DEVELOPMENT AND CHARACTERIZATION OF LAMIVUDINE LOADED NANOPARTICLES

Dissertation submitted in partial fulfillment of the requirement for the award of the

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IN

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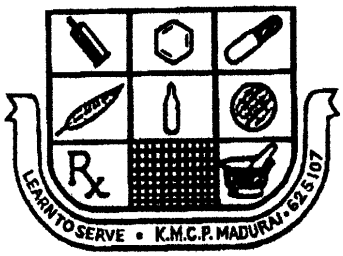
CHENNAI



DEPARTMENT OF PHARMACEUTICS

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CERTIFICATE

This is to Certify that the dissertation entitled **"DEVELOPMENT AND CHARACTERIZATION OF LAMIVUDINE LOADED NANOPARTICLES"** submitted by **Mr.S.PALANIKUMAR** to Tamilnadu Dr.M.G.R.Medical University, Chennai, in Partial fulfillment for the award of Master of Pharmacy in Pharmaceutics at K.M. College of Pharmacy, Madurai, is a bonafide work carried out by him under my guidance and supervision during the academic year **2011-2012**.

PRINCIPAL & GUIDE

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DEDICATED TO MY

FATHER AND MOTHER

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CONTENTS

1. INTRODUCTION

Most of the drugs introduced to clinical medicine exert their effects by interactive interference with cell and cell membrane related structure and functions through concentration dependent reversible interactions at specific receptor site(s). Obviously, to obtain a desirable therapeutic response, the correct amount of drug should be transported and delivered to the site of action with subsequent control of drug input rate. The distribution of other tissues therefore seems unnecessary, wasteful and a potential cause of toxicity. The developments of over past decades indicate explicit progress in the area of controlled and targeted drug delivery.

Quantitative targeted drug delivery involves a number of essential bio ligands for physiologic cell need and bio signaling. These operated through bioports referred to as receptors. The ligand-receptor interactions are highly stereo specific. Thus ligands or receptors could be exploited for site/cell specific drug delivery in a well-defined manner. Selective drug delivery or targeting seeks to improve upon the benefit/risk ratio associated with drugs. Approaches are being adopted either to control the distribution of drug by incorporating in a carrier system by altering the structure of the drug at the molecular level, or by controlling the input of the drug into the bio environment to ensure a programmed and desirable bio distribution.

The efforts to improve drug effectiveness in therapeutics have been assisted by parallel developments in molecular and cell biology. Such developments supported the successful generation of target oriented drug delivery systems.

Target therapy remains an unachieved goal; however the idea stimulated a long series of experiments that propounded the philosophy of targeting of drugs and genes are attracted present generation of researchers towards the problems and prospects associated with the concept.

A number of essential aspects should be considered for the designing of drug delivery systems to achieve this goal include target, carrier, ligand (s) and physically modulated components.

Targeted drug delivery implies for selective and effective localization pharmacologically active moiety at pre identified targets in therapeutic concentration, while restricting its access to non-target normal cellular linings, thus minimizing toxic effects and maximizing therapeutic index.

1.1. CARRIERS OF TARGETED DRUG DELIVERY SYSTEM[1]:

Targeted drug delivery sometimes called smart drug delivery. It is a method of delivering medication to a patient in a manner that in increase the concentration of medication in some part of the body relative to others. The goal of targeted drug delivery system is to prolonged, localize, target and have precede drug interaction with the diseased tissue. Advanced to targeted release system is the reduction in the frequency of the dosage taken by the patient, having more uniform effect of the drug, reduction of side effect, and reduction fluctuation in circulation drug level.

Targeted drug delivery system has been developed to optimize regenerative techniques. The system is based on method that delivers a certain amount of a therapeutic agent for a prolonged period of time to a target diseased area within the body. This helps maintain the required plasma and tissue drug levels in body. Therefore avoid any damage to the healthy tissue via drug. The drug delivery system is highly integrated and requires various disciplines, to optimize this system. **C**arrier is one of the most important entities essentially required for successful transportation of the loaded drug(s). They are drug vectors, which sequester, transport and retain drug enroute, while elute or deliver it within or in the vicinity of target. Carrier can do so either through an inherent characteristics or acquired (through structural modification), to interact selectively with biological targets, or otherwise they are engineered to release the drug in the proximity of target cell lines for effective pharmacological actions.

An ideal carrier engineered as targetable device should have following features:

- Must be able to cross anatomical barrier
- Must be recognized specifically and selectively by the target cells.
- Linkage of the drug and directing unit (ligand) should be stable in plasma.
- Should be non-toxic, non-immunogenic and biodegradable after recognition and release of drug moiety.

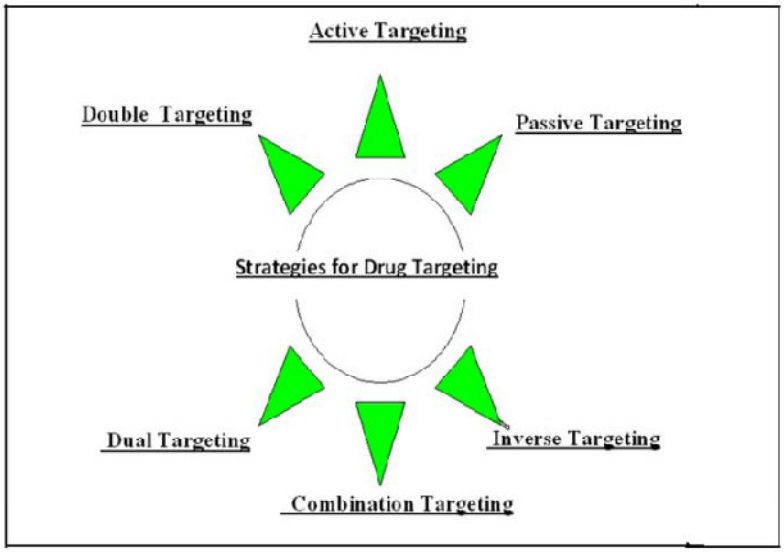
Several carriers were used as pilot molecules to selectively deliver the drug to the intended cells have been reported. Based on the nature of their origin they are categorize

Table: 1. CARRIER SYSTEMS USED FOR TARGETED DRUG DELIVERY

S. No	Category	Example
1	Colloidal Carriers	a) Vesicular systems Liposomes, Niosomes, Pharmacosomes, Virosomes, Immuno liposomes b) Micro particulate systems Microparticles, Nanoparticles, Magnetic Microspheres, Albumin Microspheres, Nanocapsules.
2	Cellular Carriers	Resealed erythrocytes, serum albumin, antibodies, platelets, leukocytes.
3	Supra molecular delivery systems	Micelles, reverse micelles, mixed micelles, polymeric micelles, liquid crystals, lipoproteins, synthetic LDL.
4	Polymer based System	Signal sensitive, Mucoadhesive, Biodegradable, Bioerodible.
5	Macro molecular carriers	a) Proteins, glycoproteins, neo glycoproteins, artificial viral envelopes. b) Glycosylated water soluble polymers (poly-lysine). c) Mabs, Immunolgical Fab fragments, antibody enzyme complex. d) Toxins, immunotoxin & rCD4 toxin conjugates. e) Lectins & polysaccharides.

1.2 LEVELS OF DRUG TARGETING:

Dependability of a carrier system relies on exploiting both, intrinsic pathway these carriers follow and the bio protection that they can offer to drugs during transit through the body. The various approaches of vectoring the drug to the target site can be classified as:



Fig; 1 level of drug targeting

- Passive targeting
- Inverse targeting
- Active targeting (ligand mediated and physical targeting)
- Dual targeting
- Double targeting
- Combination targeting

Passive targeting

Systems that target the systemic circulation are generally characterized as "passive" delivery systems. In this system targeting occurs because of the body's natural response to the physicochemical characteristics of drug carrier system. The ability of some colloids to be taken up by the RES especially in liver and spleen has made them as ideal vectors for passive hepatic targeting of drugs to these compartments. Passive capture of colloidal carriers by macrophages offers therapeutic opportunities for the delivery of anti-infective for disease conditions that involve macrophage cells of RES e.g. Leishmaniasis, brucellosis, and candidiasis.

Inverse targeting

Lazo and Hacker, 1986 developed inverse targeting. It is essentially based on successful attempts to avoid passive uptake of colloidal carriers by RES. This

effectively leads to the reversion of bio distribution trend of the carrier and hence the process is referred to as inverse targeting. One strategy to achieve inverse targeting is to suppress the function of RES by a pre-junction of colloidal carriers or macromolecules like dextrin sulphate. This leads to RES blockade and as a consequence impairment of host defence system. Alternative strategies include modification of the size, surface charge, composition, surface rigidity and hydrophilicity of carriers for desirable bio fate.

Active targeting

The facilitation of the binding of the drug-carrier to target cells through the use of ligands or engineered homing devices to increase receptor mediated localization of the drug and target specific delivery of drug (s) is referred to as active targeting. This can further be classified as

1. First order targeting (organ compartmentalization)
2. Second order targeting (cellular targeting)
3. Third order targeting (intracellular targeting)

1. First order targeting

It refers to restricted distribution of the drug-carrier system to the capillary bed of a predetermined target site, organ or tissue compartmental targeting in lymphatic, peritoneal cavity, plural cavity, cerebral ventricles, lungs, joints, eyes, etc., represents first order targeting.

2. Second order targeting

The selective delivery of drugs to a specific cell type such as tumour cells and not to the normal cells is referred as second order drug targeting e.g. the selective drug delivery to the kupffer cells in the liver.

3. Third order targeting

The third order targeting is defined as drug delivery specifically to the intracellular site of target cells e.g. Receptor based ligand-mediated entry of a drug complex into a cell by endocytosis.

Ligand mediated targeting

Ligand mediated active targeting could be achieved using specific uptake mechanisms such as receptor dependent uptake of natural low density lipoproteins (LDL) particles and synthetic lipid micro emulsions of partially reconstituted LDL particles coated with the apo proteins. The apo protein coat serves as a ligand for the LDL receptors expressed in the body.

Physical targeting (Triggered release)

The selective drug delivery programmed and monitored at the external level (ex vivo) with the help of physical means is referred to as physical targeting.

Dual targeting

The dual targeting employs carrier molecules, which have their own intrinsic antiviral effect thus synergies the antiviral effect of the loaded active drug. Drug conjugates can be prepared with fortified activity profile against the viral replication.

Double targeting

For a new future trend, drug targeting may be combined with another methodology, other than passive and active targeting for drug delivery systems. The combination is made between spatial control and temporal control of drug delivery. E.g. Mew and co-workers reported haematoporphyrin (HP)-anti-M-1antibody conjugates for the suppression of tumour following incandescent light exposure.

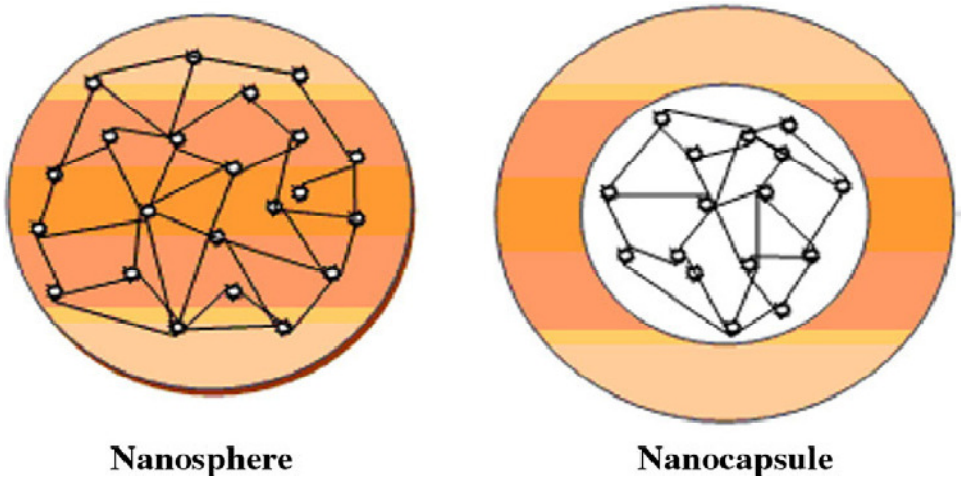
Combination targeting

Petit and Gombtz, 1998 have suggested the term combination targeting for the site-specific delivery of proteins and peptides. These targeting systems are equipped with carriers, polymers, and homing devices of molecular specificity that could provide a direct approach to target site.

1.3 PARTICULATE/COLLOIDAL DRUG CARRIER SYSTEM[2]

The colloidal carriers based on biodegradable and biocompatible polymeric systems have largely influenced the controlled and targeted drug delivery concepts. Nanoparticles are sub-nanosized colloidal structures composed of synthetic or semi synthetic polymers. Nanotechnology represents an important technological revolution in 21st century. Nanotechnology is the study of nanoparticles, nanosuspension, nanoemulsion etc. Nanoparticle is a collective name for nanospheres and nanocapsule. Nanospheres have a matrix type structure. Drugs may be adsorbed at their surface, entrapped in the particle or dissolved in it.

Fig: 2. Structures of Nanosphere and Nanocapsule



Nanocapsule has a polymeric shell and an inner liquid core. The active substance is usually dissolved in the inner core, but may also be adsorbed at their surface.

Nanosuspension consist of essentially pure drug and minimal quantities of surface stabilizing agents that may enable high dosing with reduce toxicity provided toxic drug levels are not reached in vivo. It also provides sustained release at a level below the maximum tolerated dose yet at its therapeutically effective concentration. They are mainly used for parental drug delivery. A coarse solid suspension (10-100 μm) has been produced for intramuscular and subcutaneous delivery of poor water soluble drug.

Table: 2 various carrier based dosage forms[3]

s.no	Carrier system	Size range	Features	Method of prepration
1	Nanoparticles	10-1000 nm	Submicron-sized Colloidal systems, biodegradable (or) not	Emulsion solvent evaporation method
2	Polymeric nanoparticles	10-1000 nm	Sub-nanosized colloidal structure composed of synthetic or semi- synthetic polymers	Nano precipitation method
3	Liposomes	25-100 nm	Microscopic vesicles composed of one or more concentric lipid bilayer, separated by water or aqueous buffer compartments	Nano precipitation method
4	Solidlipid nanoparticle	50-1000 nm	Submicron colloidal carriers containing solid hydrophobic core having a monolayer of phospholipids coating.	High-pressure homogenization Micro emulsion formation precipitation as lipid nanopellets
5	Lipid emulsion	Lipid globule 1-100 nm	Multi component fluid made of water. A hydrophobic liquid, or several surfactants resulting in a stable system	o/w w/o w/o/w
6	Lipidmicrotubles /microcylinders	1um	Self-organizing system in which surfactants crystallize into tightly packed bilayers that spontaneously form cylinders	Self-emulsification

7	Nanotubes and nanowires		Self-assembling sheet of atoms arranged in the form of tubes and thread-like structures of nanoscale range	Chemical evaporation dispersion
8	Functionalized nanocarriers/ quantam dots		Combination of functionalities of biomolecules and non-biologically derived molecular species	
9	Ceramic nanoparticles	50 nm	Made up of inorganic (ceramic) compounds such as silica, titania and alumina	Emulsion solvent evaporation method
10	Ethosomes		Noninvasive delivery carriers that enable drugs to reach the deep skin and systemic circulation	Ethanol injection-sonication method
11	Lipospheres	0.2-100 um	Water dispersible solid micro particles composed of solid hydrophobic fat core stabilized by a mono layers of phospholipids molecules embedded in a micro particles surface	Melt method, Co-solvent method, Pre incorporation into lipophilic carrier
12	Multi composite ultrathin capsules	50 nm few micron	Molecular assemblies of tailored architecture having layer-by-layer adsorption of oppositely charged macromolecules on to colloidal particles	Langmuir-Blodgett technique and chemisorption from solution

13	Pharmacosomes		Pure drug vesicles formed by the amphiphilic drugs	
14	Niosomes	12-16 micron	Non-ionic surfactant vesicles are bi-layered structures	Ether injection method.
15	Dendrimers		Macromolecular compounds that consist of a series of branches around an inner core	Polymerization
16	Collidosomes		Solid microcapsules which are hollow, elastic shells	Self-assembly of colloidal particles at the interface of emulsion droplets
17	Discomes	12-16 microns	Non-ionic surface active agents based discoidal vesicles.	Ether injection method
18	Aquasomes	60-300 nm	The particles core is composed of non-crystalline calcium	Self-assembling by co-precipitation method

Market Potential[4]

Nanotechnology has become the focus of immense expectation in terms of market potential and efficiency. Over 200 companies are engaged in manufacturing nanomedicine. The market amounts to $ 6.8 billion in the year 2005 about 75% of this income were from drug delivery products. It is expected to reach $ 12 billion by the end of 2012 due to the launch of more nanoproducts which is under research. Nanobased diagnostic products are about $ 12 million.

The number of products based on **polymeric microparticles** on the markets is

limited. After the introduction of the first wave of products, there was only a limited increase in the number of micro particulate products. Hence polymeric nanoparticle was introduced.

Nanoparticles,[5] are referred as nanosized colloidal particle, ranging in size from 10-1000 nm in which drug is dissolved, dispersed, attached, adsorbed or encapsulated by polymers which may be synthetic, semi-synthetic or biodegradable. Nanoparticles are ultra-fine colloidal structures, which carry drug particles that may not dissolve but remains suspended in medium. Due to their small size nanoparticles can be easily absorbed into RES and other systems of body and give a quick & better action.

1.4 POLYMERIC NANOPARTICLES[5]

These are colloidal particles ranging in the size from 10-1000 nm. They consist of macromolecular materials and can be used therapeutically, e.g. as adjuvant in vaccines, drug carriers, in which the active principle (Drug or biological active material) is dissolved, entrapped or encapsulated and the active principle is adsorbed or attached.

The concept of using nanoparticle for drug delivery was developed first by Speiser and co-workers in the late 1960 and early 1970s when cross-linked poly acrylamide nanoparticles are produced by the polymerization of acrylamide and NN – Methylene bisacrylamide after secondary solubilization in an organic solvent such as hexane. The active ingredients, drug or antigen, were incorporated into the solubilized aqueous phase. Because of the larger amount of organic solvents and surfactants used for the manufactures to develop nanoparticle, the process is now only of historical interest. Polymeric nanoparticles are composed of biodegradable or bio stable polymers and copolymers. The active agents can be;

(i) Entrapped or encapsulate within the particles
(ii) Physically adsorbed on surface,(or)
(iii) Chemically linked to the surface of the nanoparticles.

Nanoparticles have been explored for the delivery of anti-HIV molecules and it used to Targeted antiretrovirals;

(i) Macrophages/ monocytes
(ii) CNS which act as viral reservoir sites during HIV.

Polymeric nanocarrier-based antiretroviral therapy:

Polymeric nanocarriers are generally composed of polymers like gelatin, chitosan, poly (D, L-lactide-coglycolide), poly (a-caprolactone) etc. These are again classified into 3 types: polymeric micelles, nanoparticles & dendrimer type. Among polymeric nanocarriers, dendrimers and nanoparticles are important classes. Most of the nanoparticle based targeted delivery of antiretroviral drugs has been studied to target cells of the mononuclear phagocytic system (MPS), such as the monocytes/macrophages (Mo/Mac) that act as a reservoir for the HIV virus.

Advantages of polymeric nanoparticles

- Reduction of toxicity and occurrence of adverse reactions
- Better drug utilization
- Controlled rate of drug release
- Specific site of drug release
- Greater patient convenience and better patient compliance
- Enhancement of the therapeutic effectiveness of the drug i.e. the overall
- pharmacological response per unit dose
- Method of preparations are reproducible
- Easy handling of nanoparticles prepared in the powder form
- Nontoxic and biodegradable
- Relatively cheaper and stable
- No swallowing problems in case of oral administration.

Polymers used in Preparation of Polymeric Nanoparticles

Polymers used in manufacturing of polymeric nanoparticles are of two types

1. Natural hydrophilic polymers (Proteins and polysaccharides)
2. Synthetic hydrophobic polymers (Poly lactic acid and PLGA)

Natural Hydrophilic polymers:

Albumin, Gelatin, legumin or vicilin as well as polysaccharides like alginates or agarose have been extensively studied and characterized. These macromolecules are used due to their intrinsic biodegradability and biocompatibility.

Disadvantage with natural polymers are:

- Batch to batch variation

- Conditional biodegradability.
- Antigenicity.

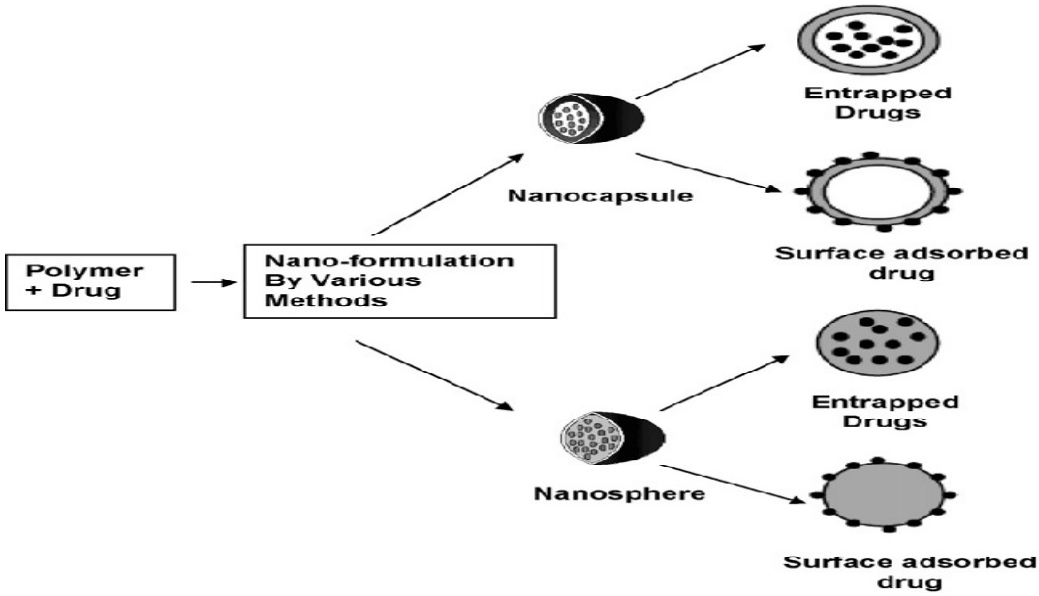
Synthetic Hydrophobic polymers:

Polymers which are used for microspheres preparation are used for nanoparticle preparation. Most of them are hydrophobic in nature. The polymers are either pre-polymerized or synthesized during nanoparticles preparation.

Table: 3. Synthetic polymers used for preparation of nanoparticles.

S.No	Classification	Examples
1	Pre-polymerized	Poly (ε-caprolactone) (PECL) Poly (lactic acid) (PLA) Poly (lactide-co-glycolide)(PLGA) Poly styrene
2	Polymerized insitu	Poly (isobutylcyanoacrylates)(PICA) Poly (butylcyanoacrylates)(PBCA) Poly hexylcyanoacrylate (PHCA) Poly methyl (methacrylate)(PMMA) Co polymer of aminoalkymethacrylate methyl methacrylate

Fig 3: Mechanism of formation of polymeric nanoparticles in drug delivery



1.5 PREPARATION TECHNIQUES OF NANOPARTICLES[6,17]:

The selection of the appropriate method for the preparation of nanoparticles depends on the physicochemical characteristics of the polymer and the drug to be loaded.

Two types of systems with different inner structures are apparently possible they are:

1. A matrix type system consisting of an entanglement of oligomer or polymer units (nanoparticles/nanospheres)
2. A reservoir type of system comprised of an oily core surrounded by an embryonic polymeric shell (nanocapsules)

The drug can either be entrapped within the reservoir or the matrix or be adsorbed on the surface of these particulate systems. The polymers are strictly structured to a nanometric size range using appropriate methodologies. They are classified as:

Amphiphilic macromolecule cross-linking

- Heat cross-linking
- Chemical cross-linking

Polymerization based methods

- Polymerization of monomers insitu
- Emulsion (micellar) polymerization
- Dispersion polymerization
- Interfacial condensation polymerization
- Interfacial complexation

Polymer precipitation methods

- Solvent extraction/evaporation
- Solvent displacement (nanoprecipitation)
- Salting out.

PREPARATION OF NANOPARTICLE USING POLYMER PRECIPITATION METHOD:

The hydrophobic polymer and hydrophobic drug is dissolved in a particular organic solvent followed by its dispersion in a continuous aqueous phase, in which the polymer is insoluble. Precipitation of the polymer produces nanoparticles with drug

loaded in it. The external phase also contains the stabilizer. Depending upon solvent miscibility techniques they are designated as solvent/evaporation method.

Polymer precipitation can be brought out by increasing the solubility of the organic solvent in the external medium by adding an alcohol

- By incorporating additional amount of water into the ultra-emulsion
- By evaporation of organic solvent at room temperature or at accelerated temperatures or by using vacuum. Using an organic solvent is completely soluble in the continuous aqueous phase – nanoprecipitation.

A. Solvent extraction method:

The preparation of nanoparticles starts with formation of conventional O/W emulsion between a partially water miscible solvent containing the polymer and the drug, and an aqueous phase containing the stabilizer. The subsequent removal of solvent (solvent evaporation method) or additions of water to the system so as to diffuse the solvent to the external phase (emulsification diffusion method) are the two variance of the solvent extraction method.

The classic procedure to prepare nanospheres is the polymer is solubilized in a solvent and dispensed in a gelatin solution by sonication to yield emulsion (O/W), then the solvent is eliminated by evaporation. For the evaporation purpose, apart from sonication, high speed/pressure homogenization methods are widely employed. The homogenizer breaks the initial coarse emulsion in nanodroplets (nano fluidization), yielding nanospheres with a narrow-size distribution.

B. Double Emulsion solvent Evaporation method:

Emulsion solvent evaporation technique has been further modified and a double emulsion (or multiple emulsion) of water in oil in water type has been used. Following evaporation of the organic solvent(s) nanoparticles are formed which are then recovered by ultracentrifugation, washed repeatedly with buffer and lyophilized.

Polymer are dissolved separately in aqueous and organic phases respectively containing stabilizer and subjected to ultra-sonication to yield water in oil emulsion (W/O). This W/O is further added to a PVA aqueous solution to yield the water in oil in water double emulsion (W/O/W). The organic solvent is allowed to evaporate

while being stirred first at atmosphere pressure for 16 h and then gradually at reduced pressure to yield nanoparticles.

C. Solvent Displacement or Nanoprecipitation

This method based on interfacial deposition of a polymer following displacement of a semi-polar solvent miscible with water from a lipophilic solution. Solvent displacement method involves the use of an organic phase, which is completely soluble in the external aqueous phase. The organic solvent diffuses instantaneously to the external aqueous phase, inducing immediate miscibility of both the phases. Consequently, neither separation nor extraction of the solvent is required for the polymer precipitation. After nanoparticles preparation, the solvent is eliminated and the free flowing nanoparticles can be obtained under reduced pressure. This method is particularly useful for drugs that are slightly soluble in water.

If the drug is highly hydrophilic, it diffuses out into the external aqueous phase, where as if the drug is highly hydrophobic, it may precipitate in the aqueous as nanocrystals, which further grown during storage. In the case of hydrophilic polymer, an aqueous solution of polymer is dispersed or emulsified in oil phase. The precipitation of polymer proceeds on addition of acetone.

D. Salting out:

The method involves incorporation of a saturated aqueous solution of polyvinyl alcohol (PVA) into an acetone solution of the polymer under magnetic stirring to form an O/W emulsion. However, the process differs from nanoprecipitation technique as in the latter the polymeric solution is completely miscible with the external aqueous medium. But in salting out technique, the miscibility of both the phases is prevented by the saturation of the external aqueous phase with PVA. The precipitation of the polymer occurs when a sufficient amount of water is added to external phase to allow complete diffusion of the acetone from internal phase into the aqueous phase.

Table 4: Methods, Polymer and drug that are used for Nanoparticles preparation[7]:

S.No	Methods and polymer employed	Incorporated or absorbed drug
1	**Emulsion polymerisation in continous aqueous phase**	
	Polymethyl methacrylate	5-flurouracil, Indomethacin
	Polymethylmethacrylateco-polymers	Doxorubicin
	Polymethyl cyanoacrylate	Actinomycin D, vinblastine, Methotrexate,
	Polybutyl cyanoacrylate	Progesterone, Pilocarpine.
2	**Emulsion polymerization in a continous organic phase**	
	Polyacrylamide	Norephedrine
	Polymethyl cyanoacrylate	Triamcinolon, fluorescein
3	**Interfacial polymerization**	
	Polyisobutyl cyanoacrylate	Indomethacin, Insulin
4	**Interfacial deposition**	
	Poly D, L –lactide	Indomethacin
5	**Denaturation of natural macromolecules in oil emulsion**	
	Serum albumin	Doxorubicin, Primaquine
6	**Carbohydrate nanoparticles**	
	Polyacrylstarch	Trimethoprim, primaquine
7	**Nanoparticle formation by desolvations of macromolecules**	
	Gelatin	Mitomycin C, Metronidazole
	Albumin	Insulin
8	**Solvent evaporation**	
	Eudragit RL	Indomethacin
	Polylactic acid	Triamcinolone.

1.6 PURIFICATION OF NANOPARTICLE:

Since most of the nanoparticles are indented for parental administration, purification of the raw nanoparticulate suspensions is essential. Depending on the method of preparation, the following potentially toxic and undesirable preparation additives can be present in the raw suspensions: organic solvents, surfactants, stabilizers, electrolytes and large polymer aggregates.

Purification is needed to separate free drug from the drug bound to the particles in order to avoid a burst effect at the time of injection. Polymer aggregates can be removed by filtration through sintered glass filters. Organic solvents especially chlorinated solvents must be absolutely eliminated below the pharmacopoeia limits. Poloxamer 188 and polysorbate 20, 40, and 80 are the surfactants approved for usage in injection form.

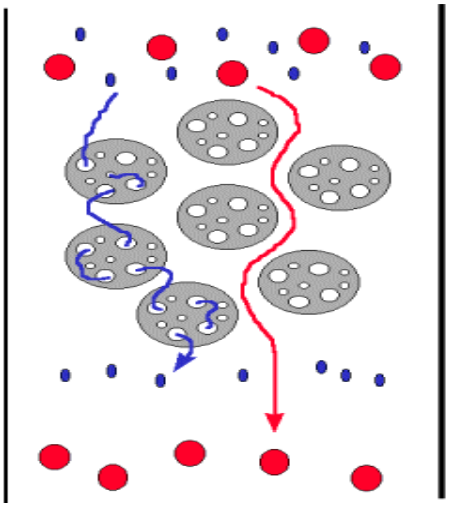
The purification technique involves the following:

1) Ultra-centrification.
2) Gel filtration.
3) Cross-flow filtration.
4) Dialysis

Gel filtration;

High molecular weight substance and impurities are difficult to remove.

Fig 4; Gel filtration chromatography



Cross flow filtration;

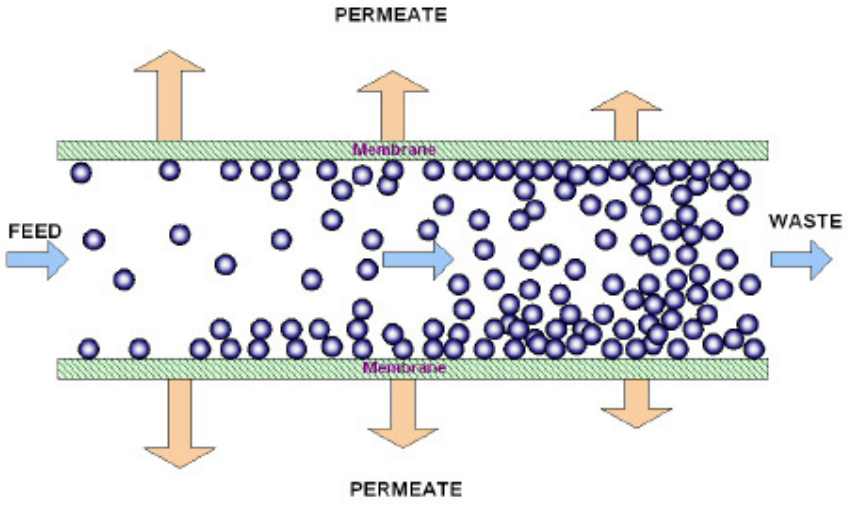


Fig 5; cross flow filtration

1.7 FREEZE – DRYING[8]:

On keeping the nanoparticles in aqueous medium during storage, biodegradation of polymer, drug leakage, and drug degradation may occur. To improve the physicochemical stability of the nanoparticles drying step is required. Heat drying may be harmful for thermoliable active compounds. Moreover due to the low glass transition temperature of poly (α- hydroxycarboxylic acid) the particles induces fusion or aggregation when heated at elevated temperatures. Therefore freeze-drying represents most applicable alternative method.

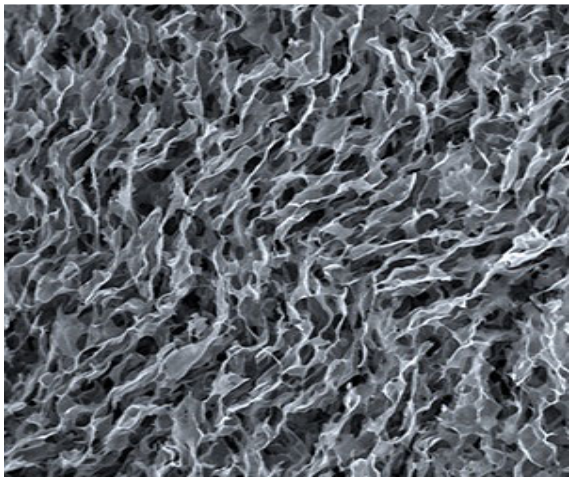


Fig 6; Freeze dried nanoparticles

The nanoparticulate suspensions are frozen at temperature ranging from -40^{o} C to -60^{o}C or in liquid nitrogen. Depending upon the amount of water to be evaporated, they are freeze-dried for periods ranging from 24 to 90 h, at a pressure below 10 Pa.

Ease of re-dispersion of freeze-dried particles depends on the particle coating. Usually particles are prepared in the presence of poloxamer, polysorbate, PVAL, or sodium dodecyl sufate and they are readily re-dispersible in water. Residues of these stabilizers or surfactants display cryoprotective characteristics and therefore facilitate aqueous reconstitution.

If the freeze- dried particles are not easily re-dispersible then addition of cryoprotective agent may be necessary. Dextrose, lactose, trehalose, sucrose and mannitol are the most widely used. Addition of cryoprotectant increases the tonicity of the final re-dispersed formulation.

1.8 STERILIZATION[30]

Nanoparticles are to be used as injections and should be sterile and apyrogenic. Aseptic processing in a clean room environment is costly, difficult to achieve and inherently risky with respect to microbial contamination of the finished product.

The nanoparticle sterilization carried by membrane filtration. The membrane filter pore size 0.22 um in some case micro-organisms and nanoparticles may be larger in size (0.25-1.0um). So this sterilization process is not more effective.

In this case the nanoparticles sterilization carried by aseptic condition with terminal sterilization process. The terminal sterilization is carried out by autoclaving. The processing under aseptic condition is not completely safe a terminal sterilization step is required to ensure the microbiological safety of final product.

Radiolytic degradation of the active compound can occur in certain cases with the formation of toxic or inactive by-products. Independent of the degradation mechanism, reduction in polymer molecular weight may significantly influence drug release patterns. Degradation of the polymer not only affects drug release, also affect the in vivo resorption of the drug carrier.

Sterile nanoparticles must meet pharmacopeia requirements relating to sterility and apyrogenicity. The nanosuspensions should be tested for sterility by membrane filtration of the test specimens followed by incubation of the membrane in a culture

medium of choice. Pyrogens are tested by measuring the rise of temperature of rabbits following intravenous administration of the product, and bacterial endotoxins are assayed by Limulus Amebocyte Lysate (LAL).

1.9 EVALUATION OF NANOPARTICLES

The prepared nanoparticles are evaluated for various parameters they are:

1. Yield
2. Drug loading
3. Entrapment efficiency
4. Size and morphology
5. In vitro drug release studies
6. Stability testing

Yield:

Percentage yield is of great importance if one considers industrial applications with costly raw materials. It plays crucial role in determining whether the preparation procedure chosen for incorporating a compound into the polymeric particles is efficient. The overall yield of a procedure is simply expressed as,

$$\text{Yield (\%)} = \frac{\text{Initial amount of raw materials}}{\text{Amount of nanoparticles}} \text{ X } 100$$

The initial amount of raw materials corresponds to the amount of active compound plus polymer however because of a certain amount of stabilizing agents or surfactants being adsorbed during the preparation, a correction factor should be introduced.

$$\text{Yield (\%)} = \frac{\text{Initial amount of raw materials}}{\text{Amount of nanoparticles X (1-Fraction of residual stabilizing agents)}} \text{ X } 100$$

Drug loading:

The drug loading or pay load or drug content is expressed as,

$$\text{Drug loading} = \frac{\text{Amount of drug in nanoparticles}}{\text{Amount of nanoparticles}} \text{ X } 100$$

Entrapment efficiency (E.E):

Drug entrapment efficiency represents the proportion of the initial amount of drug, which has been incorporated into or absorbed onto the particles. It is defined as,

$$E.E\ (\%) = \frac{\%\ \text{drug loading}}{\%\ \text{of initial content X (1-Fraction of residual stabilizing agents)}} \times 100$$

Due to the small size of the nanoparticles, the determination of drug loading is done after separation of free drug from bound drug. Drug loading can be assessed after ultracentrifugation of a nanoparticulate suspension by dissolution of the sediment in an appropriate solvent and subsequent analysis. Additional analysis of the free drug in the supernatant may be used to confirm the results. Depending upon the analytic, methods such as spectrophotometry, spectrofluorophotometry, high performance liquid chromatography or liquid scintillation counting can be used to determine the drug loading.

If the particles are purified by cross-flow filtration and subsequently freeze-dried, the particles can be directly dissolved in an adequate solvent.

Size and Morphology:

Photon Correlation Spectroscopy (PCS), Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) are most commonly used tools for studying the particle size of nanoparticles.

Photon Correlation Spectroscopy (PCS) is a laser light scattering method suitable for measurement of particles ranging from 5 nm to 5 μm. The PCS device consists of a laser, a temperature controlled cell, and a photomultiplier. The light scattered from a colloidal dispersion is detected on photomultiplier and transferred to a correlate for the calculation of a correlation function. This function is then processed to calculate the mean size of the particles. The advantage of this technique is the case of sample preparation. To be analyzed, the particles need to be diluted in a solvent, which is generally double filtered water.

Scanning Electron Microscopy is widely used for the field of nanocarriers. It has high resolution and relatively easy sample preparation. However, to be analyzed the sample should withstand a high vacuum. To visualize the particles, they have to be conductive and therefore, coating of the surface of the sample with gold is required.

The coating thickness is at least 20 nm. Removal of stabilizing agents added during the preparation of the particles is essential. Otherwise, depending on the amount of these additives, the particles are partially or completely hidden in a matrix of additives.

Photon Scanning Tunneling microscope also called as Atom force microscopy enable visualization of nanoparticles at atmospheric pressure without gold coating but the resolution is still less than SEM.

INVITRO RELEASE STUDIES[41]

In vitro release studies should be in principle is useful for quality control as well as for the prediction of in vivo kinetics. Unfortunately, due to the very small size of the particles, the release rate observed in vivo can differ greatly from the release obtained in a buffer solution. Depending on the type, drug release from nanoparticles can take place through following two processes:

- The drug may diffuse out of the carrier through solid matrix: to allow complete release from the carriers
- The solvent may penetrate the nanoparticles and dissolve the drug, which then diffuses out into the release medium.
- The carrier may be degraded by its surroundings as long as this process is faster than diffusion the release process is said to be erosion controlled.

In vitro release kinetics of a drug entrapped in nanoparticles can be evaluated by several experimental methods.

1. Dialysis
2. In situ method
3. Sample and separation techniques
4. Ultra filtration at low pressure.
5.

Dialysis:

Nanoparticles suspended in a small volume of release medium, are separated from a large bulk of sink medium by a dialysis membrane, which is permeable to the drug. The active substance is released from the nanoparticles and diffuses through the membrane. The released drug is assayed in the receptor phase.

1.10 APPLICATIONS OF NANOPARTICLES:

- Nano medicines: nanodrug, medical devices, tissue engineering etc.
- Ceramic based nanoparticles for entrapping therapeutic agent for photodynamic therapy, in this method the photosensitive drug/dye is entrapped with ceramic carrier. These ceramic nanoparticles are widely used for skin and therapeutic purpose.
- The thermo sensitive nanoparticles may be used for selective release of the content after specific localization like photodynamic therapy
- Materials: nanoparticles, carbon nanotubes, biopolymer, paints, coating.

Table; 5.Various applications of Nanoparticles/Nanocapsules

S.NO	Application	Materials	Purpose
1	Intracellular targeting	Poly(alkylcyanocrylate)poly ester, nanoparticles with anti-neoplastic or antiviral agent	Target reticulo-endothelial system for intracellular infection
2	Vaccine adjuvants	Poly(methylmethacrylate) nanoparticles with vaccines oral and intramuscular immunization	Enhances immune response, alternate acceptable adjuvant
3	DNA delivery	DNA-gelatin nanoparticles, DNA-chitosan nanoparticles, PDNA-Poly(DL-lactide-co-glycolide) nanoparticle	Enhanced delivery and significantly higher expression levels
4	Cancer therapy	Poly (alkylcyanoacrylate) nanoparticles with Anticancer agents, oligonucleotides	Targeting reduced toxicity, enhanced uptake of antitumor agents, improved in vitro and in vivo stability
5	Per oral absorption	Poly (methacrylates) nanoparticles with proteins and therapeutic agents	Enhanced bioavailability protection from G.I.T

1.11 INTRODUCTION ABOUT HIV;

Human Immunodeficiency Virus type 1 (HIV-1) infection is the major cause of impaired immune system function that leads to progression of disease and death in patients with Acquired Immunodeficiency Syndrome (AIDS). Antiretroviral therapy has been limited by several factors, such as its inherent toxicity, insufficient efficacy, and drug resistance. In 2007, it was estimated that the total number of people infected by HIV accounted for around 33 million, while another 25 million more have already died since the first reported cases in 1980. In 1980s first antiretroviral drugs were introduced and after the development of resistance when treated with single drug regimens, Highly Active Antiretroviral Therapy (HAART) was introduced in the late1990s, comprising the intense use of combination drug regimens. Several classes of ARVs including i) nucleoside reverse transcriptase inhibitors (NRTIs) ii) non-nucleoside reverse transcriptase inhibitors (NNRTIs) iii) protease inhibitors (PIs) iv) intergraded inhibitors, v) entry inhibitors. The primary rationale for using multiple agents is to disrupt HIV replication at multiple points in the lifecycle. Each of these combination regimens often comprises two nucleoside analogues and a PI to achieve synergistic effect. These therapeutic combinations are referred as the highly active ARV therapy (HAART) in general, less effective for the treatment of CNS complications than other AIDS-related illnesses. By using targeted nanoparticle drug delivery systems, anti-HIV drugs can accumulate in HIV-infected tissues or cells selectively and quantitatively, while their concentration in non-infected tissues or cells should be much lower. Therefore, side effects are reduced, lower doses are needed and drug administration regimens are simplified. Alongside, inadequate physical, chemical properties of most of these antiretroviral drugs (e.g. poor solubility, permeability, and stability) impair optimal absorption, bio-distribution, and sustained antiretroviral effect, thus contributing to poor clinical outcome. In order to solve these problems, several new and improved delivery systems and dosage form have been proposed in the literature.

Nanotechnology-based systems for HIV/AIDS treatment;

The basic concept behind the use of nanotechnology-based systems for antiretroviral drug delivery is related with the modulation of pharmacokinetics of incorporated molecules with this association, the properties that govern drug

absorption, distribution, and elimination while in the human body are determined not by the drug properties, rather by the nanosystems physical–chemical properties, particularly surface exposed molecules and electric charge, and its size. General properties of nanosystems that favor their use in antiretroviral drug delivery are well known and include versatility (virtually all drugs may be encapsulated), good toxicity profile (depending on used excipients), possibility of drug-release modulation, high drug payloads, relative low cost, easiness to produce and possible scale-up to mass production scale . Their ability to incorporate, protect and promote the absorption of non-orally administrable anti-HIV drugs, namely mono or oligonucleotides, is of importance to improve the bioavailability of several molecules. Once bioavailable, protection of incorporated drugs from metabolism is a favorable feature of nanosystems, allowing prolonged drug residence in the human body, thus reducing needed doses and prolonging time between administrations. The use of nanoparticulate systems for antiretroviral drug delivery may be particularly advantageous for targeted delivery, namely to cells or organs that are directly implicated in HIV/AIDS. This can be achieved either by passive or active targeting.

Passive targeting is based in the inherent properties of different nanosystems, namely size, particle shape, and surface charge, which can modulate its bioavailability, biodistribution and targeting, in the case of active targeting, nanotechnology-based systems are conveniently modified, most commonly by surface attachment of specific ligands that are able to recognize target cells or sites, and escape bio elimination processes. One important limitation of many current antiretroviral drugs is their unavailability to circumvent efflux pumps (particularly P-glycoprotein) that are present, for instance, in the membrane of several HIV-target cells and BBB endothelium. Nanoparticulate systems ability to escape these bio elimination processes is an added advantage in order to avoid this particular resistance mechanism to drug delivery, namely to the CNS. Thus, increasing the amount of available antiretroviral drugs and its residence time at target sites allow thinking about dose reduction and, consequently, simpler but improved regimens with less adverse effects and increased compliance. Also, the possibility of incorporating different antiretroviral drugs in the same delivery system and modulate their release individually has been shown possible. This fact may contribute to simplify drug administration schedules, being an important objective towards the reduction of

antiretroviral drug administration errors. Nanosystems seem to be able to reduce antiretroviral drugs toxicity, namely at the cellular level, providing that rigorous selection of materials and adequate preparation techniques are assured. Even if drug uptake is increased when encapsulated in nanocarriers, cell toxicity seems to be diminished, probably due to the slow-release properties of these systems. This possibility is particularly interesting taking in consideration the well-known toxicity associated with anti-HIV therapy. However, the effects of the possible bioaccumulation of nanosystems components have not been fully addressed and may point of concern for prolonged used. Lastly, nanosystems may allow obtaining antiretroviral medicines with adequate shelf-life, even if this issue has not yet been fully explored. Formulation should be carefully performed and long-term stability studied since antiretroviral drug-loaded nanotechnology-based systems may undergo several physical–chemical changes that can potentially impair efficacy and safety.

2. REVIEW OF LITERATURE

S. Tamizhrasi[18] et al., (2009) prepared and evaluated polymethacrylic acid nanoparticles containing lamivudine in different drug to polymer ratio by nanoprecipitation method. SEM indicated that nanoparticles have a discrete spherical structure without aggregation. The particle size of the nanoparticles was gradually increased with increase in the proportion of polymethacrylic acid polymer. The drug content of the nanoparticles was increasing on increasing polymer concentration up to a particular concentration. No appreciable difference was observed in the extent of degradation of product during 60 days in which, nanoparticles were stored at various temperatures. FT-IR studies indicated that there was no chemical interaction between drug and polymer and stability of drug. The in vitro release behavior from all the drug loaded batches was found to be zero order and provided sustained release over a period of 24 h. The developed formulation overcome and alleviates the drawbacks and limitations of lamivudine sustained release formulations and could possibility be advantageous in terms of increased bioavailability of lamivudine.

Ashish Deva[19] **et al**., (2010) developed Poly (lactic acid) (PLA)/chitosan (CS) nanoparticles by nanoprecipitation method for anti-HIV drug delivery applications. The prepared PLA/CS nanoparticles were characterized using DLS, SEM, and FTIR. The hydrophilic antiretroviral drug Lamivudine was loaded into PLA/CS nanoparticles. The encapsulation efficiency and in vitro drug release behavior of drug loaded PLA/CS nanoparticles were studied using UV spectrophotometer. In addition, the cytotoxicity of the PLA/CS nanoparticles using MTT assay was also studied. The in vitro drug release studies showed that drug release rate was lower in the acidic pH when compared to alkaline pH. This may due to repulsion between H^+ ions and cationic groups present in the polymeric nanoparticles. Drug release rate was found to be higher in the 6% drug loaded formulation when compared to 3% drug loaded formulation. These results indicated that the PLA/CS nanoparticles are a promising carrier system for controlled delivery of anti-HIV drugs.

Pravin Patil[20] **et al., (2009)** prepared solid lipid nanoparticles by emulsion solvent diffusion technique. The formulations were characterized for surface morphology,

size and size distribution, percent drug entrapment and drug release. The optimum rotation speed, resulting into better drug entrapment and percent yield, was in the range of 1000-1250 r/min. In vitro cumulative % drug release from optimized SLN formulation was found 40-50 % in PBS (pH-7.4) and SGF (pH-1.2) respectively for 10 h. After 24 h more than 65 % of the drug was released from all formulations in both mediums meeting the requirement for drug delivery for prolong period of time.

Rubiana M.Marnardes[21] et al., (2005) designed nanoparticles for Praziquantel using Poly (D, L-lactide-co-glycolide) (PLGA) as a carrier. The effects of some process variables on the size distribution of nanoparticles prepared by emulsion solvent evaporation method. The results showed that sonication time, PLGA and drug amounts, PVA concentration, ratio between aqueous and organic phases and the method of solvent evaporation have a significant influence on size distribution of the nanoparticles.

Kathleen Dillen[22] et al., (2006) prepared nanoparticles by water-in-oil-in-water (w/o/w) emulsification and solvent evaporation, followed by high-pressure homogenization techniques. Two non-biodegradable positively charged polymers, Eudragit RS100 and RL100, and biodegradable polymer poly (lactic-co-glycolic acid) or PLGA were used alone or in combination, with varying ratios. The formulations were evaluated in terms of particle size and zeta potential. Differential scanning calorimetric measurements were carried out on the nanoparticles and on the pure polymers Eudragit and PLGA. Drug loading and release properties of the nanoparticles were examined. The antimicrobial activity against *Pseudomonas aeruginos* and *Staphylococcus aureus* was determined. During solvent evaporation, the size and zeta potential of the nanoparticles did not change significantly. The mean diameter was dependent on the presence of Eudragit and on the viscosity of the organic phase. The zeta potential of all Eudragit containing nanoparticles was positive in ultrapure water (around + 21mV). No burst effect but a prolonged drug release was observed from all formulations. The particles activity against *P.aeruginosa* and *S.Aureus* was comparable with an equally concentrated ciprofloxacin solution.

M.C. Venier-Julienne[23] et al., (1996) prepared biodegradable poly (D, L-lactic acid-

co-glycolic acid) copolymer (PLAGA) nanoparticles by the solvent evaporation process and to incorporate an antifungal antibiotic, amphotericin B. Blank nanoparticles obtained were 130 of 27 nm in diameter. When amphotericin B was added in the organic phase, the final suspension showed two populations due to unbound drug. Free amphotericin B was removed by contacting the nanoparticle suspension with an adsorbent polymer and subsequently ultra-filtering the medium. The drug payload was between 0.7 and 1.3%.

Le Thi Mai Hoa[24] et al., (2009) developed polymeric drug nanoparticles consist of ketoprofen and Eudragit E 100. The morphology structure was done by scanning electron microscopy (SEM). The interactions between the drug and polymer can identify by Fourier transform Infrared spectroscopy (FTIR). The size distribution was measured by means of dynamic Light scattering. The nanoparticles have an average size of about 150 nm. The incorporation ability of drugs in the polymeric nanoparticles depended on the integration between polymer and drugs well as the glass transition temperature of the polymer.

R.Pignatello[25] et al., (2002) prepared nanoparticle suspensions from Eudragit RS100 and RL100 polymer resins and loaded with flurbiprofen, to improve the availability of the drug at an intra-ocular level for the prevention of the myosis induced during extra capsular cataract surgery. Nanosuspensions were prepared by a quasi-emulsion solvent diffusion technique using different formulation parameters (drug to polymer ratio, initial polymer concentration, agitation speed). The resulting nanoparticles showed mean sizes around 100 nm and a fixed positive charge (x-potential around +40/-60 mV). Stability tests after mid time storage (41^0C or room temperature) or freeze-drying was carried out to optimize a possible final pharmaceutical preparation. In vitro, dissolution tests showed a controlled release profile of FLU from the nanoparticles. In vivo anti-inflammatory efficacy was assessed in the rabbit eye after induction of ocular trauma (paracentesis) FLU-loaded nanosuspensions did not show toxicity on ocular tissues. Moreover, an inhibition of the miotic response to the surgical trauma comparable to a control eye-drop formulation was obtained, even though an actual lower concentration of free drug in the conjunctive sac was achieved from the nanoparticle system.

N. Ubricha[26] et al., (2004) evaluated effect of polymers (Eudragit RS or RL) and additives within the alcoholic phase (fatty acid esters and polyoxyethylated castor oil) on the size, zeta potential and the encapsulation efficiency of the nanoparticles. The mean diameter of the various CyA nanoparticles ranged from 170 to 310 nm the size as well as the zeta potential increased by adding fatty acid ester and polyoxyethylated castor oil within the organic phase. No significant differences in surface potential were observed for all formulations tested. Probably due to the very low water solubility of the drug, high encapsulation efficiencies were observed in a range from 70 to 85%. The oral absorption of CyA from these polymeric nanoparticles was studied in rabbits and compared to that of neoral capsule. Based on comparison of the area under the blood concentration time curve values, the relative bioavailability of CyA from each nanoparticulate formulation ranged from 20 to 35%.

Eliza Glowka[27]et al., (2009) developed and characterized polymeric nanoparticles as a sustained release system for salmon calcitonin (sCT) by a double emulsion solvent evaporation method using Eudragit RS and two types of a biodegradable poly (lactic-co-glycolic) copolymer (PLGA). The sCT was incorporated into nanoparticles with encapsulation efficiencies in the range 69–83%. In vitro release studies, unconventionally conducted in 5% acetic acid, showed great differences in sCT release time profiles. Nanoparticles with fast release profile (Eudragit RS, PLGA/Eudragit RS) released 80–100% of the encapsulated drug within a few hours. In contrast, the sCT release from pure PLGA nanoparticles was very slow, incomplete and reached only 20% after 4 weeks. In vivo study, conducted in wistar rats, proved that elevated serums CT levels could be sustained for 3 days after subcutaneous administration of PLGA nanoparticles and the achieved bioavailability was increased compared to sCT solution.

Uracha Ruktanoncha[28]et al., (2009) developed gamma-oryzanol, an antioxidant, was incorporated into three different types of solid lipid wax, triglycerides, a mixture of glycerides as solid lipid nanoparticles (SLN) and liquid lipid as nanoemulsion (NE). Instability was found only from NE due to its significant increase in particle size and decreased entrapment efficiency at a storage temperature of 45^0C. Solid lipid type in SLN plays an important role on % entrapment efficiency, but not chemical stability. A decrease in crystallinity of SLN was observed with the incorporation of

gamma oryzanol and low recrystallization index were found with two glycerides-based SLN. The in vitro release studies demonstrated that a biphasic release pattern fitted well with the Higuchi model of SLN formulations. In comparison, nearly constant release was observed in NE comprised of similar composition. Wax-based SLN demonstrated the lowest cytotoxicity NE, wax-based SLN and a mixture of glycerides based SLN were considered to enhance the antioxidant activity of gamma oryzanol.

SelvakumarKalimuthu[29] et al., (2009) prepared nanoparticles of Carvedilol with Eudragit E 100 were prepared by the nanoprecipitation method using polymeric stabilizer Poloxamer 407. Nanoparticles of Carvedilol were obtained with high encapsulation efficiency. The particles were characterized for particle size by photon correlation spectroscopy and transmission electron microscopy. The in vitro release studies were carried out by USP Type II apparatus in SGF without enzyme (pH 1.2). The particle size of the prepared nanoparticles ranged from 190 nm – 270 nm. Nanoparicles of Carvedilol were obtained with high encapsulation efficiency (85-91%). The drug release from the carvedilol nanoparticles showed within 5 minutes. These studies suggest that the feasibility of formulating carvedilol loaded Eudragit E 100 nanoparticles for the treatment of hypertension.

Rosario Pignatello[30] et al., (2002) prepared nanoparticles by nanopreciptation and modification of the quasi-emulsion solvent diffusion technique using variable formulation parameters (drug to polymer ratio, total drug and polymer amount, stirring speed). Nanosuspensions had mean sizes around 100 nm and a positive charge (z-potential of 140/160 mV), this makes them suitable for ophthalmic applications. Stability tests (up to 24 months storage at 48^0C at room temperature) or freeze-drying were carried out to optimize a suitable pharmaceutical preparation. In vitro dissolution tests indicated a controlled release profile of ibuprofen from nanoparticles. In vivo efficacy was assessed on the rabbit eye after induction of an ocular trauma (paracentesis). An inhibition of the miotic response to the surgical trauma was achieved, comparable to a control aqueous eye-drop formulation, even though a lower concentration of free drug in the conjunctive sac was reached from the nanoparticle system. Drug levels in the aqueous humor were also higher after application of the

nanosuspensions moreover Ibuprofen-loaded nanosuspensions did not show toxicity on ocular tissues.

C. Schwarz[31] et al., (1994) developed (SL) nanoparticles by high pressure homogenization of a melted lipid (Dynasan I 12) dispersed in water at increased temperature (70°C). Soy lecithin and poloxamer 188 were used as surfactants and stabilizers of the particles. The effect of homogenization parameters (pressure, cycle number) was studied and optimized to yield solid lipid nanoparticles of a quality suitable for intravenous injection. Particles were characterized by photon correlation spectroscopy (PCS) and zeta potential measurements, the fraction of large particles being the limiting factor for intravenous injection was determined using a Coulter Counter. The optimum formulation was suitable for intravenous injection (monograph 'Particulate Matter', USP XXII).

Zih-rou HUANG1[32] et al., (2008) developed Compritol (SLN-C), Precirol squalene (nano structured lipid carriers; NLC), and squalene as the lipid core material. These systems were assessed and compared by evaluating the mean diameter, surface charge, molecular environment, camptothec in release, and cell viability against a melanoma. The safety and storage stability of these systems were also preliminarily examined. The particle size ranged from 190 to 310 nm, with the NLC and LE showing the smallest and largest sizes, respectively. The in vitro drug release occurred in a sustained manner in decreasing order. It was found that varying the type of inner phase had profound effects on cell viability. The SLN-P generally showed higher cytotoxicity than the free control. The treatment of melanomas with the camptothecin-loaded SLN-C and NLC yielded cytotoxicity comparable to that of the free form. The percentage of erythrocyte hemolysis by all nanoparticles was ≤ 5%, suggesting a good tolerance to lipid nanoparticles. The results collectively suggest that the SLN-P may have the potential to serve as a delivery system for parenteral camptothecin administration because of the sustained drug release, strong cytotoxicity, limited hemolysis and good storage stability.

Swarnali Das[33] et al., (2010) prepared positively charged amphotericin-B loaded nanoparticles providing a controlled release formulation. The particles were prepared by solvent displacement or nanoprecipitation method. The non-biodegradable

positively charged polymer Eudragit RL 100 was used to prepare the different formulations with varying ratios of drug and polymer. The formulations were evaluated in terms of particle size, zeta potential, and differential scanning calorimetry measurements. Drug entrapment and release properties were examined also. The antimicrobial activity against Fusariumsolani was determined. In vivo eye irritation study was carried out by a modified Draize test. All the formulations remained within a size range of 130 to 300 nm in fresh preparation as well as after 2 months. The zeta potential was positive (+22 to -42 mV) for all the formulations were suitable for ophthalmic application. A prolonged drug release was shown by all the formulations. The formulation possesses a good antifungal activity against Fusariumsolani when tested by disk diffusion method, and no eye irritation on in vivo testing was found.

Umasankar[34] et al., (2010) developed flutamide nanoparticles by nanoprecipitation method. Using Euragit (RL100) as a copolymer with various different ratio nanoparticles were characterized by determining its particle size, drug entrapment efficiency drug release and stability studies. The particle size ranged between 335 nm to 620 nm. Drug content was found of the supportive to drug release pattern. The in vitro release of flutamide nanoparticles were carried out which exhibited sustained release of flutamide from nanoparticles up to 16 hrs.

Ahmed H. Elshafeey[35] et al., (2010) prepared Acyclovir (ACV) Eudragit (EUD) nanoparticles (NPs) different charge density. The effect of charge intensity on particle size, encapsulation efficiency and in vitro dissolution was assessed. The bioavailability of ACV NP colloids were evaluated in human volunteers, compared with commercial product using a validated LC–MS/MS method with a lower limit of quantification (LLOQ) of 0.02g/ml. EUD RL 100 with higher ammonium groups gave smaller NPs than EUD RS 100. The surface charge of the polymer did not affect encapsulation efficiency and in vitro dissolution. In human volunteers, both F2 and F5 colloidal nanosuspensions prepared with EUD RS and RL respectively in drug to polymer ratio 1:3 sustained the oral absorption of ACV, expressed by the significant lower Cmax, significant delayed Tmax and the significant higher HVD 50% Cmax The mean Cmax of F2, F5, and zovirax were 0.61 ± 0.06, 0.73 ± 0.07 and

0.92±0.21g/ml respectively. Furthermore AUC0 –12 of F2 and F5 was significantly higher than that of zovirax with values of 4.37 ± 0.885.14 ± 0.87 and 3.21 ± 0.53g/ml h respectively. The higher AUC0–12 for both F2 and F5 reflected high relative bioavailability of 136% and 159% respectively compared to commercial ACV tablets.

Romilamanchanda[36] et al., (2010) developed biodegradable poly (DLlacticcoacid) (PLGA) nanoparticle simultaneously loaded with indocyanine (ICG) and (DOX). The modified oil in water single emulsion evaporation method was used. To enhance the incorporation of both agents and control particle size, four independent processing parameters including amount of polymer, initial ICG and DOG entrapment in nanoparticles as well as the nanoparticles size was determined. The nanoparticles produced by standardized formulation were in the ranges of 171±0.0014, n=3.The entrapment efficiency was determined by spectrofluorometer measurements. The efficiency was 44.4 ± 1.6% for ICG and 74.3 ± 1.9% for DOX. Drug loading was1.015 ± 0.001%w/w, for ICG and 0.022 ± 0.001%w/w, for DOX n=3.

AtulGaikwad[37] et al., (2010) developed Eudragit RS100 nanoparticles by nanoprecipitation method for oral delivery. Formulation were prepared in ten different drugs to carrier ratio, and characterized for particle size, shape, percentage yield, drug entrapment, stability studies, zeta potential, FT-IR study, in vitro drug release and release kinetics. The shape of nanoparticles was found to be spherical by scanning electron microscopy studies, whereas size ranging from 163 nm to 378 nm. FTIR study confirmed that there was no interaction between drug and polymer. Entrapment efficiency was in the range of 14.95 ± 0.06 to 69.73 ± 0.03 W/W. No appreciable difference was observed in the extent of degradation of product during 60 days in the nanoparticles, which were stored at various temperatures. Zeta potential of formulation supports the minimum interaction between the particles. The in vitro drug release study revealed that sustained release of some formulation last up to 24 hour. The release followed Higuchi kinetics, which indicates diffusion controlled release pattern of drug.

Michele Trotta[38] et al., (2003) developed nanoparticles based on the emulsification of a butyl lactate or benzyl alcohol solution of a solid lipid in an aqueous solution of

different emulsifiers, followed by dilution of the emulsion with water, was used to prepare glyceryl monostearate nanodispersions with narrow size distribution. To increase the lipid load the process was conducted at 47 ± 2^0C and in order to reach submicron size a high-shear homogenizer was used. Particle size of the solid lipid nanoparticles (SLN) was affected by using different emulsifiers and different lipid loads. By using lecithin and taurodeoxycholic acid sodium salt, on increasing the GMS percentage from 2.5 to 10% an increase of the mean diameter from 205 to 695 nm and from 320 to 368 nm was observed for the SLN prepared using benzyl alcohol and butyl lactate, respectively. Transmission electron micrographs of SLN reveal nanospheres with a smooth surface.

Roberta CavalliTrotta[39] et al., (1997) prepared (SLN) from three oil in water microemulsions, whose internal phase was constituted of different lipid matrices. The dispersion media were two aqueous solutions of trehalose and PluronicF68 at 2% besides distilled water. SLN were sterilized by autoclaving, were stable during sterilization and maintained a spherical shape and narrow size distribution as confirmed by TEM analysis. SLN dispersions in water did not present nanoparticles larger than 1m after storage at 4°C for 1 year they were freeze dried after sterilization to obtain dry products.

Vandervoort[40] et al., (2004) developed PLGA nanoparticles were prepared by nanoprecipitation method. Different stabilizers, polyvinyl alcohol (PVA), carbopol and Poloxamer are used. The influence of the homogenized pressure and number of cycles on the properties of nanoparticles were studied. Particle size was shown to depend on the stabilizer used. An increase of the homogenization pressure or the number of cycles resulted in a decrease in particle size. The zeta potential value was influenced mainly by the nature of the stabilizer. Particles stabilized with poloxamer or PVA showed a slightly negative zeta potential value, while samples stabilized with carbopol possessed a more negative zeta potential, which become less negative after homogenization. Drug encapsulation depended strongly on the stabilizer used. The higher drug entrapment of the carbopol-stabilized particles could be explained by an electrostatic interaction between the negatively charged carboxyl groups of carbopol and the positively charged, protonated pilocarpine. The drug release patterns of the particles prepared were quite similar. Differences between the releases patterns of the

homogenized particles could be attributed both to differences in size as well as drug encapsulation. Turbidimetric measurements suggested an interaction between mucin and PLGA nanoparticles exclusively stabilized with Carbopol.

Rosario Pignatello[41] et al., (2006) developed AD6-loaded polymeric nanoparticle suspensions were made using inert polymer resins (Eudragit RS100 and RL100). By modified the quasi-emulsion solvent diffusion technique the chemical stability of AD6 in the nanosuspensions was assessed by preparing some formulations using (unbuffered) isotonic saline or a pH 7 phosphate buffer solution as the dispersing medium. The formulations were stored at 4°C, and the rate of degradation of AD6 was followed by high performance liquid chromatography (HPLC). The obtained nanosuspensions showed mean sizes and a positive surface charge (ζ-potential) that makes them suitable for an ophthalmic application these properties were maintained upon storage at 4°C for several months. In vitro dissolution tests confirmed a modified release of the drug from the polymer matrixes. Nanosuspensions prepared with saline solution and no or lower amounts of surfactant (Tween 80) showed an enhanced stability of the ester drug for several months, with respect to an AD6 aqueous solution. Based on the technological results, AD6-loaded Eudragit retard nanoparticle suspensions appear to offer promise as a means to improving the shelf life and bioavailability of this drug after ophthalmic application.

Rubiana Mara Mainardesa[42] et al., (2010) prepared nanoparticles were characterized in terms of size, zeta potential, morphology and drug entrapment efficiency. The results showed that although PLA and blend nanoparticles had the same morphology, the particle size and zeta potential were changed by the PEG. The drug entrapment efficiency was increased by PEG presence. The pharmacokinetic study showed that all the nanoparticles were able to sustain zidovudine delivery over time, but greater efficiency was obtained with PLA–PEG blend nanoparticles whose Tmax was twice that of PLA nanoparticles. The PLA and PLA–PEG nanoparticles formulations increased the zidovudine mean half-life by approximately 5.5 and 7 h, respectively, compared to zidovudine aqueous solution. The relative bioavailability of zidovudine-loaded PLA–PEG blend nanoparticles was 2.7, relative to zidovudine-loaded PLA nanoparticles and 1.3 relative to aqueous solution formulation. Thus, the PLA nanoparticles were unable to increase the zidovudine bioavailability compared to

aqueous solution formulation. The results obtained in this study indicate the potential of the PLA–PEG blend nanoparticles as carriers for zidovudine delivery by the intranasal route.

ParthaSaha[43] et al., (2010) prepared nanoparticles by ionic gelation method with the aid of sonication. Parameters such as the zeta potential, polydispersity, particle size, entrapment efficiency and in vitro drug release of the nanoparticles were assessed for optimization. The antibacterial properties of the nanoparticle formulation were evaluated and compared with that of a commercial formulation Scanning electron microscopy revealed that the nanoparticles were in the nano size range but irregular in shape. Concentrations of 0.35% w/v of chitosan and 0.40% w/v sodium tripolyphosphate (TPP) and a sonication time of 20 min constituted the optimum conditions for the preparation of nanoparticles. In vitro release data showed an initial burst followed by slow sustained drug release. Then nanoparticles demonstrated superior antimicrobial activity to plain nanoparticles and the reference, due probably to the synergistic effect of chitosan and ampicillin trihydrate. Modified ionic gelation method can be utilized for the development of chitosan nanoparticles of ampicillin trihydrate. Polymer and cross linking agent concentrations and sonication time are rate-limiting factors for the development of the optimized formulation. The chitosan nanoparticles developed would be sustained delivery of ampicillin trihydrate.

UgoBilati[44] et al., (2004) processed modifications to improve the versatility of the nanoprecipitation technique, particularly with respect to the encapsulation of hydrophilic drugs (e.g. proteins). Selected parameters of the nanoprecipitation method, such as the solvent and the non-solvent nature, the solvent/non-solvent volume ratio and the polymer concentration, were varied so as to obtain polymeric nano-carriers. Surfactants added to the dispersing medium were usually unnecessary for final suspension stabilization. Changing the solvent/non-solvent volume ratio was also not a determinant factor for nanoparticle formation and their final characteristics, provided that the final mixture itself did not become a solvent for the polymer. The nanoparticles obtained ranged from about 85–560 nm in size. The nanoparticle recovery step however needs further improvements, since bridges between particles which cause flocculation could be observed. The nanoprecipitation technique is more versatile and flexible than previously thought and that a wide range of parameters can

be modified.

Angela Lopedota[45] et al., (2009) developed glutathione (GSH) by quasi-emulsion solvent diffusion technique. The nanoparticles prepared showed homogeneous size distribution, mean diameters between 99 and 156 nm, a positive net charge and spherical morphology. Solid state FT-IR, thermal analysis (DSC), and X-ray diffraction studies suggest that the nanoencapsulation process produces a marked decrease in crystallinity of GSH. There results indicate that mean diameters, surface charges and drug-loaded nanoparticles were not markedly affected by the CD, whereas the presence of the latter influences drug release and to some extent peptide stability and absorption. Finally, it has been shown that CD/Eudragit RS 100 NPs may be used for transmucosal absorption of GSH without any cytotoxicity using the epithelial human HaCaT and murine monocyte macrophage RAW264.7 cell lines.

Yung-ChihKuo[46] et al., (2010) investigated on loading efficiency (LE) of stavudine (D4T), a human immune deficiency antiretroviral agent, on the external surfaces of poly- butyl cyano acrylate (PBCA) and methylmethacrylate, sulfopropylmethacrylate (MMA-SPM). The biodegradable PBCA NPs and MMASPM copolymer NPs were synthesized, respectively, by emulsion poly-merization and free radical polymerization larger polymeric nanoparticles (NPs), the smaller LE of D4T on the two kinds of biomaterials. Freeze drying of the two NPs, however, yields an increase in particle size and an increase in LE of D4T. These outcomes imply that for oral administration, D4T-loaded MMA-SPM NPs may be more advantageous than D4T-loaded PBCA NPs, and D4T-loaded PBCA NPs may be more favorable than D4T-loaded MMA-SPM NPs for intravenous injection.

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K.Derakhshandeh[47] et al., (2007) prepared (PLGA) nanoparticles by nanoprecipitation method and characterization. The full factorial experimental design was used to study the influence of four different independent variables on response of nanoparticle drug loading. Analysis of variance (ANOVA) was used to evaluate optimized conditions for the preparation of nanoparticles. The physical characteristics of PLGA nanoparticles were evaluated using particle size analyzer, scanning electron microscopy, differential scanning calorimetry and X-ray diffractometry. The results of optimized formulations showed a narrow size distribution with a polydispersity index

of 0.01%, an average diameter of 207 ± 26 nm, and a drug loading of more than 30%. The in vitro drug release profile showed a sustained 9-NC release up to 160 h indicating the suitability of PLGA nanoparticles in controlled 9-NC release. Thus prepared nanoparticles described here may be of clinical importance in both stabilizing and delivering camptothecins for cancer treatment.

Junping Wanga[48] et al., (2010) prepared cationic PLA based nanoparticles by the nanoprecipitation method and solvent evaporation method with polyethylenimine (PEI) and chitosan as two types of surface coating materials. Cationic poly(d,l-lactide-co-glycolide) (PLGA)–PEI, PLGA–chitosan and methoxy poly (ethylene glycol)–poly (lactide) (PEG)–PLA/PEI, PEG–PLA–chitosan nanoparticles were characterized in terms of size and size distribution by laser scattering, surface charge by zeta potential measurement, and surface chemistry by X-ray electron spectroscopy (XPS). The four type nanoparticles were compared for their interaction with RNA and nanoparticles mediated RNA transfection efficiency with a hepatitis B model, where the inhibition effects of the double strand RNA (RNA) mediated by the four types of nanoparticles were evaluated by measuring the HBs Ag expression level. The highest inhibition effect of HBs Ag (surface antigen of the hepatitis B Virus (HBV), which indicates current hepatitis B infection) expression was achieved by the PEG–PLA–PEI nanoparticles mediated RNA transfection. The results demonstrated that the RNA delivery follows a size and surface charged manner.

Tao Zhang[49] et al., (2011) prepared nanoparticles by emulsification diffusion, their size, encapsulation efficiency (EE%), drug release profile, morphology, and cytotoxicity are characterized by dynamic light scattering, spectrophotometry, transmission electron microscopy, and cellular viability assay/transepithelial electrical resistance measurement, respectively. Cellular uptake was elucidated by fluorescence spectroscopy and confocal microscopy. The NPs have an average size of 250 nm, maximal EE% of 16.1% and 37.2% for TNF and TDF, respectively. There is a 4-fold increase in the drug release rate from the 75% S-100 blend in the presence of SFS over 72 h. At a concentration up to 10 mg/ml, the PLGA/S-100 NPs are noncytotoxic for 48 h to vaginal endocervical/epithelial cells and Lactobacillus crispatus. The particle uptake (50% in 24 h) by these vaginal cell lines mostly occurred through caveolin-mediated pathway. These data suggest the promise of using PLGA/S-100

NPs as an alternative controlled drug delivery system in intra vaginal delivery of an anti-HIV/AIDS microbicide.

T. Vetrichelvan[50] et al., (2011) prepared alginate nanoparticles by in situ nano emulsion polymer cross linking approach. The nano particles were prepared using different ratios of alginates and abacavir sulfate in the ratios of (1:1, 1:2 and 1:3). The encapsulation efficiency was also studied to find out the percentage drug entrapped in the prepared nanoparticles. The result of ratio 1:3 showed a good encapsulation efficiency of 98.71%. Abacavir sulfate nanoparticle was confirmed by FT-IR, DSC and quantitated by uv prepared nanoparticle appeared spherical with a dense drug core in transmission electron microscopy studies. Hydro dynamic diameter of nanoparticles was 63 ± 0.235 nm, with a Gaussian distribution and the zeta potential – 0.6. Sustained diffusive drug release was observed in vitro, following zero order Kinetics releasing the drug pay load over a period of 16h. Embedding abacavir sulfate in alginate provided sustained release. They also offered better pharmacokinetic properties to the drug than that afforded by the free drug itself. The nanoparticle technique developed can be a good choice for the development of sustained antiretroviral drug carrier.

Kathleen Dillen[51] et al., (2008) prepared nanoparticles by w/o/w emulsification solvent evaporation. Particle size and zeta potential of the nanoparticles were measured. Nanoparticles were incubated for a short time with *P. aeruginosa* and *S. aureus* followed by measurement of the size of nanoparticles and of *P. aeruginosa* and *S. aureus* with and without adherent nanoparticles. Flow cytometric measurements were performed to detect the attachment of particles to microorganisms. Eudragit containing nanoparticles possessed a positive zeta potential, while PLGA nanoparticles were negatively charged. Following adsorption of Eudragit containing nanoparticles, a size increase for *P. aeruginosa* was observed. Flow cytometric analyses confirmed that Eudragit containing particles showed stronger interactions with the test organisms than PLGA nanoparticles. Adhesion of particles was more pronounced for *P.aeruginosa* than for *S.aureus*. Cationic Eudragit containing nanoparticles showed better adhesion to microorganisms than anionic PLGA nanoparticles, which is probably due to enhanced electrostatic interactions.

N.Jawahar[52] et al., (2009) prepared Poly (D, L-Lactide-co-Glycolide) (PLGA) nanoparticles by nanoprecipitation method using tribloere polymeric stabilizer (Pluronic RF-68). The particles were characterized for drug content, particle size and particle morphology by Transmission electron microscope (TEM). In vitro studies were determined by the bulk equilibrium reverse dialysis bag technique. The particle size of the prepared nanoparticles ranged from 20 nm to 340 nm. nanoparticles of ramipril were obtained with high encapsulation efficiency (68-75%). The drug release from the ramipril nanoparticles was sustained in Batch (F3) for more than 24 hrs with 72% drug release. This study suggests that the feasibility of formulating Ramipril loaded PLGA nanoparticles can be used to improve the therapeutic efficacy of Ramipril in the treatment of hypertensive disorder.

Jasvinder Singh[53] et al., (2006) prepared nanoparticles by water-in-oil in- water (w/o/w) double emulsion solvent evaporation method, demonstrated release proWles which were dependent on the properties of the polymers. An in vitro experiment using Caco-2 cells showed signi Wcantly higher uptake of PCL nanoparticles in comparison to polymeric PLGA, the PLGA–PCL blend and co-polymer nanoparticles. The highest uptake mediated by the most hydrophobic nanoparticles using Caco-2 cells was mirrored in the in vivo studies following nasal administration. PCL nanoparticles induced DT serum speciWc IgG antibody responses signiWcantly higher than PLGA. A signi Wcant positive correlation between hydrophobicity of the nanoparticles and the immune response was observed following intramuscular administration. The positive correlation between hydrophobicity of the nanoparticles and serum DT speciWc IgG antibody response was also observed after intranasal administration of the nanoparticles. The cytokine assays showed that the serum IgG antibody response induced is diVerent according to the route of administration, indicated by the diVerential levels of IL-6 and IFN. The nanoparticles eliciting the highest IgG antibody response did not necessarily elicit the highest levels of the cytokines IL-6 and IFN.

Liang wang[54] et al., (2006) prepared nanoparticles by melt mixing method with hydrophilic silica particles (Aerosil and Sylysia) with different particle size and specific surface areas as carriers. Powder X-ray diffraction and differential scanning

calorimetry evaluation showed that NTD in the SDs treated with the melt-mixing method was dispersed in the amorphous state. FT-IR spectroscopy obtained with the SDs indicated the presence of hydrogen bonding between the secondary amine groups of NTD and silanol groups of silica particles. The dissolution property of NTD in the SDs was remarkably improved regardless of the grade of silica. At the end of the dissolution test (60 min) the concentrations of NTD for the SDs with Aerosil 200 and Sylysia 350 were 8.88 and 10.09m g/ml, corresponding to 28 and 31 times that of the original NTD crystals, respectively. The specific surface area and the adsorbed water amount of the SDs were also significantly improved. The rapid dissolution rate from the SDs was attributed to the amorphization of drug, improved specific surface area and wet ability than the original drug crystals. In the stability test, powder X-ray diffraction pattern indicated that amorphous NTD in the SD with aerosil 200 was stable for at least 1 month under the humidy conditions (40 °C/75% RH).

S. Ramesh[55] et al., (2010) prepared nanoparticles by controlled gel-lification process. In this research work, different drug and polymer ratios were used. The prepared nanoparticles were evaluated to assess the various parameters such as drug content analysis, particle size analysis (SEM Analy-sis), Zeta potential analysis, In Vitro drug release and stability studies. The particle size of prepared nanoparticles was found to be 500nm. The drug content analysis of all the prepared formulations was estimated in the range of 57.34 ± 0.10 to 65.33 ± 0.11 %. The zeta potential of the sepia nanoparticles was estimated as 69mv. The Cumulative percentage drug release of the third formulation was found to be 90.36 %. By observing the in vitro drug release results of all formulation, we concluded that, AMN3 formulation was found to be best formulation with higher Cumulative percentage drug release.

Adlin jino nesalin j[56] et al., (2009) prepared Flutamide nanoparticles by ionic gelation technique. Nanoparticles of different core: coat ratio were formulated and analyzed for total drug content, loading efficiency, particle size and in vitro drug release studies. From the drug release studies it was observed that nanoparticles prepared with chitosan in the core: coat ratio 1:4 gives better sustained release for about 12 hrs as compared to other formulations.

J. Vandervoort[57] et al., (2002) prepared nanoparticles by w/o/w emulsification solvent evaporation method. In most cases poly (vinyl alcohol) (PVA) is used as stabilizer of the emulsion. The goal of this study was to compare a series of polymers to PVA in a 22 full factorial design study. The influence of the concentration of PVA And the polymers tested on particle size and zeta potential value was evaluated before and after freeze-drying of the prepared particles. Nanoparticles were obtained with most polymers when they were used in combination with PVA. Leaving PVA out of the formulation, however, increased the size of the particles over 1m. Two exceptions are poloxamer and carbopol, which can be considered as valuable alternatives to PVA. Zeta potential values were usually slightly negative; the most extreme zeta potential values were measured when poloxamer and carbopol were employed. The use of gelatin type A made it possible to achieve positive values. The original 22 full factorial design study was further expanded to a central composite design for poloxamer and carbopol, in order to fit the measured data to a quadratic model and to calculate response surfaces.

Manish k gupta[58] et al., (2009) prepared Cyclosporine (CYA) loaded Eudragit RL100 nanoparticles by solvent evaporation technique, with 2% PVA as stabilizer. Four batches of nanoparticles with varying drug concentrations (CYN-1, CYN-2, CYN-3 and CYN-4) were prepared. Cumulative % drug release of formulations CYN-1, CYN-2, CYN-3 and CYN-4 was 94.35%, 93.89%, 88.28% and 85.36% respectively. Formulation CYN-2, which proved to be the best showed a mean particle size of 236 nm and entrapment efficiency of 58.27%. The in vivo result of formulation CYN-2 revealed that the drug loaded nanoparticles showed preferential drug targeting to liver followed by spleen, lungs and kidneys. Stability studies showed that maximum drug content and closest in vitro release to initial data was found in the sample (formulation CYN-2) stored at 4^0C. So, in the present study Cyclosporine loaded Eudragit Nanoparticles were prepared and targeted to various organs to a satisfactory level and the prepared nanoparticles were stable at 4^0C.

Swarnali Das[59] et al., (2011) prepared AmB-loaded Eudragit nanoparticles by A solvent displacement technique. These NPs had a mean size range of 150–290 nm and a zeta potential of +19–28 mV. Even after 6 months of stability study, results were

unchanged, indicating the good potential for ocular application. In vitro release studies revealed that a maximum amount of drug was released within 24 hours (60%). The results obtained from microbial assay showed that the antifungal activity of drug-loaded NPs was equal to or slightly lower than that of free-AmB solution. In vivo experiments showed that, following topical instillation of nanosuspension to a rabbit's eye there was no irritation. From these results we can conclude that Eudragit RS 100 nanosuspension may represent an efficacious vehicle to deliver the drug into the eye.

Francesco Castelli[60] et al., (2003) prepared Nanosuspensions by a modification of the quasi-emulsion solvent diffusion technique (QESD), a particular approach to the general solvent-change method. This kind of system was planned for the ophthalmic release of non-steroidal anti-inflammatory drugs in ocular diseases associated with inflammatory processes (i.e. post-cataract surgery or uveitis). The drug release was monitored by differential scanning calorimetry (DSC), following the effects exerted by IBU on the thermotropic behaviour of DMPC multilamellar vesicles. IBU affects the main transition temperature (*T*m) of phospholipid vesicles, causing a shift towards lower values, driven by the drug fraction entering the lipid bilayer. The obtained values have been used as a calibration curve. DSC was performed on suspensions of blank liposomes added to fixed amounts of unloaded and IBU-loaded Eudragit RS100 and RL100 nanosuspensions as well as to powdered free drug. The *T*m shifts caused by the drug released from the polymer system or by the free drug, during incubation cycles at 37 ◦C, were compared to the calibration curve in order to obtain the fraction of drug released. The results were also compared with in vitro dialysis release experiments. The suitability of the two different techniques to follow the drug release as well as the differences between the RL and RS polymer systems was compared, confirming the efficacy of DSC for studying the release from polymer nanoparticulate systems. Explanation of the different rate of kinetic release could be due to void liposomes, which represent a better up-taking system than the aqueous solution phase in the dialysis experiments.

Cunxian Song[61] et al., (1998) prepared nanoparticles by oil–water emulsions using biodegradable polymers such as poly (lactic acid–co-glycolic acid) (PLGA), and specific additives after particle formation, to enhance arterial retention using either

heparin, didodecylmethylammonium bromide (DMAB), or fibrinogen, or combinations. Femoral and carotid arteries of male mongrel dogs were isolated in situ, and were then subjected to a balloon angioplasty. A NP suspension of a predetermined concentration was then infused into the artery for various durations. This was followed by a 30 min restoration of blood flow through the vessel. The arterial segments were excised and analyzed for drug levels. From the drug loading of the NP and the drug levels in the artery, the quantity of nanoparticles retained was calculated and expressed as mg per 10 mg dry arteries. In general, repeated short infusions of nanoparticle suspension (15 s34) were two-fold more effective in terms of higher arterial U-86 levels than a single prolonged infusion (60 s). A single 15 s infusion was not significantly different than a 60 s infusion on NP arterial uptake. NPs modified with either DMAB or fibrinogen had about 2.5-fold higher uptake levels compared to non-modified NPs (39.262.5 and 49.162.4 vs. 21.560.6, mg/10 mg mean6s.e. respectively). A comparably enhanced NP uptake was noted with a combined heparin /DMAB modification. Increasing the concentration of NP in 21 infusate from 5 to 30 mg ml significantly increased arterial NP uptake level (from 22.563.5 to 83.761.4 mg/10 mg). Thus, the results support the view that modified nanoparticles along with optimized infusion conditions could enhance arterial wall drug concentrations of agents to treat restenosis.

Yadav SC[62] et al., (2002) investigated on Biodegradable polymeric nanoparticles based drug delivery systems Nanoparticulate drug delivery systems seem to be a viable and promising strategy for the biopharmaceutical industry. They have advantages over conventional drug delivery systems. They can increase the bioavailability, solubility and permeability of many potent drugs which are otherwise difficult to deliver orally. Nanoparticulate drug delivery systems will also reduce the drug dosage frequency and will increase the patient compliance. In near future nanoparticulate drug delivery systems can be used for exploiting many biological drugs which have poor aqueous solubility, permeability and less bioavailability. nanoparticles provide ingenious treatment by enabling targeted delivery and con-trolled release.

M Sivabalan[63] et al., (2004) formulated and evaluated 5-Fluorouracil loaded Chitosan and Eudragit Nanoparticles for Cancer therapy. The goal of the investigation was to formulate and evaluate chitosan and Eudragit nanoparticles of 5- Fluorouracil for cancer therapy. Nanoparticles of 5- Fluorouracil were prepared using chitosan, Eudragit S 100, liquid paraffin and Tween -20 using Emulsion droplet coalescence method. The nanoparticles prepared were evaluated for morphology, loading efficiency, in vitro release and in vitro anticancer activities. The formulations CF1, CF2, EF2 and EF3 showed good drug release from the polymer. Among the four formulations EF 2 (1% Chitosan & 1.5 % Eudragit S 100) showed maximum drug release in 12 hours diffusion study and good entrapment efficiency. In vitro anticancer study revealed that the formulated nanoparticles were found to have good cidal activity on cancer cells in sustained manner.

Bivash Mandal[64] et al., (2010) prepared Sulfacetamide loaded polymeric nanosuspension by the solvent displacement method using acetone and 1% (w/v) Pluronic F108 solution. Drug to polymer ratio was chosen at four levels: 10/100 (B1), 20/100 (B2), 30/100 (B3), 40/100 (B4) (by weight). Characterization of nanosupension was performed by measuring particle size, zeta potential, Fourier Transform infrared spectra (FTIR), Differential Scanning Calorimetry (DSC), Powder X-Ray Diffraction (PXRD), drug entrapment efficiency and in vitro release. In addition, freeze drying, redispersibility and short term stability study at room temperature and at 40^0C were performed. Spherical, uniform iv particles (size below 500 nm) with polydispersity index range of 0.414 to 0.67 and positive zeta potential were obtained. Positive surface charge can allow a longer residence time of nanoparticles on the cornea surface, with a consequence slower drug release and higher drug concentrations in the aqueous humor, compared to classical eye drop. No significant drug polymer interaction was observed in the solid state characterization of freeze dried nanosuspension using DSC, PXRD, and FTIR. Drug entrapment efficiency was found to be in the range of about 28% to about 35%. In order to increase drug entrapment efficiency, selected batch was chosen to study the effect of changing polymer content, pH of external media and incorporation of polymethyl methacrylate (PMMA) on drug entrapment efficiency. Changing the external phase pH and incorporation of PMMA were significantly increased drug entrapment

efficiency of nanoparticles. No significant change in average particle size was observed after storage at room temperature and at 4ºC. Freeze dried nanosuspesnions were easily redispersed after manual hand shaking. Both batch of B3 containing 5% sucrose and 5% mannitol as cryoprotectant exhibited good redispersibility in water. The formulation of Sulfacetamide in Eudragit RL 100 nanosuspension could be utilized as potential delivery system for treating ocular bacterial infections.

Saikat Das[65] et al., (2005) prepared aspirin loaded albumin nanoparticles by coacervation method. Aspirin is a common anti-inflammatory and anti platelet agent widely used for various conditions. Albumin being both bioacceptable and biodegradable has a distinct advantage as a vehicle of drug delivery. By varying aspirin albumin ratios from 0.06 to 1.0 obtained stable nanoparticles of sizes 46.8 nm to 190.8 nm respectively with low polydispersity. Photon Correlation Spectroscopy (PCS) and Transmission Electron Microscopy (TEM) of the samples were done to characterize the nanoparticles. Drug encapsulation measured by UV spectroscopy varied from 30% to 80% for different ratios of aspirin: albumin. In vitro release study was conducted across a Spectrapor-membrane (cut off 3500 Da) precluding albumin. In contrast to simple drug solution, whose concentration peaks with in ½ to 1 hour, nanoparticle formulation releases aspirin at a sustained rate for prolonged duration (50% total cumulative percentage at the end of 20 hours, 90% at 72 hrs). From the above results we can conclude that coacervation method is well suited to produce albumin nanoparticles and the preparative variables of the procedure can be fine tuned depending on the clinical application. Nanoparticles thus produced, can be applied for intra-articular therapy in arthritis or as intraocular release agents for diabetic retinopathy.

3. AIM OF PRESENT WORK

Selective drug delivery systems or targeted drug delivery systems are designed to improve the benefit/risk ratio of any drug. They make the drug available more in the required tissue by decreasing its distribution in unwanted tissues. Innovations in the field of nanotechnology further fuel the research for the development of newer targeted drug release system which desired concentration of drug at the vicinity of action in a controlled drug release rate.

The present study was aimed to development and characterization of Lamivudine loaded nanoparticles drug delivery system using bio-degradable polymer such as Eudragit RS100 & Eudragit RL 100. The polymer enhances the binding of Lamivudine nanoparticles in specific (or) targeted site with sustained release of drug increasing therapeutic efficacy.

Lamivudine is analogue of cytidine. It inhibit both type (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B. Currently available dosage forms shows some side effect like abdominal pain, muscles pain, headache, fever, rash, alopecia, cough, nasal symptoms, arthralgia, most of which are dose dependent.

Thus the present work Development and characterization of nanoparticulate drug delivery system of antiviral drug Lamivudine aims;

- ✓ To overcome variable systemic availability.
- ✓ To overcome side effect.
- ✓ To overcome the drug resistance on long term.
- ✓ Specific site drug delivery at controlled rate.
- ✓ Prolonged systemic circulation.

PLAN AND SCOPE OF WORK

Plan of the present work involves the following process:

- Preformulation studies involve observation of physical and chemical data available. The identification of raw materials and compatibility studies between drug and polymer to be done by using Infrared spectrophotometry.
- Preparation of Lamivudine loaded Eudragit RS100 & Eudragit RL100 nanoparticles can be prepared by Emulsion solvent evaporation method.
- Formulation of Lamivudine loaded nanoparticle in various ratios of drug and polymer.

The best formulation will be selected based on the results of following parameters. The prepared nanoparticle can be evaluated by following chemical characteristic:

- Drug content determination.
- Drug entrapment efficiency.
- In vitro drug release of formulated nanoparticles.
- The morphology of nanoparticle was examined by scanning electron microscopy (SEM)
- Zeta potential analysis of the optimized formulation
- Sterilization of optimized nanoparticle formulation by Membrane Filtration method.
- Sterility test for the formulation to ensure the success of sterilization process.
- Stability studies for the best formulation at different temperature.

5. METHODOLOGY

5.1. INSTRUMENTS AND MATERIALS USED

Table: 6: Instruments used

S. No	EQUIPMENTS	SOURCE
1.	Vortex Mixer	Remi motors Ltd, Mumbai.
2.	Rotary flash evaporator	Equitron, Mumbai.
3.	Probe sonicator	Bandelin, Germany.
4.	Magnetic stirrer	Remi motors Ltd.
5.	Single beam UV spectrophotometer	Shimadzu corporation, Japan.
6.	Electronic balance	Shimadzu.
7.	Stability chamber	Osword, Mumbai.
8.	Ultra certification	Remi motors Ltd, Mumbai.
9.	pH – meter	ElicoPvt Ltd,Chennai.
10.	FTIR Spectrophotometer	Perkin Elmer, Germany.
11.	Autoclave	Kemi chem, India
12.	Double beam UV Spectro photometer	Perkin Elmer, Germany.
13.	Laminar air flow	Klenzaids, Mumbai
14.	Incubator	M.C.DALAL&CO, Mumbai
15.	Freeze drier	Allied frost, Mumbai
16.	Hot air oven	Biochemicals, Mumbai.

Table: 7: Materials used

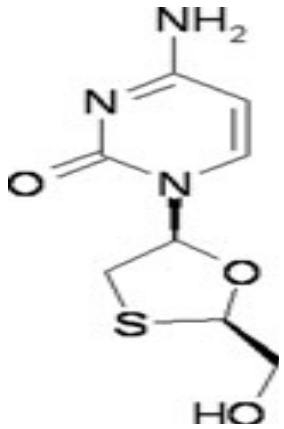
S. No	MATERIALS	SUPPLIER
1.	Lamivudine	Bafna pharmaceuticals.Mumbai.
2.	Eudragit RS 100	Micro Labs Hosur.
3.	Eudragit RL100	Micro Labs Hosur.
4.	Poly Vinyl Alcohol (PVA)	S.D.Fine Chemicals.Ltd ,Boisar
5.	Potassium dihydrogen phosphate	S.D.Fine Chemicals. Ltd,Boisar
6.	Sodium chloride	S.D.Fine Chemicals. Ltd,Boisar
7.	Disodium hydrogen phosphate	S.D.Fine Chemls. Ltd,Boisar
8.	Methanol	Merck, India
9.	Membrane filter	Gotting Ltd, West Germany

5.2. DRUG PROFILE

LAMIVUDINE[67,68]

(2', 3'-dideoxy-3'-thiacytidine, commonly called 3TC) is a potent nucleoside analog reverse transcriptase inhibitor (nRTI)

Chemical Structure:



Chemical name

(4-amino-1-[(2*R*,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)one.

Molecular formula

$C_8H_{11}N_3O_3S$

Molar mass

229.26 g/mol

Description

A white powder.

Melting point

160°C–162°C

Solubility

Soluble in water, sparingly soluble in methanol, and insoluble in acetone.

Pharmacology

Lamivudine was originally developed as an antiretroviral drug. The drug is metabolised intracellular to the active triphosphate moiety by infected by both infected and uninfected cells. Triphosphate acts as a substrate for HBV polymerase. The information of further viral DNA is blocked by incorporation of triphosphate into the viral chain and by subsequent chain termination. At the same time lamivudine not enter to normal cellular 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.

Mechanism of action:

Lamivudine is analogue of cytidine. It inhibits both type (1 and 2) of HIV reverse transcriptase and also reverse transcriptase of hepatitis B. It is phosphorylated to active metabolites that complete for incorporation in to viral DNA. It inhibits the HIV reverse transcriptase enzyme competitively and chain terminator of DNA synthesis. The lack of a 3-OH group in the incorporation 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. Several mutagenicity tests show that lamivudine should not show mutagenic activity in therapeutic doses. And some research shows lamivudine can cross the blood brain barrier.

Pharmacodynamics

Lamivudine is an antiviral agent which is highly active against hepatitis B virus in virus-transfected human hepatoma cell lines and in experimentally infected animals. Lamivudine is metabolised by both hepatitis B virus-transfected and non-transfected hepatoma cells to the triphosphate (TP) derivative which is the active form of the parent compound. The intracellular half life of the triphosphate in hepatoma cells is 17 – 19 hours in vitro. Lamivudine acts as a substrate for the HBV viral polymerase. It is considered that the formation of further viral DNA is blocked by incorporation of

lamivudine into the chain and subsequent chain termination. Lamivudine does not interfere with cellular deoxynucleotide metabolism. It is also a weak inhibitor of mammalian DNA polymerases alpha and beta. Furthermore, lamivudine has little effect on mammalian cell DNA content.

Pharmacokinetics

Absorption:

Lamivudine is well absorbed from the gut, and the bioavailability of oral lamivudine in adults is normally between 80 and 85%. Following oral administration the mean time (tmax) to maximal serum concentrations (Cmax) is about an hour. At therapeutic dose levels 100mg once daily, Cmax is in the order of 1-1.5 μg/mL and trough levels were 0.015 – 0.020 μg/mL.

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. The mean ratio CSF/serum lamivudine concentration 2 to 4 hours after oral administration was approximately 0.12.

Metabolism:

Lamivudine is predominately cleared by renal excretion of unchanged drug. The likelihood of metabolic drug interactions with lamivudine is low due to limited metabolism and plasma protein binding and almost complete renal clearance. An interaction with trimethoprim, a constituent of co-trimoxazole (trimethoprim with sulphamethoxazole) causes a 40% increase in lamivudine exposure at therapeutic doses.

Elimination:

The mean systemic clearance of lamivudine is approximately 0.3 L/h/kg. The observed half-life of elimination is 5 to 7 hours. The majority of lamivudine is excreted unchanged in the urine via glomerular filtration and active secretion (organic cationic transport system). Renal clearance is about 70% of lamivudine elimination

Side effect

Abdominal pain, vomiting, diarrhea, insomnia, cough, nasal a symptoms, muscles pain, head ach, fever, rash, alopecia, malaise, increased creatinine, phospholinase.

pKa

Lamivudine is a weak base with a p*K*a of 4.3 (protonation of the NH2 group)

Dosage

Oral chronic Hepatitis B

Chronic Hepatitis B: Adult- 100 mg once daily. Child - >2yrs: 3 mg/kg once daily. Max 100 mg/day. HIV infection: Concomitant HIV & hepatitis B infection: Adult - 150 mg bid or 300 m once daily in combination with other antiretrovirals. Child- 3months - 12 yrs: 4 mg/kg bid. Max: 300 mg/day.

Concomitant HIV& hepatitis B infection:

Adult - 150 mg or 300 mg once daily combination with other antiretroviral drugs.

Child-3 months-12years: 4 mg/kg bid. Max: 300 mg/day.

Storage

Keep container tightly closed. Keep container in a well-ventilated area and protected from light it may exhibit polymorphism

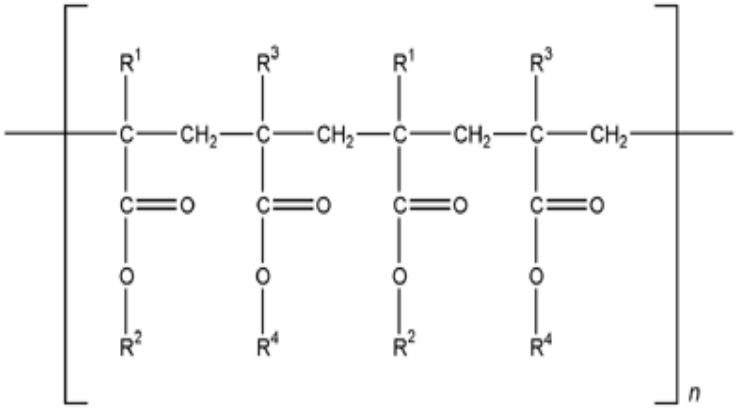
5.3 POLYMER PROFILE

EUDRAGIT RS 100

Molecular weight

150.000

Chemical Structure



R1=CH3

R2=C2H5

R3=CH3

R4=CH2CH2n(CH3)3+C1

Eudragit RS 100 is the copolymer of ethyl acrylate, methyl methacrylate and a low content of methacrylic acid ester with quaternary ammionium group. The group are present as salts and make the polymers permiable.

Chemical name

Poly (ethyl acrylate-co-methyl methacrylate-co-trimethyl ammino ethyl methacrylate chloride) 1:2:0:1.

INCI name

Acrylates/ ammonium methacrylate co polymer.

Physical properties

Colourless, clear to cloud granules with a faint amine-like odour.

Solubility

Soluble in methanol, ethanol, isopropyl alcohol, acetone, ethyl acetate, methylenechloride. Insoluble in petroleum ether and 1 N sodium hydroxide, water.

Functional Category

Tablet diluents

Application in pharmaceutical formulation and technology:

Used to form water insoluble film or film coats for sustained release product, these are also permeable to solvents.

Plasticizers

This includes dibutyl phthalate,poly ethylene glycols and tri ethyl citrate.

Storage

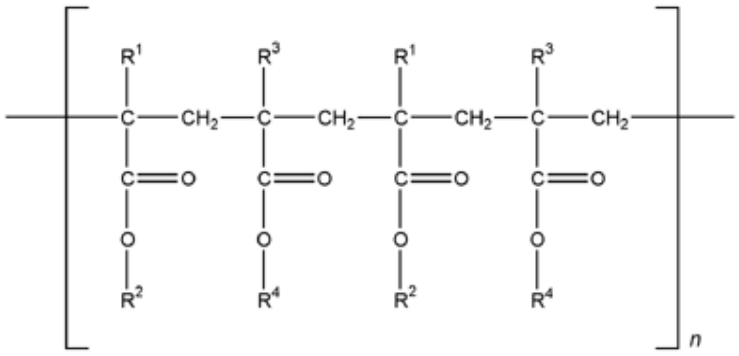
Stored at controlled room temperature (USP General noticed). Protect against moisture stored at 8degree. Avoid contacting with atmospheric moisture. Store the materials with a desiccant in original containers to maintain product quality. Keep the container closed until it is ready for use.

5.4 EUDRAGIT RL 100

Molecular weight

150.000

Chemical Structure



R1=H

R2=CH3

R3=CH3

R4=CH2CH2.N(CH3)3C1

Eudragit RL100 is the copolymer of ethyl acrylate, methyl methacrylate and a low content of methacrylic acid ester with quaternary ammionium group.The group are present as salts and make the polymers permiable.

Chemical name

Poly (ethyl acrylate-co-methyl methacrylate-co-trimethyl ammino ethyl methacrylate chloride)1:2:0:2.

INCI name

Acrylates/ ammonium methacrylate co polymer.

Physical properties

Colourless, clear to cloud granules with a faint amine-like odour.

Solubility

Soluble in methanol, ethanol and isopropyl alcohol, acetone, ethyl acetate and methylene chloride. Insoluble in petroleum ether and 1 N sodium hydroxide, water.

Functional Category

Film former, Tablet diluents.

Application in pharmaceutical formulation and technology:

Used to form water insoluble film or film coats for sustained release product, these are also permeable to solvents.

Plasticizers

This includes dibutyl phthalate,poly ethylene glycols and tri ethyl citrate.

Storage

Stored at controlled room temperature (USP General Notice) protect against moisture. Avoid contacting with atmospheric moisture. Store the materials with a desiccant in original containers to maintain product quality. Keep the container closed until it is ready for use.

5.5 POLYVINYL ALCOHOL[72]

Synonym

Airvol, Elvanol,Polyviol, Poval, PVA, Vinyl alcohol polymer

Chemical name

Ethanol, Homopolymer

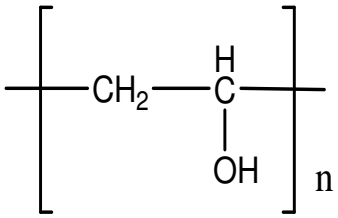
Empirical formula

$(C_2H_4O)_n$

Molecular weight

30, 000 to 200,000

Chemical Structure



Description

Colour	: white to cream-colored granular powder.
Odour	: odourless
Melting point	: 228° C for fully hydrolyzed grades, 80-190°C for partially hydrolyzed grades.
Refractive index	: 1.49-1.53

Solubility

Soluble in hot or cold water, slightly soluble in some polyhydroxy compounds, certain amines and amides. Insoluble in aliphatic, aromatic and chlorinated hydrocarbons, esters, ketones, oils.

Functional category

Coating agent, Nonionic Surfactant, Viscosity builder

Applications

It is used primarily in topical pharmaceutical formulations, particularly in ophthalmic products as a viscosity increasing agent, as a stabilizing agent for emulsions.

Pharmacopeia Specifications:

pH (4% aqueous solution)	= 5 to 8
Loss on drying	= 5%
Residue on ignition	= 2%
Water – insoluble substances	= 0.1%
Degree on hydrolysis	= 85 to 89%

Stability and Storage Conditions:

PVA undergoes slow degradation at 190˚C and rapid degradation at 200˚C, stable on exposure to light. Aqueous solutions are stable and should be stored in corrosion resistant containers. For extended storage preservative should be added. The bulk should be stored in a well closed container, in a cool, dry place.

6. EXPERIMENTAL INVESTIGATIONS

6.1. CONSTRUCTION OF STANDARD CURVE FOR LAMIVUDINE[69,75]

A. By UV Spectroscopy Method

Lamivudine is estimated spectrophotometrically at 270 nm and it obeys Beer-Lambert's Law in the range of 2 – 20 µg/ml.

Determination of Absorbance maximum (λ_{max})

Lamivudine was dissolved in phosphate buffer saline pH 7.4. Solution with 20 µg/ml concentration was prepared by suitable dilution. The solution was scanned in UV spectrophotometer at 200 to 400 nm using phosphate buffer saline pH 7.4 as blank. Absorbance maximum was determined as 270 nm. The drug was later quantified by measuring the absorbance at 270 nm in phosphate buffer saline pH7.4.

Preparation of pH 7.4 Phosphate Buffer Saline[56]

Disodium hydrogen phosphate 2.38 gm, potassium di-hydrogen phosphate 0.19 gm, sodium chloride 8 gm accurately weighed and it is transformed in to 1000 ml volumetric flask and volume is made up with distilled water. The pH was adjusted if necessary.

Preparation of Stock solution

Stock solution was prepared by dissolving 100 mg of Lamivudine drug in 100 ml of solvent medium, so as to get a solution of 1000 µg/ml concentration (Primary stock solution) From primary stock solution 2 ml was taken in 100 ml standard flask and it diluted to100 ml with solvent medium PBS pH 7.4 (secondary stock solution) to get the concentration of 2-20 mcg/ml.

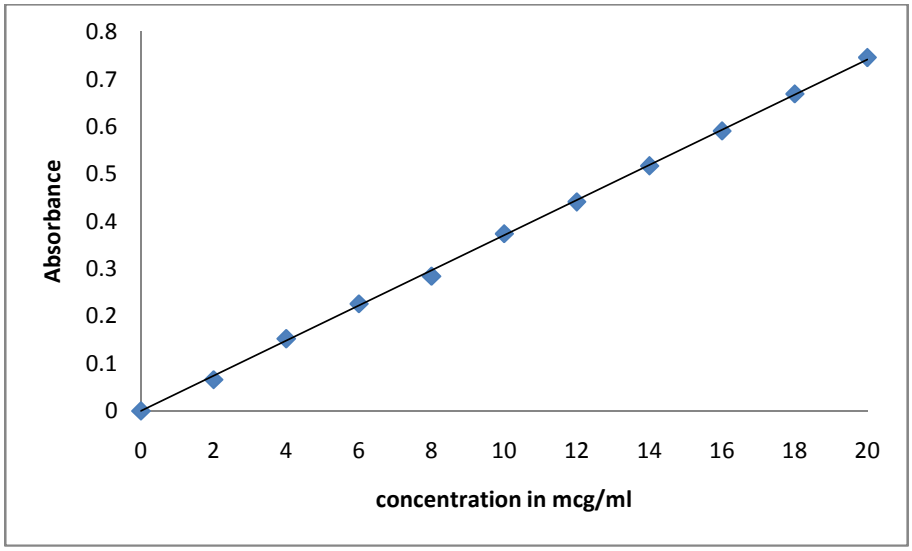
Preparation of Standard solution

From the secondary stock solution aliquots ranging from 1to10 ml with PBS to get the final concentration ranges from 2 to 20 µg per ml. Absorbance of the solution was measured at 270 nm UV spectrophotometrically against drug free PBS pH 7.4 media as blank.

Table: 8: CALIBRATION CURVE OF LAMIVUDINE AT 270 nm

S.NO	Concentration (µg/ml)	Absorbance at 270nm
1	2	0.066
2	4	0.152
3	6	0.226
4	8	0.284
5	10	0.374
6	12	0.441
7	14	0.517
8	16	0.591
9	18	0.669
10	20	0.746

FIGURE: 7: STANDARD CURVE FOR LAMIVUDINE



Slope = 0.037

$r^2 = 0.9995$

PREFORMULATION STUDY

6.2 DRUG AND POLYMER COMPATABLITY STUDY BY FTIR[18,24,35]

One of the requirements for the selection of suitable excipient (or) carrier for pharmaceutical formulations is its compatibility. Therefore in the present work, a study was carried out using Fourier Transformed Infrared (FT-IR) spectrophotometer (using Perkin Elmer) to confirm the absence of any possible chemical interactions between the Lamivudine and Eudragit RS 100 & Eudragit RL 100.

Infrared spectroscopy by potassium bromide pellet method was carried out on pure substances (Lamivudine and Eudragit RS 100 & Eudragit RL 100) separately and their physical mixtures. They are compressed under 15 tonnes pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000 to 400 cm-1 in a spectrophotometer.

The spectrum of physical mixtures was compared with the original spectra to determine any possible molecular interactions between the drug and polymer. Fourier Transformer Infrared Spectroscopy (FTIR) analysis measures the selective absorption of light by the vibration modes of specific chemical bonds in the sample. The observation of vibration spectrum of encapsulated drug evaluates the kind of interaction occurring between the drug and polymer.

In the present work, Lamivudine pure drug, pure Eudragit RS 100 & Eudragit RL 100 was submitted to FTIR and spectra are obtained. They are compressed under 10 tonnes pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000 to 400 cm^{-1} in a spectrophotometer.

IR SPECTRA DATA FOR PURE DRUG LAMIVUDINE

Table: 9:

Frequency cm^{-1}	Groups Assigned
1637.71	C=O - Stretching
3328.32	NH - Stretching
1286.72	C-O - Stretching
3214.01	O-H - Stretching
2924.56	C-H - Stretching

IR SPECTRA DATA FOR EUDRAGIT RS 100

Table: 10

Frequency cm^{-1}	Groups Assigned
2928	CH -Stretching
1740	C =O-Stretching
1466	CH -Stretching
1021	CO-Stretching

IR SPECTRA DATA FOR EUDRAGIT RL 100

Table: 11

Frequency cm^{-1}	Groups Assigned
2932	CH -Stretching
1740	C =O-Stretching
1466	CH -Stretching
1021	CO-Stretching

IR SPECTRA DATA FOR PHYSICAL MIXTURE

Table: 12

Frequency cm^{-1}	Groups Assigned
1637.71	C=O - Stretching
3328.32	NH - Stretching
1286.72	C-O - Stretching
3214.01	O-H - Stretching
2924.56	C-H - Stretching

6.3. METHOD OF PREPARATION OF LAMIVUDINE NANOPARTICLES:

Emulsion Solvent Evaporation Method[21,37,378,49]

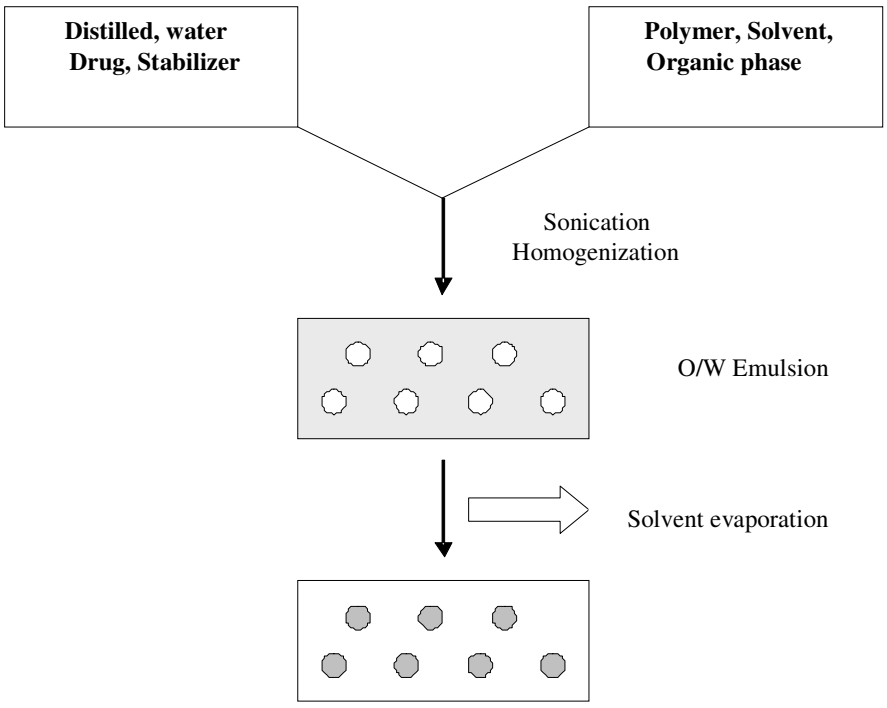
All batches of nanoparticles were prepared by Emulsion Solvent Evaporation method. The required quantity of polymer was dissolved in 4 ml methanol and 2 ml chloroform in (2:1) ratio as organic phase. The organic phase was then mixed with an aqueous phase containing drug and 0.2% polyvinyl alcohol (4 ml). The polymer concentration differs in various batches formulation as given in table; 15

Table 15: Various Composition of Nanoparticles Formulation

S.NO	Formulation Code	Drug (Lamivudine) in mg	Polymer Eudragit RS 100	Polymer Eudragit RL100	Drug: Polymer Ratio
1	F1	10	30	-	1:3
2	F2	10	20	-	1:2
3	F3	10	10	-	1:1
4	F4	10	-	30	1:3
5	F5	10	-	20	1:2
6	F6	10	-	10	1:1
7	F7	10	15	15	1:1
8	F8	10	20	10	2:1
9	F9	10	25	5	2.5:0.5
10	F10	10	27.5	2.5	2.75:0.25

This mixture was homogenized by vortex mixture for 1 min and then sonicated using a probe sonicator set at 55W of energy output for 1min to form an oil-in-water emulsion. The emulsion thus formed was further evaporated by flash rotatory evaporator for 20 min. The nanoparticle was collected by ultra-centrifugation (15,000 rpm).

Fig: 8: Schematic representation of Emulsion Solvent Evaporation method



The prepared nanoparticles are washed with water. The washed liquid was eliminated by centrifugation and purified nanoparticle was collected.

6.4. EVALUATION OF NANOPARTICLES[42]

6.4.1 DRUG ENTRAPMENT STUDY

The entrapment efficiency study was determined by free drug content in the supernatant which is obtained after centrifuging the solid lipid suspension at (15,000rpm for 20 min at zero degree using ultra centrifuge) The absorbance was measured at 270 nm by UV spectrophotometrically.

6.4.2 INVITRO DRUG RELEASE STUDIES[29,35]

By UV Spectrophotometric Method[45]

The in vitro drug release study was carried out by using the diffusion membrane technique. The nanoparticles preparation was placed in a dialysis membrane and it is dropped in to a beaker containing 200 ml of diffusion medium (phosphate buffer saline pH 7.4) the medium was maintained at 37^0 C under magnetic stirring at constant speed. At fixed time interval 1ml of sample was taken from the diffusion medium for every 1 hour and it was replaced by 1ml fresh medium. This process was carried out for 24 hours. The sample was measured UV spectrophotometrically at 270 nm. The percentage of drug released at various time intervals was calculated from calibration graph.

6.4.3 MORPOLOGY OF NANOPARTICLES BY SIMPLE MICROSCOPY:

The optimized formulation was morphologically characterized by microscopy. The small amount sample was placed in a glass slide and investigated in microscopy.

6.4.4 SCANNING ELECTRON MICROSCOPY[39]

The optimized formulation was morphologically characterized by scanning electron microscopy (SEM). The sample for SEM analysis was mounted in the specimen using an adhesive small sample was mounted directly in scotsch double adhesive tape. The sample was analyzed in hitachi scanning electron microscope operated at 15 kv and photograph was taken.

6.4.5 SURFACE CHARGE (ZETA POTENTIAL) DETERMINATION[43]

Zeta potential is an important parameter to evaluate and establish an optimum condition for stability of colloidal or dispersed systems. The prepared nanoparticle suspensions were characterized with respect to zeta potential by using zeta potential analyser (Malvern Zeta seizer). Zeta potential is electrical charges on particles surface it create electrical barrier it is very important for drug stability. The effect of Eudragit RS100 & Eudragit RL100 (polymer) on the surface characteristics of the nanoparticle was studied.

6.4.6 pH AND PHYSICAL APPEARANCE:

The pH of the formulation was measured using pH meter. It plays a vital role

in process of stability and formulation activity. The physical appearance of the formulation such as colour and suspended foreign particulate matter were to be examined.

STERILIZATION OF NANOPARTICLES

The prepared nanoparticles were administered as injection, so it needs to sterilize. The sterilization process was carried out by membrane filter placed with 0.4 micron membrane. The prepared nanoparticle suspension was allowed to flow through the membrane filter. This whole process can carry out in aseptic condition maintained by laminar air Flow Bench. The sterilized nanoparticle suspension was stored in suitable container.

6.4.7 STERILITY TESTING OF NANOPARTICLES[73]:

STERILITY TEST PROCEDURE:

The sterility test is a important for nanoparticle because it's a parental formulation. The sterility test for nanoparticles is done by various medium as per IP.

PREPARATION OF SOYABEAN CASEIN MEDIUM (SCDM);

25 gms of dehydrated media was weighed and mix with small amount of freshly prepared hot water and made up to 1000 ml with hot water in a 1000 ml beaker. The medium was cooled to room temperature and pH adjusted to 7.3 ± 0.2. The medium was dispensed in suitable container and sterilized at 121^{O}C for 15 min.

PREPARATION OF FLUID THIOGLYCOLLATE MEDIUM (FTM)**:**

26 gm of dehydrated media was weighed and mix with small amount of freshly prepared hot water and made up to 1000 ml with hot water in a 1000 ml beaker. The medium was adjusted to pH7.1 ± 0.2. The sterilized media should not have more than upper one third of the medium in pink color.

Preparation of rinsing fluid (Fluid A):

1 gm of peptic digest of animal tissue was weighed and taken and mixed up with small amount of freshly prepared hot water and made up to one 1000 ml in a beaker. The solution was filtered and pH adjusted to pH 7.1 ± 0.2. The solution was then dispensed in suitable container and autoclaved at 121^{0} C 15 min for sterilization.

Procedure:

The vials containing Lamivudine loaded nanoparticles were broken open under aseptic condition provided by laminar air flow works. All precautions and preventive measures were taken to avoid contamination by the process or by the analyst. The drug solution was then passed through sterile membrane lodged on a membrane holder assembly. After passing through the solution the membrane was rinsed with thrice with 100 ml of sterile peptone (Fluid A). The membrane was then cut into two halves using sterile scissors. One half of the filter paper was introduced into the container with SCDM and the other half in to the container with FTM.

This prepared medium is placed in different temperature. Soybean casein digest medium (SCDM) is incubated at $22.5^{o}C \pm 2.5^{0}C$ and Fluid thioglycollate medium (FTM) is incubated at $32.5^{0}c \pm 2.5^{0}C$. The containers were observed for turbidity or appearance growth of microorganism for 14 days. Positive control and negative control test were done to validate the sterility testing procedure.

Negative control:

Negative control confirms the sterility of the sterilized media. It was the uninnoculated sterile media and observed for 14 days. Negative control was maintained for both (FTM) and (SCDM) Medias and incubated in the appropriate temperature.

Positive control:

Positive control confirms the suitability of the media for the growth of microorganism. The positive control for SCDM and FTM was inoculated with Bacillus subtilis suspension with less than 100 CFUs and incubated recommended temperature respectively for 14 days. The growth of micro organism witnessed by the turbidity of the medium confirms the presence of nourishments favoring Microorganism.

6.4.8 STABILITY STUDIES OF NANOPARTICLES[59,74]

The Stability studies of nanoparticles involves observing the formulation at 45°C/70% RH which constitutes Accelerated condition and 4°C on refrigerator and room temperature. The formulations were kept in both the temperature for 3 months and sufficient amount of samples were taken at periodic intervals for performing the

following tests.

a. Physical appearance

b. pH of the solution

c. In vitro drug release (Dissolution)

d. Percentage of drug entrapment

7. RESULTS AND DISCUSSION

7.1. DEVELOPMENT OF LAMIVUDINE NANOPARTICLES[21]

In this study, Lamivudine loaded nanoparticles were prepared by Emulsion solvent evaporation method using Eudragit RS 100 & Eudragit RL100, methanol and chloroform solvent are used in the ratio of (2:1). The organic phase was then mixed with an aqueous phase containing drug and 0.2% polyvinyl alcohol. This mixture was homogenized by vortex for 1 min and then sonicated using a probe sonicator to form an oil-in-water emulsion. The emulsion thus formed was further evaporated by flash rotatory evaporator for 20 min.

Formulations with different ratios of polymer were prepared. Several physicochemical characteristics of nanoparticles such as morphology, particle size determination, drug release profile were investigated and stability of optimized formulation at various temperatures was evaluated.

7.2 DRUG & POLYMER COMPATIBILITY STUDIES BY FTIR[25, 48]

Pressed Pellet Technique was used to handle the sample in FTIR spectrometer. In this technique a pinch of sample was mixed with potassium bromide and the mixture was pressed with special discs under high pressure into a transparent pellet and then inserted in to special holder of IR spectrometer.

IR spectrums for pure drug alone and physical mixture of drug and polymers are taken. The spectrum of physical mixture was compared with spectrum of pure drug. Bands seen in pure drug also recognized in physical mixture. Hence there was no significant interaction between drug and excipients.

7.3 ENTRAPMENT EFFICIENCY OF NANOPARTICLE[44]

The Entrapment efficiency of Lamivudine loaded nanoparticles was prepared by Emulsion solvent evaporation method. The formulation F1 (Lamivudine 10 mg with 30mg of Eudragit RS 100) which shows less entrapment value of 61% was represented in table no.16. This is due to repulsive force between drug and the polymer.

Table: 16: Entrapment efficiency formulations with Drug and polymer:

S.NO	Formulation Code	Drug (mg)	Eudragit RS 100 (mg)	Eudragit RL 100 (mg)	Entrapment Efficiency (%)
1	F1	10	30	-	61±0.12
2	F2	10	20	-	68±0.09
3	F3	10	10	-	72± 0.17
4	F4	10	-	30	49±0.14
5	F5	10	-	20	56±0.11
6	F6	10	-	10	60±0.08
7	F7	10	15	15	73±0.12
8	F8	10	20	10	83±0.17
9	F9	10	25	5	94±0.05
10	F10	10	27.5	2.5	85±0.08

In formulation (F2) polymer concentration was decreased (Lamivudine 10 mg Eudragit 20 mg) the entrapment efficiency was increased to 68%. Further decrease in polymer concentration in formulation F3 (Lamivudine 10 mg Eudragit 10 mg) entrapment efficiency was 72%. Formulation F4, F5, F6, carried out by same process as like first three formulation but changes in polymer concentration 30 mg, 20 mg, 10 mg of (Eudragit RL 100) is taken. The entrapment efficiency was 49 ± 0.03 for F4, 56 % for F5, 60% for F6. Based on above result.

Formulation F7 was carried out by combination of both polymers intially with same concentration (Lamivudine 10 mg Eudragit RS100 15 mg RL 100 15 mg) were taken. Entrapment efficiency was increased in great number F7 73% and also in F8 83%. In order to study the polymer concentration the formulation F9 was carried out in different concentration (Lamivudine 10 mg with 25 mg of Eudragit RS 100 and 5 mg of Eudragit RL 100) it give high percentage of entrapment efficiency 94%, which indicate the steady increasea in entrapment efficiency.

Further study was carried out in F10 changes in polymer concentration (Lamivudine 10 mg with 27.5 mg of Eudragit 100 and 2.5 mg of Eudragit RL 100) entrapment efficiency is 85% was not as like F9. From the above result formulation

F9 shows highest percentage of entrapment efficency of 94%. So this formulation was selected for further studies .

The drug entrapment efficiency is based on chemicals structure of drug and polymer used. Eudragit RS100 contains more number of non polar group but Eudragit RL 100 contain more number of polar group, at the same time Lamivudine have more number of non polar. So Eudragit RS 100 has less repulsive force with Lamivudine drug molecules. So the entrapment efficiency is high inFormulation F9.

7.4 INVITRO DRUG RELEASE PROFILE OF NANOPARTICLES[30,40,41]

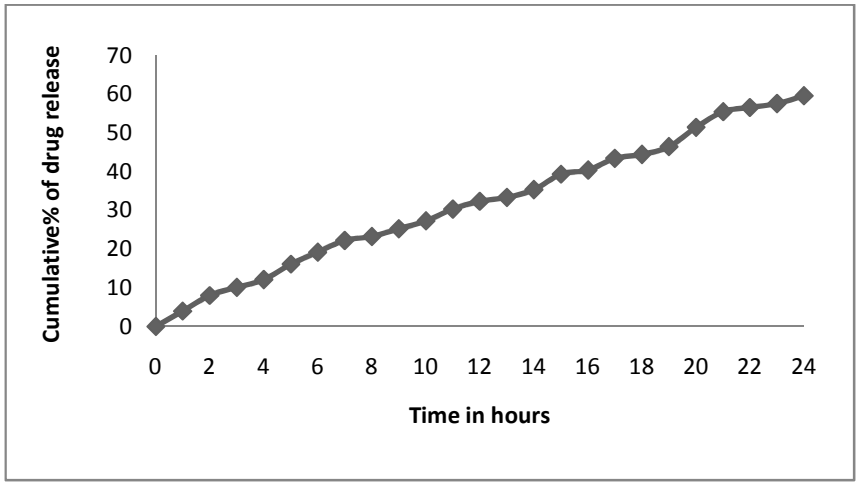
- The in vitro drug release of lamivudine nanoparticle can carried out by membrane diffusion method and in vitro drug release study was carried out for 24 hours.
- The in vitro drug release of lamivudine loaded nanoparticles with various polymers (Eudragit RS 100 & Eudragit RL 100).
- The in vitro drug release of formulation F1 (Lamivudine 10 mg with 30 mg of Eudragit RS 100). The percentage of in vitro drug release was 59.57% in 24 hours.
- The formulation F2 was carried out by decreasing the polymer concentration (Lamivudine 10 mg with 20 mg of Eudragit RS 100). The percentage of in vitro drug release was found to be 66.65%
- The formulation F3 was carried out by further decrease in polymer concentration (Lamivudine 10 mg with10mg of Eudragit RS 100). The percentage of drug release was 70.68 % shown in the table 13.
- The formulation F4 was prepared by replacing the polymer with Eudragit RL 100. (Lamivudine 10 mg with 30 mg of Eudragit RL 100) The in vitro drug release was found to be 52.50% in 24 hours. The formulation F5 and F6 was done by further decrease in polymer concentration. The in vitro drug release was 60.58% and 69.61 % respectively.
- The formulation F7 was carried out by combination of two polymers in same concentration (Lamivudine 10 mg Eudragit RS 100 15 mg & Eudragit RL 100 15 mg) due to the combination of polymer it intent to modified the surface characteristics. So the percentage of drug release was found to be 71.65% in 24 hours as given table: 23

- The formulation F8 was done by increase the polymer concentration of Eudragit RS100 and decreases the polymer concentration of Eudragit RL100. (Lamivudine 10 mg with 20 mg of Eudragit RS 100& 10 mg of Eudragit RL 100) as the result of these changes, in vitro drug release was increased 81.97% when comparing with other formulation.
- The formulation F9 was carried out by same process as like F7 & F8 but changes in the polymer concentration (Lamivudine 10 mg with 25 mg of Eudragit RS 100 & 5 mg Eudragit RL 100) in vitro drug release was found to be 92.89% it will show the highest percentage of drug release when comparing with other formulation
- Further study was carried out by increasing the polymer concentration of Eudragit RS 100 (Lamivudine 10 mg with 27.5 mg of Eudragit RS 100 & 2.5 mg Eudragit RL 100) percentage of drug release was found to be 83.78%, which shows less in vitro drug release when compared with F9.
- From the above formulation (F1 to F10) it confirms that the percentage of drug release was satisfactory in formulation F9 and it shows higher percentage of drug release of 92.89 %. So it was decided to be the best formulation. So it optimized for further study.

Table: 17: In vitro drug release for F1

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.4	0.4	4.0
2	0.8	0.804	8.04
3	1.0	1.008	10.08
4	1.2	1.210	12.10
5	1.6	1.612	16.12
6	1.9	1.916	19.16
7	2.2	2.219	22.19
8	2.3	2.322	23.22
9	2.5	2.523	25.23
10	2.7	2.725	27.25
11	3.0	3.027	30.27
12	3.2	3.230	32.30
13	3.3	3.332	33.32
14	3.5	3.533	35.33
15	3.9	3.935	39.35
16	4.0	4.039	40.39
17	4.3	4.340	43.40
18	4.4	4.443	44.43
19	4.6	4.644	46.44
20	5.1	5.146	51.46
21	5.5	5.551	55.51
22	5.6	5.655	56.55
23	5.7	5.756	57.56
24	5.9	5.957	59.57

Figure No: 9: IN VITRO DRUG RELEASE FOR FORMULATION F1

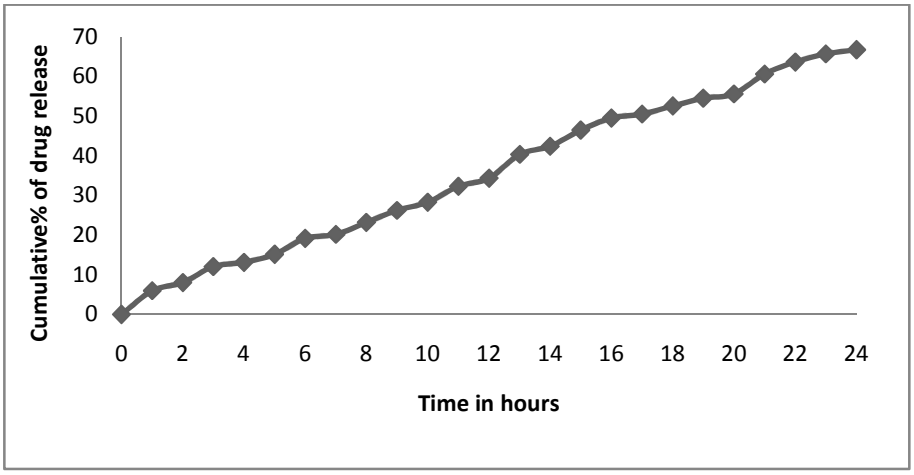


Slope = 2.369 r^2 = 0.994

Table: 18: In vitro drug release for F2

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.6	0.6	6.0
2	0.8	0.806	8.06
3	1.2	1.208	12.08
4	1.3	1.312	13.12
5	1.5	1.513	15.13
6	1.9	1.915	19.15
7	2.0	2.019	20.19
8	2.3	2.320	23.20
9	2.6	2.623	26.23
10	2.8	2.826	28.26
11	3.2	3.228	32.28
12	3.4	3.432	34.32
13	4.0	4.034	40.34
14	4.2	4.240	42.40
15	4.6	4.642	46.42
16	4.9	4.946	49.46
17	5.0	5.049	50.49
18	5.2	5.250	52.50
19	5.4	5.452	54.52
20	5.5	5.554	55.54
21	6.0	6.055	60.55
22	6.3	6.360	63.60
23	6.5	6.563	65.63
24	6.6	6.665	66.65

Figure No: 9: IN VITRO DRUG RELEASE FOR FORMULATION F2

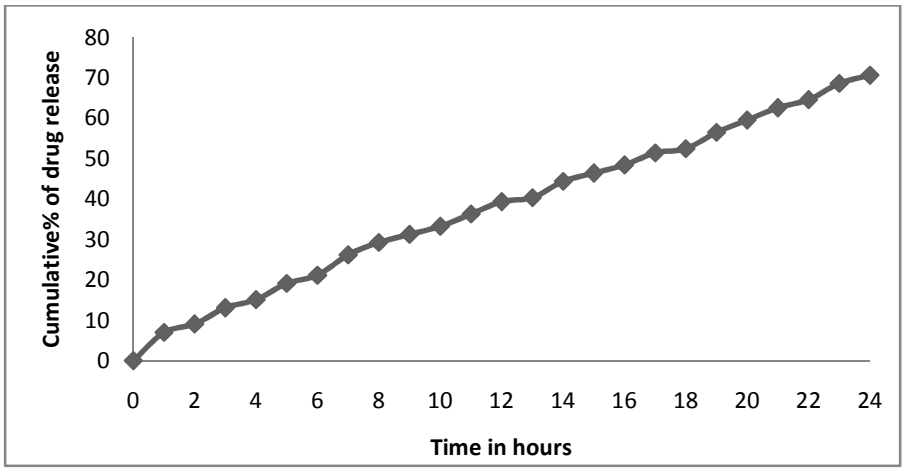


Slop = 2.759 $r^2 = 0.994$

Table: 19: In vitro drug release for F3

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.7	0.7	7.0
2	0.9	0.907	9.07
3	1.3	1.309	13.09
4	1.5	1.513	15.13
5	1.9	1.915	19.15
6	2.1	2.119	21.19
7	2.6	2.621	26.21
8	2.9	2.926	29.26
9	3.1	3.129	31.29
10	3.3	3.331	33.31
11	3.6	3.633	36.33
12	3.9	3.936	39.36
13	4.0	4.039	40.39
14	4.4	4.440	44.40
15	4.6	4.644	46.44
16	4..8	4.846	48.46
17	5.1	5.148	51.48
18	5.2	5.251	52.51
19	5.6	5.652	56.52
20	5.9	5.956	59.56
21	6.2	6.259	62.59
22	6.4	6.462	64.62
23	6.8	6.864	68.64
24	7.0	7.068	70.68

Figure No: 10: IN VITRO DRUG RELEASE FOR FORMULATION F3

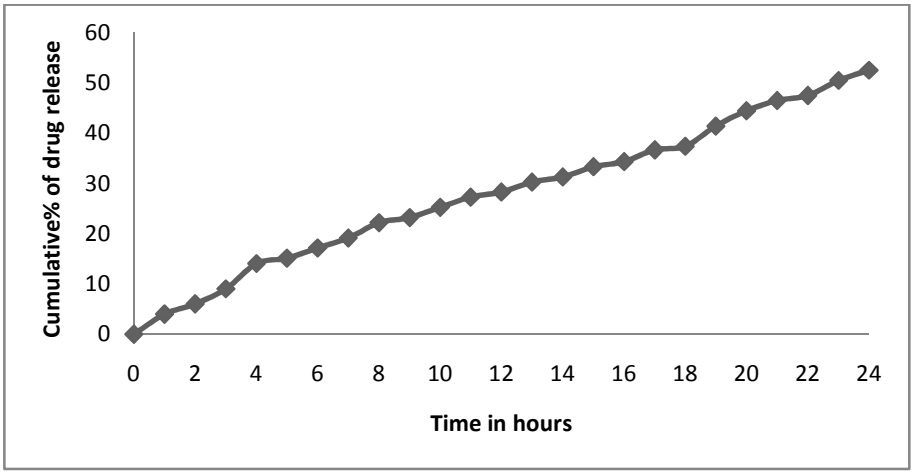


Slop = 2.777 $r^2 = 0.993$

Table: 20: In vitro drug release for F4

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.4	0.4	4
2	0.6	0.604	6.04
3	0.9	0.906	9.06
4	1.4	1.409	14.09
5	1.5	1.514	15.14
6	1.7	1.715	17.15
7	1.9	1.917	19.17
8	2.2	2.219	22.19
9	2.3	2.322	23.22
10	2.5	2.523	25.23
11	2.7	2.725	27.25
12	2.8	2.827	28.27
13	3.0	3.028	3.28
14	3.1	3.130	31.30
15	3.3	3.331	33.31
16	3.4	3.433	34.33
17	3.6	3.634	36.64
18	3.7	3.736	37.36
19	4.1	4.137	41.37
20	4.4	4.441	44.41
21	4.6	4.644	46.44
22	4.7	4.746	47.46
23	5.0	5.047	50.47
24	5.2	5.250	52.50

Figure No: 11: IN VITRO DRUG RELEASE FOR FORMULATION F4

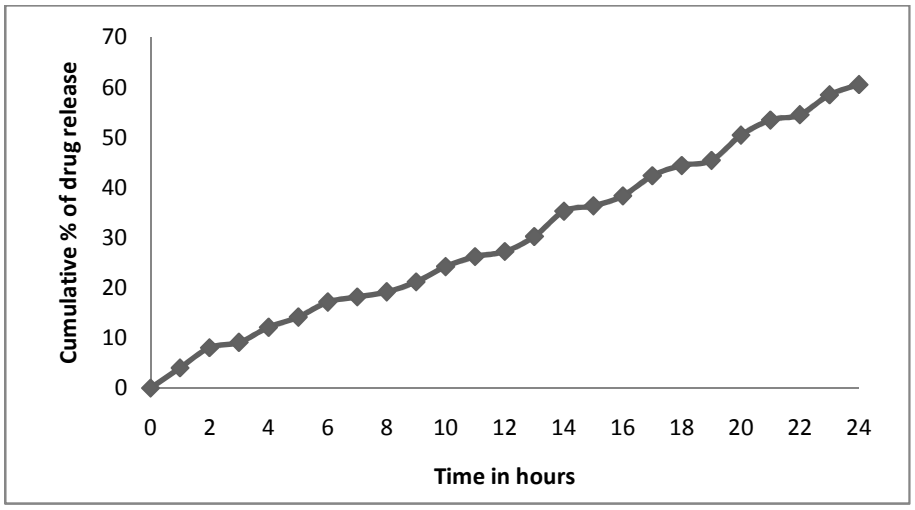


Slope = 2.015 $r^2 = 0.989$

Table: 21: In vitro drug release for F5

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.4	0.4	4
2	0.8	0.804	8.04
3	0.9	0.908	9.08
4	1.2	1.209	12.09
5	1.4	1.412	14.12
6	1.7	1.714	17.14
7	1.8	1.817	18.17
8	1.9	1.918	19.18
9	2.1	2.119	21.19
10	2.4	2.421	24.21
11	2.6	2.624	26.24
12	2.7	2.726	27.26
13	3.0	3.027	30.27
14	3.5	3.530	35.30
15	3.6	3.635	36.35
16	3.8	3.836	38.36
17	4.2	4.238	42.38
18	4.4	4.442	44.42
19	4.5	4.544	45.44
20	5.0	5.045	50.45
21	5.3	5.350	53.50
22	5.4	5.453	54.53
23	5.8	5.854	58.54
24	6.0	6.058	60.58

Figure No: 12: IN VITRO DRUG RELEASE FOR FORMULATION F5

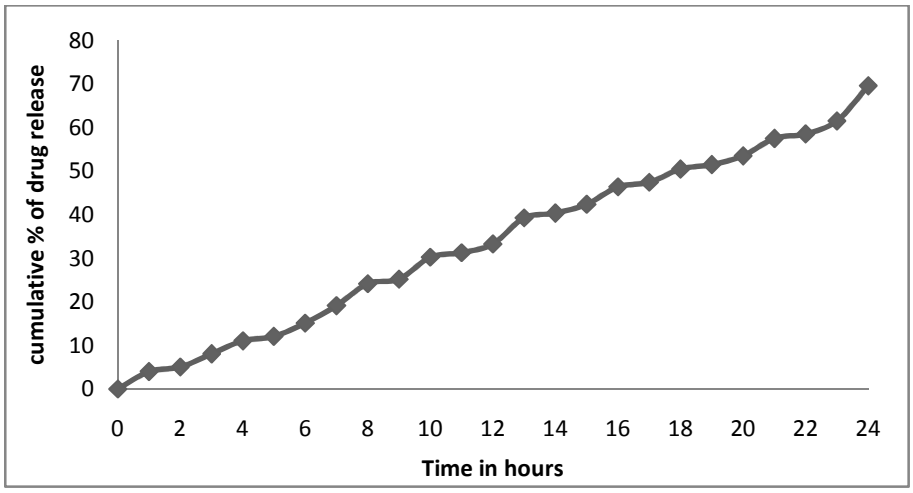


Slop = 2.423 $r^2 = 0.993$

Table: 21: In vitro drug release for F6

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.4	0.40	0.40
2	0.5	0.504	05.04
3	0.8	0.805	08.05
4	1.1	1.108	11.08
5	1.2	1.211	12.11
6	1.5	1.512	15.12
7	1.9	1.915	19.15
8	2.4	2.419	24.19
9	2.5	2.524	25.24
10	3.0	3.025	30.25
11	3.1	3.104	31.30
12	3.3	3.331	33.31
13	3.9	3.933	39.33
14	4.0	4.039	40.39
15	4.2	4.240	42.40
16	4.6	4.642	46.42
17	4.7	4.746	47.46
18	5.0	5.047	50.47
19	5.1	5.150	51.50
20	5.3	5.351	53.51
21	5.7	5.753	57.53
22	5.8	5.857	58.57
23	6.1	6.158	61.58
24	6.7	6.761	67.61

Figure No: 13: IN VITRO DRUG RELEASE FOR FORMULATION F6

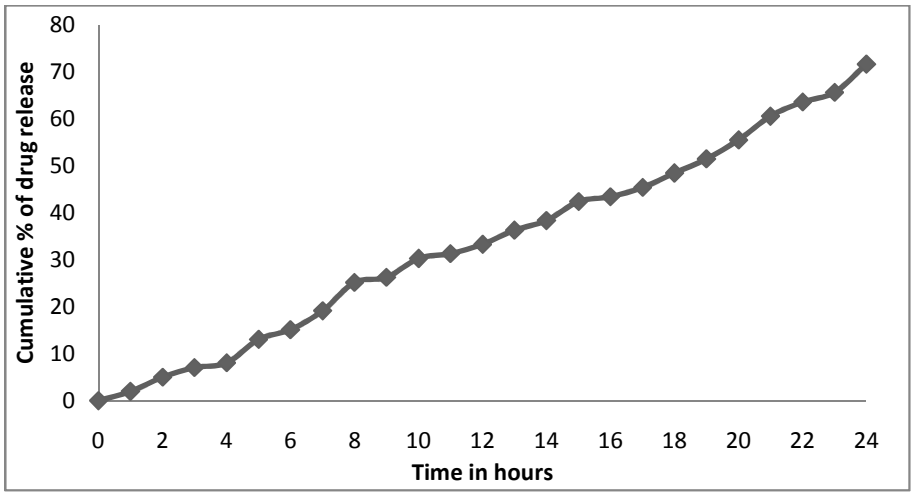


Slope = 2.759 r^2 = 0.993

Table: 23: In vitro drug release for F7

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.2	0.2	2.0
2	0.5	0.502	5.02
3	0.7	0.705	7.05
4	0.8	0.807	8.07
5	1.3	1.308	13.08
6	1.5	1.513	15.13
7	1.9	1.915	19.15
8	2.5	2.519	25.19
9	2.6	2.625	26.25
10	3.0	3.026	30.26
11	3.1	3.130	31.30
12	3.3	3.331	33.31
13	3.6	3.633	36.33
14	3.8	3.836	38.36
15	4.2	4.238	42.38
16	4.3	4.342	43.42
17	4.5	4.543	45.43
18	4.8	4.845	48.45
19	5.1	5.148	51.48
20	5.5	5.551	55.51
21	6.0	6.055	60.55
22	6.3	6.360	63.60
23	6.5	6.563	65.63
24	7.1	7.165	71.65

Figure No: 14: IN VITRO DRUG RELEASE FOR FORMULATION F7

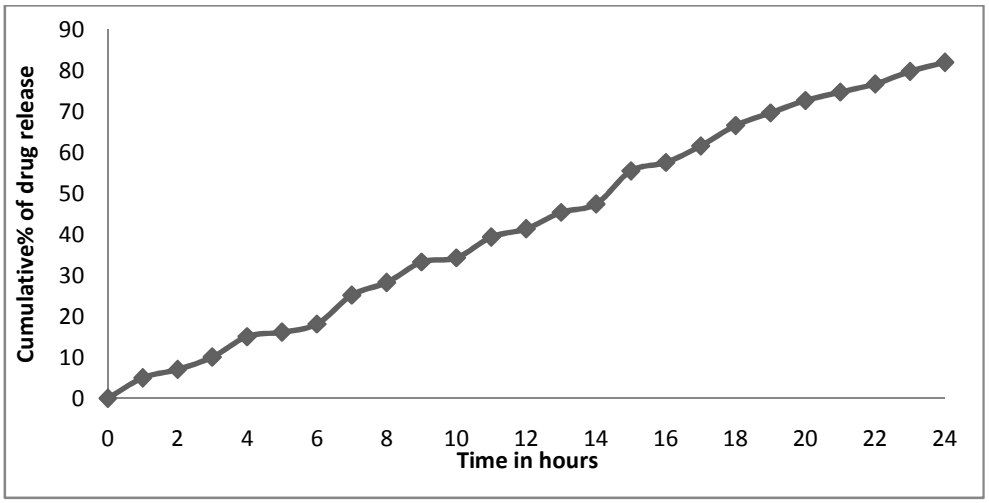


Slope = 2.884 $r^2 = 0.994$

Table: 24: In vitro drug release for F8

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.5	0.5	0.5
2	0.7	0.705	07.05
3	1.0	1.007	10.07
4	1.5	1.510	15.10
5	1.6	1.615	16.15
6	1.8	1.816	18.16
7	2.5	2.518	25.18
8	2.8	2.825	28.25
9	3.3	3.328	33.28
10	3.4	3.433	34.33
11	3.9	3.934	39.34
12	4.1	4.139	41.39
13	4.5	4.541	45.41
14	4.7	4.745	47.45
15	5.5	5.547	55.47
16	5.7	5.755	57.55
17	6.1	6.157	61.57
18	6.6	6.661	66.61
19	6.9	6.966	69.66
20	7.2	7.269	72.69
21	7.4	7.472	74.72
22	7.6	7.674	76.74
23	7.9	7.976	79.76
24	8.1	8.197	81.97

Figure No: 15: IN VITRO DRUG RELEASE FOR FORMULATION F8

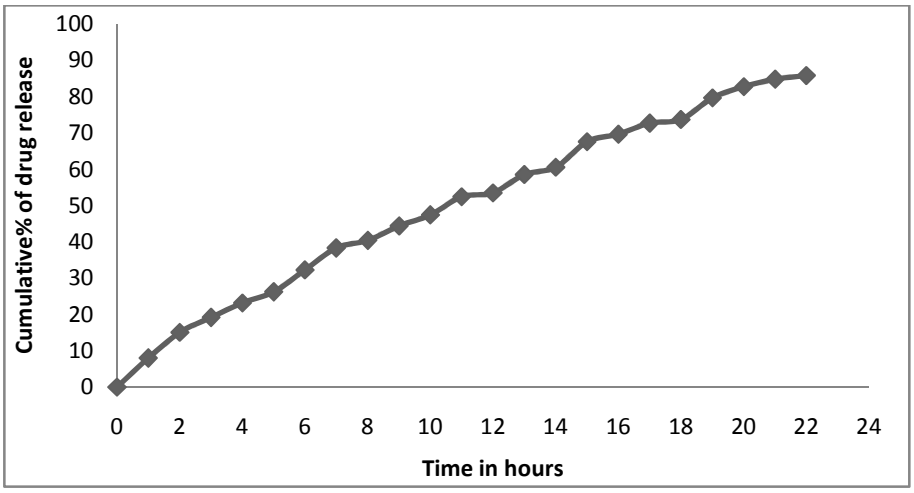


Slope = 3.245 r^2 = 0.996.

Table: 25: In vitro drug release for F9

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.8	0.8	8.0
2	1.5	1.508	15.08
3	1.9	1.918	19.18
4	2.3	2.319	23.19
5	2.6	2.623	26.23
6	3.2	3.226	32.26
7	3.8	3.832	38.32
8	4.0	4.038	40.38
9	4.4	4.440	44.40
10	4.7	4.744	47.44
11	5.2	5.247	52.47
12	5.3	5.352	53.52
13	5.8	5.853	58.53
14	6.0	6.058	60.58
15	6.7	6.760	67.60
16	6.9	6.967	69.67
17	7.2	7.269	72.69
18	7.3	7.372	73.72
19	7.9	7.973	79.73
20	8.2	8.279	82.79
21	8.4	8.482	84.82
22	8.5	8.584	85.84
23	8.9	8.985	89.85
24	9.2	9.289	92.89

Figure No: 16: IN VITRO DRUG RELEASE FOR FORMULATION F9

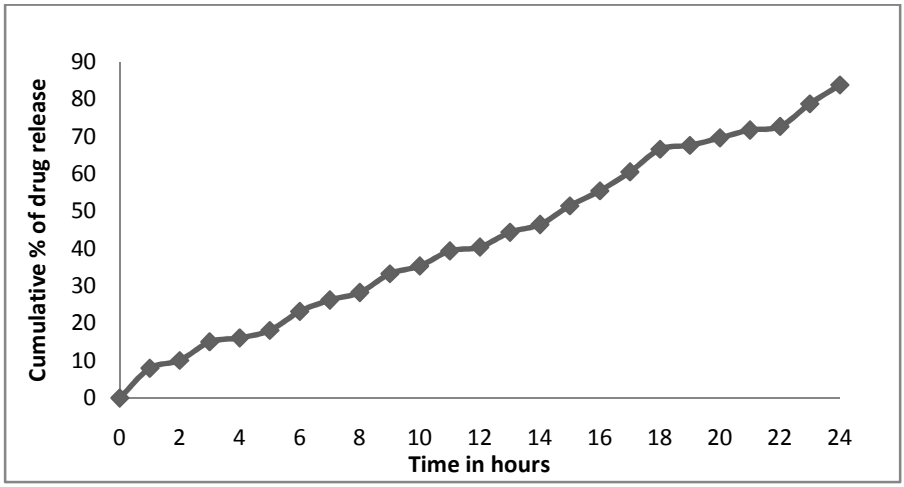


Slope = 3.704 $r^2 = 0.988$

Table: 26: In vitro drug release for F10

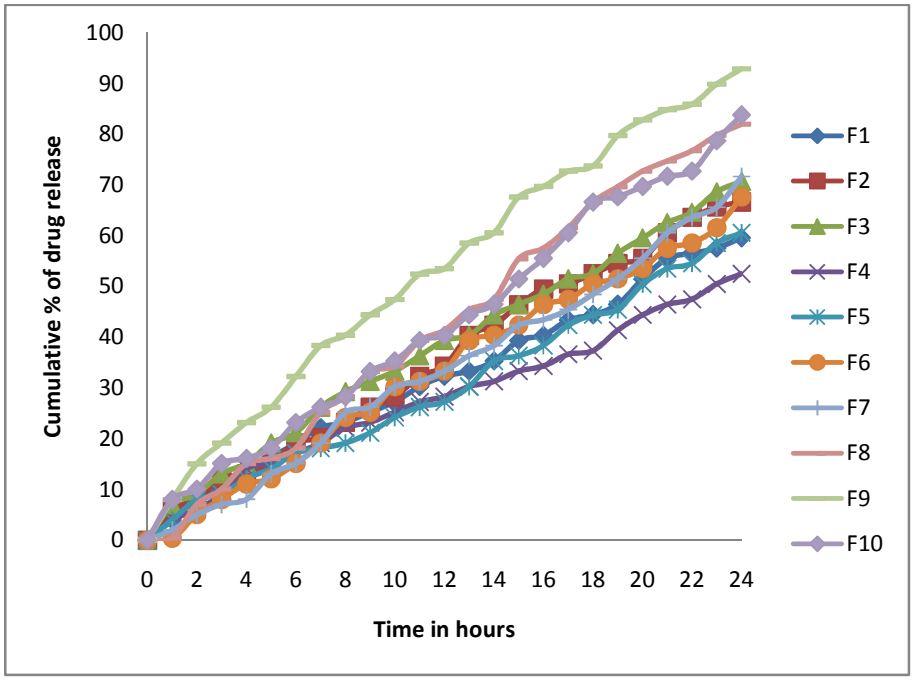
Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.8	0.8	8.0
2	1.5	1.508	15.08
3	1.9	1.918	19.18
4	2.3	2.319	23.19
5	2.6	2.623	26.23
6	3.2	3.226	32.26
7	3.8	3.832	38.32
8	4.0	4.038	40.38
9	4.4	4.440	44.40
10	4.7	4.744	47.44
11	5.2	5.247	52.47
12	5.3	5.352	53.52
13	5.8	5.853	58.53
14	6.0	6.058	60.58
15	6.7	6.760	67.60
16	6.9	6.967	69.67
17	7.2	7.269	72.69
18	7.3	7.372	73.72
19	7.9	7.973	79.73
20	8.2	8.279	82.79
21	8.4	8.482	84.82
22	8.5	8.584	85.84
23	8.9	8.985	89.85
24	9.2	9.289	92.89

Figure No: 17: IN VITRO DRUG RELEASE OF FORMULATION F10



Slope = 3.320 r^2 = 0.995

Fig: 18: SUMMARIZED IN VITRO DRUG RELEASES OF NANOPARTICLES FORMULATION (F1-F10)



7.5 MORPHOLOGY OF NANOPARTICLES:

The characteristics of optimized nanoparticles formulation (F9) particle size were studied by simple microscopy. Small amount of sample was placed in glass slide and placed in simple microscope. Image of prepared nanoparticle formulation shows the encapsulation of polymer mixture on drug particles.

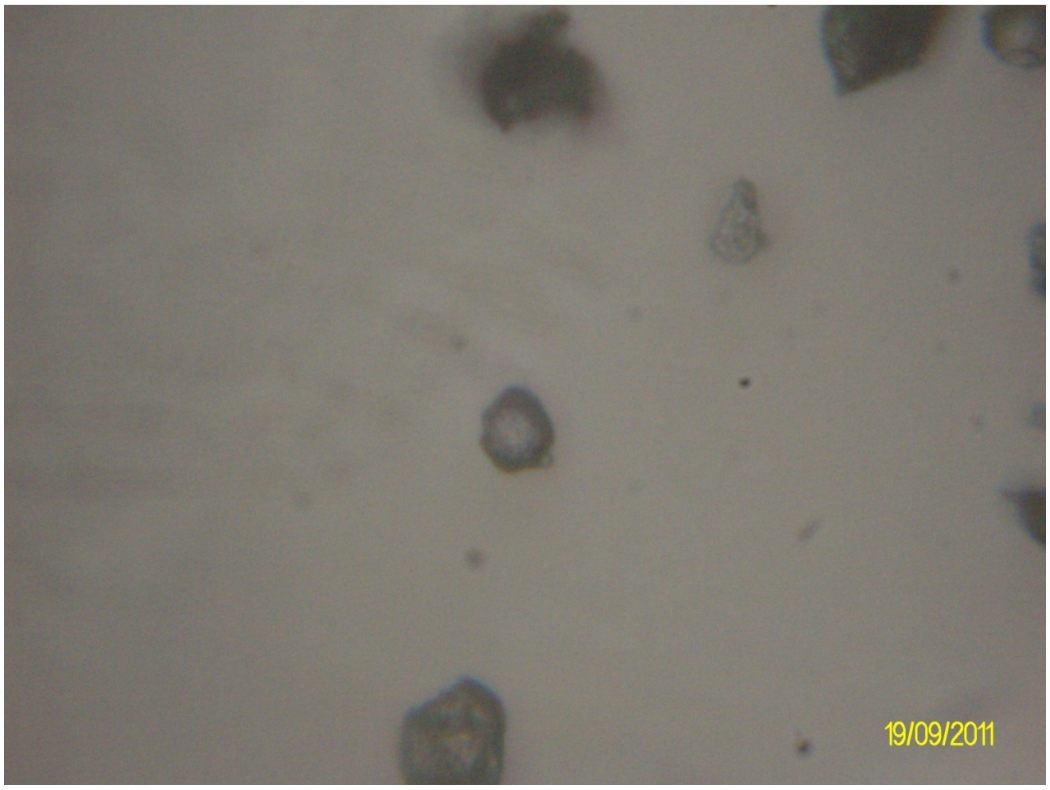


Fig: 19: MICROSCOPIC IMAGE OF NANOPARTICLES

7.6. SCANNING ELECTRON MICROCOPY[41, 42, 44]

The surface characteristics of optimized formulation (F9) particle size were studied by scanning electron microscopy. SEM image of prepared nanoparticle formulation shows the coating of polymer mixture on drug particles. The size distribution of nanoparticles in SEM is 400 nm, which indicates a thin and uniform coating over the drug.

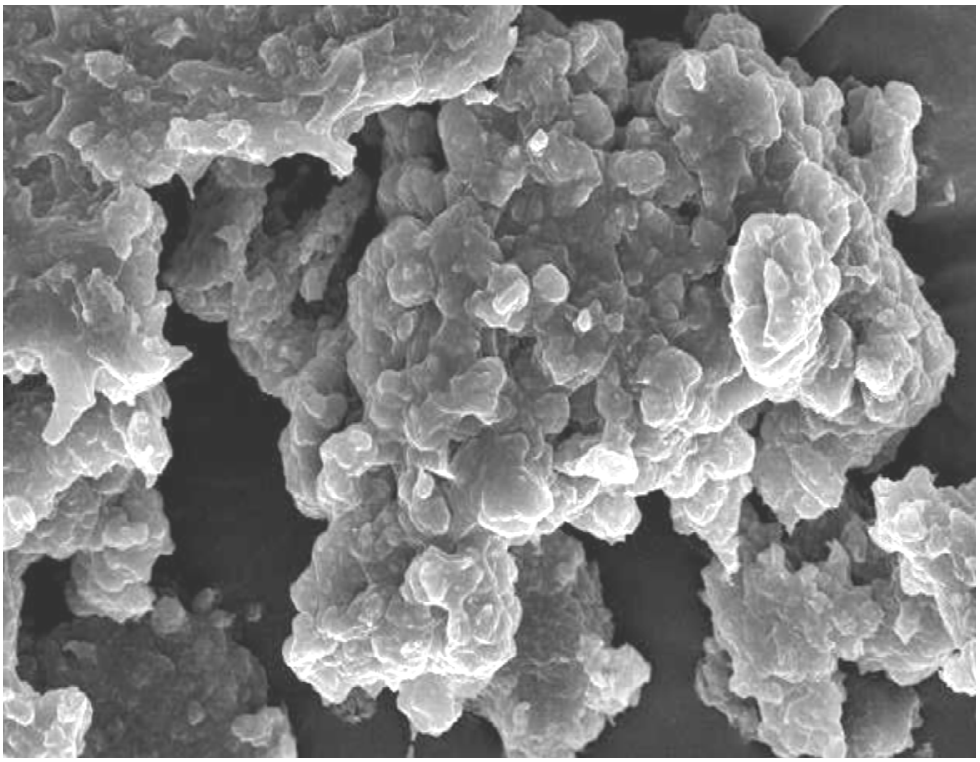


Fig: 20: SEM IMAGE OF NANOPARTICLES

7.7 SURFACE CHARGE (ZETA POTENTIAL)[36]

The zeta potential of a nanoparticle is commonly used to characterize the surface charges property of nanoparticles. It reflects the electrical potential of particles is influenced by the composition of the particles and the medium in which it is dispersed. When nanoparticle formulations are administered through intravenous route they are easily identified and detected by the phagocytes. The particle size and the hydrophobicity surface of the nanoparticle determine the adsorption of blood components (proteins) called as opsonins. This opsonin in turn decides the fate of the nanoparticles. Binding of these opsonins on to the surface is known as Opsonization. Non modified nanoparticles were rapidly opsonized and gets easily eliminated from the body. Hence, to increase the likelihood of the success in drug targeting by nanoparticles, it is necessary to minimize the opsonization and to prolong the circulation of nanoparticles in vivo.

The zeta potential of the nanoparticle formulation with Eudragit (RS 100 & RL 100) (formulation F9) particles which present in the formulation are de-aggregated and remain same and more stable in the suspension and zeta potential (mV) is 59.0 and Zeta Deviation (mV) is 5.29 and conductivity (mS/cm) is 0.0866. So this polymer is more suitable for nanoparticle preparation and the result shows smooth surface character and efficient repelled action and it decreases the opsonization.

7.8. STERILITY TEST[73]

The optimized Lamivudine loaded nanoparticle formulation (F9) was subjected to sterility test. The test was carried out as per specification Indian pharmacopeia. Both Soya bean casein digest medium (SCDM) and Fluid thioglycollate medium (FTM) were used. The method followed is Method A – Membrane filtration method. The positive control was prepared from standardized Bacillus subtilis suspension. The prepared nanoparticle samples dipped in SCDM and FTM incubated for 14 days. The result shows absence of turbidity. So it indicates the sterility of formulation passes the sterility test.

Fig: 21: Observation of Sterility test done in Soya bean Casein Digest medium (SCDM)

Fig: 22: Observations of sterility test done in Fluid Thioglycolate medium (FTM)

7.9 STABILITY STUDIES OF LAMIVUDINE NANOPARTICLES[25,74]

The stability studies of the optimized nanoparticle formulation F9 was carried out for 3 months. The test was performed in three condition 4^0C, room temperature and 45^0C/70%RH. At the time interval of one month the nanoparticle formulation were evaluated for entrapment efficiency. The stability of nanoparticle formulation was more stable in refrigerator (4°C) when compared to room temperature and at (45^0C/70%RH).

Table.27: Stability studies of nanoparticles

S.No	Storage Condition	Test parameters	1st month	2nd month	3rd month
1	4^0C	pH Colour Sterility	7.5 Clear& colour less Passes	7.5 Clear& colour less passes	7.5 Clear& colour less passes
2	Room temperature	pH Colour Sterility	7.4 Clear& colour less Passes	7.4 Clear& colour less passes	7.3 Clear& colour less passes
3	Acceleration Conditions at 45°C/70% RH	pH Colour Sterility	7.5 Clear& colour less Passes	7.3 Clear& colour less passes	7.3 Clear& colour less passes

Table: **28: In vitro release for optimized formulation F9 stability study at 4^0C**

Time (Hrs)	Cumulative % drug release		
	1st month (%)	2nd month (%)	3rd month (%)
1	8.0	12.0	7.0
2	15.08	19.12	9.07
3	19.18	21.19	13.09
4	23.19	23.21	19.13
5	26.23	24.23	24.19
6	32.26	29.24	25.24
7	38.32	31.29	32.24
8	40.38	39.31	39.32
9	40.40	41.39	48.39
10	47.44	44.41	50.48
11	52.47	48.44	54.50
12	53.52	50.48	56.54
13	58.53	52.50	60.56
14	60.58	54.52	63.60
15	67.60	57.54	65.63
16	69.67	58.57	69.65
17	72.69	64.58	72.69
18	73.72	68.64	75.72
19	79.73	69.68	77.75
20	82.79	75.69	81.77
21	84.82	79.75	82.81
22	85.84	84.79	83.82
23	89.85	86.84	87.83
24	92.89	92.86	91.87

Fig: 21: STABILITY STUDY RELEASE DATA FOR FORMULATION F9 AFTER THREE MONTHS AT 4°C

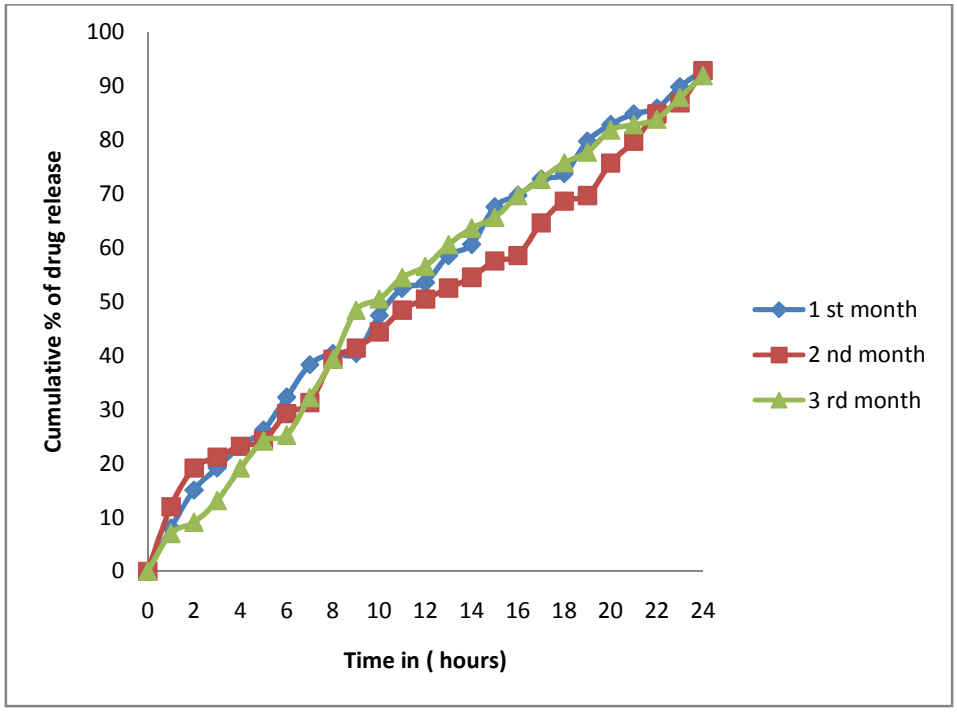


Table: 29: In vitro data for optimized formulation F9 stability study at room temperature

Time (Hrs)	Cumulative % drug release		
	1st month	2nd month	3rd month
1	8.0	5.0	0.5
2	10.08	7.05	07.05
3	15.10	13.07	10.07
4	16.15	17.13	15.10
5	18.16	24.17	16.15
6	23.18	29.24	18.16
7	26.23	31.29	25.18
8	28.26	34.31	28.25
9	33.28	39.34	33.28
10	35.33	45.39	34.33
11	39.35	47.45	39.34
12	40.39	49.47	41.39
13	44.40	51.49	45.41
14	46.44	53.51	47.45
15	51.46	56.53	55.47
16	55.51	61.56	57.55
17	60.55	63.61	61.57
18	66.60	68.63	66.61
19	67.66	69.68	69.66
20	69.67	71.69	72.69
21	71.69	72.71	74.72
22	72.71	77.72	76.74
23	78.72	80.77	79.76
24	83.78	82.80	81.97

Fig: 22: STABILITY STUDY RELEASE DATA FOR FORMULATION F9 AFTER THREE MONTHS AT ROOM TEMPERATURE

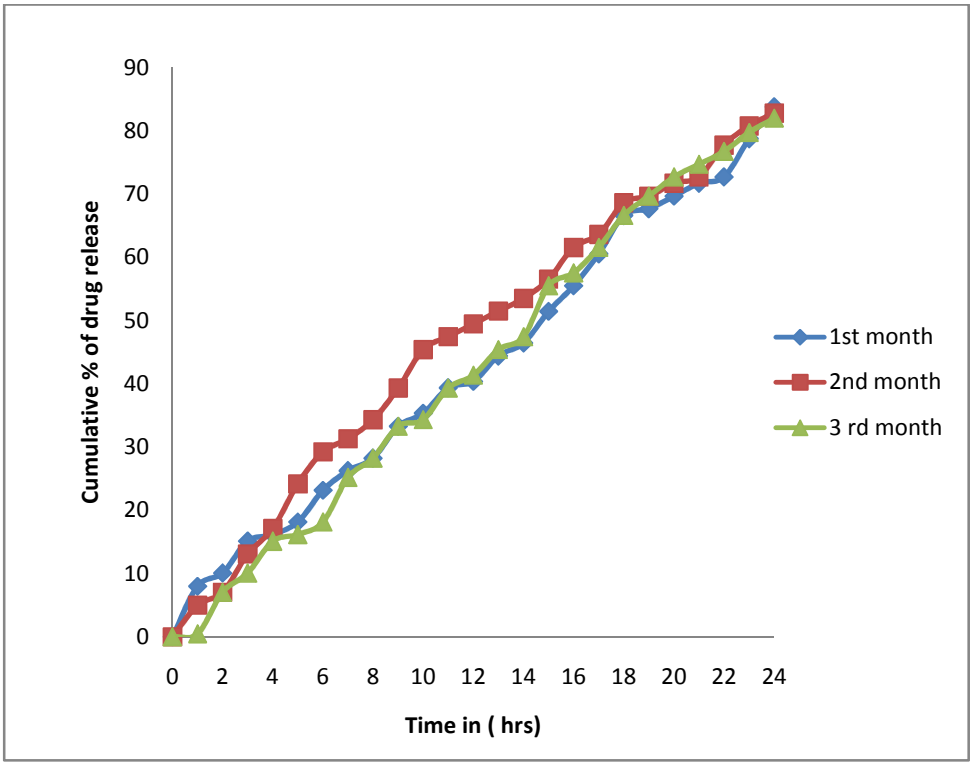
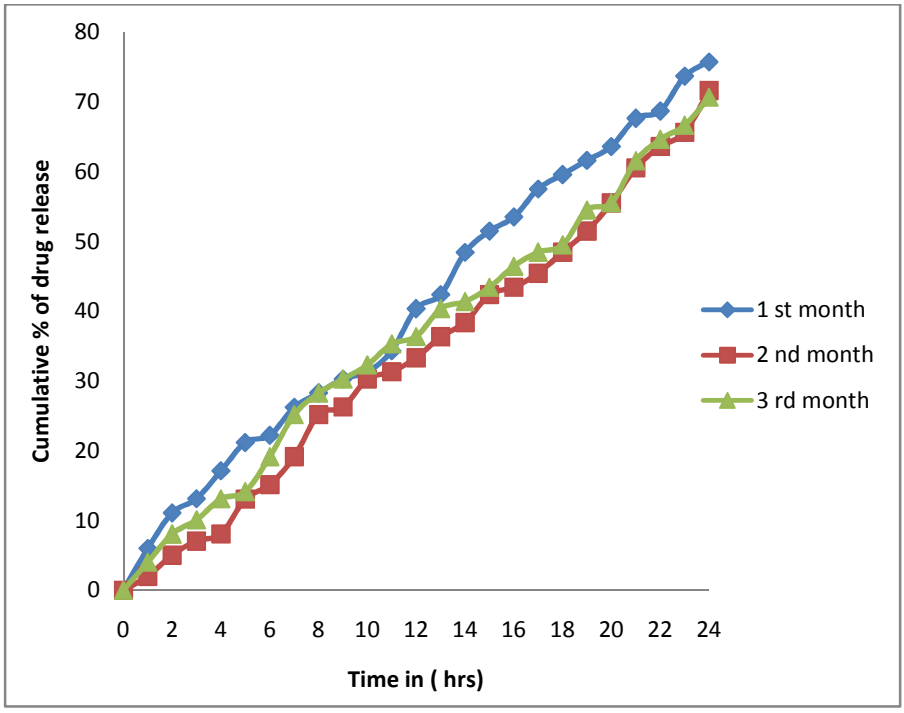


Table: 30: In vitro data for optimized formulation F9 stability study at 45°c/75%RH

Time (Hrs)	Cumulative % drug release		
	1st month	2nd month	3rd month
1	6.0	2.0	4.0
2	11.06	5.02	8.04
3	13.11	7.05	10.08
4	17.13	8.07	13.10
5	21.17	13.08	14.13
6	22.21	15.13	19.14
7	26.22	19.15	25.19
8	28.26	25.19	28.25
9	30.28	26.25	30.28
10	31.30	30.26	32.30
11	34.31	31.30	35.32
12	40.34	33.31	36.35
13	42.40	36.33	40.36
14	48.42	38.36	41.40
15	51.48	42.38	43.41
16	53.51	43.42	46.43
17	57.53	45.43	48.45
18	59.57	48.45	49.48
19	61.59	51.48	54.49
20	63.61	55.51	55.54
21	67.63	60.55	61.55
22	68.67	63.60	64.61
23	73.68	65.63	66.64
24	75.73	71.65	70.66

Fig: 23: STABILITY STUDY RELEASE DATA FOR FORMULATION F9 AFTER THREE MONTHS AT 45°C /75% RH



Kinetics of drug release for optimized formulation F9[37]

The optimized formulation F9was introduced in to graphical treatment for kinetics of drug release.

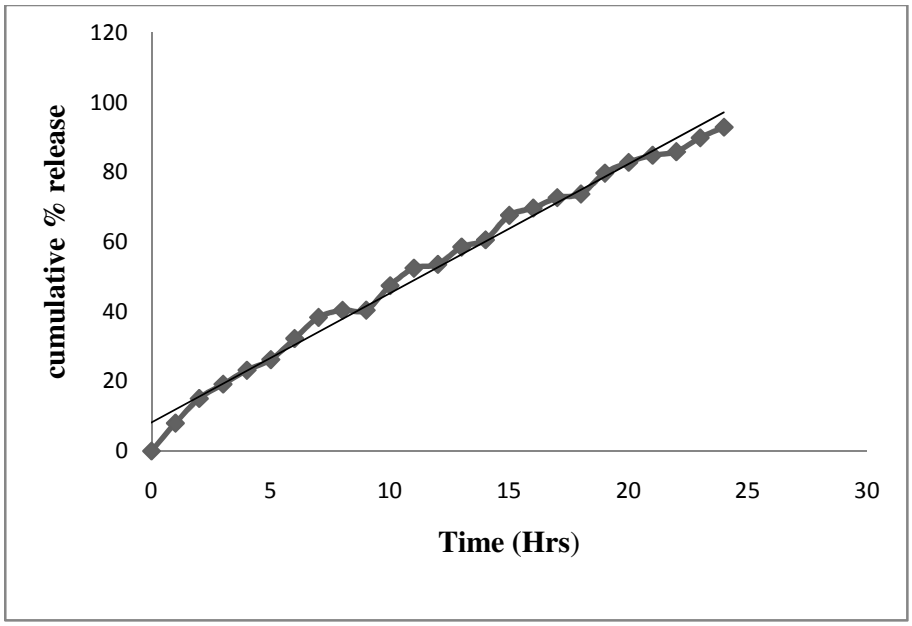


Fig: 24: Zero order Plot for formulation F_9

Slope = 3.704

Regression = 0.988

The optimized formulation F9 of nanoparticle is more suitable for parenteral administration it shows good in the in vitro release kinetic study. The zero order plots were obtained by plotting cumulative percentage drug release versus time. The regression value is 0.988.

HIGUCHI'S PLOT:

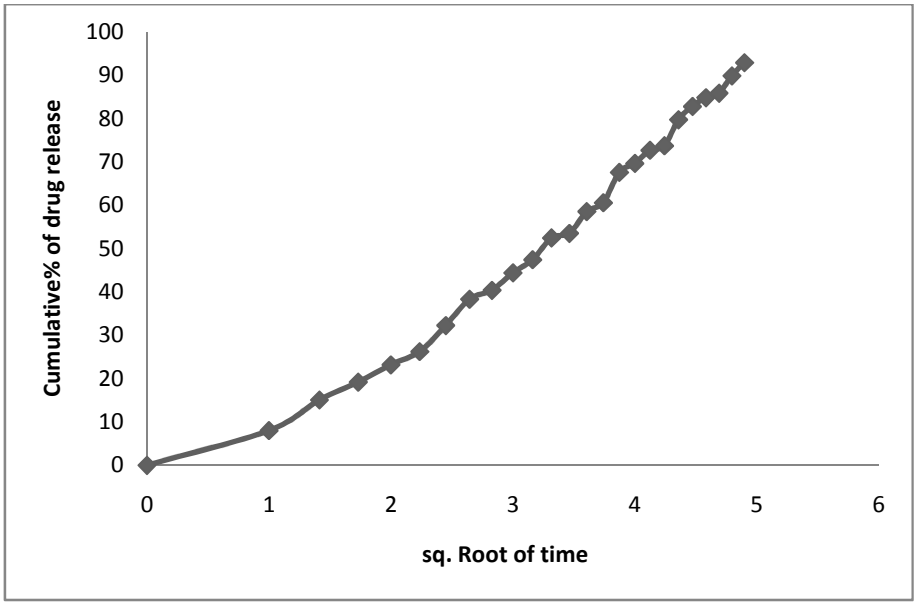


Fig: 25: HIGUCHI'S PLOT FOR FORMULATION F9

Slope = 20.93

Regression = 0.973

Higuchi plot was made by plotting cumulative % drug release against square root of time. The regression value was found to be 0.973. This indicates that diffusion is one of the mechanisms of drug release.

KORSEMEYER PLOT:

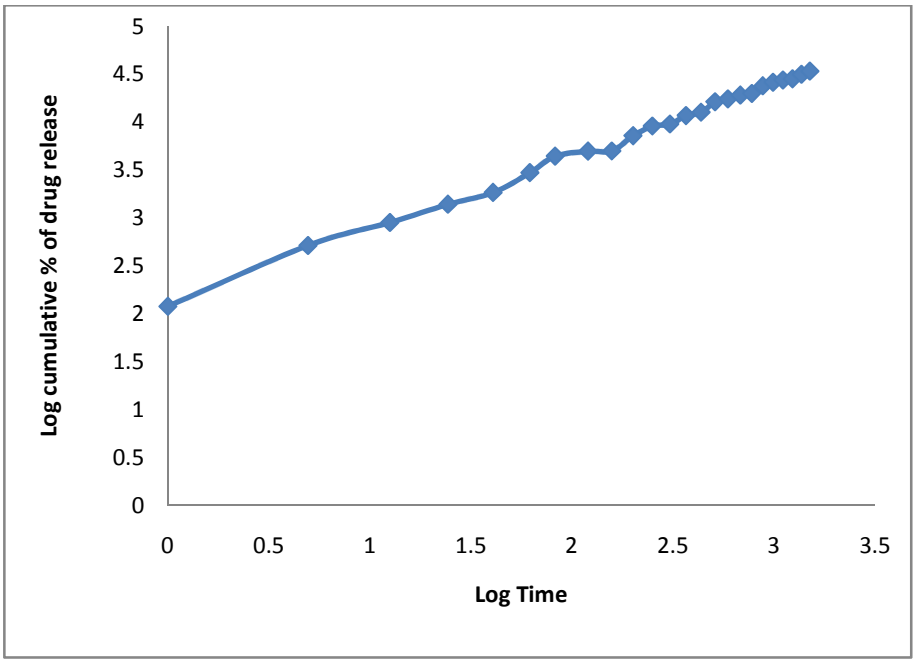


Fig: 26: KORSEMEYER'S PLOT FOR FORMULATION F9

n = 0.764

The graph was plotted between log cumulative % of drug release and log time. The value was found to be 0.45 < n < 0.89 anomalous (non – fickian) diffusion. This indicates that the diffusion and erosion could be the reason for the mechanism of drug release.

8. SUMMARY AND CONCLUSION

The present study Lamivudine nanoparticles aimed to develop a nanoparticulate drug delivery system of antiviral drug Lamivudine using biodegradable polymer Eudragit RS 100 & RL 100.

The polymer enhances the binding of Lamivudine nanoparticles in specific or targeted site with sustained release of drug increasing therapeutic efficacy. These nanoparticles may also reduce the dose & dose frequency with desired therapeutic response.

The pre-formulation studies were performed by using FTIR. The spectra of pure drug, pure polymer and nanoparticle formulation were examined. The study revealed the absence of significant interactions between drug and polymer.

All batch of nanoparticles (F1-F10) were prepared by emulsion solvent evaporation method, formulation was subjected to evaluation involving following tests they are;

- Entrapment efficiency.
- In vitro drug release studies.
- Microscopic determination.
- Particle size determination
- Sterility test.
- Stability test.

The entrapment efficiency of the optimized formulation was 94 ± 0.05% and invitro drug release was 92.89% after 24 hours. It also obeys the zero order,follows diffusion and erosion mechanism of release. Particle size determination by Scanning Electron Microscope shows the best formulation containing size of about 100 nm.The formulation passed the sterility test performed as per specifications of Indian pharmacopoeia. The stability test performed revealed that the formulation was good. The best formulation was examined for zeta potential determinations. The formulation (F9) showed maximum deviation of -27mV which demonstrated that the particles are separate and highly repelling. This repelling property found to be more useful in decreasing opsonization and favors target specificity. The prepared formulation was sterilized by Membrane filtration technique. It was an aseptic technique involving the use of laminar air flow workstation.

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