportive throughout my work undertaken and endowed me with the most precious knowledge to see success in my endeavor. My work bears the imprint of all those people, I am grateful to.

At this moment of accomplishment, first of all I would like to show my deep-felt gratitude to my dearest parents ***Venkateswara rao (Nanna), Nirmala (amma),*** *without whom I am nothing and to my sweet sister* ***Chandana*** *for her utmost encouragement and care for their guidance right from childhood who were always there for me in each and every stage of my life.*

I am short of words to express my love and deep sense of gratitude to my brother ***Ravi Chowdary*** *for supporting and guiding me to shape up my career.*

I take this opportunity to express my deep sincere gratitude, indebtedness and heart-felt thanks to my esteemed research guide, **Abdul Nasir Kurnool** Assistant Professor, Department of Pharmaceutics for his intellectual supervision, constant guidance, perpetual encouragement and constructive suggestions which helped me immensely throughout my project work.. His belief in my abilities and me is deeply treasured.

I owe my warmest and humble thanks to Dr. B. Ramesh , Dr. Mohammed Gulzar Ahmed, Vedamurthy Joshi, Kiran Kumar, Nagesh ,Senthil kumar and other faculty members of SAC college of Pharmacy, B.G NAGARA for their valuable help and guidance during the course of my research work.

Friends during a ray of sunshine to our lives

"There is nothing better than the encouragement of a good friend". I am really grateful to my friends Manjula, Kiran, Nayana, Suman, Ashwini, Guru, Ravi, Akshatha, Promod Dhakal, Suyash, Bullet Bro, Ankit, Chethana, Pavithra, Sachin, Amrit, Hansiya, Vinay who have been with me through these years during crucial juncture at B.G. Nagara.

I shall forever remember my friends Sreekanth, Siddhu, Mounika, Tittu, Madhukar, Nagaraj, for their inspiration, moral support, encouragement that helped me to grow and expand my thinking.

Thank you to one and all.....

Ms. JASTI HARIKA

CONTENTS

ABSTRACT

The current study concern with the preparation and evaluation of microspheres of naturally occurring gums in the view of effectiveness, biodegradable, easy of availability, cost effectiveness with Lamivudine as model drug. Lamivudine is an active anti-retroviral drug having biological half life of 4-6 hours and 86% bioavailability and licensed for the treatment of HIV and chronic Hepatitis B. Microspheres of Lamuvidine were prepared using xanthan gum and guar gum by solvent evaporation technique. Compatibility study was carried out by using FTIR at the range of 4000 to 400 cm^{-1} shows no significant change in the characteristic peaks of Lamuvidine and excipients in all the formulation, which indicates the compatibility of Lamuvidine with excipients. The prepared microspheres were analyzed for particle size, surface morphology, % yield, % drug entrapment efficiency, *in-vitro* drug release studies, *in-vitro* drug release kinetics and stability studies. Microspheres thus obtained were found to be pale yellow color and free flowing. Micromeritic studies of the prepared formulations are found within the prescribed limits and indicated good flow property. The Scanning Electron Microscopy (SEM) studies inferred the spherical shape and size range of 100µm to 200µm. *In-vitro* drug release shows decreases as concentration of xanthan gum increases. The release kinetics study revealed that the prepared microspheres were best fitted to the zero order and indicates that drug release from microspheres was diffusion-controlled and that the microspheres were stable. We conclude that, microspheres offer a practical and suitable approach to prepare controlled release of Lamuvidine with natural occurring xanthan gum as rate controlling agent to enhance bioavailability and reduction in dose frequency.

Keywords: Lamivudine; Xanthan gum; microspheres; Solvent evaporation; and oral controlled drug delivery.

LIST OF ABBREVIATIONS

ABBREVIATIONS	EXPANSIONS
R& D	Research and Development
NDDS	Novel drug delivery system
CDDS	Controlled drug delivery system
GIT	Gastro intestinal tract
pH	Negative logarithm of hydrogen ion concentration
AUC	Area under the curve
Vit	Vitamin
IgG	Immunoglobulin G
DNA	Deoxyribonucleic acid,
RNA	Ribonucleic acid
HIV	Human immunodeficiency virus
AIDS	Acquired immune deficiency syndrome
UNAIDS	United Nations programme on HIV and AIDS
α	Alpha
B	Beta
γ	Gamma
o/w	Oil in water
w/o	Water in oil
λ_{max}	Absorption maxima
%	Percentage
%CDR	Percentage cumulative drug release

°C	Degree Centigrade
µm	Micrometer
Hr	Hour
Hrs	Hours
Avg	Average
IP	Indian Pharmacopoeia
USP	United States of Pharmacopoeia
Cm	Centimeter
C_{max}	Maximum concentration
Conc	Concentration
CRDFs	Controlled release dosage forms
$C_{SS\ max}$	Steady state concentration
Cum	Cumulative
Nm	Nanometer
Ml	Milliliter
Wt	Weight
w/v	Weight by volume
w/w	Weight by weight
FTIR	Fourier transform infrared
G	Gram
Rpm	Revolutions per minute
UV	Ultraviolet
$t_{1/2}$	Half Life

Std	Standard
SEM	Scanning electron microscopy
HCl	Hydrochloric acid
ICH	International conference on harmonization
RH	Relative humidity
HPMC	Hydroxy propyl methyl cellulose
µg	Microgram
mg	Milligram
Min	Minute
Vs	Versus
MW	Molecular weight
N	Normality
No	Number

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Introduction

1. INTRODUCTION

New drug delivery technologies are revolutionizing the drug discovery, development and creating R&D focused pharmaceutical industries to increase the momentum of global advancements. In this view novel drug delivery systems (NDDS) have many benefits, which include improved therapy by increasing the efficacy and duration of drug activity, increased patient compliance through decreased dosing frequency and convenient routes of administration and improved site specific delivery to reduce unwanted adverse effects.[1]

Oral route is the most commonly employed route of drug administration. Although different route of administration are used for the delivery of drugs, oral route remain the preferred mode. The popularity of the oral route is attributed patient acceptance, ease of administration, accurate dosing, cost effective manufacturing method and generally improved shelf-life of the product.[2]

1.1. Controlled Drug Delivery[3]

Controlled drug delivery is one which delivers the drug at a predetermined rate, locally or systemically, for a specified period of time. Continuous oral delivery of drugs at predictable and reproducible kinetics for predetermined period throughout the course of GIT. Recently, a new generation of pharmaceutical products, called controlled release drug delivery systems, such as those developed from the osmotic pressure activated drug delivery system, have recently received regulatory approval for marketing, and their pharmaceutical superiority and clinical benefits over the sustained release and immediate release pharmaceutical products have been increased.

Advantages of controlled drug delivery system[4]

Controlled release drug delivery systems have received much attention in past two decades as they overcome the disadvantages of conventional therapy and offer some benefits like:

- Controlled administration of a therapeutic dose at the desired delivery rate.
- Constant blood levels of the drug, reduction of side effects.
- Minimization of dosing frequency.
- Enhancement of patient compliance.
- To obtain better therapeutic efficacy and diminished toxicity.
- Increased safety margin of high potency drugs due to control of plasma levels.
- Maximum utilization of drug enabling reduction in total amount of dose administered.
- Reductions in health care costs through improved therapy, shorter treatment period less frequency of dosing and reduction in personnel time to dispense, administer and monitor the patients.

Disadvantages of controlled drug delivery system

Decreased systemic availability in comparison to immediate release conventional dosage forms; this may be due to incomplete release, increased first pass metabolism, increased instability, insufficient residence time for complete release, site-specific absorption, pH dependent solubility.

- Poor *in vitro-in vivo* correlation.
- Possibility of dose dumping due to food.
- Retrieval of drug is difficult in case of toxicity, poisoning or hypersensitivity reactions.
- Reduced potential for dosage adjustment of drugs normally administered in varying strengths.
- Higher cost of formulation.

1.2. Oral Controlled Drug Delivery[5, 6]

Oral controlled – release drug delivery is thus a drug delivery system that provides the continuous oral delivery of drugs at predictable and reproducible kinetics for a predetermined

period throughout the course of GI transit.

In the exploration of oral controlled – release drug administration, one encounters three areas of potential challenges:

1. Development of a drug delivery system: To develop a viable oral controlled – release drug delivery system capable of delivering a drug at a therapeutically effective rate to a desirable site for duration required for optimal treatment.
2. Modulation of gastrointestinal transit time: To modulate the GI transit time so that the drug delivery system developed can be transported to a target site or to the vicinity of an absorption site and reside there for prolonged period of time to maximize the delivery of a drug dose.
3. Minimization of hepatic first pass metabolism: If the drug to be delivered is subjected to extensive hepatic first pass metabolism preventive measures should be devised to either bypass or minimize the extent of hepatic metabolic effect.

The controlled release systems for oral use are mostly solid and based on dissolution or diffusion or a combination of both the mechanisms in the control of release rate of drug. Depending upon the manner of drug release, they are classified as:

A. Continuous release system:

These systems release the drug for a prolonged period of time along the entire length of GI tract with normal transit of the dosage form. The various systems under this category are:

I. Dissolution controlled release systems

II. Diffusion controlled release systems

III. Dissolution and diffusion controlled release systems

IV. Ion-Exchange resins – drug complexes

V. Slow dissolving salts and complexes

VI. pH–dependent formulations

VII. Osmotic pressure controlled systems

VIII. Hydrodynamic pressure controlled systems

B. Delayed transit and continuous release system:

These systems are designed to prolong their residence in the GI tract along with their release. Often, the dosage form is fabricated to retain in the stomach and hence the drug present therein should be stable at gastric pH. Systems included in this category are:

I. Altered density systems

II. Muco-adhesive systems

III. Size-based systems

C. Delayed release systems:

The design of such system involves release of drug only at a specific site in the GI tract.

The drugs contained in such systems are:

I. Intestinal release systems

II. Colonic release systems

With recent advances in oral delivery technology, many more opportunities for treatment are available such as local therapy in the GI tract, delivery of macromolecules using muco adhesive compounds and permeation enhancers with site specific targeting. Absorption rates may be altered by using controlled release formulations to increase the residence time of a drug.

Advantages of oral controlled-release drug delivery systems:

Therapeutic advantage: Reduction in drug plasma level fluctuations; maintenance of a steady plasma level of the drug over a prolonged period, ideally simulating an intravenous infusion of a drug.

Reduction in adverse side effects and improvement in tolerability: Drug plasma levels are maintained within a narrow therapeutic window with no sharp peaks and with AUC of plasma concentration versus time comparable with total AUC from multiple dosing with immediate release dosage form.

Patient comfort and compliance: Oral drug delivery is the most common and convenient for patients, and a reduction in dosing frequency enhances compliance.

Reduction in health care cost: The total cost of the controlled release product could be lower than the immediate release product. With reduction in side effects the overall expense in disease management also would be reduced.

Table 1: Disadvantages of oral controlled-release drug delivery systems: [7, 8]

Disadvantage	Reason
Over dose	There is always possibility of sudden release of the total dose administered i.e. dose dumping, which may result in toxic manifestations.
Less flexibility in dose adjustments	The adjustment of dosage for controlled release dosage form is very difficult. The physician has less flexibility in adjusting the dosage regimens.
Side effects	Along with longer duration of action controlled release preparations shows long duration of side effects, especially if the patient is hypersensitive to the given medication.
Cost	The cost of unit dose of controlled therapeutic system is higher than the conventional dosage forms.

1.3. MICROENCAPSULATION[9]

Microencapsulation is a process by which solids, liquids or even gases may be enclosed in microscopic particles formation of thin coatings of wall material around the substances. There are

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

Applications of microencapsulation

The technology has been used widely in the design of controlles release and sustained release dosage forms.

- To mask the bitter taste of drugs like Paracetamols, Nitrofurantion etc.
- Many drugs have been microencapsulated to reduce gastric and other G.I tract irritations. Sustained release Aspirin preparations have been reported to cause significantly less G.I. bleeding than conventional preparations.
- A liquid can be converted to a pseudo-solid for easy handling and storage. E.g. Eprazinone.
- Hygroscopic properties of core material may be reduced by microencapsulation. E.g. Sodium chloride.
- Carbon tetra chlorides and a number of other substances have been microencapsulated to reduce their odor and volatility.
- Microencapsulation has been employed to provide protection to the core materials against atmospheric effects. e.g:Vit.A.Palmitate.
- Separation of incompatible has been achieved by encapsulation.

1.4. MICROSPHERES[10]

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000µm.They are made of polymeric, waxy or other protective materials that are biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. The solvents used to dissolve the polymeric materials are chosen according to the polymer and drug solubility, process safety and economic considerations. Microspheres are small

and have large surface-to-volume ratio. At the lower end of their size they have colloidal properties. The interfacial properties of microspheres are extremely important, oftens including their activity.

The potential use of microspheres in pharmaceutical industry

- The conversion of oils and other liquids to solids for ease of handling
- Taste and odour masking
- To delay the volatilization
- Separation of incompatible materials.
- Improvement of flow properties of powders
- Safe handling of toxic substances
- Improve the solubility of water insoluble substances by adding in dispersion of such material in aqueous media
- Production of sustained, controlled release and targeted medications.
- Reduce the dose dumping potential compared to large implantable devices.

ADVANTAGES[11]

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.

DISADVANTAGES

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.

APPLICATIONS

Pharmaceutical applications in drug delivery system

Ophthalmic Drug Delivery

Polymer exhibits favorable biological behavior such as bioadhesion, permeability-enhancing properties, and interesting physico-chemical characteristics, which make it a unique material for the design of ocular drug delivery vehicles. Due to their elastic properties, polymer hydro gels offer better acceptability, with respect to solid or semisolid formulation, for ophthalmic delivery such as suspensions or ointments, ophthalmic chitosan gels containing paclitaxels were obtained by casting method with high loading efficiencies and the chemical integrity of molecule was unaltered during preparation according to study.

Oral drug delivery

The potential of polymer films containing diazepam as an oral drug delivery was investigated in rabbits. The results indicated that a film composed of a 1:0.5 drug polymer mixture might be an effective dosage form that is equivalent to the commercial tablet dosage forms. The ability of polymer to form films may permit its use in the formulation of film dosage forms, as an

alternative to pharmaceutical tablets. The pH sensitivity, coupled with the reactivity of the primary amine groups, make polymer a unique polymer for oral drug delivery applications.

Nasal drug delivery

The nasal mucosa presents an ideal site for bioadhesive drug delivery systems. Polymer based drug delivery systems such as microspheres, liposomes and gels have been demonstrated to have good bioadhesive characteristics and swell easily when in contact with the nasal mucosa increasing the bioavailability and residence time of the drugs to the nasal route. Various polymer salts such as chitosan lactate, chitosan aspartate, and chitosan glutamate and chitosan hydrochloride are good candidates for nasal sustained release of vancomycin hydrochloride. Nasal administration of Diphtheria Toxoid incorporated into chitosan micro particles results in a protective systemic and local immune response against Diphtheria Toxoid with enhanced IgG production. Nasal formulations have induced significant serum IgG responses similar to secretory IgA levels, which are superior to parenteral administration of the vaccine. Nasal absorption of insulin after administration into polymer powder were found to be the most effective formulation for nasal drug delivery of insulin in sheep compared to chitosan nanoparticles and chitosan solution.

Gastrointestinal drug delivery:

Polymer granules having internal cavities prepared by de acidification when added to acidic and neutral media are found buoyant and provided a controlled release of the drug prednisolone. Floating hollow microcapsules of melatonin showed gastro retentive controlled-release delivery system. Release of the drug from these microcapsules is greatly retarded with release lasting for 1.75 to 6.7 hours in simulated gastric fluid. Most of the mucoadhesive microcapsules are retained

in the stomach for more than 10 hours e.g., Metoclopramide and glipizide loaded chitosan microspheres.

Transdermal drug delivery

Polymer has good film-forming properties. The drug release from the devices is affected by the membrane thickness and crosslinking of the film.Chitosan-alginate polyelectrolyte complex has been prepared in-situ in beads and microspheres for potential applications in packaging, controlled release systems and wound dressings. Polymer gel beads are a promising biocompatible and biodegradable vehicle for treatment of local inflammation for drugs like prednisolone which showed sustained release action improving therapeutic efficacy. The rate of drug release was found to be dependent on the type of membrane used. A combination of chitosan membrane and chitosan hydro gel containing lidocaine hydrochloride, a local anesthetic, is a good transparent system for controlled drug delivery and release kinetics.

Colonic drug delivery

Polymer has been used for the specific delivery of insulin to the colon. The chitosan capsules were coated with enteric coating (Hydroxy propyl methyl cellulose phthalate) and contained, apart from insulin, various additional absorption enhancer and enzyme inhibitor. It was found that capsules specifically disintegrated in the colonic region. It was suggested that this disintegration was due to either the lower pH in the ascending colon as compared to the terminal ileum or to the presence bacterial enzyme, which can degrade the polymer.

Per oral drug delivery

As polymer and most of its derivatives has a mucoadhesive property, a presystemic metabolism of peptides can be strongly reduced leading to a strongly improved bioavailability of many per orally given peptide drugs, such as insulin, calcitonin, and buserelin. Unmodified chitosan has a

permeation-enhancing effect for peptide drugs. A protective effect for polymer embedded peptides towards degradation by intestinal peptidases can be achieved by the immobilization of enzyme inhibitors on the polymer. The mucoadhesive property of polymer gel can be enhanced by threefold to sevenfold by admixing chitosan glyceryl mono-oleate. Drug release from the gel followed a matrix diffusion controlled mechanism.

Gene delivery

Gene delivery systems include viral vectors, cationic liposomes, poly cation complexes, and microencapsulated systems. Viral vectors are advantageous for gene delivery because they are highly efficient and have a wide range of cell targets. However, when used in vivo they cause immune responses and oncogenic effects. To overcome the limitations of viral vectors, non-viral delivery systems are considered for gene therapy. Non-viral delivery system has advantages such as ease of preparation, cell/tissue targeting, low immune response, unrestricted plasmid size, and large-scale reproducible production. Polymer has been used as a carrier of DNA for gene delivery applications.

Criteria for the Preparation of microspheres[12]

Preparation of microspheres should satisfy certain criteria:

1. The ability to incorporate reasonably high concentrations of the drug.
2. Stability of the preparation after synthesis with a clinically acceptable shelf life.
3. Controlled particle size and dispersability in aqueous vehicles for injection.
4. Release of active reagent with a good control over a wide time scale.
5. Biocompatibility with a controllable biodegradability and
6. Susceptibility to chemical modification.

1.4.1 TYPES OF MICROSPHERES[13]

Bioadhesive microspheres Adhesion can be defined as sticking of drug to the membrane by using the sticking property of the water soluble polymers. Adhesion of drug delivery device to the mucosal membrane such as buccal, ocular, rectal, nasal etc can be termed as bioadhesion. These kinds of microspheres exhibit a prolonged residence time at the site of application and causes intimate contact with the absorption site and produces better therapeutic action.

Magnetic microspheres This kind of delivery system is very much important which localizes the drug to the disease site. In this larger amount of freely circulating drug can be replaced by smaller amount of magnetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres are chitosan, dextran etc.The different types are

a) Therapeutic magnetic microspheres**:** Are used to deliver chemotherapeutic agent to liver tumour.Drugs like proteins and peptides can also be targeted through this system.

b) Diagnostic microspheres**:** Can be used for imaging liver metastases and also can be used to distinguish bowel loops from other abdominal structures by forming nano size particles supra magnetic iron oxides.

Floating microspheres In floating types the bulk density is less than the gastric fluid and so remains buoyant in stomach without affecting gastric emptying rate. The drug is released slowly at the desired rate, if the system is floating on gastric content and increases gastric residence and increases fluctuation in plasma concentration. Moreover it also reduces chances of striking and dose dumping. One another way it produces prolonged therapeutic effect and therefore reduces dosing frequencies. Drug (ketoprofen) given through this form.

Radioactive microspheres Radio emobilisation therapy microspheres sized10-30 nm are of larger than capillaries and gets tapped in first capillary bed when they come across. They are injected to the arteries that lead to tumour of interest. So all these condition sadcio active microspheres deliver high radiation dose to the targeted areas without damaging the normal surrounding tissues.9It differs from drug delivery system, as radio activity is not released from microspheres but acts from within a radioisotope typical distance and the different kinds of radioactive microspheres are α emitters, β emitters, γ emitters.

Polymeric microspheres The different types of polymeric microspheres can be classified as follows and they are biodegradable polymeric microspheres and Synthetic polymeric microspheres.

Biodegradable polymeric microspheres Natural polymers such as starch are used with the concept that they are biodegradable, biocompatible, and also bioadhesive in nature Biodegradable polymers prolongs the residence time when contact with mucous membrane due to its high degree of swelling property with aqueous medium , results gel formation. The rate and extent of drug release is controlled by concentration of polymers and the release pattern in a sustained manner. The main drawback is, in clinical use drug loading efficiency of biodegradable microspheres is complex and is difficult to control the drug release .However they provide wide range of application in microsphere based treatment.

Synthetic polymeric microspheres The interest of synthetic polymeric microspheres are widely used in clinical application, moreover that also used as bulking agent, fillers, embolic particles, drug delivery vehicles etc and proved to be safe and biocompatible. But the main disadvantages of these kind of microspheres are tend to migrate away from injection site and lead to potential risk, embolism and further organ damage.

Different methods of microspheres manufacturing are[11]

Spray Drying

In Spray Drying the polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, Acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporate instantaneously leading the formation of the microspheres in a size range 1-100μm. Micro particles are separated from the hot air by means of the cyclone separator while the trace of solvent is removed by vacuum drying. One of the major advantages of process is feasibility of operation under aseptic conditions this process is rapid and this leads to the formation of porous micro particles.

Solvent Evaporation

The processes are carried out in a liquid manufacturing vehicle. The microcapsule coating is dispersed in a volatile solvent which is immiscible with the liquid manufacturing vehicle phase. A core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation the core material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size microcapsule. The mixture is then heated if necessary to evaporate the solvent for the polymer of the core material is disperse in the polymer solution, polymer shrinks around the core. If the core material is dissolved in the coating polymer solution, matrix – type microcapsules are formed. The core materials may be either water soluble or water in soluble materials. Solvent evaporation involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous.

Wet Inversion Technique

Chitosan solution in acetic acid was dropped in to an aqueous solution of counter ion sodium tripolyposphate through a nozzle. Microspheres formed were allowed to stand for 1 hr and cross linked with 5% ethylene glycol diglysidyl ether. Microspheres were then washed and freeze dried. Changing the pH of the coagulation medium could modify the pore structure of Chitosan solution microspheres.

Hot Melt Microencapsulation

The polymer is first melted and then mixed with solid particles of the drug that have been sieved to less than 50 μm. The mixture is suspended in a non-miscible solvent (like silicone oil), continuously stirred, and heated to 5°C above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microspheres are washed by decantation with petroleum ether. The primary objective for developing this method is to develop a microencapsulation process suitable for the water labile polymers, *e.g.* poly anhydrides. Microspheres with diameter of 1- 1000 μm can be obtained and the size distribution can be easily controlled by altering the stirring rate. The only disadvantage of this method is moderate temperature to which the drug is exposed.

Polymerization techniques

The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as:

a) Normal polymerization

b) Interfacial polymerization.

Both are carried out in liquid phase.

Normal polymerization

It is carried out using different techniques as bulk, suspension, precipitation, emulsion and micellar polymerization processes. In bulk, a monomer or a mixture of monomers along with the initiator or catalyst is usually heated to initiate polymerization. Polymer so obtained may be moulded as microspheres. Drug loading may be done during the process of polymerization. Suspension polymerization also referred as bead or pearl polymerization. Here it is carried out by heating the monomer or mixture of monomers as droplets dispersion in a continuous aqueous phase. The droplets may also contain an initiator and other additives. Emulsion polymerization differs from suspension polymerization as due to the presence initiator in the aqueous phase, which later on diffuses to the surface of micelles. Bulk polymerization has an advantage of formation of pure polymers.

Interfacial polymerization

It involves the reaction of various monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase. This process is based on the principle of decreasing the solubility of the polymer in organic phase to affect the formation of polymer rich phase called the coacervates. In this method, the drug particles are dispersed in a solution of the polymer and an incompatible polymer is added to the system which makes first polymer to phase separate and engulf the drug particles. Addition of non-solvent results in the solidification of polymer. Poly lactic acid (PLA) microspheres have been prepared by this method by using butadiene as incompatible polymer. The process variables are very important since the rate of achieving the coacervates determines the distribution of the polymer film, the particle size and agglomeration of the formed particles. The agglomeration must be avoided by stirring the suspension using a suitable speed stirrer since as the process of

microspheres formation begins the formed polymerize globules start to stick and form the agglomerates. Therefore the process variables are critical as they control the kinetic of the formed particles since there is no defined state of equilibrium attainment.

Solvent extraction

Solvent evaporation method is used for the preparation of microparticles, involves removal of the organic phase by extraction of the organic solvent. The method involves water miscible organic solvents such as isopropanol. Organic phase is removed by extraction with water. This process decreases the hardening time for then microspheres. One variation of the process involve direct addition of the drug or protein to polymer organic solution. The rate of solvent removal by extraction method depends on the temperature of water, ratio of emulsion volume to the water and the solubility profile of the polymer.

Preparation of Microspheres by Thermal cross-linking

Citric acid, as a cross-linking agent was added to 30 ml of an aqueous acetic acid solution of chitosan (2.5% wt/vol) maintaining a constant molar ratio between chitosan and citric acid(6.90× 10−3 mol chitosan: 1 mol citric acid). The chitosan cross-linker solution was cooled to 0°C and then added to 25 ml of corn oil previously maintained at 0°C, with stirring for 2 minutes. This emulsion was then added to 175 ml of corn oil maintained at 120°C, and cross linking was performed in a glass beaker under vigorous stirring (1000 rpm) for 40 minutes. The microspheres obtained were filtered and then washed with diethyl ether, dried and sieved.

Preparation of Microspheres by Glutaraldehyde cross linking method

A 2.5% (w/v) chitosan solution in aqueous acetic acid was prepared. This dispersed phase was added to continuous phase (125 ml) consisting of light liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing 0.5% (wt/vol) Span 85 to form a water in oil (w/o) emulsion.

Stirring was continued at 2000 rpm using a 3- blade propeller stirrer). A drop-by-drop solution of a measured quantity (2.5 ml each) of aqueous gluteraldehyde (25%v/v) was added at 15, 30, 45, and 60 minutes. Stirring was continued for 2.5 hours and separated by filtration under vacuum and washed, first with petroleum ether (60°C- 80°C) and then with distilled water to remove the adhered liquid paraffin and gluteraldehyde, respectively. The microspheres were then finally dried in vacuum desiccators.

1.4.2. POLYMERS USED IN MICROSPHERES[14]:

A number of different substances both biodegradable as well as non-biodegradable have been investigated for the preparation of microspheres. These materials include the polymers of natural and synthetic origin and also modified natural substances. Synthetic polymers employed as carrier materials are methyl methacrylate, acrolein, lactide, glycolide and their copolymers, ethylene vinyl acetate copolymer, polyanhydrides, etc. The natural polymers used for the purpose are albumin, gelatin, starch, collagen and carrageenan.

Classification of polymers

A) Synthetic Polymers: divided into two types;

1) Non-biodegradable- Acrolein, Glycidyl methacrylate, Epoxy polymers, etc.

2) Biodegradable- Polyanhydrides, Polyalkyl cyano acryalates Lactides and glycolides and their copolymers.

B) Natural materials: Obtained from different sources like proteins, carbohydrates and chemically modified carbohydrates.

i) Proteins (albumin, gelatin, collagen)

ii) Carbohydrate (starch, agarose, carrageenan)

iii) Chemically modified carbohydrates [poly (acryl dextran), Poly (acryl starch)]

Natural Gums[15]

Natural gums (gums obtained from plants) are hydrophilic carbohydrate polymers of high molecular weights, generally composed of monosaccharide units joined by glucocidic bonds. They are generally insoluble in oils or organic solvents such as hydrocarbons, ether, or alcohols. Gums are either water soluble or absorb water and swell up or disperse in cold water to give a viscous solution or jelly. On hydrolysis they yield arabinose, galactose, mannose and glucuronic acid. Natural gums including acacia, ghatti, karaya, locust bean, albizia, khaya,guar,tragacanth and xanthan, are obtained as exudates or extractives from the bark of stems, branches and roots of various plants. The plant based polymers have been studied for their application in different pharmaceutical dosage forms like matrix controlled system, film coating agents, buccal films, microspheres, nanoparticles, viscous liquid formulations like ophthalmic solutions, suspensions, implants and their applicability and efficacy has been proven . These have also been utilized as viscosity enhancers, stabilisers, disintegrants, solubilisers, emulsifiers, suspending agents, gelling agents, bioadhesives & binders.

Advantages of natural gums in pharmaceutical sciences[16]

The following are a number of the advantages of natural plant–based materials.

Biodegradable:- Naturally available biodegradable polymers are produced by all living organisms. They represent truly renewable source and they have no adverse impact on humans or environmental health (*e.g.*, skin and eye irritation).

Biocompatible and non-toxic:- Chemically, nearly all of these plant materials are carbohydrates composed of repeating sugar (monosaccharides) units. Hence, they are non- toxic.

Low cost:- It is always cheaper to use natural sources.The production cost is also much lower compared with that for synthetic material. India and many developing countries are dependent on agriculture.

Environmental:-Friendly processing- Gums and mucilages from different sources are easily collected in different seasons in large quantities due to the simple production processes involved.

Local availability:- (especially in developing countries) -In developing countries, governments promote the production of plant like guar gum and tragacanth because of the wide applications in a variety of industries.

Disadvantages of natural gums:

Microbial contamination:-The equilibrium moisture content present in the gums and mucilages is normally 10% or more and, structurally, they are carbohydrates and, during production, they are exposed to the external environment and, so there is a chance of microbial contamination. However, this can be prevented by proper handling and the use of preservatives.

Batch to batch variation:-Synthetic manufacturing is a controlled procedure with fixed quantities of ingredients, while the production of gums and mucilages is dependent on environmental and seasonal factors.

Uncontrolled rate of hydration:-Due to differences in the collection of natural materials at different times, as well as differences in region, species, and climate conditions the percentage of chemical constituents present in a given material may vary. There is a need to develop suitable monographs on available gums.

Reduced viscosity on storage:-Normally, when gums and mucilages come into contact with water there is an increase in the viscosity of the formulations. Due to the complex nature of gums

and mucilages (monosaccharides to polysaccharides and their derivatives), it has been found that after storage there is reduced in viscosity.

Classification of gums:

A] According to the charge:

Non-ionic seed gums: guar, locust bean, tamarind, xanthan, amylose, arabinans, cellulose, galactomannans.

Anionic gums: arabic, karaya, tragacant, gellan, agar, algin, carrageenans, pectic acid.

B] According to the source:

Marine origin/algal (seaweed) gums: agar, carrageenans, alginic acid, laminarin.

Plant origin:

(1) shrubs/tree exudates— gum arabica, gum ghatti, gum karaya, gum tragacanth,khaya and albizia gums;

(2) Seed gums—guar gum, locust bean gum, starch, amylose, cellulose;

(3) Extracts— pectin, larch gum;

(4) Tuber and roots—potato starch.

Animal origin: chitin and chitosan, chondroitin sulfate, hyaluronic acid.

Microbial origin (bacterial and fungal): xanthan, dextran, curdian, pullulan, zanflo, emulsan, Baker's yeast glycan, schizophyllan, lentinan, krestin, scleroglucan.

C] Semi-synthetic

Starch derivatives—hetastarch, starch acetate, starch phosphates.

Cellulose derivatives- Carboxy methyl cellulose(CMC), Hydroxy ethyl cellulose,Hydroxy propyl methyl cellulose (HPMC), Methyl cellulose (MC), Microcrystalline cellulose(MCC).

D] According to shape

Linear: algins, amylose, cellulose, pectins.

Branched:

(1) short branches—xanthan, xylan, galactomanan;

(2) branch-on-branch—amylopectin, gum arabic, tragacanth.

1.5. Viruses[17]

Viruses are small (commonly range from 20 to 30 nm) obligate intracellular parasites consist of either double or single-stranded DNA or RNA enclosed in a protein coat called capsid.

Viral DNA enters into the host cell nucleus; further transcription of this viral DNA occurs into mRNA with the help of host cell RNA polymerase followed by translation of the mRNA into virus-specific proteins. The proteins formed contain some enzymes which aids in synthesizing more viral DNA as well as proteins of coat and envelope. On complete assembly of coat proteins around the viral DNA, virions are released by budding or after cell lysis.

The retrovirus virion consists of reverse transcriptase. This reverse transcriptase makes a DNA copy of the viral RNA. The DNA copy is then integrated into the genome of the host cell, which is termed as provirus and is transcribed into both new genomic RNA and mRNA for translation into viral proteins. The formed viruses are released by budding. HIV is an RNA retrovirus. Some of the RNA retroviruses can also transform normal cells into malignant cells.

According to Baltimore classification system, viruses are classified into families depending on their type of genome and their method of replication.

Table 2: Classification of viruses on the basis of their method of replication

Class	Method of replication	Example
I	double- stranded DNA viruses	Adenoviruses, Herpesviruses, Poxviruses
II	single-stranded DNA viruses	Parvoviruses
III	double- stranded RNA viruses	Reoviruses
IV	single-stranded positive sense RNA viruses	Picornaviruses, Togaviruses
V	single-stranded negative sense RNA viruses	Orthomyxoviruses, Rhabdoviruses
VI	positive-sense single-stranded RNA viruses that replicate through a DNA intermediate	Retroviruses
VII	double-stranded DNA viruses that replicate through a single stranded RNA intermediate	Hepadnaviruses

1.5. Introduction of HIV[18, 19]:

Human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS), commonly referred to as HIV/AIDS, constitute one of the most serious infectious

disease challenges to public health globally. AIDS is considered to be an epidemic according to estimates from the UNAIDS/WHO AIDS Epidemic Update, July 2008. Globally, there were an estimated 33 million people living with HIV in 2007. Moreover, in Sub-Saharan Africa remains most heavily affected by HIV, accounting for 67% of all people living with HIV and for 72% of AIDS deaths in 2007 and globally the percentage of women among people living with HIV has remained stable at 50% for several years. HIV-1 is the globally common infection while HIV-2 is more prevalent in West Africa, and takes a longer time to develop into immunodeficiency from infection than HIV-1. HIV infection in the human body results mainly from integration of the viral genome into the host cell for the purpose of cell replication, and AIDS is the advanced stage of the disease caused by HIV infection. The end stage of the disease may be characterized by a spectrum of diseases opportunistic infections (such as Pnuemocystis carinii and Mycobacterium tuberculosis), dementia and cancer. Interestingly, HIV has been referred to as a ‘‘master regulator” of cellular gene expression as a means to augment expression of its own genome. The development of drugs for HIV infection has undergone substantial progress, currently various drugs are used as anti retroviral therapy and has contributed significantly to improved patient/disease management.

When HIV infects a cell, a viral enzyme, reverse transcriptase copies the viral single stranded RNA genome into a double-stranded viral DNA. The viral DNA is then integrated into the host chromosomal DNA, which then allows host cellular processes, such as transcription and translation to reproduce the virus. Reverse Transcriptase Inhibitors blocks the reverse transcriptase's enzymatic function and prevent completion of synthesis of the double-stranded viral DNA, thus preventing HIV from multiplying**.**

1.5.1. Life cycle of HIV[20]:

HIV infection is diagnosed by the presence of antibodies to HIV in the plasma. Various serological tests such as ELISA (Enzyme Linked Immune Sorbent Assay), Orasure western blot, SUDS (single used diagnostic system), Orasure HIV-118 are used for the diagnostic purpose. The U.S Center for Disease Control and Prevention (CDC) defines the signs or symptoms of AIDS. People are diagnosed with AIDS when they show certain systems defined.

The CDC's definition of AIDS includes:

- CD4+ T cell count less than 200 per cubic mm of blood compared with about 1,000 CD4+ T cells (healthy people).
- CD4+ T cells count lees than 14% of all lymphocytes.

Recommendations of CDC include of testing of CD4+T cell count for every three to six months in all HIV-infected persons, though the need may vary by patient to patient.

The steps of the HIV life cycle:

- HIV Binds to a specific type of CD4 receptor and to co-receptors present on the surface of a CD4+ T lymphocyte.
- Once binding over Virus can fuse with the host cell (CD4 cell) and release its genetic material into the host cell.
- The next step is reverse transcription of the genetic material of the virus by special enzyme reverse transcriptase and its integration into the host DNA by HIV enzyme integrase.
- Once integrated virus begins to produce new viral RNA and proteins, turning the cell into a HIV reservoir.
- This production leads to assembly, budding, and maturation, by which the new HIV particles are packaged and migrate out to infect new cells.

Each step in the life cycle of retroviral infection can be used as a potential target for antiviral therapy.

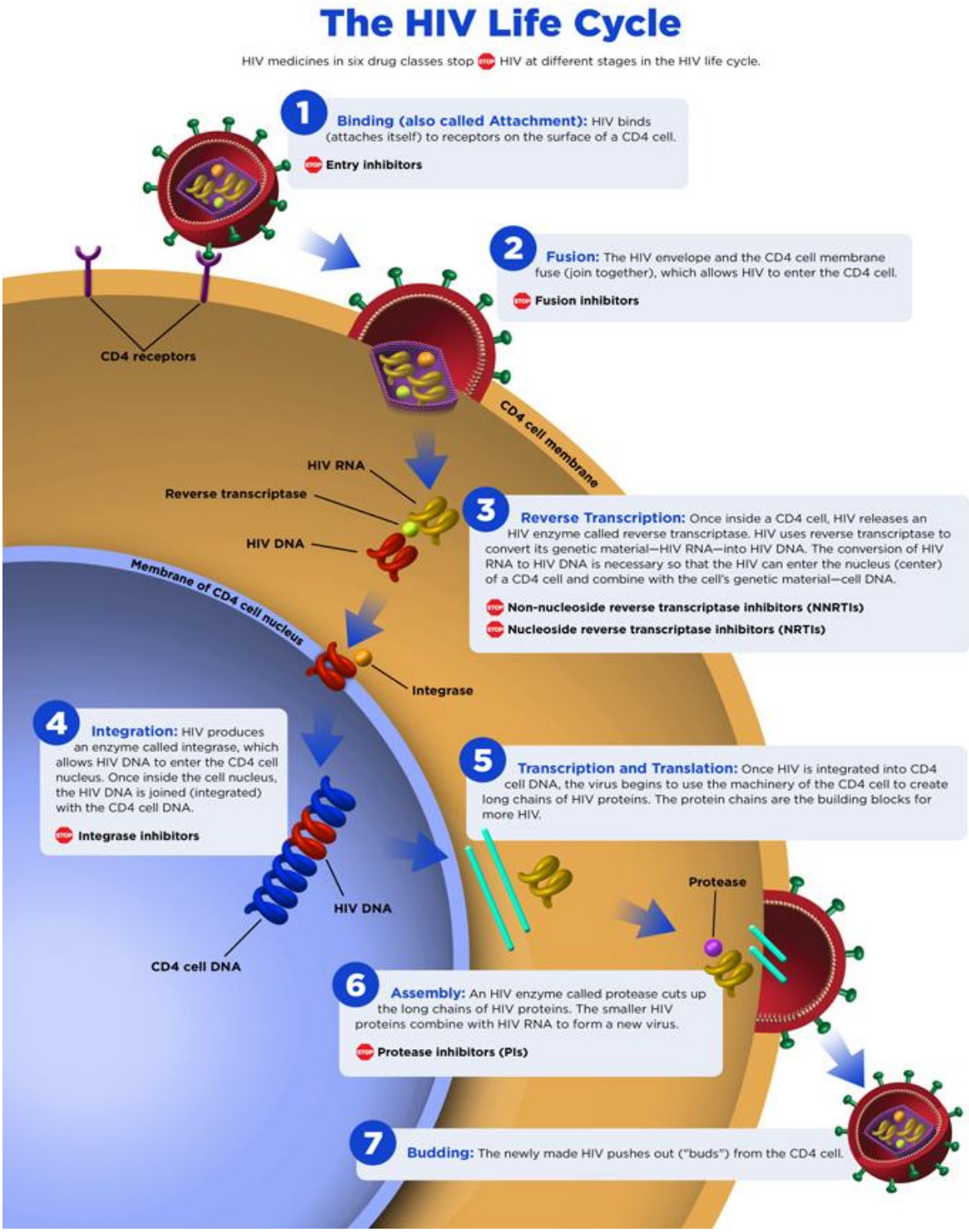


Figure 1: Schematic Diagram of HIV Life cycle[21]:

1.6. Anti Viral Agents[22]:

Antiviral drugs are a class of medication used specifically for the treating viral infections. Like antibiotics for bacteria, specific antivirals are used for specific viruses. Unlike most antibiotics, antiviral drugs do not destroy their target pathogen; instead they inhibit their development. Most of the antiviral drugs now available are designed to help deal with HIV, herpes viruses, the hepatitis B and C viruses, and influenza A and B viruses.

The emergence of antivirals is the product of a greatly expanded knowledge of the genetic and molecular function of organisms, allowing biomedical researchers to understand the structure and function of viruses, major advances in the techniques for finding new drugs, and the intense pressure placed on the medical profession to deal with the human immunodeficiency virus (HIV), the cause of the deadly acquired immunodeficiency syndrome (AIDS) pandemic.

The first experimental antivirals were developed in the 1960s, mostly to deal with herpes viruses, and were found using traditional trial-and-error drug discovery methods. Researchers grew cultures of cells and infected them with the target virus. They then introduced into the cultures chemicals which they thought might inhibit viral activity, and observed whether the level of virus in the cultures rose or fell. Chemicals that seemed to have an effect were selected for closer study.

CLASSIFICATION OF ANTI VIRAL DRUGS[23]:

1. **Anti-Herpes virus**

   Idoxuridine, Acyclovir, Valacyclovir, Famciclovir, Ganciclovir, Foscarnet

2. **Anti-Retrovirus**

   (a) Nucleoside reverse transcriptase inhibitors (NRTIs)

   Zidovudine (AZT), Didanosine, Zalcitabine, Stavudine, Lamivudine, Abacavir.

(b) Nonnucleoside reverse transcriptase inhibitors (NNRTIs)

Nevirapine, Efavirenz, Delaviridine.

(C) Protease inhibitors

Ritonavir, Indinavir,Nelfinavir, Saquinavir, Amprenavir,Lopinavir.

3. Anti-Influenza virus

Amantadine, Rimantadine

4. Nonselective antiviral drugs

Ribavirin, Adefovir dipivoxil,Interferon alpha.

Chapter 2

2. OBJECTIVES

Lamivudine is a Nucleoside Reverse Transcriptase Inhibitor (NRTI), used for the treatment of HIV and chronic Hepatitis B. Long-term AIDS therapy with the conventional tablets of Lamivudine found to have some drawbacks, accumulation of drug in multi-dose long-term therapy, poor patient compliance and high cost. Designing of controlled and sustained release once-daily formulations of Lamivudine can overcome these problems and maintaining of systemic drug levels consistently above its target antiretroviral concentration throughout the course of the treatment. Controlled release matrix tablets, floating tablets, nanoparticles, microparticles, liposomes, and niosomes; which may possibly suitable for the controlled and/or sustained release of Lamivudine useful in developing the more effective AIDS therapy with very less or no adverse side effects.

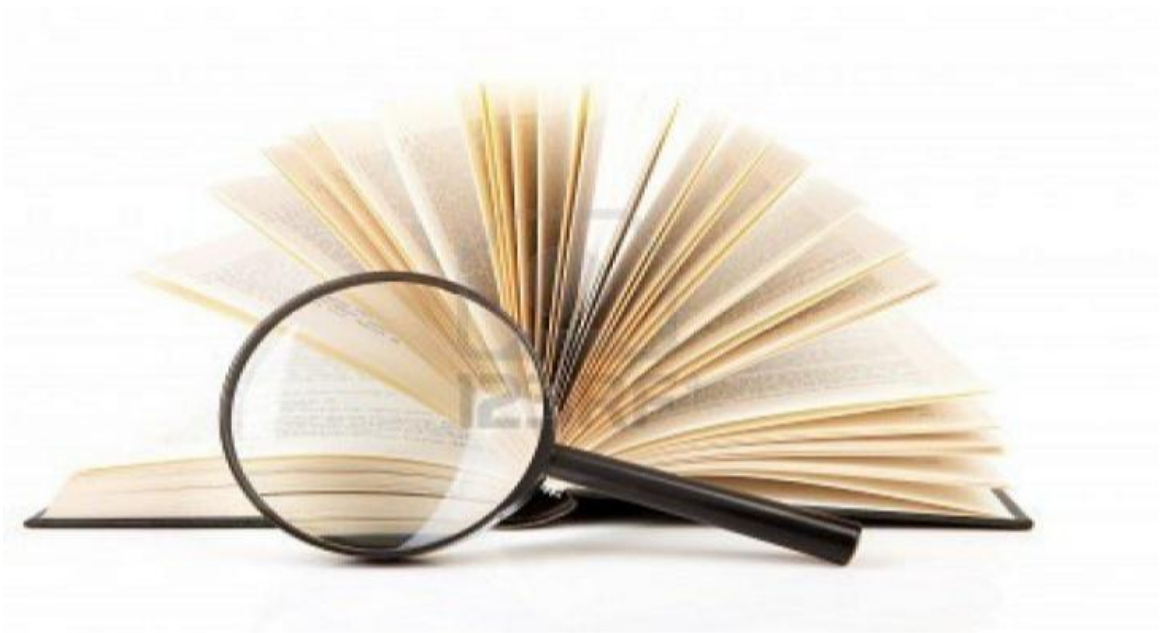
For the treatment of AIDS, the dosage of conventional oral formulations of Lamivudine is 300mg per day (i.e. 150 mg twice daily, multiple times a day) with an absolute bioavailability of 86% ± 16%, peak serum concentration of Lamivudine (C*max*) of 1.5 ± 0.5 mcg/ml and mean elimination half-life (t½) of 4 to 6 hours, thus necessitating frequent.

Keeping the above fact in view the present work is aimed to prepare different formulations of Lamivudine microspheres using different polymers. The composition of these formulations was selected by using optimization technique. Effect of various factors like drug to polymer ratio, polymer to polymer ratio and polymer grade on the response parameters.

Objectives of the study:

- To perform pre-formulation studies on drug and polymers and to establish their compatibility in formulation using FTIR.
- Determination of λ_{max} for Lamivudine.
- To prepare microspheres of Lamivudine using Xanthan gum and guar gum using gluteraldehyde as cross-linking agent by using solvent evaporation technique.
- Evaluation of the formulation consists of
    - Physical characterization of microspheres which includes
        - Particle size analysis.
        - Determination of particle shape and surface morphology.
    - Percentage yield.
    - Drug loading.
    - Drug entrapment efficiency.
    - *In-vitro* drug release studies.
    - *In-vitro* drug release kinetics.
    - To carry out the accelerated stability studies on selected formulations.

Chapter 3



Literature Review

3. REVIEW OF LITERATURE

Yashavantsinh Chavda et al., [24] have formulated and evaluated Lamivudine microspheres using a combination of Eudragit RS 100 and Eudragit RL 100 polymers by solvent evaporation method. The parameters determined were bulk density, tapped density, angle of repose, particle size, drug content, % drug encapsulation efficiency & in vitro dissolution. *In-Vitro* drug release was carried out using phosphate buffer pH (6.8). Larger microspheres showed greater drug loading and smaller microspheres showed a faster drug release.SEM study shows that particles made of Eudragit RL100 and RS100 were spherical and not aggregated. As the concentration of Eudragit RL 100 increases the release rate of drug also increases. F3 batch shows that the cumulative % drug release (95%) was higher than the other batches. The effect of polymer ratio on % drug encapsulation efficiency was investigated using 3^2 full factorial designs. Release profile followed Higuchi model (R2=0.924). It appears that mechanism of drug release from microspheres was diffusion controlled.

Aliya Parveen et al., [25] have designed and evaluated mucoadhesive microspheres of Lamivudine by using Ionic gelation technique using sodium alginate, guar gum, Sodium CMC and HPMC. Percentage drug content, Entrapment efficiency and *in-vitro* dissolution studies were also carried out. *In-vitro* Dissolution Study was carried out by using phosphate buffer pH (6.8). The surface morphology of Lamivudine microspheres were discrete, uniform and spherical with a smooth textural surface by using SEM analysis. The results indicate that the drug released from formulation (F6) is in the range of 84.31±0.12% to 85.11±0.11 up to 12 hours. Sodium CMC showed higher mucoadhesion and degree of swelling than other

polymers. Therefore, M3C3 formulation may be used for reducing the dosing frequency thereby improving the effectiveness of the drug.

Kumar Darapu B.N et al., [26] have formulated and evaluated floating microspheres of Ranitidine hydrochloride with Xanthan gum and Eudragit polymers by solvent evaporation. The formulations were evaluated by FTIR, drug loading, % entrapment, particle size, SEM, drug release kinetics. Ranitidine HCl has 50% bioavailability, low half life of 2.2 hours, exhibits poor bioavailability when given in conventional dosage form due to degradation in lower GIT. This may be attributed to the acrylic polymer property of Eudragit S 100 which gave lower release and hydrophilic nature of HPMC showed higher release.The dissolution rates of HPMC microspheres batches were higher than Xanthan gum and Eudragit S 100 batches. It was found that with increase in polymer ratio there was an increase in the particle size range and due to lower density of microspheres buoyancy was 80% till 12 hours for both the polymers. The release kinetics of Ranitidine HCl microspheres followed super case II transport diffusion.

Anand Kumar M.A et al., [27] formulated Lamivudine microspheres by w/o/w multiple emulsion solvent evaporation technique using osmogen like sodium chloride and polymers like ethyl cellulose, cellulose acetate, and poly vinyl alcohol as a continuous phase. FTIR studies indicated that there was no drug polymer interaction. Morphological characterization of the microcapsules was carried out by using scanning electron microscopy and shows that the microspheres prepared with ethyl cellulose had a formed smooth surface and spherical. *In -vitro* dissolution studies were performed using USP type I dissolution apparatus using phosphate buffer pH 7.4. The release kinetics data and characterization studies indicate that drug release from microspheres was diffusion-controlled and that the microspheres were

stable. The encapsulation, drug loading and desired release was achieved when increasing the concentration of polymer. It can be concluded that microspheres of Lamivudine formulation F – IV prepared by Ethyl cellulose polymer in the ratio of 1:0.9 shows desirable stability, prolonged drug release following zero order .

Brahmaiah.B et al., [28] have prepared and evaluated mucoadhesive microspheres of Simvastin by Orifice-ionic gelation method using polymers such as HPMC (K100M), carbopol 940p, Sodium CMC, guar gum, sodium alginate, ethyl cellulose, methyl cellulose, and Xanthan gum. The microspheres were characterized for drug content, mucoadhesive property by *in- vitro* wash-off test and in-vitro drug release. The microspheres were smooth and elegant in appearance showed no visible cracks as confirmed by SEM and FT-IR studies indicated the lack of drug-polymer interactions in the ideal formulation. The formulation F10 was selected as an ideal formulation based on the in vitro release profile which shows an extended drug release of 97.11%. Result of in vitro wash off test studies indicate that the formulation F10, F13, F14, and F15 having considerable mucoadhesive property. Among all the fabricated formulation, F10 was chosen as an ideal formulation showing an extended drug release over a period of 8h (97.11%) and mucoadhesive property (78%). Drug release was diffusion controlled and followed Higuchi kinetics.

Abdur Rouf Al Mamun Md et al., [29] have developed glipizide microspheres with natural gums using guar gum and xanthan gum by orifice ionic gelation technique and they were characterized by scanning electron microscopy and particle size analysis. Microspheres were formulated successfully with good spherecity except formula F5 and F6, which contained the highest amount of gum. They were very irregular in shape and their sizes were over 1000 μm. The performed tests indicated that formulations, containing lower amount of gum, had

smaller particle size than the formulations that contained higher amount of gum. The SEM images illustrated that guar gum-containing microspheres were slightly more spherical than xanthan gum containing microspheres. Sphericity decreased with increasing amount of gum. In case of guar gum, microspheres had smoother surface with lower amount of gum. On the other hand, xanthan gum-containing formulations showed better smoothness in increased amount of gum .Among six formulations, four formulations were found to be spherical and free flowing. F1 and F4 possessed the best results among all the formulations in terms of surface smoothness and shape.

Patil PB et al., [30] prepared and evaluated mucoadhesive microspheres of atenolol and propranolol by solvent diffusion and an interpolymer complexation poly acrylic acid (PAA) with polyvinylpyrrolidone (PVP) to increase gastric residence time. A mixture of ethanol/water was used as the internal phase, corn oil was used as the external phase of emulsion, and span80 was used as the surfactant. The results shows that complexation between poly acrylic acid and polyvinylpyrrolidone as a result of hydrogen bonding was confirmed by the shift in the carbonyl absorption bands of poly acrylic acid using FTIR. The mean particle size increased with the increase in polymer concentration. The release rate of atenolol from the complex microspheres was slower than the PVP microspheres at pH 2.0 and 6.8.

Subbiah Ganesh et al., [31] have characterized Idarubicin microspheres using ethyl cellulose and HPMC by solvent evaporation technique. Proportions of ethyl cellulose and HPMC were subjected to measurement of morphology, mean particle size, particle size distribution, percentage drug entrapment, drug loading and drug release (*in-vitro*). *In –vitro* release of the prepared microspheres were performed in phosphate buffer pH 7.8 over a period of 12 Hrs.

The release of idarubicin increased with increase in ethyl cellulose. By increasing the concentration of ethyl cellulose the mean particle size also increased. The entrapment efficiency of idarubicin microsphere prepared was shown to be approximately 65 %. The percentage yield of various microspheres was found to fall between 54 % and 62 %. The *in - vitro* release showed the highest regression coefficient values for Highchi's model, indicating discussion to be the predominant mechanism of drug release. Diffusion was found to be the main release mechanism.

Priyadarshini M.K et al., [32] formulated and evaluated Zidovudine microspheres by using chitosan as a polymer were prepared by ionic gelation method. The formulations were evaluated by FTIR, drug loading, % entrapment, particle size, SEM, drug release kinetics. The microspheres were smooth and elegant in appearance showed no visible cracks as confirmed by SEM and FT-IR studies indicated the lack of drug-polymer interactions in the ideal formulation. The *in-vitro* drug release studies were carried out by using phosphate buffer pH (7.4). The formed microspheres showed prolonged in vitro drug release. And follows drug release by Higuchi model. Formulation F4 showed high entrapment efficiency (87 %), particle size (95 μm) and drug release (67.69 %) over 24 hrs. Hence it was considered to be good microsphere formulation with greater bioavailability and lesser side effects.

J.s.Patil et al., [33] have formulated and evaluated Zidovudine microspheres by spray drying technique using olibanum gum resin as a rate controlling polymer. The FTIR and DSC study confirmed that no chemical interaction took place during entrapment process. The X-ray diffraction study indicates the amorphous dispersion of the drug after entrapment into microspheres. The resin-coated microspheres were spherical, discrete, free flowing and

multinucleate monolithic type. Zidovudine release from the resin coated microspheres was slow over 24hr and dependant on core: coat ratio. Entrapment efficiency was in the range of 67.41 to 80.32%.The cumulative in-vitro release studies of different formulations were performed in phosphate buffer pH (7.4).The cumulative release of Zidovudine significantly decreased with increasing polymer concentration Drug release from the microspheres was by non-fickian diffusion mechanism.

Kesari Asha et al., [34] formulated and evaluated Zidovudine loaded chitosan microspheres by emulsification method using gluteraldehyde as crosslinking agent. The prepared microspheres were characterized for FTIR, X-ray powder diffractometry and Scanning electron microscopy. The infrared spectra showed stable character of Zidovudine in the drug loaded microspheres and revealed the absence of drug polymer interactions.SEM study revealed that the microspheres were spherical and porous in nature. The *in-vitro* release studies were performed in pH(7.4) Phosphate buffer. The drug loaded microspheres showed 72-94% of entrapment and release was extended upto 12hrs. From the *in-vitro* dissolution study of pure drug and optimized formulation it was observed that pure drug (zidovudine) was released at faster rate. About 97% drug was released within 6 minutes but when encapsulated in chitosan microspheres, 96% drug was released from the formulation in 12 hrs indicating controlled release of the drug from the optimized formulation.

Santhosh Kumar Mankala et al.,[35] prepared and characterized mucoadhesive microcapsules of gliclazide with natural gums by orifice-ionic gelation and emulsification ionic gelation techniques. Formulations were then evaluated for surface morphology, particle shape, Carr's index, microencapsulation efficiency, drug release, mucoadhesion studies. Compatibility studies were performed by FTIR, DSC, and XRD techniques and no interactions were found

between drug and excipients used. The microspheres were found spherical and free flowing with emulsion ionic gelation technique with a size range 400-600μm. All microspheres showed good mucoadhesive property in in-vitro wash of test. *In-vitro* drug release studies showed that the guar gum has more potentiality to retard the drug release compared to other gums and concentrations. Microspheres containing Xanthan gum showed good mucoadhesion and rate was found faster at gastric pH than at intestinal pH indicating good mucoadhesive property in intestinal pH. Drug release from the microspheres was found slow following zero order release kinetics with non-fickian release mechanism stating release.

K Prakash et al., [36] prepared and characterized Lamivudine microcapsules using various cellulose polymers by solvent evaporation technique. The prepared microcapsules were characterized for the percent drug content, entrapment efficiency, FTIR, DSC, scanning electron microscopy (SEM) and in vitro dissolution studies. FTIR and DSC thermograms showed the stable character of Lamivudine in the microcapsules.The microcapsules were spherical and free flowing revealed that the microcapsules were porous in nature. *In vitro* dissolution studies were performed using USP type I dissolution apparatus using phosphate buffer pH 7.4.The release of drug from the microcapsules extended up to 8 to 12 hours. The release kinetics data and characteristion studies indicate that drug release from microcapsules was diffusion – controlled and that the micro capsules were stable.

T.Sudhamani et al., [37] prepared and evaluated ethyl cellulose microspheres of ibuprofen by solvent evaporation technique. The prepared microspheres were subjected to various evaluation and *in-vitro* release studies. The prepared microspheres had good spherical geometry with smooth surface as evidence by SEM. The *in-vitro* release studies showed that ibuprofen microspheres of 1:2 ratios showed better sustained effect over a period of 8 hours.

The average particle size of ibuprofen loaded microspheres was found to be in the range of 224 to 361μm. The particle size of a microsphere was determined by optical microscopy and all the batches of microspheres show uniform size distribution. The *in-vitro* dissolution studies showed that ibuprofen microspheres formulation F3 showed better sustained effect (93%) over a period of 8 hours than other formulations. Highest percentage of loading was obtained by increasing the amount of ibuprofen with respect to polymer.

J Josephine LJ et al., [38] formulated and evaluated floating microspheres of stavudine as a model drug for prolongation of gastric retention time for oral delivery by emulsion solvent diffusion using eudragit RS 100 as a rate controlling polymer. The floating microspheres were evaluated for micromeritic properties, particle size, % yield, *in vitro* buoyancy, incorporation efficiency and drug release. Drug–polymer interaction was studied using FTIR analysis and showed that there were no changes in the IR spectra of pure Stavudine in the presence of Eudragit RS 100. The prepared microspheres were found to be spherical and free flowing and remain buoyant for more than 12 hrs. The drug-loaded microspheres (A1) showed encapsulation efficiencies up to 88% and also showed good micromeritic properties for their suitability as oral dosage forms. The microspheres having lower densities exhibited good buoyancy effect and hence, these could be retained in the gastric environment for more than12 h. The mechanism of drug released was found to be diffusion controlled.

Parul K Patel et al., [39] prepared and characterized gum acacia microspheres by single step emulsion in-situ polymer crosslinking method using gluteraldehyde (GL) as the crosslinking agent and Hydrochloric acid (HCl) as the catalyst. Surface and shape characteristics of microspheres were evaluated by means of scanning electron microscopy. Crosslinking with higher amount of gluteraldehyde produced microspheres with lower swelling degree. These

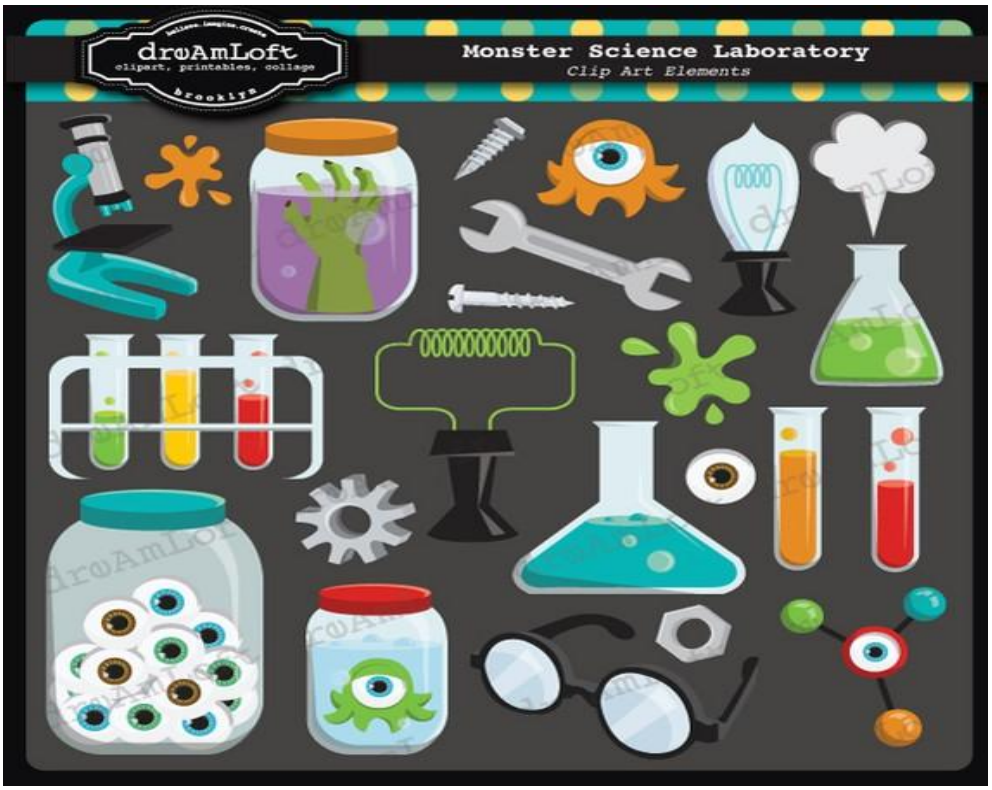
results lead to the conclusion that cross linked GA presents good perspectives for its use in modified release pharmaceutical formulations. Crosslinking reduced the solubility of the natural gum which is indicated by decrease in weight loss on increasing temperature and reaction time. The increasing amount of glutaraldehyde will increase the crosslinked density, indicated based on the reduction of swelling ability and % weight loss of obtained gum acacia microspheres. Crosslinked gum acacia microspheres can be a potential carrier for colon specific drug delivery since reduced hydrophilicity due to crosslinking can prevent premature drug release.

Syed Ershad et al.,[40] prepared and evaluated floating microspheres of ritonavir by ionic gelation method by using sodium alginate and Xanthan gum as polymers. The prepared floating microspheres were evaluated with respect to particle size distribution, floating behavior, drug content, entrapment efficiency, morphology and in vitro release study. The results indicated that the release rate was found to decrease with increase in concentration of coating material applied. The wall thickness of microspheres was found to be increased with the increase in concentration of coating material applied. The microspheres were subjected to *In-vitro* release studies by employing 0.1N Hydro chloric acid and the data. The floating microspheres followed zero order kinetics and the mechanism of drug release was governed by peppas model indicating non fickian diffusion controlled release mechanism.

Brahmaiah B et al.,[41]formulated and evaluated extended release mucoadhesive microspheres of rosuvastatin by orifice-ionic gelation method using polymers such as HPMC K 100 M, Carbopol 940P, sodium CMC, guar gum, sodium alginate, ethyl cellulose, methyl cellulose and Xanthan gum. The microspheres were characterized for drug content, entrapment efficiency, mucoadhesive property by *in vitro* wash-off test and *in-vitro* drug

release. The microspheres were smooth and elegant in appearance showed no visible cracks as confirmed by SEM and FT-IR studies indicated the lack of drug-polymer interactions. Rosuvastatin release from the microspheres was studied in phosphate buffer (pH 7.0) for 8 hours. The microspheres exhibited good mucoadhesive properties for optimized formulation (F10) in the in vitro wash off test. Drug release was diffusion controlled and followed Higuchi kinetics.

Chapter 4



Materials & Methods

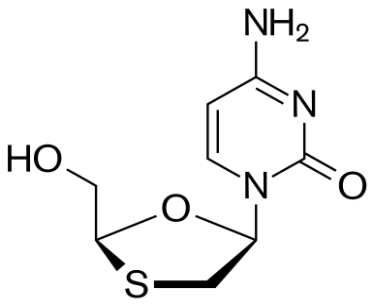
4. MATERIALS AND METHODS

4.1. DRUG PROFILE

LAMIVUDINE [42, 43, 44]

Lamivudine is nucleoside reverse transcriptase inhibiters (NRTIs). It is a Nucleoside analogue, which was originally licensed for the treatment of HIV. It is now additionally licensed for the treatment of chronic hepatitis B with evidence of viral replication.

Chemical Structure:



Chemical Name: 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2 dihydropyrimidin-2-one

CAS Number: 134678-17-4

Brand Name: Epivir-HBV, Hepavir, Hepitec, Heptavir, Avolam.

Chemical Formula: $C_8H_{11}N_3O_3S$

Molecular weight: 229.256

Description: White to off-white crystalline solid.

Melting point: 160-162^0 C

Solubility: Soluble in water; sparingly soluble in methanol; practically insoluble in acetone.

Storage: Lamivudine should be kept in a well-closed container, protected from light.

MECHANISM OF ACTION: Lamivudine is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B virus. It is

phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated. Some research suggests that Lamivudine can cross the blood–brain barrier. Lamivudine treatment has been shown to restore Zidovudine sensitivity of previously resistant HIV.

Pharmacokinetics:

Absorption: As the aqueous solubility profile (70 mg/ml at 20°C) of Lamivudine is good, it dissolves easily in gastric fluids. Lamivudine is well absorbed from the gut, and the bioavailability of oral Lamivudine is normally between 86% ± 16%. Following oral administration the mean time (t*max*) to maximal serum concentrations (C*max*) is about an hour.

Distribution: From intravenous studies, the mean volume of distribution is 1.3 L/kg. Lamivudine exhibits linear pharmacokinetics over the therapeutic dose range and displays low plasma protein binding to albumin. Limited data show relatively low penetration of Lamivudine into the central nervous system.

Metabolism: Lamivudine is predominately cleared by renal excretion of unchanged drug. No evidence of first pass effect. The likelihood of metabolic drug interactions with Lamivudine is low due to limited metabolism and plasma protein binding and almost complete renal clearance.

Elimination: The mean systemic clearance of Lamivudine is approximately 0.3 L/h/kg. The observed half-life of elimination is 4 to 6 hours. The majority of Lamivudine is excreted unchanged in the urine via glomerular filtration and active secretion (organic cationic transport system). Renal clearance accounts for about 70% of Lamivudine elimination.

Half life: 4-6 hours.

Bioavailability: 86 % (oral)

Dose: 300mg per day (i.e. 150 mg twice daily)

Adverse Effects: Depression, Pancreatitis, Guillain-Barre syndrome(such as difficulty breathing/swallowing/moving your eyes, drooping face, paralysis, slurred speech).

4.2. POLYMER PROFILE:

Xanthan Gum [45, 46]

Nonproprietary Names:

- BP: Xanthan gum
- PhEur: Xanthani gummi
- USPNF: Xanthan gum

Synonyms:

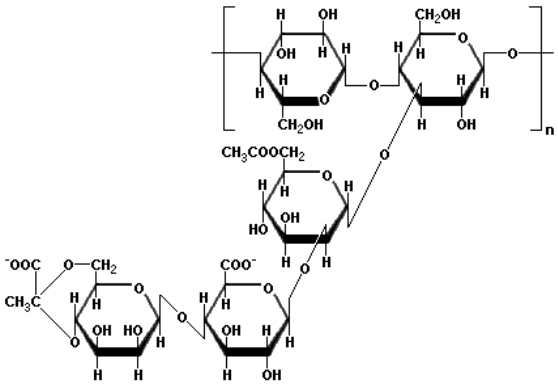
Corn sugar gum; E415; Keltrol; polysaccharide B-1459; Rhodigel; Vanzan NF; Xantural.

Chemical Name: Xanthan gum

Empirical Formula and Molecular Weight:

Empirical Formula: $(C_{35}H_{49}O_{29})_n$

Structural Formula:



Molecular weight is approximately 2×10^6

Functional Category:

Stabilizing agent, suspending agent, Viscosity-increasing agent.

Applications in Pharmaceutical Formulation or Technology:

Xanthan gum is widely used in oral and topical pharmaceutical formulations, cosmetics, and foods as a suspending and stabilizing agent.

It is also used as a thickening and emulsifying agent. It is nontoxic, compatible with most other pharmaceutical ingredients, and has good stability and viscosity properties over a wide pH and temperature range.

Xanthan gum has also been used to prepare sustained-release matrix tablets. Controlled-release tablets of diltiazem hydrochloride prepared using xanthan gum have been reported to sustain the drug release in a predictable manner and the drug release profiles of these tablets were not affected by pH and agitation rate.

xanthan gum can also be used as an excipient for spray-drying and freeze-drying processes for better results.

Xanthan gum can be used to increase the bioadhesive strength in vaginal formulations and as a binder in colon specific drug delivery systems.

Description:

Xanthan gum occurs as a cream- or white-colored, odourless, free-flowing, fine powder

Typical Properties:

Acidity/alkalinity : pH 6.0–8.0 for a 1% w/v aqueous solution.

Freezing point : 08 for a 1% w/v aqueous solution.

Heat of combustion : 14.6 J/g (3.5 cal/g)

Melting point : chars at 270 ^{0}C.

Refractive index: 1.333 for a 1% w/v aqueous solution.

Solubility:

Practically insoluble in ethanol and ether; soluble in cold or warm water

Specific gravity: 1.600 at 25^0C

Viscosity (dynamic):

1200–1600m Pas (1200–1600 cP) for a 1% w/v aqueous solution at 25^0C.

Stability and Storage Conditions:

Xanthan gum is a stable material. Aqueous solutions are stable over a wide pH range (pH 3–12), although they demonstrate maximum stability at pH 4–10 and temperatures of 10–608C.

Xanthan gum solutions of less than 1% w/v concentration may be adversely affected by higher than ambient temperatures: for example, viscosity is reduced. Solutions are also stable in the presence of enzymes, salts, acids and bases.

The bulk material should be stored in a well-closed container in a cool, dry place.

Incompatibilities:

Xanthan gum is an anionic material and is not usually compatible with cationic surfactants, polymers, or preservatives as precipitation occurs. Anionic and amphoteric surfactants at concentrations above 15% w/v cause precipitation of xanthan gum from a solution.

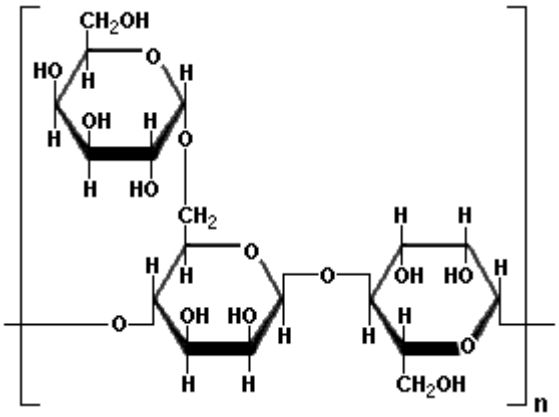
Xanthan gum is compatible with most synthetic and natural viscosity-increasing agents. If it is to be combined with cellulose derivatives, then xanthan gum free of cellulose should be used to prevent depolymerization of the cellulose derivative.

Xanthan gum solutions are stable in the presence of up to 60% water-miscible organic solvents such as acetone, methanol, ethanol, or propan-2-ol. However, above this concentration precipitation or gelation occurs.

Xanthan gum is incompatible with oxidizing agents, some tablet film-coatings, carboxymethylcellulose sodium, dried aluminum hydroxide gel, and some active ingredients such as amitriptyline, tamoxifen, and verapamil.

Guar gum [47, 48]

Structural formula:



Synonyms: Guar flour, Jaguar gum.

Biological source: Guar gum is the powder of the endosperm of the seeds of Cyamopsis tetragonolobus

Family: Leguminoceae

Category: Pharmaceutical aid (tablet binder; tablet disintegrate; suspending Agent).

Description: Almost white to pale yellowish white powder; Odour characteristic.

Empirical formula: $(C_6H_{12}O_6)_n$

Solubility: When stirred with 50 parts of water, a thick jelly is formed which, with further addition of 150 parts of water, yields a thick transparent suspension; practically insoluble in ethanol (95%).

Incompatibility: Acetone, alcohol, tannins, strong acids and alkalis, borate ions, if present

in the dispersing water, will prevent hydration of guar gum. The addition borate ions to hydrated solutions produce cohesive gels, which prevents further hydration. The gel can be liquefied by reducing the pH below 7

Uses: Guar Gum is used as a bulk-forming laxative and as a thickening agent, a tablet binder and a disintegrator in pharmaceuticals. Guar Gum is a good emulsifying agent.

Industrially, this is used in paper manufacturing, printing, polishing, textiles and also in food and cosmetic industries. It is very important as flocculent in ore-dressing and treatment of water.

Safety: Guar gum is widely used in foods and oral and topical pharmaceutical formulations. Excessive consumption may cause gastrointestinal disturbance such as flatulence, diarrhea or nausea. Therapeutically, daily oral doses of up to 25 g of Guar gum have been administered to patients with diabetes mellitus.

Table 3: List of Chemicals

Sl No.	Chemical name	Source
1	Lamivudine	Yarrow chem products, Mumbai
2	Xanthan gum	Yarrow chem products, Mumbai
3	Guar gum	Mahalakshmi chem. Bangalore
4	Span 80	S D Fine Chem Limited, Mumbai
5	Paraffin Liquid (light & heavy)	S D Fine Chem Limited, Mumbai
6	Glutaraldehyde	S D Fine Chem Limited, Mumbai
7	n-hexane	S D Fine Chem Limited, Mumbai

All other reagents used were of analytical grade. Distilled water was used throughout the study.

Table No.4: List of Instruments Used with Manufacturer

Sl No	Instruments	Manufacturer
1	Electronic analytical balance	ACCULAB, EUROPE, Germany
2	UV-Visible spectrometer	Shimadzu UV-1800, Japan
3	Cary 630 FT IR	Agilent technologies
4	Dissolution apparatus	Lab India, Mumbai, India
5	Magnetic stirrer	Almicro, Bangalore
6	pH meter	Techno scientific products, Bangalore
7	Hot air oven	Kadavil electro mechanical industries, kerala
8	Microscope	Pilot products, Bombay
9	Scanning electron microscopy	HITACHI, Japan
10	Stability chamber	LABTOP, SKY Lab Instruments & Engineering Pvt. Ltd.

4.3. PRE-FORMULATION STUDIES:

Pre-formulation testing is the first step in the rational development of dosage forms of a drug. It can be defined as an investigation of Physical and chemical properties of drug substance, alone and when combined with excipients. The overall objective of pre-formulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be produced at large scale. A thorough understanding of physico-chemical properties may ultimately provide a rationale for formulation design or support the need for molecular modification or merely confirm that there are no significant barriers to the compounds development. The goals of the program therefore are:

1. To establish the necessary physico-chemical characteristics of a new drug substance.
2. To determine its kinetic release rate profile.
3. To establish its compatibility with different excipients.

Hence, pre-formulation studies on the obtained sample of drug include physical tests and compatibility studies.

4.3.1. IR Spectroscopy:

FR-IR spectroscopy was carried out to check the compatibility between drug and excipients. Infrared spectroscopy was conducted using s thermo Nicolet FTIR and the spectrum was recorded in the region of 4000 to 400 cm^{-1}.The sample (drug and drug-excipient mixture in 1:1 ratio) in KBR (200-400mg) was compressed in to discs by applying a pressure of 5 tons for 5 min in hydraulic press. The interaction between drug-excipients was observed from IR-spectral studies by observing any shift in peaks of drug in the spectrum of physical mixture of drug-excipients.

4.3.2. Solubility Analysis:

Pre-formulation solubility analysis was done to select a suitable solvent system to dissolve the drug as well as various excipients used for formulation of microspheres.

4.3.3 Melting Point Determination:

Melting point determination of the obtained drug sample was done as it is a first indication of purity of the sample. The presence of relatively small amount of impurity can be detected by a lowering as well as widening in the melting point range. The melting point of Lamivudine was measured by Thiele's tube apparatus.

4.3.4. Determination of λmax:

Lamivudine was dissolved in 0.1N HCL and phosphate buffer pH 6.8, further diluted with the same and scanned for maximum absorbance in UV double beam spectrophotometer (shimadzu 1800) in the range from 200 to 400 nm, 0.1 N HCL and phosphate buffer pH 6.8 as blank.

4.3.5. Standard Calibration Curve of Lamivudine:

Accurately weighed 100 mg of Lamivudine was dissolved in 100 ml of 0.1 N HCl (pH 1.2) (Conc. 1000 µg/ml) to prepare first stock solution. 1 ml of above solution was pipetted out into 100 ml volumetric flask and volume was made up to with 0.1 N HCl (pH 1.2) (Conc. 10 µg/ml) to prepare stock II solution. The aliquot solution of stock II solution was further diluted with pH 1.2 to get 5µg, 10µg, 15µg, 20µg, 25µg and 30µg of drug in the final solution. Then the absorbance was measured in a double beam UV spectrophotometer at 270 nm against pH 1.2 as blank. The same procedure was repeated by using phosphate buffer pH 6.8. The absorbances obtained were tabulated as in Table 8 and Table 9. Calibration curve was plotted and shown in Figure 6 and 7 respectively. The maximum obtained in the graph was considered as λmax for the pure drug.

Table 5: Formulation of Microspheres

Formulation	Drug (mg)	Xanthan Gum (mg)	Guar Gum (mg)	Liquid Paraffin(ml)	Span 80 (v/v)
F1	100	15	-	200	0.5
F2	100	20	-	200	0.5
F3	100	25	-	200	0.5
F4	100	30	-	200	0.5
F5	100	-	15	200	0.5
F6	100	-	20	200	0.5
F7	100	-	25	200	0.5
F8	100	-	30	200	0.5
F9	100	-	35	200	0.5

4.3.6. Preparation of Lamivudine microspheres by Solvent evaporation technique [49]

Preparation of Microspheres

Microspheres were prepared by using different ratios of drug: natural gum (1:1.15, 1:1.20, 1:1.25). Gums were allowed to hydrate in 20 ml water for 3 hrs. weighed quantity of drug (100mg) was dispersed in 10 ml of methylene chloride and add the aqueous solution of gum. The above drug-gum dispersion was acidulated with 0.5 ml of concentrated sulphuric acid to give a clear viscous solution. The resultant solution was emulsified into the oily phase by poured into 200 ml of paraffin liquid containing 0.5 % w/w span 80 as an emulsifying agent. Stirred mechanically at 1800 rpm for 210 min using a stirrer and heated by a hot plate at 50^0C. 1.2 %

w/v dichloromethane was added as encapsulating agent and 0.15 % w/v of gluteraldehyde as crosslinking agent, stirring and heating were maintained for 2.5 hrs until the aqueous phase was completely removed by evaporation. The oil was decanted and collected microspheres were washed with water to remove surfactant residue and three times with 100 ml aliquots of n-hexane, filtered through whatman filter paper, dried in an oven at 80^0C for 2 hr to collect discrete, solid, free flowing microspheres and stored in a desiccators at room temperature.

4.4. EVALUATION OF LAMIVUDINE MICROSPHERES

Micromeritic Studies [50, 51]

The prepared microspheres are characterized by their micromeritic properties such as microsphere size, tapped density, Carr's compressibility index, Hausner's ratio and angle of repose.

Bulk Density:

The bulk density is defined as the mass of powder divided by bulk volume.

The bulk density was calculated by dividing the weight of the samples in grams by the final volume in cm

$$\text{Bulk density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres before tapping}}$$

Tapped Density:

Tapped density is the volume of powder determined by tapping by using a measuring cylinder containing weighed amount of sample. The cylinder containing Known amount of microspheres was tapped for about 1 minute on a tapped density apparatus until it gives constant volume.

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres before tapping}}$$

Carr's Compressibility Index

This is an important property in maintaining uniform weight. It is calculated using following

equation.

$$\% \text{ Compressibility Index} = \frac{Tapped\ density - Bulk\ density}{Tapped\ density} \times 100$$

Lower the compressibility values indicate better flow

Table 6: Relationship between % Compressibility and Flowability

% Compressibility	Flowability
5-15	Excellent
12 – 16	Good
18 – 21	Fair to passable
23 – 35	Poor
33 – 38	Very poor
> 40	Extremely poor

Hausner's ratio

A similar index like percentage compressibility index has been defined by Hausner. Values less than 1.25 indicate good flow, where as greater than 1.25 indicates poor flow. Added glident normally improves flow of the material under study. Hausner's ratio can be calculated by formula,

$$\text{Hausner's ratio} = \frac{Tapped\ density}{Bulk\ density}$$

Angle of Repose (θ):

Good flow properties are critical for the development of any pharmaceutical tablet, capsules or powder formulation. It is essential that an accurate assessment of flow properties be made as early in the development process as possible so that an optimum formulation can be quickly identified. Interparticle forces between particles as well as flow characteristics of powders are evaluated by

angle of repose. Angle of repose is defined as the maximum angle possible between the surface and the horizontal plane.

PROCEDURE: The angle of repose of each powder blend was determined by glass funnel method. Powders were weighed accurately and passed freely through the funnel so as to form a heap. The height of funnel was so adjusted that the tip of the funnel just touched the apex of the heap. The diameter of the powder cone so formed was measured and the angle of repose was calculated using the following equation,

$$\tan \theta = h/r$$

$$\theta = \tan^{-1}(h/r)$$

Where, θ = angle of repose

h = height of the pile and,

r = radius of the powder cone respectively.

Angle of repose affects particle size distribution, as larger the particle size, it will flow freely and vice-versa. It is a helpful parameter to monitor quality of powdered or granular pharmaceutical formulations. For good flowing materials, the angle of repose should be less than 30°.

Table 7: Relationship between Angle of Repose and Flowability

Angle of Repose	**Flowability**
< 25	Excellent
25-30	Good
30-40	Passable
> 40	Very Poor

Particle Size Determination [52]: The particle size of the microspheres was determined by using optical microscopy method. Approximately 100 microspheres were counted for particle size using a calibrated optical microscope.

Morphological Study using SEM: The morphological study was carried out by Scanning Electron Microscope (SEM). Microspheres were scanned and examined under Electron Microscope HITACHI SU 1500, Japan connected with Fine coat, JEOL JFC-1100E Ion sputter. The sample was loaded on copper sample holder and sputter coated with carbon followed by Gold.

Drug Loading and Drug Entrapment: Microspheres equivalent to 50 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl (pH-1.2) repeatedly. The extract was transferred to a 100 mL volumetric flask and the volume was made up using 0.1N HCl (pH-1.2). The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically (UV 1700, Shimadzu, Japan) at 212 nm against appropriate blank. The amount of drug loaded and entrapped in the microspheres was calculated by the following formulas:

$$\% \text{ Drug loading} = \frac{\text{Weight of the drug loaded in the microspheres(DC)}}{\text{Total weight of the microspheres}} \text{ X } 100$$

$$\% \text{ Drug entrapment} = \frac{\text{Amount of drug actually prsent(DC)}}{\text{Theoretical drug loaded expected}} \text{ X } 100$$

(DC- Actual Drug Content)

Percentage yield

$$\% \text{ yield determined by following equation} = \frac{\text{Practical yield}}{\text{Theoritical yield}} \text{X} 100$$

In vitro drug release Study [24]

The prepared microspheres were subjected to *in vitro* drug release sequentially in three different suitable dissolution media. USP type II dissolution apparatus was used. The dissolution medium for the first 2 hr was 900 ml of 0.1 N HCl (pH 1.2) and continued in phosphate buffer pH 6.8 for the next 7 hrs The temperature of dissolution medium was maintained at 37 ±0.5 °C and the basket was rotated at 50 rpm. An aliquot of 5 ml was withdrawn at predetermined time intervals and replaced with an equal volume of the fresh dissolution medium to maintain sink conditions. The samples were analyzed at 272 nm, for the percentage drug release using an UV Visible double beam spectrophotometer. The release study was performed in triplicates.

Dissolution studies:

Apparatus: LABINDIA USP Type II

Dissolution media: 0.1 N HCl (pH-1.2)

Speed: 100 rpm

Volume of medium: 900 mL

Aliquots taken at each time interval: 5 mL

Temperature: 37±0.5°C

Wavelength: 272 nm

4.6. Release Kinetics [53]

The matrix systems were reported to follow the Peppas release rate and the diffusion mechanism for the release of the drug. To analyze the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to, Zero order, First order, Higuchi matrix, Peppas and Hixson Crowell model. In this by comparing the r-values obtained, the best-fit model was selected.

Zero Order Kinetics:

Drug dissolution from Pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation:

$$Qt = Qo + Kot$$

Where,

Qt = Amount of drug dissolved in time t

Qo = Initial amount of drug in the solution and

Ko = Zero order release constant

First Order Kinetics:

To study the first order release kinetics the release rate data were fitted to the following equation.

$$\text{Log } Qt = \log Qo + K1t / 2.303$$

Where,

Qt = Amount of drug released in time t

Qo = Initial amount of drug in the solution and

K1 = First order release constant.

Higuchi Model:

Higuchi developed several theoretical models to study the release of water soluble and low soluble drugs incorporated in semi-solid and/or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The Higuchi equation is

$$Qt = KH \times t1/2$$

Where,

Qt = amount of drug released in time t and

KH = Higuchi dissolution constant

Korsmeyer-Peppas Model:

To study this model, the release rate data is fitted to the following equation.

$$Mt / M = K. tn$$

Where,

Mt / M = Fraction of drug release,

K = Release constant

t = Drug release time and

n = Diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form

The values of 'n' are,

n = 0.45 Fickian (case I) release

0.45 < n < 0.89 Non-Fickian (Anomalous) release

n = 0.89 Case II (Zero order) release

> 0.89 Super case II type release

Hixson–Crowell Model:

To study the Hixson–Crowell model, the release rate data are fitted to the following equation.

$$Wo\ 1/3 - Wt\ 1/3 / Ks\ t$$

Where,

Wo = Amount of drug in the pharmaceutical dosage form

Wt = Remaining amount of drug in the pharmaceutical dosage form

Ks = Constant incorporating the surface-volume relation

4.7. Stability Studies [54, 55]

Stability of a drug has been defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications.The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light, and enables recommended storage conditions.

ICH guidelines the length of study and storage conditions:

Accelerated testing - 40°C/75% RH for six months.

The accelerated stability study of the best formulations was carried out as per the ICH guidelines.

Procedure:

In the present study, stability study was carried out for a period up to the 60 days for selected formulations. The selected formulations were analyzed for the physical appearance, drug entrapment, and *in-vitro* release study.

Chapter 5

Results & Discussion

5. RESULTS AND DISCUSSION

5.1. PRE-FORMULATION STUDIES

5.1.1. COMPATIBILITY STUDY:

- **IR Spectroscopy:**

The FT-IR spectrum of the Lamivudine pure drug was found to be similar to the standard spectrum of Lamivudine as in I.P. The individual FT-IR spectra of the pure drug Lamivudine, as well as the combination spectra of the drug and polymers are shown in the figures 2-5. All the characteristic of peaks of Lamivudine were present in spectrum of drug and polymers, indicating compatibility between drug and polymers.

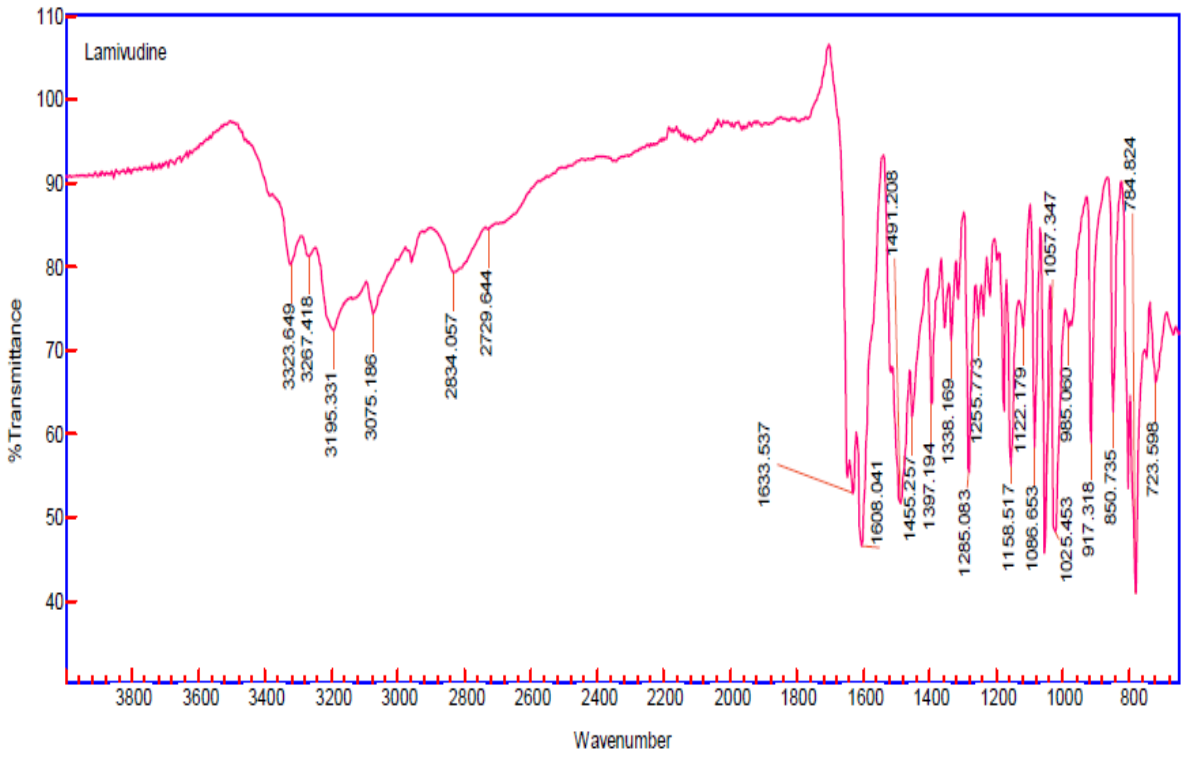


Figure 2: IR Spectrum of pure drug Lamivudine

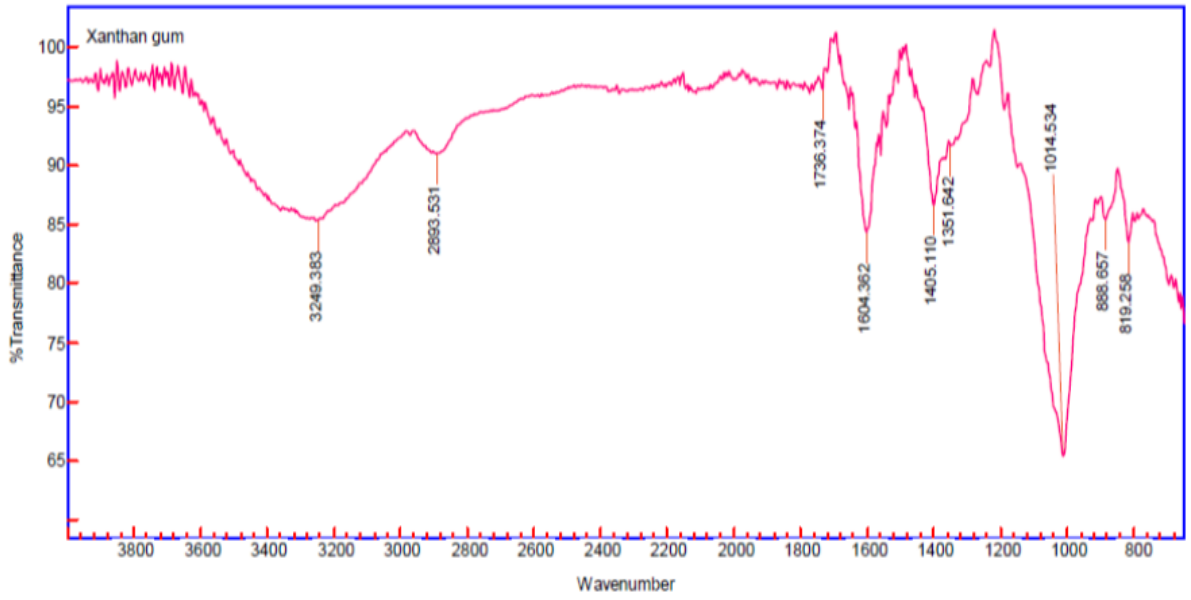


Figure 3: IR spectrum of Xanthan gum

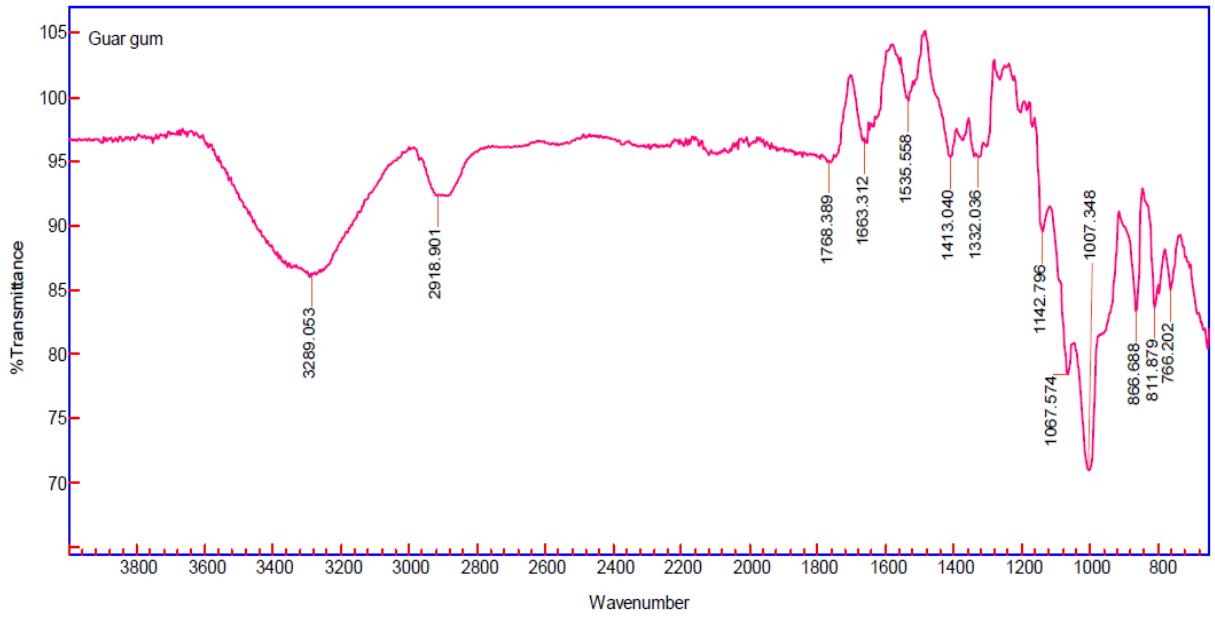


Figure 4: IR spectrum of Guar gum

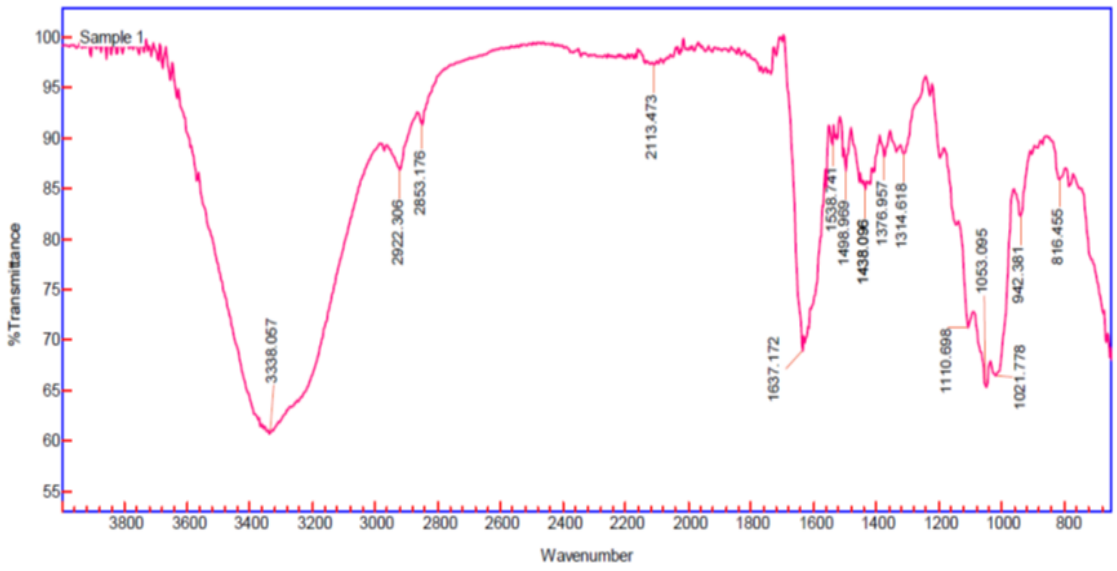


Figure 5: IR Spectrum of Lamivudine with xanthan gum + guar gum

5.1.2. Solubility analysis:

The Lamivudine is freely soluble in water; sparingly soluble in methanol; practically insoluble in acetone. It was soluble in 0.1N HCL (pH 1.2) and phosphate buffer (pH 6.8).

Solubility analysis is important because the drug has to dissolve in the solvents and also in the dissolution medium used.

5.1.3. Melting point determination:

The melting point of the obtained drug sample was found to be 161^0C which is within the reported range of 160-162^0C. It complies with the purity of the drug sample.

5.1.4. Determination of λmax in 0.1N HCl (pH 1.2) and phosphate buffer (pH 6.8):

Lamivudine was dissolved in 0.1N HCL(pH 1.2) and phosphate buffer(pH 6.8), further diluted with the same and scanned for maximum absorbance in UV double beam spectrophotometer (shimadzu 1800) in the range from 200 to 400 nm, using pH 1.2 and pH 6.8 as blank. The λmax of drug was found to be 272 nm.

5.1.5. Standard calibration curve of Lamivudine:

The absorbance was measured in a UV spectrophotometer at 272 nm against 0.1N HCl pH(1.2) and phosphate buffer (pH 6.8). The absorbances of standard solutions of Lamivudine ranging from 5-30 µg/ml so obtained were tabulated as in Table 8 and 9. Calibration curves were plotted and shown in Figure 6 and7 respectively. The curves were found to be linear in the range of 5-30 µg/ml at λmax 272 nm. The regression values were found to be 0.999 and 0.994 in pH 1.2 and pH 6.8 respectively.

Table 8: Spectrophotometric Data for the Estimation of Lamivudine in 0.1N HCL (pH 1.2) at 272 nm

Sl No	Concentration (µg/ml)	Absorbance
1	0	0
2	5	0.068
3	10	0.132
4	15	0.202
5	20	0.264
6	25	0.34
7	30	0.409

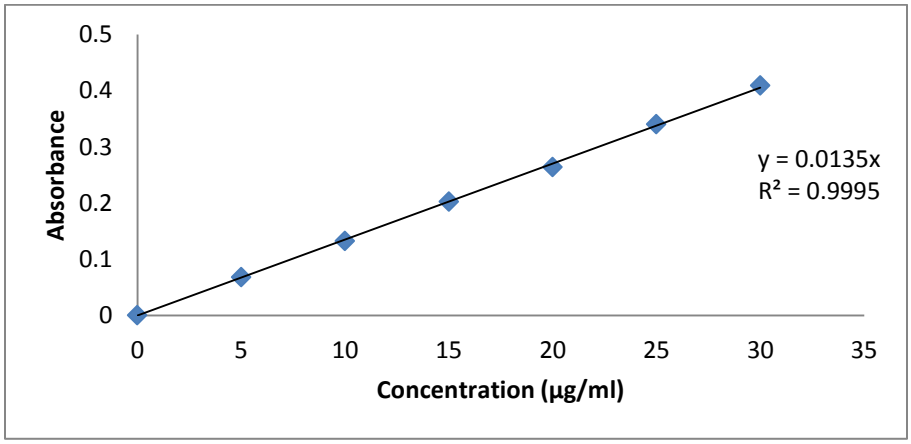


Figure 6: Standard Calibration Curve of Lamivudine in 0.1N HCl

Table 9: Spectrophotometric Data for the Estimation of Lamivudine in pH 6.8 Phosphate buffer at 272 nm

Sl No	Concentration (µg/ml)	Absorbance
1	0	0
2	5	0.142
3	10	0.268
4	15	0.453
5	20	0.614
6	25	0.801
7	30	0.969

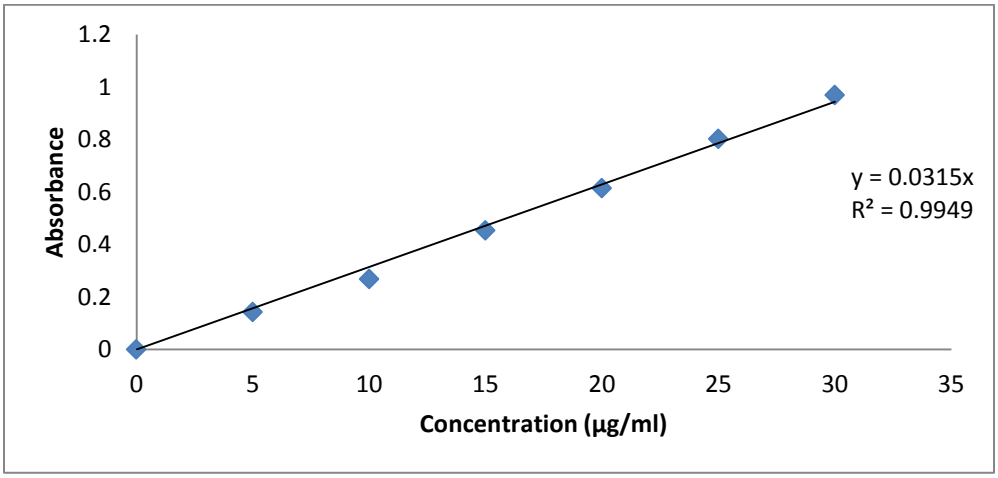


Figure 7: Standard Calibration Curve of Lamivudine in pH 6.8

5.2. Micromeritic Properties:

The results of all formulations F1 to F9 of Lamivudine microsphere are shown in Table10, which were evaluated for variable parameters such as bulk density, tapped density, % Compressibility index, Hausner's ratio and angle of repose.

The % Compressibility index was in the range of 11-18 for all the formulations F1 to F9 indicating good flow property. The values of angle of repose for formulations F1,F2, F5 and F6

was found to be in the range of 25-30 which indicated the good flow potential.

Table 10: Micromeritic properties of Lamivudine microspheres

Formulation Code	Bulk Density ($g/cm^{3)}$	Tapped Density (g/cm^3)	Compressibility Index (%)	Hausner's Ratio	Angle of Repose (θ)
F1	0.4426±0.005	0.5126±0.009	13.65±1.21	1.158±0.02	26.93±0.23
F2	0.4986±0.008	0.5814±0.004	14.24±1.32	1.166±0.05	25.74±0.24
F3	0.5234±0.015	0.6243±0.008	16.16±1.27	1.193±0.011	32.94±0.17
F4	0.4813±0.009	0.5446±0.005	11.94±1.34	1.131±0.019	33.81±0.14
F5	0.5418±0.013	0.6183±0.001	12.36±1.04	1.141±0.02	28.67±0.36
F6	0.6168±0.011	0.7136±0.012	13.56±1.02	1.156±0.08	27.08±0.16
F7	0.4576±0.014	0.5228±0.008	12.47±1.21	1.142±0.03	33.61±0.64
F8	0.4754±0.013	0.5845±0.011	15.24±1.03	1.229±0.023	34.54±1.07
F9	0.5438±0.016	0.6432±0.014	15.45±0.84	1.183±0.026	37.12±1.51

Particle Size Analysis:

Average particle size of microspheres as determined by optical microscopy by using stage micrometer and ocular micrometer are shown in Table 11 and in Figure 8.The mean particle size for the formulation F1 to F4 containing Xanthan gum was found to be in range from278±7.14µm to 913±6.35µm.For formulation F4 to F9 containing Guar gum the mean particle size was found to be in range from 572±12.51µm to 991±10.73 µm respectively. With increase in polymers concentration in the microspheres from F1 to F9, the particle size of microspheres increases respectively. This is because the viscosity of the polymer solution increases with increasing polymer concentration, which in turn decreases the stirring efficiency.

Table 11: Average Particle Size of Lamivudine Microspheres

Formulation code	Average particle size (µm)±SD
F1	913±6.35
F2	940±11.28
F3	456±12.42
F4	278±7.14
F5	991±10.73
F6	743±12.24
F7	650±8.69
F8	590±11.46
F9	572±12.51

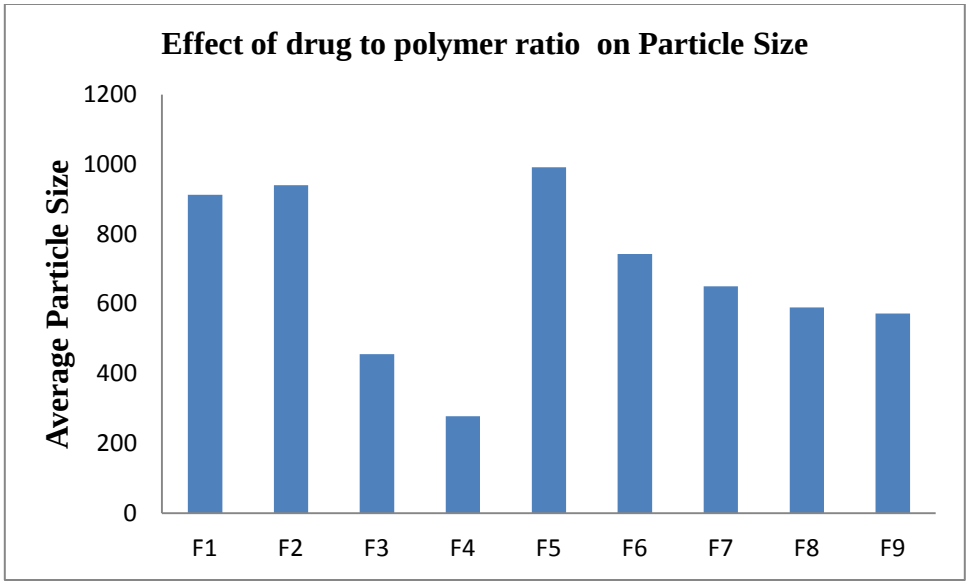


Figure 8: Comparison of Avg. Particle Size of the Prepared Microspheres

Scanning Electron Microscopy

The determination of shape and surface morphology was done by scanning electron microscope HITACHI SU 1500, Japan. SEM analysis of the samples revealed that all microspheres prepared were spherical in shape. The microspheres of Lamivudine with Guar gum were smooth, spherical and slightly aggregated particles when compared with the microspheres of xanthan gum which

were porous, rough, grossly, discrete spherical. Scanning electron photomicrographs of the formulations F1 and F5 are shown in Figure 9.

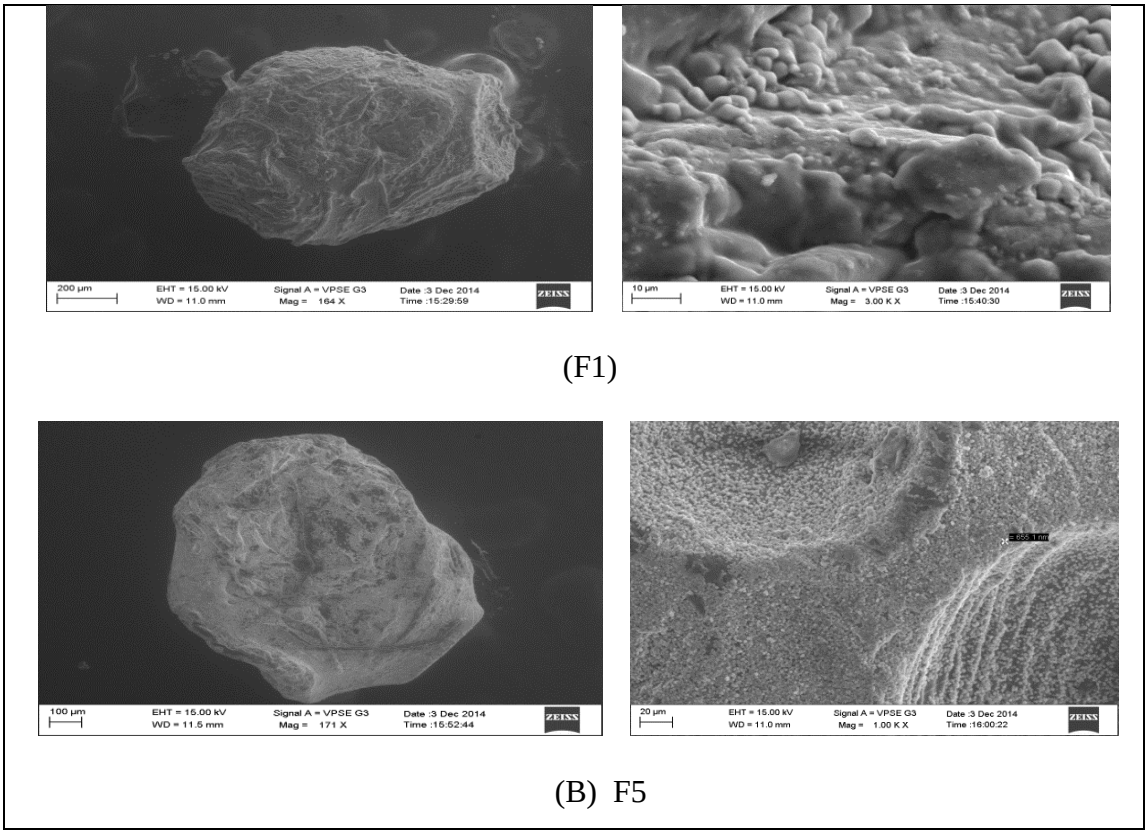


Figure 9: SEM images of F1 and F5 formulation

Drug Loading and Drug Entrapment:

The values of %drug loading and %entrapment efficiency are shown in Table12 .As the polymer concentration was increased the %drug loading decreased and %entrapment efficiency was increased due to increase in the viscosity of the solution. This can be attributed to the permeation characteristics of each polymer used, that could facilitate the diffusion of part of entrapped drug to the surrounding medium during preparation of microspheres

Comparison of %drug loading and %entrapment efficiency are shown in Figure 10 and 11.

Table 12: Drug Loading and Drug Entrapment of Lamivudine Microspheres

Formulation Code	Actual Drug Content (mg)	Theoretical Drug Content (mg)	Total Weight of Microspheres (mg)	% Drug Loading	%Drug Entrapment
F1	19.28	25	50	38.56	78.12
F2	13.75	16.67	50	27.5	82.48
F3	11.30	12.5	50	22.60	90.40
F4	19.53	25	50	21.06	92.12
F5	13.94	16.67	50	37.88	73.62
F6	11.43	12.5	50	32.86	78.44
F7	19.72	25	50	29.44	84.88
F8	14.16	16.67	50	28.32	86.94
F9	11.56	12.5	50	23.12	92.48

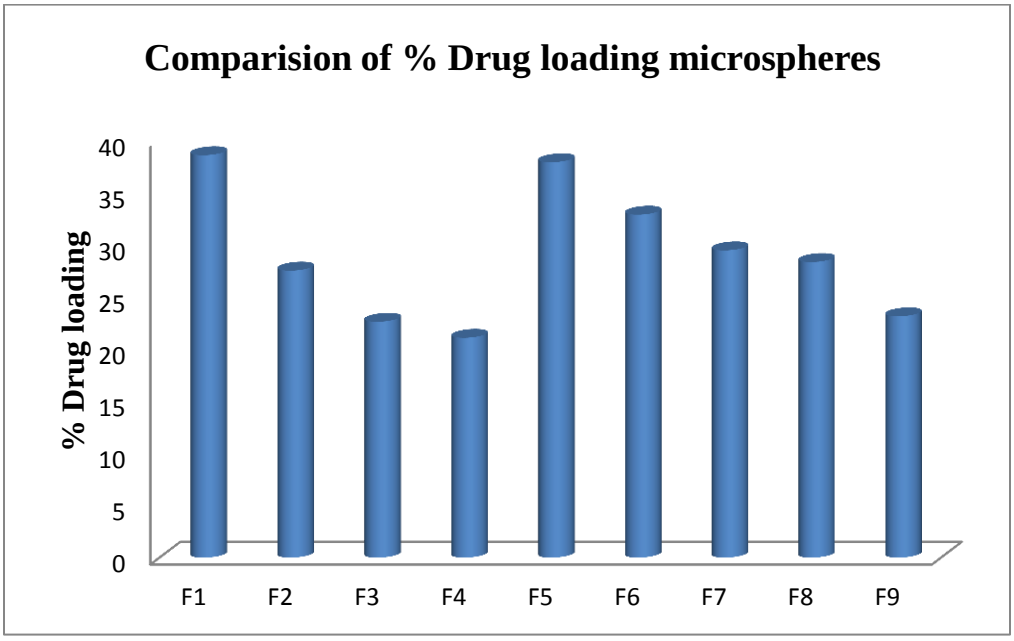


Figure10: Comparison of % Drug Loading of the Prepared Microspheres

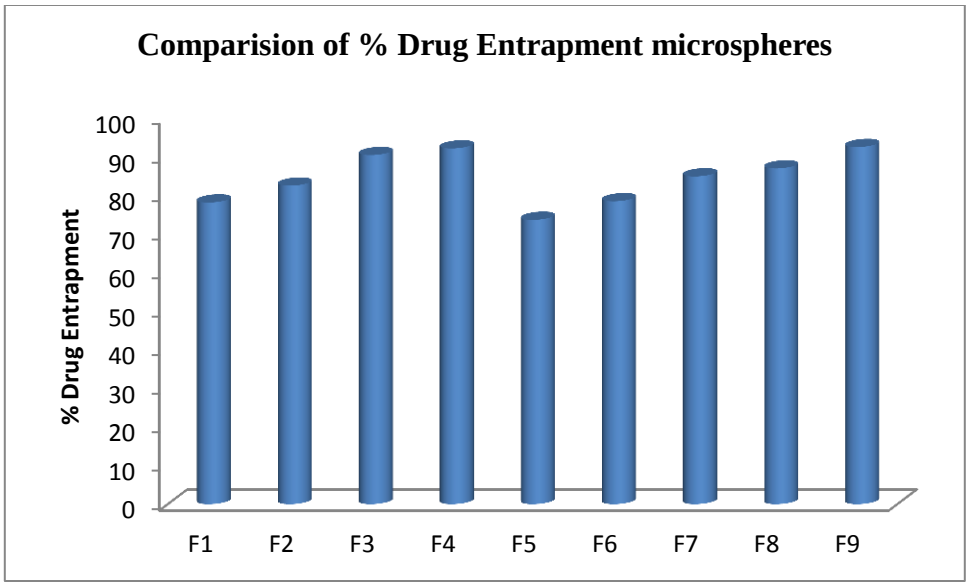


Figure 11: Comparison of % Drug Entrapment of the Prepared Microspheres

Percentage yield:

Percentage yield of different formulation F1 to F9 were calculated and the yield was found to be 79.21%, 75.73%, 67.68%, 61.54%, 81.13%, 71.4%, 70.70%, 59.68% and 55.50% respectively. The percentage practical yield slightly decreased as the polymer ratio increased. The results of all formulations F1 to F9 of microsphere are shown in Table 13 and Figure 12.

Table 13: Practical Yield of Lamivudine Microspheres

Formulation Code	Theoretical Wt (mg)	Practical Yield (mg)	%Yield
F1	1000	752	79.21
F2	1500	1095	75.73
F3	2000	1296	67.68
F4	1000	764	61.54
F5	1500	1097	81.13
F6	2000	1288	71.4
F7	1000	747	70.7
F8	1500	1089	59.68
F9	2000	1310	55.50

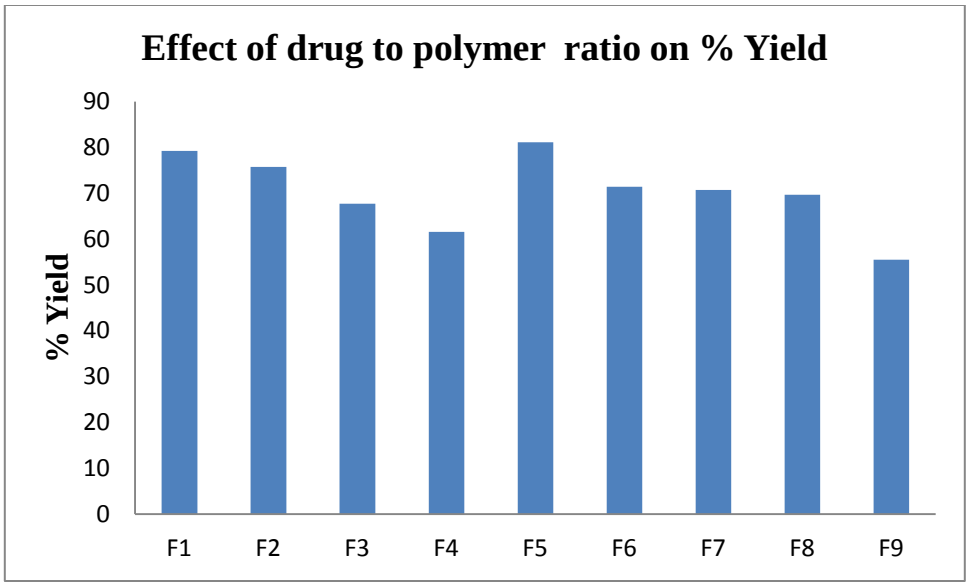


Figure 12: Comparison of % Yield of the Prepared Microspheres

***In-vitro* drug release studies:**

Dissolution studies on all the nine formulations of Lamivudine microspheres were carried out using a USP dissolution apparatus Type II. 0.1N HCl (pH 1.2) and pH 6.8 was used as the dissolution medium. The *in-vitro* drug release data of different formulations are shown in Table. No.14 and Figure.No.13.The cumulative percent drug release after 12 hours was found to be in the range of 81.723, 79.038,76.389 and 71.558% for the formulations F1, F2, F3 and F4 respectively whereas cumulative percent drug release after 12 hours was 82.14,80.57,74.474,69.093,63.568% for formulations F5 to F9 respectively. The cumulative drug release significantly decreased with increase in polymer concentration. The increased density of the polymer matrix at higher concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at a lower polymer concentration and have a larger surface area exposed to dissolution medium, giving rise to faster drug release.

Table 14: *In–vitro* drug release for Lamivudine Microspheres in 0.1N HCL (pH 1.2) and (pH 6.8) phosphate buffer

Time (hrs)	CUMULATIVE % DRUG RELEASE OF FORMULATION								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	17.215	16.557	14.472	13.959	17.215	16.959	16.553	14.141	13.093
2	28.410	25.765	24.433	22.557	35.765	26.557	20.535	18.370	17.085
3	34.714	32.406	31.723	29.146	42.406	39.146	25.844	23.465	22.431
4	47.375	38.389	37.073	31.811	48.389	38.811	31.817	29.634	27.563
5	52.038	43.050	41.369	34.477	53.050	44.477	32.497	31.050	30.774
6	59.000	54.998	49.069	49.063	64.998	51.063	39.136	39.360	37.124
7	63.306	62.318	53.716	45.712	72.318	56.712	54.469	48.156	44.744
8	69.320	68.331	65.697	64.350	76.331	63.350	61.803	59.691	49.424
9	75.633	72.994	69.020	67.669	79.994	71.669	68.447	66.313	65.768
10	81.723	79.038	76.389	71.558	82.146	80.574	74.474	69.093	63.568

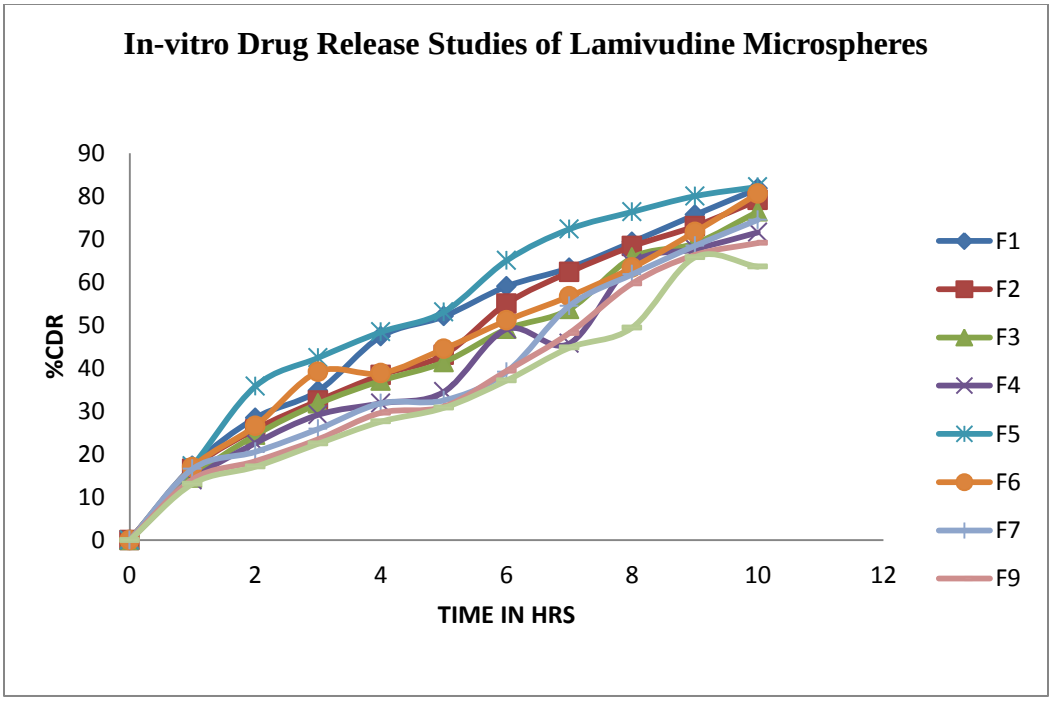


Figure 13: Comparative *In-vitro* Dissolution Profile of Lamivudine Microspheres

5.3. Release Kinetics:

The results obtained from in-vitro drug release were plotted adopting five different mathematical models of data treatment as follows:

% Cum. Drug Release Vs. Time (Zero order rate kinetics).

Log % Cum. Drug Retained Vs. Time (First order rate kinetics).

% Cum. Drug release was plotted against √T (root time). (Higuchi model)

Log % Cum. Drug Release Vs. Log Time (Peppas exponential equation).

Hixson-Crowell's erosion equation, (% Cum. Drug Retained)$^{1/3}$ Vs. Time.

The curve fitting results of the release rate profile of the designed formulation are shown in the Figure 23-27 which gave an idea on the release rate and the mechanism of release. The values were compared with each other for model and drug equation as shown in Table 15 based on the highest regression values (r^2), fitting of the release rate data to various models revealed that all the formulations (F1 to F9) follow zero order release kinetics with regression values ranging from 0.9552 to 0.9961.

All the formulations were subjected to Korsmeyer-Peppas plots, 'n' value ranges from 0.4196 to 0.4596 indicating that the drug release was by non-fickian diffusion mechanism.

Table 15: Model Fitting Release Profile of Lamivudine Microspheres

Formulation code	Mathematical Models (Kinetics)						Best Fit Model
	Korsmeyer–Peppas		Higuchi	Hixson–Crowell	First order	Zero order	
	R^2	n	R^2	R^2	R^2	R^2	
F1	0.9405	0.4596	0.9633	0.9668	0.8892	0.9893	Zero order
F2	0.9352	0.4480	0.9687	0.9636	0.8979	0.9834	Zero order
F3	0.9239	0.4408	0.9499	0.9308	0.8678	0.9552	Zero order
F4	0.943	0.4374	0.9748	0.9731	0.9084	0.9914	Zero order
F5	0.9466	0.4574	0.9748	0.9686	0.9103	0.9868	Zero order
F6	0.9349	0.4231	0.9618	0.9422	0.8874	0.9636	Zero order
F7	0.967	0.4460	0.9908	0.9899	0.9304	0.9961	Zero order
F8	0.972	0.4192	0.9885	0.9868	0.9479	0.9942	Zero order
F9	0.9676	0.4358	0.9855	0.9809	0.9471	0.9896	Zero order

Korsmeyer-Peppas model for prepared Microspheres

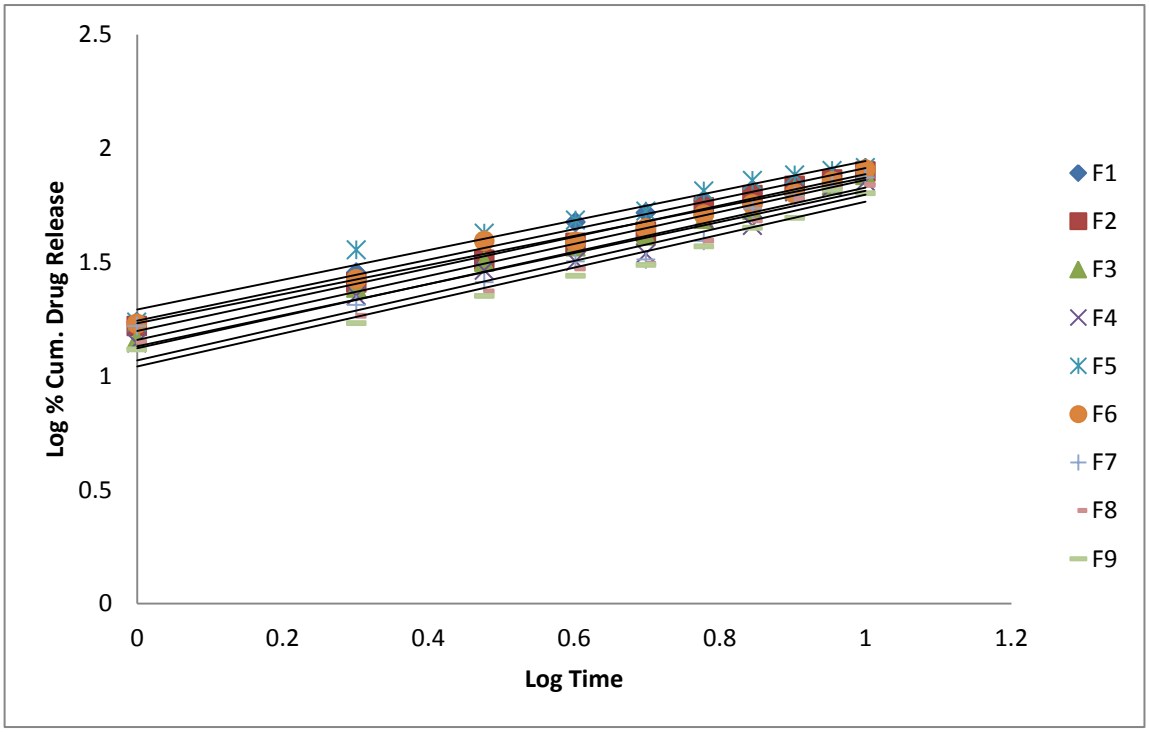


Figure 14: Korsmeyer-Peppas release Kinetics for prepared microspheres

Higuchi model for prepared microspheres

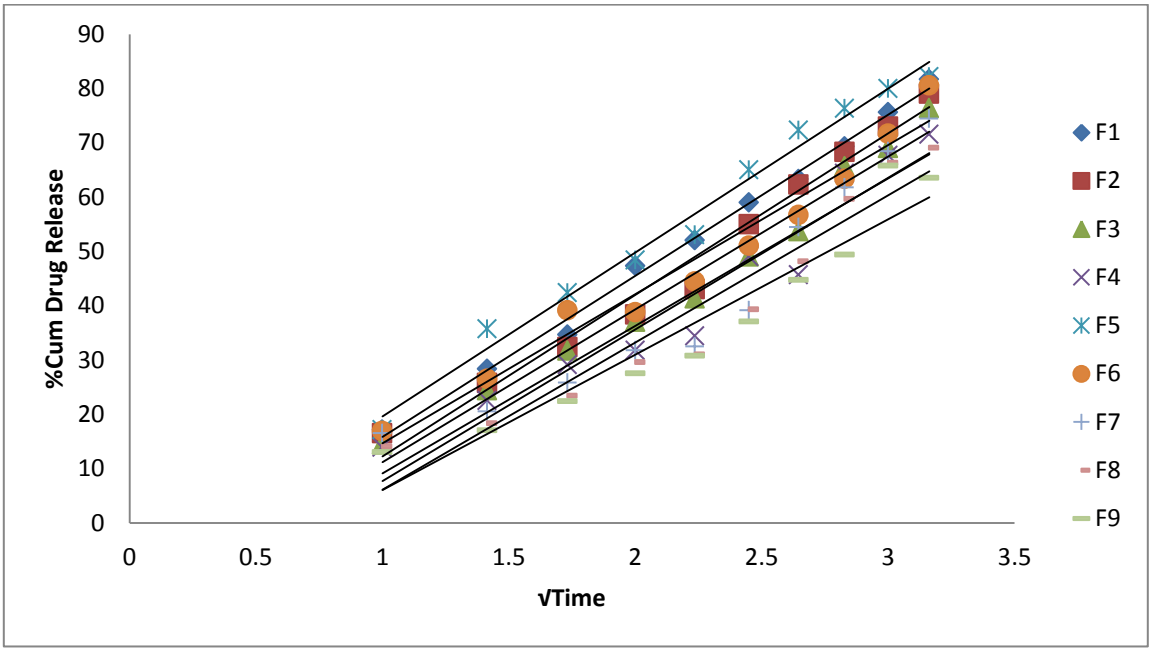


Figure 15: Higuchi release kinertics for prepared microspheres

Hixson-crowell model for prepared microspheres

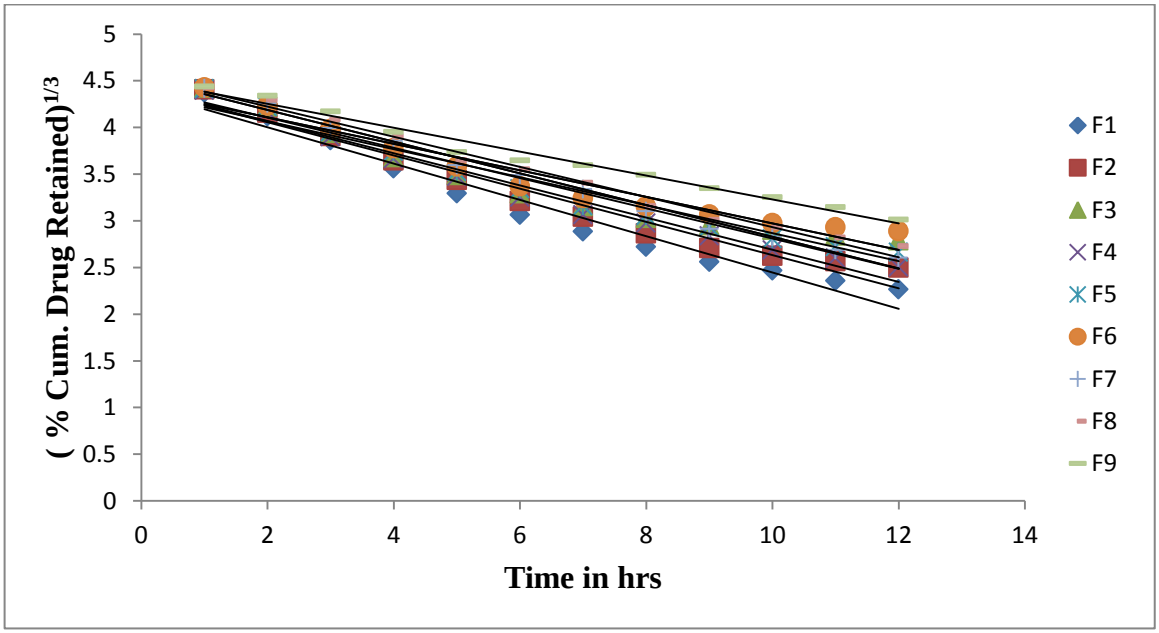


Figure 16: Hixon-crowel release kinetics for prepared microspheres

First order Model for prepared microspheres

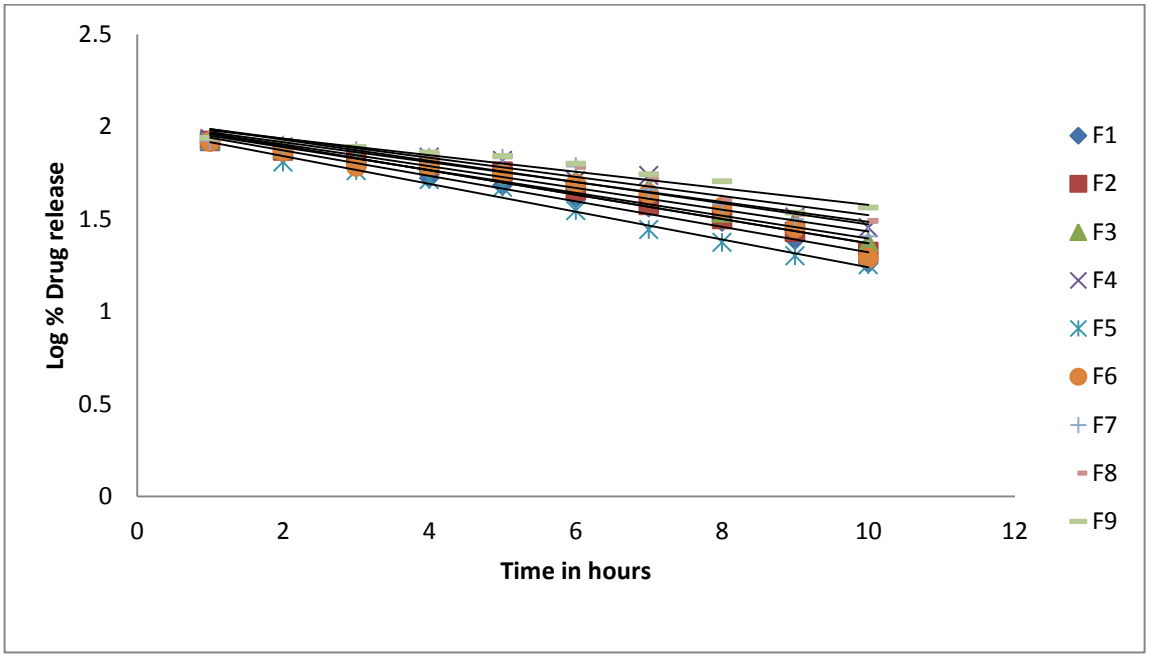


Figure 17: First order release kinetics for prepared Microspheres

Zero order for prepared microspheres

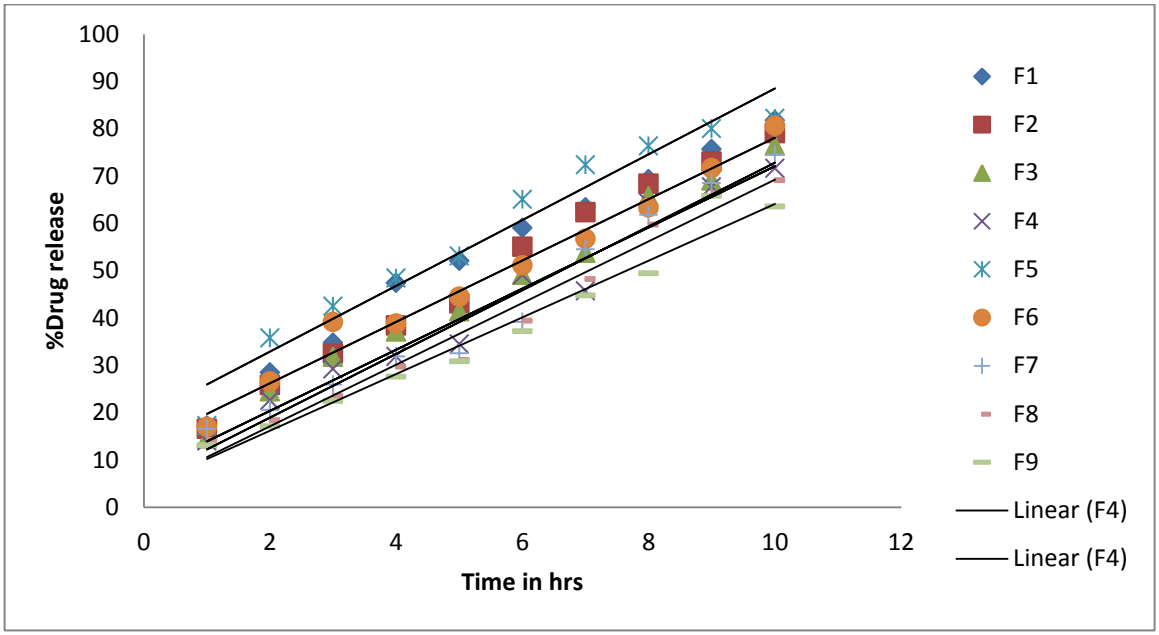


Figure 18: Zero order release Kinetics for prepared Microspheres

5.4. Stability study

Stability study was conducted for the prepared Lamivudine microspheres of formulation F1 and F5 at 40°C/75% RH respectively for a period of 60 days. Then, the sample was analyzed for physical appearance, entrapment efficiency, and drug release studies of the microsphere at the end of 15, 30, 45 and 60 days. The results of stability studies are given in the Table 16.There was no significant change in the physical appearance, drug entrapment, and *in-vitro* release study of the microspheres.

Table 16: Stability Studies for Formulations Stored at 40ºC/75% RH

Tested after days	% Drug entrapment		% CDR	
	F1	F5	F1	F5
15	78.12	73.26	81.723	82.175
30	77.37	73.29	80.234	82.147
45	77.41	72.23	81.173	81.765
60	78.26	72.32	81.69	82.251

Chapter 6

Conclusion

6. CONCLUSION

The present study reports a novel attempt to formulate microspheres of the Lamivudine by using natural gums like xanthan gum and guar gum as carrier for better treatment of HIV and chronic hepatitis B. Microspheres of Lamivudine were prepared by solvent evaporation method. Various evaluation parameters were assessed, with a view to obtain controlled release of Lamivudine.

Details regarding preparation and evaluation of formulations have been discussed in previous chapters. From the study following conclusions could be drawn,

- FTIR study indicated that the drug is compatible with all the excipients.
- Natural gums like xanthan gum and guar gum can be used to formulate microspheres.
- Micromeritic studies revealed that the mean particle size of the prepared microspheres was within the range of 278±7.14 to 991±10.73 µm.
- SEM analysis of the microspheres revealed that guar gum containing microspheres were smooth, spherical and slightly aggregated particles when compared with the microspheres of xanthan gum which were porous, rough, grossly, discrete spherical.
- Good percentage of drug entrapment and practical yields were obtained with all the polymers. As the polymer concentration was increased the % drug loading decreased and % entrapment efficiency was increased due to increase in the viscosity of the solution.
- Cumulative percentage drug release significantly decreased with increase in polymer concentration.
- The overall curve fitting into various mathematical models was found to be on an average. The formulations F1 to F9 were best fitted to zero order kinetic model and the drug release from the formulation was by non-Fickian diffusion mechanism.

- Selected F1 and F5 formulated microspheres were stable and compatible at the selected temperature and humidity in storage for 60 days.
- From the stability studies it was found that there was no significant change in the drug entrapment, and *in-vitro* drug release characteristics of the microspheres.

Thus, the formulated microspheres seem to be a potential candidate as an oral controlled drug delivery system in prolonging the drug retention in GIT.

SCOPE OF THE STUDY:

- Further detailed stability studies and in-vivo bioavailability studies are to be done to establish the efficacy of these formulations.
- *In-vitro–in-vivo* correlations are to be done to establish the guarantee of efficacy and bioavailability of the formulation.

Chapter 7

Summary

7. SUMMARY

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body and also achieve and maintain the desired plasma concentration of the drug for a particular period of time. However, incomplete release of the dug, shorter residence times of dosage forms in the upper GIT leads to lower oral bioavailability. Such limitations of the conventional dosage forms have paved way to an era of controlled and novel drug delivery systems.

Lamivudine, an anti-viral drug has been chosen as a model drug in the formulation of controlled drug delivery drug delivery systems for the present work. It is a drug of choice in treatment of HIV. However, it has been reported that absolute bioavailability of Lamivudine when given orally is (86%) and half life of 4-6 hrs. A microparticulate drug delivery system was planned for Lamivudine as such a system when administered would remain adheres on the gastric mucosa for a prolonged period of time and drug would be available in the dissolved form. This would lead to improvement in the bioavailability of the drug. In this way, it stands an advantage over conventional dosage form.

The scheme of work has been divided into various parts. The collection of theoretical and technical data by extensive literature survey, review of literature and drug profile is presented in chapter 3 and 4 respectively. This was followed by procurement of materials and standardization of all materials used in the formulation of microspheres.

Microspheres formulations were prepared using two polymers (Xanthan gum and Guar gum) by using Solvent evaporation technique.

The prepared microspheres were characterized for their percentage yield, particle size, morphology, drug entrapment, *in-vitro* release and drug release studies. Almost all the formulations showed fairy acceptable values for all the parameters evaluated.

Microspheres of different sizes and improved drug entrapment efficiency could be obtained by varying the drug to polymer ratio. Further the analysis of release mechanism was carried out by fitting the drug diffusion data to various kinetic equations. The overall curve fitting into various mathematical models was found to be average and best fitted into zero order kinetic model. The drug release from the formulations was by non-fickian diffusion mechanism.

Stability study was conducted for the prepared microspheres of selected formulations for 60 days. There was no significant change in the physical appearance, drug entrapment, and *in-vitro* release study of the microspheres.

From the above results F1 and F5 were found to be best formulations for the oral delivery of Lamivudine that complied with all the parameters. However, *in-vivo* experiments need to be carried out to know the absorption pattern and bioavailability of drug from the microspheres and thus enabling us to establish *in-vitro* and *in-vivo* correlation.

Thus, the prepared microspheres proved to be a potential candidate as a microparticulate controlled release drug delivery device in this era of patenting novel and controlled release formulations.

Chapter 8

Bibliography

8. BIBLIOGRAPHY

1. Bhabani Shankar N, Uday Kumar N. Lamivudine loaded microspheres for oral use; Design, Development and establishment of *In Vivo-In Vitro* Correlation. Asian J of Pharma and Clinical Research.**2009;2(1):55-60.**
2. Bijank G, Rabindra D, Soumyadeep G, Manas C, Amitava B. Formulation Development Studies of Bilayer Tablet Glipizide: A novel and Evolutionary Approach in the Treatment of Diabetes. Asian J Pharm Cli Res. **2013;6(4):131–137.**
3. Srikanth P,Raju N,Wasim Raja S, Brito Raj S. A review on Oral controlled drug delivery.Int J of Adv Pharmaceutics.**2013;3(1):51-58.**
4. Brahmankar DM and Jaiswal SB. Biopharmaceutics and Pharmacokinetics - A treatise: 1st edition. Delhi: Vallabh Prakashan; **2000.P: 337.**
5. Chein YW. Novel Drug Delivery Systems. 2nd edition. New York: Marcel Dekker**; 2005**. **P.139-41.** (Drug and the Pharmaceutical Sciences; vol.29).
6. McConville JT. Recent trends in oral drug delivery: Drug Del Re Autumn /winter ;**2005.24-26.**
7. G. Robinson JR, Swarbrick J. Sustained and controlled drug delivery systems: Secondary education. New York: Marcel Dekker. inc.; **1987.**
8. H.Talukder R, Fassihi R. Gastroretentive delivery systems: A mini review, Drug Development and Industrial Pharmacy. **2004;30 (10):1019-1028.**
9. Venkatesan P,Manavalan R and Valliappan K. Microencapsulation: a vital technique in Novel drug delivery system.J of Pharmaceutical Sciences and Res:**2009;1(4):26-35**
10. Gunja c, Prakash B, Saha R.N.Microspheres technology and its applications.(PDF).http://docstoc.com/docs/38166355/review-on-microspheres,**Nov 2010**

11. Sahil K,Akanksha M, Premjeet S, Bilandi A and Kapoor B.Microsphere:A Review.Int J of Res in Pharmacy and Chemistry.**2011;1(4):2231-2781.**
12. Alagusundaram M, Madhu Sudana Chetty.C, Umashankari K, Venkata Badarinath A, Lavanya C and Ramkanth S.Int J of ChemTech Res.**2009;1(3):526-534.**
13. Deshmukh V,Warad S,Solunke R, Walunj S,Palve S, Jagdale G.Microspheres:As New Drug Delivery system.**2013;2(6):4504-4519.**
14. Vikrant K Nikam, Gudsoorkar VR, Hiremath SN, Dolas R.T and Kashid VA.Microspheres- A Novel Drug Delivery :An Overview.Int J of Pharmaceutical and Chem Sciences.**2012;1(1):113-128.**
15. Vamsi Krishna LN, Kulkarni PK, Mudit Dixit, Lavanya D, Raavi PK. Brief introduction of Natural gums, Mucilages and their applications in Novel drug delivery systems-A Review.**2011;2(6):54-71.**
16. Girish k jani, Dhiren p shah, Vipul d prajapati, Vineet c jain.Gums and mucilages: versatile excipients for pharmaceutical formulations.Asian J of pharmaceutical sciences.**2009;4(5):308-322.**
17. Sharma P, Chawla A, Sandeep A, Pravin P . Novel Drug Delivery approaches on Antiviral and Antiretroviral agents. J of advanced Pharm Tech & Research. **2012;3(3):151–9.**
18. M.D.Dhanaraju , R.Mani Kumar, P.Nithya, J.V.V.N.Kishan, G.Thirumurugan. Controlled Delivery of Antiretroviral Drug loaded Chitosan Cross linked Microspheres. Scholars Res Library .**2009;1(2):279-86.**
19. Vamshi Allam K, Dr Praveen Kumar G, Vishvasa Cheruku, Anand Jannu, Chaitanya Kumari Bairi.Controlled and sustained release approaches in developing suitable dosage forms for the Antiretroviral drug Lamivudine.**2011;8(1):21-27.**

20. Velmurugan S, Ashraf Ali M, Praveen Kumar. Microparticulate Drug Carriers: A Promising Approach for the delivery of Anti Hiv Drugs.Int J of Pharmacy and Pharmaceutical sciences.**2014;6(2):31-39.**
21. .http://aidsinfo.nih.gov/education-materials/fact-sheets/19/73/the-hiv-life-cycle
22. http://en.wikipedia.org/wiki/Antiviral_drug
23. KD Tripathi. Essentials of medical pharmacology-6th Edition. Jaypee brothers medical publishers (p) LTD, page no; **767.**
24. Chavda Y, Bhimani B, Patel G, Daslaniya D. IJPRBS Preparation and evaluation of Lamivudine Microspheres with eudragit ® polymers by solvent evaporation method for lymphatic system. ijprbs. **2013;2(3):89–106.**
25. Parveen A, Syed IA. Formulation and Evaluation of Mucoadhesive Microspheres of Lamivudine. Int J Pharm Sci Drug Res. **2014;6(2):102–8.**
26. Kumar Darapu B.N, Moorthy KS, Vetrichelvan T. Research Article Formulation and *In-Vitro* Evaluation of Gastroretensive Floating Microspheres of Ranitidine Hydrochloride. Pharmanest. **2010;1(2):298–306**.
27. A AKM, R KR, Ramanathan G, J HSAM, Rohini SG. Available online http://www.ijddr.in Covered in Official Product of Elsevier , The Netherlands © 2010 IJDDR Formulation of Lamivudine Microspheres in Multiple Emulsion Form Using Osmogen And Different Polymers - Studying The Release Profiles. Int J Drug Dev Res. **2011;3(3):277–84.**
28. Brahmaiah B, Kumar Desu P, Nama S, Sd.Khalilullah, S.Satish Babu. Formulation and evaluation of extended release Mucoadhesive microspheres of Simvastatin. Int J Pharm Biomed Res **2013, 4(1), 57-64.**

29. Rouf A, Mamun A, Bagchi M, Amin L, Sutradhar KB, Huda NH. Development of natural gum based glipizide mucoadhesive microsphere. J Appl Pharm Sci. **2014;4(01):66–9.**
30. Patil PB, Gawali VU, Patil HN, Hardikar SR, Bhosale A V. Preparation and Evaluation of Mucoadhesive Microspheres of Atenolol and Propranolol. Int J Pham Tech Res. **2009;1(3):639–43.**
31. Raj P, Mohammed I, Pravalika T. Controlled release Formulation and Evaluation of Idarubicin Microsphere using Biodegradable Hydrophilic and Hydrophobic Polymer Mixtures. Asian J of Pharma and Clinical Research. **2010;3(3):3–6.**
32. Priyadarshini M.K, S.Parthiban,Kumar GPS, Tamizh Mani T.Formulation and evaluation of Microspheres encapsulating Zidovudine by ionic gelation techniques. Int J Res Pharm Nano Sci. **2014;3(5):461–8.**
33. Patil JS, Kadam D V, Shiralashetti SS, Marapur SC, Kamalapur M V. Utilization of Natural Olibanum Gum Resin as a Rate Controlling Polymer in Design and Evaluation of Microspheres of an Antiretroviral Drug by using a Unique Spray Drying Technique. Indian J Pharm Educ Reseach. **2012;46(2):155–60.**
34. Value SJRI, Asha K, Vikash D. Available online http://www.ijddr.in Covered in Official Product of Elsevier , The Netherlands Formulation and evaluation of Zidovudine loaded chitosan Microspheres for controlled release. Int J Drug Dev Res. **2012;4(1):96–105.**
35. Mankala SK, Nagamalli NK, Raprla R, Kommula R. Preparation and Characterization of Mucoadhesive Microcapsules of Gliclazide with Natural Gums. Stamford J Phamaceutical Sci. **2011;4(1):38–48.**

36. Prakash K, Raju PN, Shanta KK, Lakshmi MN. Preparation and characterization of lamivudine microcapsules using various cellulose polymers. Trop J Pharm Res. **2007;6(December):841–7.**

37. Sudhamani T. Preparation And Evaluation Of Ethyl Cellulose Microspheres Of Ibuprofen. Int J Pharma Res Dev. **2010;2(8):119–25.**

38. Lj JJ, Rt M, Wilson B, Shanaz B, Bincy R. Formulation And In Vitro Evaluation Of Floating Microspheres Of Anti-Retro Viral Drug As A Gastro Retentive Dosage Form. Int J Res Pharm Chem. **2011;1(3):519–27.**

39. Patel PK. Research and Reviews : Journal of Pharmacy and Pharmaceutical Sciences Preparation and Characterization of Crosslinked Gum Acacia Microspheres by Single Step Emulsion In-Situ Polymer Crosslinking Method : A Potential Vehicle for Controlled Drug. J Pharm Pharm Sci. **2013;2(1):40–8.**

40. Ershad S, Kishore VS, Sandeep M, Kartheek U, Rizwana SK, Tejaswi L. Preparation And Evaluation Of Floating Microspheres Of Ritonavir. Int J Res Pharm Chem. **2013;3(4):834–41.**

41. Brahmaiah B, Desu PK, Nama S, Khalilullah S, Babu SS. Formulation and evaluation of extended release mucoadhesive microspheres of simvastatin. Indian J Pharm Biomed Res. **2013;4(1):57–64.**

42. http://www.drugbank.ca/drugs/DB00709

43. http://en.wikipedia.org/wiki/Lamivudine

44. Allam KV, Kumar GP, Cheruku V, Jannu A, Bairi CK. Review Article. Int J Pharm Sci Rev Res. **2011;8(1):21–7.**

45. Hand book of pharmaceutical excipients, 5th edn. Rowe RC, Sheskey PJ and Owen SC (Eds.,) Pharmaceuticl press, Washington DC, USA. **2006:297-300,237-241, 97-100.**
46. http://en.wikipedia.org/wiki/Xanthan gum.
47. Ainley Wade, Paul J Weller. Hand book of pharmaceutical excipients. **2th edn. Page no 215**.
48. http://en.wikipedia.org/wiki/Guar gum.
49. Anjana J, Hukuman Z, D.V.Gowda.Preparation,Evaluation and vioavailability studies of propafenonr hydrochloride loaded with gum microspheres for controlled study:Int J of pharma and Bio science.**2013;4(2):1036-1049.**
50. Liberman H, Lachman L. The Theory and Practice of Industrial Pharmacy. 3rd ed. Bombay: Verghese Publication House; **1991. p. 171-93**.
51. Martin A, Micromeretics. In: Martin A, ed. Physical Pharmacy. Baltimores, MD: Lippincott Williams and Wilkins; **2001. p. 423-54.**
52. Krishnan SK,Gulzar Ahmed M,B.Ramesh, Kiran kumar P.Design and evaluation of chitosan based mucoadhesive microspheres of captopril;Int J of univ pharmacy and bio sciences.**2012;1(2):67-77.**
53. Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. Eur J Pharm Sci. **2001;13:123-33**.
54. Shyamala Bhaskaran.Industrial pharmacy. 1st ed. New Delhi. Birla Publications Pvt. Ltd; **2010. p. 46-69.**
55. ICH Q1A (R2) Stability testing guidelines: Stability Testing of new drug substances and products. [Online]. [Cited 2008 Nov 10]; Available from:URL:http://www.tga.health.gov.au/docs/pdf/euguide/inch/273699r2en.pdf