DEVELOPMENT AND CHARACTERIZATION OF IRBESARTAN NANOPARTICLES

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CHENNAI

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K.M.COLLEGE OF PHARMACY

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APRIL-2016

CERTIFICATE

This is to certify that the dissertation entitled "**DEVELOPMENT AND CHARACTERIZATION OF IRBESARTAN NANOPARTICLES"** submitted by **Mr.E.S.MOHAMED JALALUTHEEN** in partial fulfillment for the award of **Master of Pharmacy** in **Pharmaceutics** under The Tamilnadu Dr.M.G.R Medical University, Chennai, done at **K.M.COLLEGE OF PHARMACY**, Madurai-625107, is a bonafide work carried out by him under my guidance and supervision during the academic year **2015-2016.** The dissertation partially or fully has not been submitted for any other degree or diploma of this university or other universities.

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CONTENTS

1. INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly(ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes [1].

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties [2].

ADVANTAGES OF NANOPARTICLES [3]

- Suitable for different routes of administration.
- Carrying capacity of nanoparticles is high.
- Shelf stability of drug increase.
- Ability of sustain and control drug release pattern.
- System increases the bioavailability drugs.
- Used for targeted drug delivery of drugs.
- Development of new medicine which are safer.

1.1. TARGETED DRUG DELIVERY SYSTEM[1]

Targeted delivery can be achieved by either active or passive targeting. Active targeting of a therapeutic agent is achieved by conjugating the therapeutic agent or the carrier system to a tissue or cell-specific ligand. Passive targeting is achieved by incorporating the therapeutic agent into a macromolecule or nanoparticle that passively reaches the target organ. Target drug delivery system has been developed to optimize regenerative technique. The system is based on method that delivers a certain amount of a therapeutic agent for prolonged period of time to a target diseased area within the body. This helps maintain in the required plasma and tissue drug level in body. Therefore avoid any damage to the healthy tissue via drug.

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, nonimmunogenic and biodegradable after recognition and release drug moiety.

1.2. NANOPARTICLES DRUG DELIVERY SYSTEM[4]

Nanoparticles are solid, colloidal particles consisting of macromolecular substances that vary in size from 10 nm to 1000 nm. The drug of interest is dissolved, entrapped, adsorbed, attached or encapsulated into the nanoparticle matrix. Depending on the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained with different properties and release characteristics for the encapsulated therapeutic agent. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a polymer membrane, whereas nanospheres are matrix systems in which the drug is physically and uniformly dispersed.

- Matrix type nanosphere, drug molecules are evenly dispersed in the polymer matrix.
- Core shell nanocapsule, drug molecule is presented in a core covered with a polymer shell.
- Matrix type nanosphere where drug crystals are embedded in a polymer matrix.

POLYMERIC NANOPARTICLES[4]

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 polyacrylamide nanoparticles are produced by the polymerization of acrylamide and NN-Methylene bisacrylamide after secondary solubilisation in an organic solvent such as hexane. The active ingredients, drug or antigen, were incorporated into the solubilised aqueous phase because of the larger amount of organic solvents and surfactant used for the manufactures to develop nanoparticles, the process is now only of historical interest. Polymeric nanoparticles are composed of biodegradable or bio stable polymer and copolymer. The active agents can be;

- Entrapped or encapsulate within the particles
- physically adsorbed on surface
- Chemically linked to the surface of the nanoparticles.
- Nanoparticles have been explored for the delivery of anti-HIV molecules

And it used to target anti retrovirals;

(i) Macrophage/monocytes

(ii) CNS which act as viral reservoir sites during HIV

Polymer used in Preparation of polymeric Nanoparticles

Polymer used in manufacturing of polymeric nanoparticles is of two types:

1. Natural hydrophilic polymers (protein and polysaccharides)
2. Synthetic hydrophobic polymers (poly lactic acid and PLGA)

Natural Hydrophilic polymers;

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

Disadvantages with natural polymers are;

- Batch to batch variation
- Conditional biodegradability
- Antigenicity

Synthetic Hydrophobic polymers;

Polymers which are used for microspheres preparation are used for nanoparticles preparation. Most of them are hydrophobic in nature. The polymers are either pre –polymerized or synthesized during nanoparticles preparation. Eg; Poly (€-caprolactone) (PECL), Poly(lactic acid)(PLA), poly (lactic de-co glycolide)(PLGA), poly styrene etc.,

1.3. FORMULATION TECHNIQUE OF NANOPARTICLES

The selection of the appropriate method for the preparation of nanoparticles depends on the physiochemical characteristics of the polymer and the drug to be loaded[4, 5]

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

1. A matrix type system containing of an entanglement of oligomer or polymer units (nanoparticles/nanocapsules)

2. A reservoir type of system comprised of an oily core surrounded by an embryonic polymeric shell (nanocapsule)

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

Amphiphilic macromolecules 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
- Solvent evaporation

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. Organic solvent is completely soluble in the continuous phase-nanoprecipitation.

A. Extraction method Solvent;

The preparation of nanoparticles starts with formulation 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 addition of water to the system so as to diffuse the solvent to the external phase (emulsification diffusion method) is the two variance of the solvent extraction method.

The classical procedure nanospheres is the polymer, solubilized in a solvent and dispensed in a gelatin solution by sonication to yield emulsion (O/W) then the solvent is eliminated by evaporation. The homogenizer breaks the initial coarse emulsion in nanodroplets (nanofludization) 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

It is based on interfacial deposition of a polymer following displacement of 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, inducing immediate miscibility of both the phases. After nanoparticles preparation, the solvent is eliminated and the free flowing nanoparticles can be obtained under pressure. The method is particularly useful for drugs that are slightly soluble in water. If the drug is highly hydrophilic, It is diffused out into external aqueous phase, where as if the drug is highly hydrophobic, it may precipitate in the aqueous as nanocrystals, which further grow during storage. In the case of hydrophilic polymer, an aqueous solution of polymer is dispersed or emulsified in oil phase.

D. Salting out

It is one commonly, used method used for preparation of nanoparticle. This method, involves the mixing of saturated aqueous solution of polyvinyl alcohol (PVA) into an acetone solution of the polymer under magnetic stirring resulting in the formation of o/w emulsion. The precipitation of the polymer occurs when sufficient amount of water is added to external phase to allow complete diffusion of the acetone from internal phase into aqueous phase.

E. Solvent evaporation

The solvent evaporation method is a well-established and frequently used method for the manufacturing of particles with sizes above 1 nm and also sizes of less than 1000 nm. In this process the preformed polymer and the drug are dissolved in a volatile, water-immiscible organic solvent. This organic phase is then added to the aqueous phase under stirring, and the organic solvent is removed by heating and/or under reduced pressure. The polymer precipitates and forms micro- or nanospheres instantaneously containing the drug dispersed in the polymer matrix.

1.4. MECHANISM OF DRUG RELEASE

The polymeric drug carriers deliver the drug at the tissue site by any one of the three general physico-chemical mechanisms.

1. By the swelling of the polymer nanoparticles by hydration followed by release through diffusion.

2. By an enzymatic reaction resulting in rupture or cleavage or degradation of the polymer at site of delivery, there by releasing the drug from the entrapped inner core.

3. Dissociation of the drug from the polymer and its de-adsorption/release from the swelled nanoparticles.

1.5. Characteristics of Nanoparticles

Particle size and size distribution are the most important characteristics of nanoparticle systems. They determine the in vivo distribution, biological fate, toxicity and the targeting ability

of nanoparticle systems. In addition, they can also influence the drug loading, drug release and stability of nanoparticles.

Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system[6]. Generally nanoparticles have relatively higher intracellular uptake compared to microparticles and available to a wider range of biological targets due to their small size and relative mobility. Desai et al found that 100 nm nanoparticles had a 2.5 fold greater uptake than 1 μm microparticles, and 6 fold greater uptake than 10 μm microparticles in a Caco-2 cell line [7].

In a subsequent study, the nanoparticles penetrated throughout the sub mucosal layers in a rat in situ intestinal loop model, while micro particles were predominantly localized in the epithelial lining. It was also reported that nanoparticles can across the blood-brain barrier following the opening of tight junctions by hyper osmotic mannitol, which may provide sustained delivery of therapeutic agents for difficult-to-treat diseases like brain tumors.

Tween 80 coated nanoparticles have been shown to cross the blood-brain barrier. In some cell lines, only submicron nanoparticles can be taken up efficiently but not the larger size microparticles [8].

Drug release is affected by particle size. Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. Whereas, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out. Smaller particles also have greater risk of aggregation of particles during storage and transportation of nanoparticle dispersion. It is always a challenge to formulate nanoparticles with the smallest size possible but maximum stability.

Polymer degradation can also be affected by the particle size. For instance, the rate of PLGA polymer degradation was found to increase with increasing particle size in vitro [9]. It was thought that in smaller particles, degradation products of PLGA formed can diffuse out of the particles easily while in large particles, degradation products are more likely remained within the polymer matrix for a longer period to cause autocatalytic degradation of the polymer material. Therefore, it was hypothesized that larger particles will contribute to faster polymer degradation

as well as the drug release. However, Panyam et al prepared PLGA particles with different size ranges and found that the polymer degradation rates in vitro were not substantially different for different size particles.

Currently, the fastest and most routine method of determining particle size is by photon-correlation spectroscopy or dynamic light scattering. Photon-correlation spectroscopy requires the viscosity of the medium to be known and determines the diameter of the particle by Brownian motion and light scattering properties. The results obtained by photon-correlation spectroscopy are usually verified by scanning or transmission electron microscopy (SEM or TEM).

1.6. Surface properties of nanoparticles

When nanoparticles are administered intravenously, they are easily recognized by the body immune systems, and are then cleared by phagocytes from the circulation [10]. Apart from the size of nanoparticles, their surface hydrophobicity determines the amount of adsorbed blood components, mainly proteins (opsonins). This in turn influences the in vivo fate of nanoparticles. Binding of these opsonins onto the surface of nanoparticles called opsonization acts as a bridge between nanoparticles and phagocytes. The association of a drug to conventional carriers leads to modification of the drug biodistribution profile, as it is mainly delivered to the mononuclear phagocytes system (MPS) such as liver, spleen, lungs and bone marrow.

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. This can be achieved by (a) surface coating of nanoparticles with hydrophilic polymers/surfactants; (b) formulation of nanoparticles with biodegradable copolymers with hydrophilic segments such as polyethylene glycol (PEG), polyethylene oxide, polyoxamer, poloxamine and polysorbate 80 (Tween 80).

The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge

prevents aggregation of the particles. The zeta potential can also be used to determine whether a charged active material is encapsulated within the centre of the nanocapsule or adsorbed onto the surface.

1.7. Drug loading

Ideally, a successful nanoparticulate system should have a high drug-loading capacity thereby reduce the quantity of matrix materials for administration. Drug loading can be done by two methods:

• Incorporating at the time of nanoparticles production (incorporation method)

• Absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption /absorption technique).

Drug loading and entrapment efficiency very much depend on the solid-state drug solubility in matrix material or polymer (solid dissolution or dispersion), which is related to the polymer composition, the molecular weight, the drug polymer interaction and the presence of functional groups (ester or carboxyl) [11,12,13]. The PEG moiety has no or little effect on drug loading. The macromolecule or protein shows greatest loading efficiency when it is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption 19 For small molecules, studies show the use of ionic interaction between the drug and matrix materials can be a very effective way to increase the drug loading[14].

1.8. Applications of Nanoparticulate Delivery Systems

Tumor targeting using nanoparticulate delivery systems

Nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active targeting by ligands on the surface of nanoparticles. Nanoparticles will reduce the drug exposure of health tissues by limiting drug distribution to target organ.

Recently Bibby et al reported the biodistribution and pharmacokinetics (PK) of a cyclic RGD doxorubicin-nanoparticle formulation in tumorbearing mice. Their biodistribution studies

revealed decreasing drug concentrations over time in the heart, lung, kidney and plasma and accumulating drug concentrations in the liver, spleen and tumor. The majority injected dose appeared in the liver (56%) and only 1.6% in the tumour at 48 hrs post injection, confirming that nanoparticles have a great tendency to be captured by liver. This indicates the greatest challenge of using nanoparticles for tumour targeting is to avoid particle uptake by mononuclear phagocytic system (MPS) in liver and spleen.

Long circulating nanoparticles

To be successful as a drug delivery system, nanoparticles must be able to target tumors which are localized outside MPS-rich organs. In the past decade, a great deal of work has been devoted to developing so-called "stealth" particles or PEGylated nanoparticles, which are invisible to macrophages or phagocytes. A major breakthrough in the field came when the use of hydrophilic polymers (such as polyethylene glycol, poloxamines, poloxamers, and polysaccharides) to efficiently coat conventional nanoparticle surface produced an opposing effect to the uptake by the MPS. These coatings provide a dynamic "cloud" of hydrophilic and neutral chains at the particle surface which repel plasma proteins. As a result, those coated nanoparticles become invisible to MPS, therefore, remained in the circulation for a longer period of time. Hydrophilic polymers can be introduced at the surface in two ways, either by adsorption of surfactants or by use of block or branched copolymers for production of nanoparticles [15, 16].

Studies show nanoparticles containing a coat of PEG not only have a prolonged half-life in the blood compartment but also be able to selectively extravasate in pathological sites such as tumors or inflamed regions with a leaky vasculature. As a result, such long-circulating nanoparticles have increased the potential to directly target tumors located outside MPS-rich regions.

Targeting with small ligands appears more likely to succeed since they are easier to handle and manufacture. Furthermore, it could be advantageous when the active targeting ligands are used in combination with the long-circulating nanoparticles to maximize the likelihood of the success in active targeting of nanoparticles.

Reversion of multidrug resistance in tumour cells

Anticancer drugs, even if they are located in the tumour interstitium, can turn out to be of limited efficacy against numerous solid tumour types, because cancer cells are able to develop mechanisms of resistance[17]. These mechanisms allow tumours to evade chemotherapy. Multidrug resistance (MDR) is one of the most serious problems in chemotherapy. MDR occurs mainly due to the over expression of the plasma membrane pglycoprotein (Pgp), which is capable of extruding various positively charged xenobiotics, including some anticancer drugs, out of cells. In order to restore the tumoral cells' sensitivity to anticancer drugs by circumventing Pgp-mediated MDR, several strategies including the use of colloidal carriers have been applied. The rationale behind the association of drugs with colloidal carriers, such as nanoparticles, against drug resistance derives from the fact that Pgp probably recognizes the drug to be effluxed out of the tumoral cells only when this drug is present in the plasma membrane, and not when it is located in the cytoplasm or lysosomes after endocytosis.

Nanoparticles for oral delivery of peptides and proteins

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides and proteins. Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation. For instance, it has been found that insulin-loaded nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration.

The surface area of human mucosa extends to 200 times that of skin. The gastrointestinal tract provides a variety of physiological and morphological barriers against protein or peptide delivery, e.g., (a) proteolytic enzymes in the gut lumen like pepsin, trypsin and chymotrypsin; (b) proteolytic enzymes at the brush border membrane (endopeptidases); (c) bacterial gut flora; and (d) mucus layer and epithelial cell lining itself. The histological architecture of the mucosa is

designed to efficiently prevent uptake of particulate matter from the environment. One important strategy to overcome the gastrointestinal barrier is to deliver the drug in a colloidal carrier system, such as nanoparticles, which is capable of enhancing the interaction mechanisms of the drug delivery system and the epithelia cells in the GI tract [19].

Targeting of nanoparticles to epithelial cells in the GI tract using ligands

Targeting strategies to improve the interaction of nanoparticles with adsorptive enterocytes and M-cells of Peyer's patches in the GI tract can be classified into those utilizing specific binding to ligands or receptors and those based on nonspecific adsorptive mechanism. The surface of enterocytes and M cells display cell-specific carbohydrates, which may serve as binding sites to colloidal drug carriers containing appropriate ligands. Certain glycoproteins and lectins bind selectively to this type of surface structure by specific receptor-mediated mechanism. Different lectins, such as bean lectin and tomato lectin, have been studied to enhance oral peptide adsorption. Vitamin B-12 absorption from the gut under physiological conditions occurs via receptor-mediated endocytosis. The ability to increase oral bioavailability of various peptides (e.g., granulocyte colony stimulating factor, erythropoietin) and particles by covalent coupling to vitamin B-12 has been studied. For this intrinsic process, mucoprotein is required, which is prepared by the mucus membrane in the stomach and binds specifically to cobalamin. The mucoprotein completely reaches the ileum where resorption is mediated by specific receptors [20].Absorption enhancement using non-specific interactions.

In general, the gastrointestinal absorption of macromolecules and particulate materials involves either paracellular route or endocytotic pathway. The paracellular route of absorption of nanoparticles utilises less than 1% of mucosal surface area. Using polymers such as chitosan, starch or poly (acrylate) can increase the paracellular permeability of macromolecules. Endocytotic pathway for absorption of nanoparticles is either by receptor-mediated endocytosis, that is, active targeting, or adsorptive endocytosis which does not need any ligands. This process is initiated by an unspecific physical adsorption of material to the cell surface by electrostatic forces such as hydrogen bonding or hydrophobic interactions. Adsorptive endocytosis depends primarily on the size and surface properties of the material. If the surface charge of the nanoparticles is positive or uncharged, it will provide an affinity to adsorptive enterocytes though

hydrophobic, whereas if it is negatively charged and hydrophilic, it shows greater affinity to adsorptive enterocytes and M cells. This shows that a combination of size, surface charge and hydrophilicity play a major role in affinity. This is demonstrated with poly (styrene) nanoparticles and when it is carboxylated [21].

Nanoparticles for gene delivery

Polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells where they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such vaccines produce both humoral and cell-mediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system. The key ingredient of polynucleotide vaccines, DNA, can be produced cheaply and has much better storage and handling properties than the ingredients of the majority of protein-based vaccines. Hence, polynucleotide vaccines are set to supersede many conventional vaccines particularly for immunotherapy. However, there are several issues related to the delivery of poly nucleotides which limit their application. These issues include efficient delivery of the polynucleotide to the target cell population and its localization to the nucleus of these cells, and ensuring that the integrity of the polynucleotide is maintained during delivery to the target site.

Nanoparticles loaded with plasmid DNA could also serve as an efficient sustained release gene delivery system due to their rapid escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment. Hedley *et al* reported that following their intracellular uptake and endolysosomal escape, nanoparticles could release DNA at a sustained rate resulting in sustained gene expression. This gene delivery strategy could be applied to facilitate bone healing by using PLGA nanoparticles containing therapeutic genes such as bone morphogenic protein.

Nanoparticles for drug delivery into the brain

The blood-brain barrier (BBB) is the most important factor limiting the development of new drugs for the central nervous system. The BBB is characterized by relatively impermeable endothelial cells with tight junctions, enzymatic activity and active efflux transport systems. It

effectively prevents the passage of water-soluble molecules from the blood circulation into the CNS, and can also reduce the brain concentration of lipid-soluble molecules by the function of enzymes or efflux pumps. Consequently, the BBB only permits selective transport of molecules that are essential for brain function.

Strategies for nanoparticle targeting to the brain rely on the presence of and nanoparticle interaction with specific receptor-mediated transport systems in the BBB. For example polysorbate 80/LDL, transferrin receptor binding antibody (such as OX26), lactoferrin, cellpenetrating peptides and melanotransferrin have been shown capable of delivery of a self non transportable drug into the brain via the chimeric construct that can undergo receptor-mediated transcytosis. It has been reported poly (butylcyanoacrylate) nanoparticles was able to deliver hexapeptide dalargin, doxorubicin and other agents into the brain which is significant because of the great difficulty for drugs to cross the BBB. Despite some reported success with polysorbate coated NPs, this system does have many shortcomings including desorption of polysorbate coating, rapid NP degradation and toxicity caused by presence of high concentration of polysorbate. OX26 MAbs (anti-transferrin receptor MAbs), the most studied BBB targeting antibody, have been used to enhance the BBB penetration of lipsosomes. However, recently, Ji et al. demonstrated that brain uptake of lactoferrin, an iron-binding glycoprotein belonging to the transferrin (Tf) family, is twice that of OX26 and transferrrin in vivo. It is possible soon we will see these BBB specific molecules used for targeting nanoparticles to the brain [22].

1.9. APPLICATION OF NANOPARTICLES IN DIFFERENT FIELD

- Nanomedicines: Nano drugs medical devices, Tissue engineering etc.
- Chemical and cosmetics: Nano scale chemicals and compounds, paints, coating etc.
- Material: Nanoparticles, carbonnanotubes, biopolymer, paints, coating.
  Food sciences and: Processing, nutraceticalsfood, nanocapsules, water air.
- Environment energy: purification filters,fuelcells,photovoltaic.
- Military and Energy: Biosensor,weapons,sensory enhancement.
- Electronic: Semi conductorchips,memorystorage,photonica,optoelectronics.
- Scientific Tools:Atomic force, microscopic scanning and tunnelling microscope

1.10. DISEASE OVER REVIEW –HYPERTENSION[23]

The drugs used to lower BP in Hypertension is a very common disorder,particularly as middle age. It is not a disease in itself, but is an important risk factor for cardiovascular mortality and morbidity. For practical purposes hypertension could be that level of BP at or above which long-term antihypertensive treatment will reduce cardiovascular mortality. The JNC (2003) and WHO-ISH guidelines (2003) have defined it to be 140 mm Hg systolic and 90 mm Hg diastolic,though risk appear to increase even above 120/80 mm Hg. Epidemiological studies have confirmed that higher pressure (systolic or diastolic or both) greater risk of cardiovascular disease.

1.11. TREATMENT OF HYPERTENSION

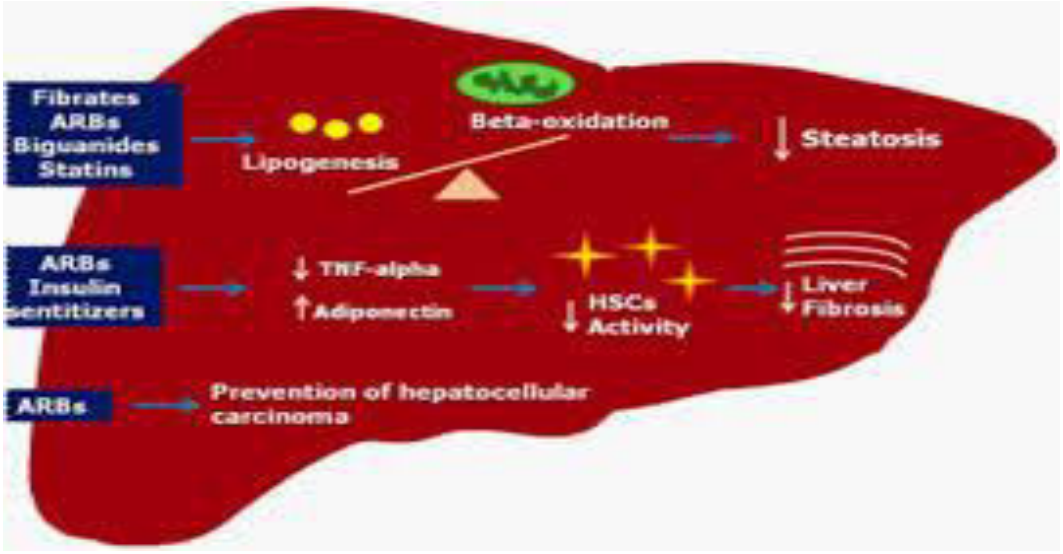
The aim of antihypertensive therapy is to prevent morbidity and mortality associated with persistently raised BP by lowering it to an acceptable level, with minimum inconvenience to the patient. Both systolic and diastolic BP predict the target organ damage and complications such as:

(a) Cerebro vasculardisease, transient schaemic attacks, storke,

(b) Hypertension heart disease –left ventricular hypertrophy, CHF.

(c) Glomerulopathy, Renal failure.

(d) Coronary artery disease (CAD), angina, myo-cardialinfaraction, sudden cardiac death.



1.12. Future opportunities and challenges

Nanoparticles have already been applied as drug delivery systems with great success. Nanoparticles provide massive advantages regarding drug targeting, delivery and with their potential for combine diagnosis and therapy and one of the major tools in Nanomedicine. These are many technical, challenges in developing the following techniques:- Virus-like systems for intracellular systems, Architecting of biomimetic polymers, control of sensitive drugs, functions (of active drug targeting, bioresponsive triggered systems, systems interacting with me body smart elivery), nanochips for nanoparticle release, carriers for advanced polymers for the delivery of therapeutic peptide / proteins. Drug delivery techniques were established to deliver or control the amount & rate. Most major and established internal research programmes on drug delivery that are formulations and dispersion containing components down to nano sizes.

2. REVIEW OF LITERATURE

Muhammed Rafeeq[24] et al., (2010) formulated Isoniazid in chitosan Nanoparticles inorder to enhance bioavailability and to reduce dose frequency. Chitosan was dissolved in acetic acid aqueous solution at various concentrations Drug was dispersed in above Chitosan solution kept over magnetic stirrer at room temperature for a period of 30 minute. The Tripolyphosphate aqueous solution with various concentrations added drop wise to the above solution and followed by sonication for 5min. The resulting Chitosan nanoparticles suspension was centrifuged at 16,000 rpm for 30 min. After freeze drying the Nanoparticles were collected. Zeta potential shows good positive potentials. It shows good encapsulation efficiency. And good release profile follows first order release kinetics. The optimized formulation was recommended for future studies like Nano dry powder preparation.

Rahul Paruchuri[25] et al., (2010) formulated polylactide -o - glcolic acid (PLGA) nanoparticles of Irinotecan for cancer therapy. The analytical method development is carried out using acetonitrile and phosphate buffer saline. Different organic solvents were tried and various surfactants were used to optimize the nanoparticulate formulation. The size range and zeta potential was measured using Malvern zeta seizer. The lyophilization was carriedout using two different cryoprotectants. The maximum percent drug entrapment was found out to be 37.2%. The in *vitro* drug release of IRN NP was also found out using dialysis method in phosphate buffer saline pH 7.4. The *invitro* drug release showed sustained release of drug over 24 hours. Hence the IRN loaded PLGA nanoparticles have potential as a drug delivery system. Furthermore, they may have utility for site-specific drug delivery since the small size of the particles may allow their delivery to extravascular target sites through the leaky endothelia of inflamed and cancerous area.

Archana Nerella[26] et al., (2014). designed and evaluated Letrozole Solid lipid nanoparticles (SLN) of LTZ. SLNs were prepared by hot homogenization followed by ultrasonication. Trimyristin was used as solid lipid core, Soyphosphatidyl choline, Tween 80 as surfactant mixture. Process and

formulation variables were studied and optimized. LTZ-SLN were characterized for mean particle size, polydispersity index (PDI) and zeta potential for all the formulations. The mean particles size, PDI, zeta potential and entrapment efficiency of optimized LTZ-SLN optimized formulation was found to be 28.54 nm, 0.162, 11.80 mV, 85.64 %, respectively. *In vitro* release profiles are performed in 0.1N HCl using modified franz diffusion cell showed controlled drug release behavior over a period of 24h. LTZ-SLN formulations are subjected to stability study over a period of 1 month in terms of particle size, zeta potential, PDI, entrapment efficiency and are found to be stable. Differential scanning calorimetry (DSC) and transmission electron microscopy (TEM) analysis was performed to characterize the state of drug, lipid modification, shape and surface morphology of prepared LTZ-SLN formulations.

Sovan Lal Pal[27] et al., **(2011)** developed novel drug delivery systems using nanoparticles. Nanoparticles can offer significant advantages over the conventional drug delivery in terms of high stability, high specificity, high drug carrying capacity, ability for controlled release, possibility to use in different route of administration and the capability to deliver both hydrophilic and hydrophobic drug molecules.

Dinda[28] et al., (2013) investigated Solid Lipid Nanoparticles (SLNs) are important because of their size and stability. SLNs have been reported as an alternative drug delivery device to traditional polymeric nanoparticles. SLNs are in submicron range (50-1000nm) and are composed of physiologically tolerated lipid components. At room temperature the particles are in solid state. They are made up of bio-compatible and bio-degradable materials capable of incorporating lipophilic and hydrophilic drugs. Paclitaxel is an effective drug against Aggressive Cancer's because it adversely affect the process of cell division by preventing restructuring. The incorporating paclitaxel in SLNs using Glyceryl Mono-stearate (GMS) as a lipid matrix, poly-oxy ethylene as a surfactant, soya-lecithin as a co-emulsifier. Paclitaxel loaded SLNs are prepared by Solvent emulsification and evaporation method using ultra sonication and optimization of critical process variables were carried out to develop stable SLNs. The average particle size of SLNs was found to be 63nm ±5.77 with Poly dispersity index (PDI) 0.272 ± 0.02 and entrapment efficiency was found 94.58%. The stability studies and zeta potential were performed at refrigerated temperature (2-8C) indicating no significant

increase in particle size after one month storage. *In-vitro* release studies showed initial burst release followed by controlled release for 48hrs (about 73%). The release profile was fitted into Higuchi's model .The drug diffuses from SLNs at a comparatively slower rate as the distance for diffusion increases.

Rahul Nair[29] et al., (2012) prepared aqueous suspension of Solid lipid Nanoparticles containing Chitosan (CT) which is a biopolymer that exhibits a number of interesting properties which include controlled drug delivery. Carbamezapine (CBZ) is a lipophilic drug which shows it antiepileptic activity by inactivating sodium channels. The solid lipid Nanoparticles (SLN) of Chitosan-CBZ were prepared by using solvent injection method using ethanol as organic solvent. The prepared SLN formulations exhibited high encapsulation efficiency, high physical stability. The drug incorporated SLNs have demonstrated that the controlled release patterns of the drug for prolonged period. The prepared SLNs were characterized for surface morphology by SEM analysis, entrapment efficiency, zeta potential, FTIR, DSC and *In-vitro* diffusion studies. The hydrodynamic mean diameter and zeta potential were 168.7±1.8 nm and −28.9 ±2.0 mV for SLN-chitosan-CBZ respectively. Therefore chitosan-SLN can be good candidates to encapsulate CBZ and to increase its therapeutic efficacy in the treatment of Epilepsy.

Amalendu P Ranjan[30] et al., (2012) formulated curcumin loaded poly (lactic acid-co-glycolic acid) nanoparticles (PLGA-CURC). This improved the bioavailability of curcumin, a potent natural anticancer drug, making it suitable for cancer therapy. The nanoparticles formed after scale-up process were characterized for particle size, drug loading and encapsulation efficiency, surface morphology, in vitro release kinetics and pharmacokinetics. Stability analysis and gamma sterilization were also carried out. It revealed that process scale-up is being mastered for elaboration to 5 g level. The mean nanoparticle size of the scaled up batch was found to be 158.5 ± 9.8 nm and the drug loading was determined to be 10.32 ± 1.4%. The *in vitro* release study illustrated a slow sustained release corresponding to 75% drug over a period of 10 days. The pharmacokinetic profile of PLGA-CURC in rats following i.v. administration showed two compartmental model with the area under the curve (AUC0-∞) being 6.139 mg/L h. Gamma sterilization showed no significant

change in the particle size or drug loading of the nanoparticles. Stability analysis revealed long term physiochemical stability of the PLGA-CURC formulation.

Zahoor Ahmad[31] et al., (2006) developed Alginate (a natural polymer) based nanoparticulate delivery system. Determination of frontline ATDs (Rifampicin, Isoniazid, Pyrazinamide and Ethambutol). Alginate nanoparticles were prepared by the controlled cation induced gelification method and administered orally to mice. The drug levels were analysed by high performance liquid chromatography (HPLC) in plasma/tissues. The therapeutic efficacy was evaluated in M. tuberculosis H37Rv infected mice. High drug encapsulation efficiency was achieved in alginate nanoparticles, ranging from 70%-90%. A single oral dose resulted in therapeutic drug concentrations in the plasma for 7-11 days and in the organs (lungs, liver and spleen) for 15 days. In comparison to free drugs (which were cleared from plasma/organs within 12-24 h), there was a significant enhancement in the relative bioavailability of encapsulated drugs. In TB-infected mice three oral doses of the formulation spaced 15 days apart resulted in complete bacterial clearance from the organs, compared to 45 conventional doses of orally administered free drugs.

Anbarasan[32] et al., (2011) optimized formulation and *in vitro* evaluated Chloroquine Phosphate loaded Chitosan Nanoparticles. Chloroquine loaded Chitosan–tripolyphosphate Nanoparticles were prepared by ionic gelation method with variable drug to polymer ratios (1:3, 1:4, 1:5, 1:6 and1:7). The drug follows linearity in the concentration range 5-30 µg/ml with regression coefficient value of 0.994. The drug content of Nanoparticles increases on increasing the polymer concentration up to a particular level. Entrapment efficiency of 92.87% was achieved with drug to polymer ratio 1:6. *In-vitro* release of Chloroquine Phosphate from Chitosan Nanoparticles was 85.13% within 24 h. TEM image indicates that the nanoparticles have a discrete spherical structure and particle size was in the range nanometer. FTIR studies show the evidence of cross linking between positively charged amino group of Chitosan and negatively charged Phosphate group of TPP (TriPolyPhosphate) without any significant interaction between Chloroquine Phosphate and Chitosan Nanoparticles after encapsulation. Good stability is observed at refrigeration condition compared to other temperature conditions during eight weeks of storage.

Partha Saha[33] et al., (2010) developed Ampicillin trihydrate-loaded chitosan nanoparticles were prepared 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 nanosize 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 the nanoparticles. *In vitro* release data showed an initial burst followed by slow sustained drug release. The 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 crosslinking agent concentrations and sonication time are rate-limiting factors for the development of the optimized formulation. The chitosan nanoparticles developed would be capable of sustained delivery of Ampicillin trihydrate.

Tamizhrasil[34] 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 average particle size was found to be 121.8- 403.4 nm. 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 behaviour 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.

Shagufta Khan[35] et al., (2012) formulated Dithranol in the form of solid lipid nanoparticle. Solid lipid nanoparticles of dithranol were obtained by adaption of lipid dispersion method. Solubility study, partition coefficient determination, UV analysis, HPLC study, FTIR study were also performed. After the preformulation studies loaded solid lipid nanoparticles was also prepared.

Priyanka patil[36] et al., (2014) optimized and evaluated Doxorubicin loaded spray dried chitosan nanocarriers as a sustained release by using ionotropic gelation technique. Spray-drying becomes a good technique to improve the stability of colloidal nanoparticles. The influences of the 4 decision variables (i.e. chitosan concentration, cross-linking concentration, stirring speed, stirring time on the mean particle size, Entrapment efficiency, *in-vitro* release a four factorial / two level experimental design was carried out by the design expert software. The prepared nanoparticles were evaluated for particle size, scanning electron microscopy (SEM), percentage yield, drug entrapment, zeta potential, Differential scanning calorimetry and in-vitro release study. Among all batches have high drug loading 66% and particle size 126-1392 nm. Based on *in-vitro* release study formulations show biphasic pattern characterized by initial burst release followed by a slower and sustained release.

Vivek Kumar Gupta[37] et al., (2010) investigated the nanoparticle formulations enhanced dissolution properties and the potential for intracellular drug delivery. Specifically, pure drug nanoparticles, polymeric nano- particles and polyelectrolyte complexes offer some encouraging results for delivering drugs to various organs and through various routes. Traditional techniques such as spray drying and grinding, and more recent advances in supercritical fluid extraction, precipitation, and double solvent evaporation have been employed to produce nanoparticle formulations for delivery of hydrophilic & hydrophobic drugs here, the benefits of nanoparticle formulations and current progress are compared in light of the practical encumbrances of producing formulations, and possible toxicological effects of these materials.

Srinivas[38] et al., (2012) prepared controlled release formulation of Moxifloxacin Hydrochloride ocular nanoparticles. The nanoparticles were prepared by solvent displacement method using Eudragit RL 100 as a polymer. Different formulations were prepared by varying the ratios of drug and polymer and varying the ratios of organic and aqueous phase. The formulations were evaluated in terms of particle size, FTIR, drug entrapment efficiency and in vitro drug release profile was examined. The anti bacterial activity against gram positive and gram negative bacteria were determined. *In vivo* studies were carried out by Draize test. The mean particle size for drug loaded formulations was found to be below 200 nm. The zeta potential remained in the range of positive values for all batches +10 mV to +40mV. The formulation possesses good antibiotic activity against Escherichia coli, Bacillus subtilus and Staphylococcus aureus microorganism and no eye irritation on *in-vivo* testing.

Adlin Jino Nesalina[39] et al., (2012) formulated zidovudine loaded nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions (TPP). Nanoparticles of different core: coat ratio were formulated and evaluated for process yield, loading efficiency, particle size, zeta potential, in vitro drug release, kinetic studies and stability studies. The chitosan nanoparticles have a particle diameter ranging approximately 342–468 nm and a zeta potential 20.4 to 37.08 mV. There was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulations. The *in vitro* release behaviour from all the drug loaded batches were found to follow first order and provided sustained release over a period of 24 h. No appreciable difference was observed in the drug content of product during 60 in which nanoparticles were stored at 4˚C and room temperature. According to the data obtained, this chitosan- based delivery system opens new and interesting perspectives as drug carriers.

Mohamed Ali Attia Shafie[40] et al., (2013) formulated and investigated mucoadhesive chitosan-sodium alginate nanoparticles as new vehicle for the prolonged topical ophthalmic delivery of Betamethasone sodium phosphate. Ionotropic gelation method was used to produce Betamethasone loaded chitosan alginate nanoreservoir system. The effect of changing different

formulation parameters (pH of chitosan solution, sodium alginate concentration, calcium chloride concentration, chitosan concentration, drug concentration and the addition of tween 80) on the physicochemical properties and in vitro release of the drug loaded nanoparticles was studied. The mean particle size ranged from 16.8 to 692 nm and the zeta potential generally ranged from +18.49 to +29.83 mV depending on the formulation conditions. The highest encapsulating efficiency obtained was 64%. *In vitro* release studies showed an initial burst release of the drug followed by slow sustained release over 24, 48 or 72 hours depending on the formulation parameters. The *in vivo* studies carried out for two selected formulations showed the release of 84%, 59.5% of the drug over 12 hours showed good stability at both 25°C and 40°C as the drug content was within the accepted range, the pH was (5–7) and the mean particle size for both formulations over the three months was still interesting for ophthalmic application. chitosan alginate nanoparticles would be a promising system for the sustained release delivery of Betamethasone sodium phosphate to the posterior segment of the eye.

Sagar[41] et al., (2013) formulated and evaluated intranasal mucoadhesive nanoparticles of Rizatriptan benzoate (RZB). loaded Chitosan (CS) nanoparticles were prepared by ionic gelation of CS with tripolyphosphate anions (TPP). The ionic gelation method is easy, reproducible and led to efficient entrapment. Some process variables like effect of CS concentration, TPP concentration were also evaluated with respect to % entrapment. The maximum entrapment efficiency and drug content was 69.1% and 60.63%, shown by optimized formulation. Particle size of optimized formulation was 0.248 µ shown by Zetasizer. Spray drying of optimized formulation was carried out and process yield was determined, which was found to be 38.78%. Spray dried nanoparticles was spherical in shape and varied surface roughness was found in Scanning electron microscopy (SEM) images. Spray dried nanoparticles were evaluated by Differential scanning calorimetry (DSC), X-ray diffraction (XRD) pattern to study crystalline/amorphous nature of nanoparticles, and mucoadhesive test. The percentage mucoadhesion on nasal mucosa of goat was found to be 29.4%. The release behavior of CS nanoparticles were evaluated in phosphate buffer pH 6.5, revealed that RZB loaded CS nanoparticles is most suitable for intranasal drug delivery.

Panakanti Pavan Kumar[42] et al., (2012) formulated Atorvastatin (ATRS) loaded solid lipid nanoparticles by hot homogenization fallowed by ultrasonication technique, and optimization of formulation and process parameters to formulate preferred SLN dispersions. The effects of composition of lipid materials, surfactant mixture and sonication time on particle size, PDI, zeta potential, drug entrapment efficiency, and *in vitro* drug release behavior were investigated The mean particles size, PDI, zeta potential and entrapment efficiency of optimized formulation (A5) was found to be 50.0± 6.12 nm, 0.08±0.011,10.40± 4.68mV, 88.7± 6.08 % respectively. Shape and surface morphology was determined by Transmission Electron Microscopy (TEM) which revealed fairly spherical shape of nanoparticles. The *in-vitro* drug release study demonstrated that ATRS-SLN It possessed controlled drug release over a period of 24 hrs than dispersion of pure drug. Stability studies performed on the selected formulations revealed that there was no physical instability of the developed formulation for a period of 3 months at room and refrigerated temperatures.

Amar singh[43] et al., (2011) prepared Losartan potassium loaded chitosan nanoparticles by ionic gelation of chitosan with tripolyphosphate ions. Nanoparticles of different core coat ratio were formulated and evaluated for drug content, loading efficiency, particles size, zeta potential, *invitro* drug release and stability studies. Scanning electron microscopy indicated that the nano-particles were found to be in nanometer range and showed ideal surface morphology. Differential scanning calorimetry analysis indicated that there were no chemical interactions between drug and polymer and stability of drug. *Invitro* release behavior from all the drug loaded batches were found to follow zero order and provided sustained release over a period of 24 hrs. The developed formulation overcomes and could possibility be advantageous in terms of sustained release dosage forms of Losartan potassium.

Yukio[44] et al., (2012) designed novel nanoparticles, which possess nitroxide radicals in the core for novel bioimaging and nanotherapy. Nitroxide radical containing nanoparticles (RNP) shows high safety, long blood circulation, magnetic resonance imaging and ESR imaging sensitive character and efficient therapeutic effects to several diseases such as cerebral and renal ischemia

reperfusions, ulcerative colitis and Alzheimer's disease models. RNPs are, thus, promising as new nanotherapeutic materials.

Magdalena Stevanovi[45] et al., (2009) preparaed Poly (lactide-co-glycolide) based micro and nanoparticles for the controlled drug delivery**.** Polymers like polylactides (PLA), polyglycolides (PGA), (lactide-co-glycolides) (PLGA), are approved by the World Health Organization (WHO) and Food and Drug Administration (FDA) as materials that can be used in medicine and pharmacy. Owing to their biodegradable nature, polymer materials, such as copolymer poly (DL-lactide-co-glycolide), are widely used in various medical applications; controlled release of delivering drugs, carriers in the tissue engineering, fixation of bone fractures, strings, etc.

Ping Lil[46] et al., (2008) formulated chitosan–alginate nanoparticles by ionotropic pre-gelation of an alginate core followed by chitosan polyelectrolyte complexation. Morphology and structure characterization of nanoparticles by transmission electron microscope (TEM) and Fourier transform infrared spectra (FTIR), respectively. Nifedipine released from chitosan–alginate nanoparticles was 26.52% at pH1.5, 69.69% at pH6.8 and 56.50% at pH 7.4 within 24hrs. The release of nifedipine from nanoparticles was pH-responsive. Quick release occurred in simulated intestinal fluid (SIF, pH6.8) and phosphate buffer solution (pH7.4), while the release was slow in simulated gastric fluid (SGF, pH1.5). The release profile was characterized by an initial burst effect in three media, followed by a continuous and controlled release phase, the drug release mechanism from polymer was due to Fickian.

Mariangela de Burgos[47] et al., (2011) investigated physicochemical characterization and *invivo* ACE inhibition evaluation of seven Captopril from cyclodextrin nanoparticles CAP/CD complexes. The physicochemical analysis demonstrated complete Amorphization and complexation between CAP and CDs, indicating the substitution of water molecules inside the CD cavity with CAP. During the infusion of angiotensin I, the administration of all CAP/CD complexes induced a reduction in mean arterial pressure similar to that observed upon CAP administration. The

nanoparticles obtained by the kneading method showed a potent and long-lasting inhibitory activity (~22 hrs) on the angiotensin I pressor effect. The inclusion complex of CAP and CD can function as a novel antihypertensive formulation that may improve therapeutic use of CAP by reducing its oral dose administration to once per day, thus providing better quality of life for almost 25% of the world's population who suffer from hypertension.

Catarina pinto reis[48] et al., (2006) formulated the drug-loaded polymeric nanoparticles, because they show promise as drug delivery systems as a result of their controlled and sustained release properties, subcellular size, and biocompatibility with tissue and cells. Several methods to prepare nanoparticles have been developed during the last two decades, classified according to whether the particle formation involves a polymerization reaction or arises from a macromolecule or preformed polymer. the most important preparation methods are described, specially those that make use of natural polymers.

Lulina Florentina[49] et al., (2012) studied the drug carriers were tested in the controlled drug release process and the influence of the silica pore morphology and geometry on drug release profiles the *invitro* release studies and to evaluate the kinetic release mechanism, the Korsmeyer and Peppas equation was used. The obtained drug delivery system based on MgO–SBA-15 matrix exhibits exciting structural features and is therefore promising for its use as antihypertensive drug delivery system, having potential therapeutic benefits resulting in safe and effective management of Captopril and adsorption and *invitro* release.

Mohammed M. Rahman[50] *et al.,* (2013) prepared Nebivolol drug based on Silver oxide nanoparticles by a wet chemical method**.** The morphological, structural, elemental, and optical properties of nanoparticles are investigated by UV/vis. and FT-IR spectroscopy, powder X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and field-emission scanning electron microscopy (FESEM) etc. fabricated on a glassy carbon electrode (GCE) to give a fast response

towards Nebivolol drug. The Nebivolol drug sensor also displays good sensitivity and long-term stability, and enhanced electrochemical I-V response.

Vikram S Shenoy[51] et al., (2013) investigated the formulation and *invitro* anticancer activities of solid lipid nanoparticles (SLNs) of 5-Fluorouracil (5-FU) using glyceryl monostearate (GMS) and cetyl palmitate (CP) by hot homogenization method. The lipids were selected based on the partition coefficient of 5-FU in lipids. The optimized nanoparticles were characterized for their zeta potential, morphology, release kinetics, and anticancer activity. Higher entrapments were achieved using a combination of emulsifiers. The zeta potential of the optimized CP and GMS SLN formulation were −8.26 and −9.35 mV, respectively. Both the optimized formulations were spherical. Subtoxic concentration of 5-FU-loaded CP SLNs (0.12 μg/mL) possessed comparable antimigrational activity, colony inhibition activity, and cytopathic as that of 5-FU solution effects. The encapsulating 5-FU in CP would be a promising delivery system for delivering 5-FU.

Suganeswari[52] et al., (2011) prepared and evaluated nanoparticles containing hypolipidaemic drug (Atorvastatin calcium: D1N) antihypertensive agent (Amlodipine besylate D2N) loaded by nanoprecipitation method using tribloere polymeric stabilizer (Pluronic F68). Biodegradable nanoparticles formulated from poly (D,L-lactide-co-glycolide) (PLGA) polymers are being extensively investigated for various drug delivery applications. Nanoparticles using PLGA polymers were formulated using nanoprecipitation technique, and were characterized for size, drug loading, and *invitro* release. Atorvastatin calcium is a second generation 3- hydroxy-3-methyl glutaryl CoA reducatase inhibitor approved for clinical use as a lipid lowering agent. Atorvastatin calcium, the world's best selling drug is associated with poor oral bioavailability (12%) and serious adverse effects like rhabdomyolysis on chronic administration. Side effect of Atorvastatin was reduced 60% by combining with amlodipine. The Amlodipine has potency to promote the activity of atorvastatin. A biodegradable nanoparticulate approach was introduced here with a view to improving the efficacy and safety of Atorvastatin calcium. Particulate systems like nanoparticles have been used as a physical approach to alter and pharmacodynamic properties of various types of drug molecules. The nanoparticluate suspension of Amlodipine is to improve its absorption rate and therapeutic efficacy.

Subramanian[53] et al., (2012) formulated and evaluated of Ofloxacin nanoparticles by emulsion Polymerization method to release the drugs in the vicinity of the target tissue. Nanoparticles are solid colloidal particles with diameter ranging from 1nm – 1000nm. Ofloxacin is a first generation fluroquinolones antimicrobials it active against gram negative bacteria. Ofloxacin nanoparticles was prepared by emulsion polymerization method by using Eudragit S-100 polymer as a continuous phase for four different ratios such as (Drug: Polymer) (1:1), (1:2), (1:3) and (1:4). The polymer was dissolved in Dichloromethane and the solution was emulsified with the aqueous solution of Ofloxacin containing 2% Tween 80 by stirring for 1 hrs at 15^{o}C with the aid of mechanical stirrer at 3000rpm.The nanoparticles was formed and separated by centrifugation. The formulated nanoparticles are evaluated for external morphological studies using scanning electron microscope. The *invitro* release study was performed in phosphate buffer pH 7.4. The IR study reveals that in no major shifting as well as non less of functional groups peak between the drug, polymer and drug loaded nanoparticles. The *invitro r*elease study showed that the formulation ratio (1:1) gives the better sustained effect over 88.13 % of drug was released at 8 hrs. the Ofloxacin nanoparticles are suitable candidates to produce good antibiotic prolong action of the drug nanoparticles.

Adlin Jino Nesalin[54] et al., (2009) developed Flutamide nanoparticles formulated using chitosan polymer by ionic gelation technique. Nanoparticles of different core coat ratio were formulated and analyzed for total drug content, loading efficiency, particle size and *invitro* drug release studies. The nanoparticles prepared with chitosan in the core: coat ratio 1:4 gives better sustained release for about 12 hrs as compared to other formulations.

Karthikeyan[55] et al., (2013) formulated Stavudine nanoparticles specially designed to release the drug in the vicinity of target sites. The surface modified Stavudine entrapped low molecular weight chitosan (CS) nanoparticles as potential drug delivery system for anti-HIV chemotherapy. The particle size and the surface morphology results revealed that Stavudine nanoparticles (SNPs) were smooth spheroidal with a size ranging from 260 nm-632 nm. The drug entrapment efficiency was found to be near 83%. *In vitro* release studies revealed that the rate of

drug release from SNP5 was 93% in 24 hrs. Release of drug follows zero order and show sustained release behavior. Koresmeyer models shows that the drug follow non-Fickian transport as the value of n>0.5. The results suggest that chitosan polymer based nanoparticulate formulations are potential means to achieve release of stavudine for the prolonged period of time for effective therapy.

Selvakumar[56] et al., (2009) prepared the 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 *invitro* 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. The feasibility of formulating carvedilol – loaded Eudragit E 100 nanoparticles for the treatment of hypertension.

Riddhi Dave Patel[57] et al., (2012) investigated cryoprotectant on lyophlisation of Doxorubicin–Hcl loaded chitosan nanoparticles. Nanoparticles stability of formulation is very poor when it is in form of aqueous suspension. So, Freeze-drying becomes a good technique to improve the stability of colloidal nanoparticles. Shelf life of colloidal particles can be enhanced by lyophilisation by several folds. cryoprotectants are added during lyophilisation to protect the intactness of particles in injectable products. The role and influence of type of cryoprotectant and its concentration in the lyophilization of Doxorubicin- HCl Chitosan Nanoparticles.

Jameel Ahmed[58] et al., (2012) designed Repaglinide loaded solid lipid nanoparticles design and characterization solid lipid nanoparticles (SLNs) have attracted increasing attention during recent years. prolonged release SLNs of repaglinide (RPG) for oral delivery and to improve bioavailability of RPG. SLNs were formulated using tristearin as the lipid core and poloxamer 188 and egg lecithin as a mixture of emulsifiers by microemulsion method. Formulations were characterized by infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), particle size analysis, entrapment

efficiency , scanning electron microscopy (SEM), short term storage stability at 40 ± 2 °C/75 ± 5% RH and *invitro* release studies. IR and DSC studies showed that when the drug was incorporated into the solid matrix there was no possible interaction between drug and lipid carrier.Variables such as concentrations of lipid and emulsifiers and stirring speed showed great impact on particle size and entrapment efficiency Optimized SLNs with 5% lipid, 2% emulsifier mixture and stirring speed of 21,500rpm showed mean particle size of 440 nm and EE% 74.66. SEM confirms that the SLNs are in circular shape.

Hequn[59] et al., (2013) studied the *invivo* evaluation of Doxorubicin loaded BSA nanoparticles with folic acid modified dextran surface biocompatible and biodegradable doxorubicin loaded nanoparticles with targeting ability were prepared from BSA–dextran–folic acid conjugate via a pH adjustment and heating process. The BSA–dextran–folic acid conjugate was produced by an esterification reaction between folic acid and dextran and then the maillard reaction between the modified dextran and BSA. The nanoparticles have a size about 90 nm and excellent dispersibility at pH 7.4 aqueous solution. The Doxorubicin loading efficiency and loading amount of the nanoparticles are larger than 90% and 14%, respectively. The antitumor activity and toxicity of the nanoparticles were evaluated through murine ascites hepatoma H22 tumor-bearing mice. Importantly, the nanoparticles can decrease the toxicity of Doxorubicin that results in a significant increase of the average life time in comparison with the free Doxorubicin as well as the nanoparticles without folic acid.

Vyjayanthimala[60] et al., (2014) formulated and evaluated chitosan Nanoparticles of Zidovudine for antiviral therapy. Nanoparticles of Zidovudine were prepared using chitosan, liquid paraffin and Tween-20 using Emulsion droplet coalescence method. The concentration of the polymer Chitosan was selected based on the results on preliminary screening. The nanoparticles prepared were evaluated for morphology, loading efficiency and *invitro* release. The particle shape and morphology of the prepared Zidovudine nanoparticles were determined by SEM analysis. The amount of Zidovudine entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticle and the amount of non-entrapped drug remaining in

the aqueous supernatant. A Franz diffusion cell was used to monitor Zidovudine release from the nanoparticles. Drug released 75.89% of Zidovudine in 12 hrs with a burst drug release nearly 14.86% of drug within the initial 1 hrs. Among the four formulations showed maximum drug release in 12 hrs diffusion study and good entrapment efficiency. *In-vitro* antiviral study revealed that the formulated nanoparticles were found to have good viral activity.

Kumar Vikas[61] et al., (2011) investigated on novel drug delivery system for delivery of antihypertensive drugs novel drug delivery systems present an opportunity for formulation scientists to overcome the many challenges associated with antihypertensive drug therapy, thereby improving the management of patients with hypertension. Currently available Anti-hypertensive drugs can be classified into these categories: ACEinhibitors, angiotensin antagonist, calcium channel blocker, diuretics, central sympathomimetics, a- adernergic blocker, vasodilator, â-adernergic blocker. Most of these drugs bear some significant drawback such as relatively short half-life, low bioavailability, poor permeability and undesirable side effects. efforts have been made to design drug delivery systems for antihypertensive drugs to a reduce the dosing frequency, increase the bioavailability, deliver them to the target cells selectively with minimal side effects. The various anti hypertensive drug delivery systems that have been developed for achieving sustained drug release kinetics,and for addressing formulation difficulties such as poor solubility, stability and drug entrapment. The physicochemical lproperties and the *invitro/ invivo* performances of various system such as such a sustained release tablets, ceramic implants, nanoparticles, nanocontainers, liposomes, emulsomes, aspasomes, microemulsions,nanopowders and Pheroid TM are summarised. This review highlights the significant potential that novel drug delivery system have for the future effective treatment of hypertensive**.**

Kumar[62] et al., (2007) developed and evaluated a Nitrendipine loaded solid lipid nanoparticles: influence of wax and glyceride lipids on plasma pharmacokinetics. Nitrendipine is an antihypertensive drug with poor oral bioavailability ranging from 10 to 20% due to the first pass metabolism. For improving the oral bioavailability of nitrendipine, nitrendipine loaded solid lipid

nanoparticles have been developed using triglyceride (tripalmitin), monoglyceride (glyceryl monostearate) and wax (cetyl palmitate). Poloxamer 188 was used as surfactant. Hot homogenization of melted lipids and aqueous phase followed by ultrasonication at temperature above the melting point of lipid was used to prepare SLN dispersions. SLN were characterized for particle size, zeta potential, entrapment efficiency and crystallinity of lipid and drug. *invitro* release studies were performed in phosphate buffer of pH 6.8 using Franz diffusion cell. Pharmacokinetics of nitrendipine loaded solid lipid nanoparticles after intraduodenal administration to conscious male wistar rats was studied. Bioavailability of nitrendipine was increased three- to four-fold after intraduodenal administration compared to that of nitrendipine suspension. solid lipid nanoparticles as carriers for improving the bioavailability of lipophilic drugs such as nitrendipine by minimizing first pass metabolism. Nitrendipine is an antihypertensive drug with poor oral bioavailability ranging from 10 to 20% due to the first pass metabolism. For improving the oral bioavailability of nitrendipine, nitrendipine loaded solid lipid nanoparticles have been developed using triglyceride (tripalmitin), monoglyceride (glyceryl monostearate) and wax (cetyl palmitate). Poloxamer 188 was used as surfactant. Hot homogenization of melted lipids and aqueous phase followed by ultrasonication at temperature above the melting point of lipid was used to prepare SLN dispersions. Pharmacokinetics of nitrendipine loaded solid lipid nanoparticles after intraduodenal administration to conscious male wistar rats was studied. Bioavailability of nitrendipine was increased three- to four-fold after intraduodenal administration compared to that of nitrendipine suspension.

Krishna sailaja[63] et al., (2011) prepared a nanoparticle using natural polymer and their application targeting delivery of drugs to the diseased lesions is one of the most important aspects of drug delivery system. The *invivo* to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects. In order to see functionality and toxicity of nanoparticles in various food and drug applications, it is important to establish procedures to prepare nanoparticles are controlled size. Natural polymers have been classified into polysaccharides and proteins. Chitosan is a natural polymer obtained by deacetylation of chitin. After

cellulose chitin is the second most abundant polysaccharide in nature. It is biologically safe, nontoxic, biocompatible and biodegradable polysaccharide. Chitosan nanoparticles have gained more attention as drug delivery carriers because protein nanoparticle are nontoxicity, stability for long duration,non-antigen also posseses biodegradability. In fact protein is biopolymer, which is commonly used for preparation of nano structured molecules for drug delivery.

Anjali Goel[64] et al.,(2012) synthesized colloidal iridium nanoparticles by chemical oxidation method with different surfactants like poly vinyl pyrrolidone (PVP), poly vinyl alcohol (PVA) and poly oxyethylene lauryl ether (POLE). It was found that shape and size of Ir-nano particles resulted were related to kind of capping agent (surfactant) used. The characterization of the synthesized nano particle has been carried out by UV-vis, X-ray diffraction (XRD), FT-IR, scanning electron microscopy (SEM) and transmission electron microscopic (TEM) techniques. UV-vis and FT-IR con- firm the oxidation of IrCl3 into IrO2 while XRD con-firms the amorphous nature of the iridium nanoparticles synthesized. The morphology and size of the particle were confirmed by TEM.

Tapas Kumar Kundu [65] et al., (2011) prepared ZnO nanoparticles in support of poly(vinyl alcohol) (PVA) molecules. PVA molecules offer plenty of active OH groups and a metal ion-polymer complex is formed via a kind of ligand reaction.The particle sizes lie in the range of 23 nm - 43 nm. The Electronparamagnetic resonance (EPR) spectra of the powders are characterized by a broad resonance peak owing to presence of zn+ defects in the specimens.The nanopowders show an intense violet emission along with the emission in blue and green band. In comparison, ZnO specimens having micron sized grain which are prepared without using PVA do not show any emission with significant intensity. zn+ defects play a role in improving the optical emission of ZnO nanoparticles prepared by this method.

Zhang[66] et al., (2010) developed nanoparticles for antimicrobial drug delivery Numerous antimicrobial drugs have been prescribed to kill or inhibit the growth of microbes such as bacteria, fungi and viruses. Even though the therapeutic efficacy of these drugs has been well established,

inefficient delivery could result in inadequate therapeutic index and local and systemic side effects including cutaneous irritation, peeling, scaling and gut flora reduction. Nanostructured biomaterials, nanoparticles in particular, have unique physico chemical properties such as ultra small and controllable size, large surface area to mass ratio, high reactivity, and functionalizable structure. Encapsulation of antimicrobial drugs in nanoparticle systems has emerged as an innovative and promising alternative that enhances therapeutic effectiveness and minimizes undesirable side effects of the drugs. the current progress and challenges in synthesizing nanoparticle platforms for delivering various antimicrobial drugs are reviewed.

Che-ming[67] et al., (2010) investigated that nanoparticle assisted combination therapies for effective cancer treatment combination chemotherapy and nanoparticle drug delivery are two areas that have shown significant promise in cancer treatment. Combined therapy of two or more drugs promotes synergism among the different drugs against cancer cells and suppresses drug resistance through distinct mechanisms of action. Nanoparticle drug delivery, on the other hand, enhances therapeutic effectiveness and reduces side effects of the drug pay loads by improving their pharmacokinetics. These two active research fields have been recently merged to further improve the efficacy of cancer therapeutics. the recent efforts in developing nanoparticle platforms to concurrently deliver multiple types of drugs for combination chemotherapy. the challenges and design specifications that need to be considered in optimizing nanoparticle-based combination chemotherapy.

Afifa Bathool[68] et al., (2012) developed Atorvastatin calcium chitosan loaded nanoparticles prepared by solvent evaporation method for sustained release. Low oral bioavailability of Atorvastatin calcium (14%) due to an extensive high first-pass effect makes it as prime target for oral sustained drug delivery. Weighed amount of drug and polymer were dissolved in suitable organic solvent DMSO and 2% acetic acid as an organic phase. This solution is added drop wise to aqueous solution of Lutrol F68 and homogenized at 25000rpm followed by magnetic stirring for 4hrs. Nanoparticles were evaluated for its particle size, scanning electron microscopy (SEM), Fourier-Transform infrared spectroscopy (FTIR), percentage yield, drug entrapment and for *invitro* release

kinetics. Among the four different ratios, 1:4 ratio showed high drug loading and encapsulation efficiency. SEM studies shows that prepared nanoparticles were spherical in shape with a smooth surface. Particle size of prepared nanoparticles was found to be in range between 142 nm to 221 nm. FTIR and DSC shows drug to polymer compatibility ruling out any interactions. *invitro* release study showed that the drug release was sustained up to 7 days. Hence, prepared nanoparticles proved to be promising dosage form for sustained drug delivery of Atorvastatin reducing dosing frequency, thus increasing the patient compliance.

Vivek Kumar Gupta[69] et al., (2010) formulated hydrophilic & hydrophobic drugs nanoparticle have many advantages over traditional dosage forms, such as enhanced dissolution properties and the potential for intracellular drug delivery. Specifically, pure drug nanoparticles, polymeric nano-particles and polyelectrolyte Traditional techniques such as spray drying and grinding, and more recent advances in supercritical fluid extraction, precipitation, and double solvent evaporation have been employed to produce nanoparticle formulations for delivery of hydrophilic & hydrophobic drugs here, the benefits of nanoparticle formulations and current progress are compared in light of the practical encumbrances of producing formulations, and possible toxicological effects of these materials.

Peng Guo[70] et al., (2012) prepared amorphous hydrophobic drug nanoparticles by nanoporous membrane extrusion method for formulating hydrophobic drugs into nanoparticulate form in a scalable and inexpensive manner. The nanoporous membrane extrusion (NME) method was used to prepare hydrophobic drug nanoparticles. NME is based on the induced precipitation of drug-loaded nanoparticles at the exits of nanopores. Three common hydrophobic drug models (silymarin, beta carotene and butylated hydroxytoluene) were tested. The authors carefully investigated the morphology, crystallinity and dissolution profile of the resulting nanoparticles. Using NME, the authors successfully prepared rather uniform drug nanoparticles (~100 nm in diameter). These nanoparticles were amorphous and show an improved dissolution profile compared with untreated drug powders. NME could be used as a general method to produce nanoparticles of hydrophobic drugs.

Parmar[71] et al., (2011) designed Valsartan nanoparticles is an antihypertensive drug with poor oral bioavailability range from 10-35% because poor solubility dissolution and most importantly extensive first pass hepatic metabolism.The present study deals with the development and characterization of valsartan loaded solid lipid nanoparticles to enhance the solubility by pass hepatic metabolism and enhance the lymphatic absorption leading to improved bioavailability.

Vaibhav Shukla[72] et al., (2012) developed Dilitiazem Hcl nanoparticles it is an antihypertensive agent that antagonizes the action of beta 1- Receptor, DTZ when gives orally is well absorbed from the gastro intestinal tract and is subjected to an extensive first pass effect. DTZ undergoes extensive metabolism in which only 2% to 4% of the unchanged drug appear in the urine. Drug which induce or inhibit hepatic microsomal enzymes may alte DTZ disposition. It has been reported that the absolute bioavailability of DTZ when the given orally is 30-40% the biological half life of DTZ is 4-6 hrs and the main site of absorption is proximal small intenstine. A muco adhesive nanoparticles delivery system was envisioned for DTZ as such a system when administered would adhere on the gastric mucosa for a prolong period of time and the drug would be available at the main site of absorption.

Madhushri[73] et al., (2012) formulated solid lipid nanoparticles containing Clotrimazole for treatment of fungal infections like eczema, itching, pruritis etc. Topical formulation enriched with SLN of Clotrimazole was prepared. The solid lipid nanoparticulate dispersion of Clotrimazole was prepared by hot homogenization technique using polymers like Carbopol 934, mannitol and PEG 6000. The nanoparticulate dispersion was evaluated for various parameters such as physical evaluations, particle size, diffusion studies, DSC, SEM, stability studies. The solid lipid nanoparticulate dispersion showed mean particle size less than 1000 nm. Differential scanning Calorimetry studies revealed no drug excipient compatibility. Diffusion studies release profile of clotrimazole from nanoparticulate dispersion showed prolonged drug release and all other evaluations were found to be complied the limits. formulation of SLN containing clotrimazole can be successfully formulated to localize the drug in the skin for to treat topical fungal infections.

Mohammed Khan[74] et al., (2012) developed and evaluated mucoadhesive nanoparticles of chitosan using Tramadol Hcl. Spray drying method was employed for producing nanoparticles using different drug to polymer ratio. Nanoparticles were evaluated for variables like yield, drug loading, entrapment efficiency, swelling, *invitro* mucoadhesion, particle size, polydispersity index & zeta potential, scanning electron microscopy, transmission electron microscopy, X-ray diffraction study and drug polymer compatibility by differential scanning calorimetry & Fourier Transform Infrared Radiation studies. Tramadol HCl loaded chitosan nanoparticles is a promising delivery through nasal route for relief of pain.

Arjun Dedakia[75] et al., (2013) prepared Poly caprolactone composite microparticles by w/o/w emulsion method**.** The drug as Theophylline from Xanthine derivative is still widely used as an effective bronchodilator in the disease of Asthma .Theophylline is used as a prophylactic drug and to prevent acute exacerbations of asthma also. The drug release in different dosage forms and find out the different burst effect in different dosage forms. The different batches of different dosage forms like drug containing microparticles, blank microparticles, and drug containing nano particles, blank nanoparticles, blank Composite Microparticles and drug containing the composite microparticles. Furthermore Double Emulsion Solvent Evaporation Method(W1/O/W2) was used to prepare these all type of dosage forms. The prepared Polycaprolactone composite microparticles was characterized by % Yield ,Particle size analysis, Zeta Potential, drug loading efficiency, X ray diffraction study and scanning Electron Microscopy. The different types of ratio of polymers to drugs are used.

Srinivas[76] et al., (2012) formulated of Moxifloxacin hydrochloride ocular nanoparticles. The nanoparticles were prepared by solvent displacement method using Eudragit RL 100 as a polymer. Different formulations were prepared by varying the ratios of drug and polymer and varying the ratios of organic and aqueous phase. The formulations were evaluated in terms of particle size, FTIR, drug entrapment efficiency and *invitro* drug release profile was examined. The antibacterial activity against gram positive and gram negative bacteria were determined. *Invivo* studies were carried out by

Draize test. The mean particle size for drug loaded formulations was found to be below 200 nm. The formulation possesses good antibiotic activity against *Escherichia coli, Bacillus subtilus* and *Staphylococcus aureus* microorganism and no eye irritation on *invivo* testing.

Jun Sung Kim[77] et al., (2007) developed the antimicrobial effects of silver (Ag) ion or salts are well known, Stable Ag nanoparticles were prepared and their shape and size distribution characterized by particle characterizer and transmission electron microscopic study. The antimicrobial activity of Ag nanoparticles was investigated against yeast, *Escherichia coli,* and *Staphylococcus aureus*. In these tests, Muller Hinton agar plates were used and Ag results, yeast and E. coli were inhibited at the low concentration of Ag nanoparticles, whereas the growth-inhibitory effects on S. aureus were mild. The free-radical generation effect of Ag nanoparticles on microbial growth inhibition was investigated by electron spin resonance spectroscopy. Ag nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems.

Dastagiri[78] et al., (2013) prepared antineoplastic drug loaded polymeric nanoparticles using biodegradable polymers (Chitosan and Eudragit RS 100) by emulsion droplet coalescence method. The model drug used here is 5-fluorouracil which is a pyrimidine analogue that is mainly used to treat colonic carcinoma, under the category of anti-neoplastic drugs. Tween 20 was used as emulsifier and colloidal stabilizer. The prepared nanoparticles were evaluated for particle size, surface morphology by TEM, surface charge, drug loading and entrapment efficiency, and for drug release by diffusion. Results show that the prepared nanoparticles are in nanosize, below 1000 nm, having appropriate zeta potential values with better entrapment of drug and controlled release of drug for a period of 12 hrs. with high entrapment efficiency, optimum zeta potential, and showing more controlled release of drug.

Rajkumari[79] et al., (2011) prepared the oral mucoadhesive sustained release nanoparticles of Clarithromycin in order to improve its therapeutic effect and reducing dosing frequency and its

dose related side effects. Clarithromycin containing chitosan nanoparticles were prepared by ionotropic gelation method. The method is reproducible easy and led to the efficient entrapment. Formulation had spherical particles in the particle range from 100 - 1500nm. Some process variables like effect of chitosan concentration, TPP concentration, acetic acid concentration were also evaluated with respect to drug content and encapsulation efficiency. The maximum encapsulation efficiency and drug content were 67.43 % and 6.13. The sustained release behavior of chitosan nanoparticles were evaluated both in phosphate buffer saline and simulated gastric fluid and results revealed that clarithromycin loaded chitosan nanoparticles are most suitable mode of delivery of drug for promising therapeutic action.

Umasankar[80] et al., (2010) formulated of Cytarabine nanoparticles. Cytarabine is a synthetic pyrimidine nucleoside. Cytarabine is most commonly used to treat acute myeloid leukaemia. minimize the frequency of doses and toxicity and to improve the therapeutic efficacy by formulating cytarabine nanoparticle. Cytarabine nanoparticles were formulating by ionic gelation method using polymer chitosan with three different ratios. Nanparticles were characterized by determining its particle size, drug entrapment efficiency, drug release and stability studies. The particle size ranged between 350nm to 600nm. Drug content was found to be supportive to the drug release pattern. The *invitro* release of cytarabine nanoparticles were carried out which exhibited a sustained release of cytarabine from nanoparticles up to 16 hrs. The results showed that nanoparticles were more beneficial in providing drug delivery system

Vimal Kumar Varma[81] et al., (2009) prepared Diclofenac Sodium loaded ethyl cellulose composite magnetic microspheres**.** Diclofenac sodium-containing ethyl cellulose micro particles were prepared by the Emulsion-solvent evaporation method with a view for use in the application of magnetic carrier technology. The properties of these magnetic microspheres, such as morphological, magnetic susceptibility and polymer-drug interactions were characterized by different techniques .The loading efficiency and swelling kinetics magnetic microspheres were also studied. The formulated microspheres were below 5μm and spherical in nature as evidenced from SEM. The *invitro* release profile was studied in normal saline medium up to 7 hrs using USP XXII dissolution

apparatus. Drug release in the first hour was found to increase and reached a maximum, releasing approximately 57.46% to 81.44% of the total drug content from the microspheres within 7 hrs. A third order equation for the drug release was also calculated. Microspheres showed greater retention time under the influence of magnetic field created by an electromagnet with field strength 8000 G, when compared to the retention in the absence of magnetic field. Magnetic ethyl cellulose microspheres could be retained at their target site *invivo*,following the application of the magnetic field and are being capable of releasing the drug for an extended period of time, thus making them a suitable depot for delivering chemotherapeutic agent *invivo*.

Thiagarajan Madheswaran[82] et al., (2013) designed of Finasteride-Loaded Liquid crystalline nanoparticles for topical delivery liquid crystalline nanoparticles in the treatment of androgenetic alopecia. The potential of this nanocarrier, FNS-loaded LCN was prepared by ultrasonication method and characterized for size, shape, *invitro* release, and skin permeation retention properties. The particle size ranged from 153.8 to 170.2 nm with a cubical shape and exhibited controlled release profile with less than 20% of the drug released in the first 24 hrs. The release profile was significantly altered with addition of different additives. Formulation with lower monolein exhibited higher skin permeation with a flux rate of 0.0610.005 μg cm 24 hrs. The permeation however, significantly increased with glycerol, propylene glycol, and polyethylene glycol 400, while it declined for the addition of oleic acid.

Azza A Hasan[83] et al., (2012) prepared anti-glucomatous Dorzolamide hydrochloride-(Dorzo) loaded nanoparticles as a controlled release system. Eudragit RS 100 (RS) and/or RL 100 (RL) were used in formulations by an opportunely adapted Quasi-emulsion solvent diffusion technique. The formulations were evaluated in terms of particle size, zeta potential, drug entrapment, and release profile. All formulations showed tiny particle size varying from 114 to 395 nm for RS and 65 to 277 nm for RL. Positive zeta potential was 19 to 32 mV for RS and 23 to 42 mV for RL formulations. Increasing polymer concentration lead to increase the percentage of drug entrapped in all batches, to a certain extent (drug: polymer 1:4). Nanoparticles prepared using RL showed lower

entrapment efficiency than RS. In contrast, increasing the stirring rate resulted in an increase in the percentage of Dorzo entrapped. A prolonged drug release was shown by all the formulations. Increasing the polymer concentration caused a decrease in the release rate. Dorzo-loaded nanoparticles could represent promising drug ophthalmic carriers, due to small particle size, positive zeta potential, and sustained release profile.

Beny Baby[84] et al., (2012) prepared nanoparticles of Levofloxacin by ionic gelation method using chitosan as a biodegradable polymer and tripolyphosphate as the cross linking agent. The particle size of the prepared formulations varied between 190 and 632 nm. The nanoparticles showed favorable drug entrapment efficiency which varied between 60.06 ± 0.06 % and 74.29 ± 0.04 % and the drug content ranged between 67.20 ± 0.30 % and 76.10 ± 0.61 %. The FTIR spectral studies and DSC thermogram indicated that there was no interaction between the drug and polymers used. The scanning electron microscopy indicated that prepared nanoparticles were discrete, uniform and spherical with a smooth surface. According to this model, the drug releases from these formulations may be controlled by diffusion through the micropores. During and at the end of the stability study, the tested formulation showed non-significantly different drug content, entrapment efficiency and invitro drug release from that observed at the beginning of the study. No color changes were also observed during the study period.

Ahmed Abushrida[85] et al., (2011) investigated with the potential for long circulation times or the ability to preferentially reach particular tissues. The preparation of iron oxide nanoparticles was achieved using inorganic solution methods to prepare particles of small size using a narrow size distribution. The nanoparticles were coated with dextran and carboxymethyl dextran as reference materials using the same method as in the preparation of the iron oxide nanoparticles. the biodegradable polymer poly(glycerol adipate) (PGA) as a coating for iron oxide nanoparticles. PGA is already used in drug delivery systems and showed an ability to control the rate of release of the drug.

Khemariya[86] et al., (2010) prepared solid lipid nanoparticles for Nateglinide method and characterization-Solid lipid nanoparticles (SLNPs) based on different lipidic components have been produced by modified solvent injection method and characterized for Nateglinide encapsulation efficiency, morphology, zeta potential, particle size, and drug release. Morphology and dimensional distribution have been investigated by electron microscopy and Photon Correlation Spectroscopy. Cell viability experiments demonstrate that SLNPs exhibit no toxicity. Spherical SLNPs with an average particle size of ~173 nm were formulated. Delivery of Nateglinide by SLNPs led to a significantly higher accumulation by the endothelial cell monolayer as compared to the drug in aqueous solution. *invitro* release kinetics based on a dialysis method demonstrated that Nateglinide was released in a prolonged fashion for 24 hrs. Both free and encapsulated drug reduced the time spent on the blocks in the bar test, although the action of encapsulated Nateglinide was more rapid in onset and prolonged than free drug. SLNPs would be promising drug delivery carriers to enhance delivery of Nateglinide.

Nagavarma[87] et al.,(2010) prepared Polymeric nanoparticles (PNPs) defined as particulate dispersions or solid particles with size in the range of 10-1000nm. There has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. Polymeric nanoparticles have been extensively studied as particulate carriers in the pharmaceutical and medical fields, because they show promise as drug delivery systems as a result of their controlled and sustained release properties, subcellular size, biocompatibility with tissue and cells.

G. Nabiyouni [88] et al., (2011) investigated the surface adsorption of PEG and polyvinyl alcohol (PVA) with different molecular weights onto the Zinc oxide (ZnO) nanoparticles The ZnO nanoparticles are then analyzed using Fourier transform infra red (FTIR), X-ray diffraction (XRD) and thermogravimetric analysis (TGA) techniques. The aggregation of nano particles before and after polymer adsorption is also investigated by scanning electron

microscopy (SEM). Adsorbing the PEG and PVA, due to a relatively large electrostatic repulsive force between ZnO nano particles, the size of aggregated nanoparticles decreases. The low molecular weight polymers exhibit a higher adsorption rate on the particles' surfaces comparison to the polymers with high molecular weight.

3.0 RESEARCH ENVISAGED

3.1 AIM OF WORK

Development of nanoparticles are one of the emerging fields of nanotechnology with several potential application in drug delivery, clinical medicine and research as well as in other discipline. The use of nanoparticles as drug carrier system is a very attractive controlled drug release.

Irbesartan is an angiotensin receptor blockers used mainly for the treatment of hypertension. It belongs to class Π drug according to biopharmaceutical classification system.

The bioavailability of the valsartan after oral administration is low (60%) with higher variability. The present study was aimed at developing nanoparticle of irbesartan in order to improve the bioavailability and efficacy in treatment of hypertension.

Irbesartan is a poorly water soluble drug. To increase solubility of the drug and reduce the dose frequency and improve the bioavailability the study was aimed at nanoparticle of Irbesartan.

Thus the present work is to Develop and characterize a nanoparticulate drug delivery system of antihypertensive drug (Irbesartan) and also

- To increase the solubility
- 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.

3.2. PLAN AND SCOPE OF WORK

Plan of the present work involves the following;

1. Preformulation studies involve observation of physical and chemical data available. The identification of raw materials and compatibility studies between drug and polymer is to be done by using Infrared spectrophotometry.

2. Preparation of Irbesatran Nanoparticles prepared by Precipitation Method.

3. Formulation of Irbesartan Nanoparticles in various ratios of drug and polymer.

4. The best formulation will be selected based on the results of following parameters. The prepared nanoparticles is to be evaluated by following chemical characteristics
   - Drug entrapment efficiency.
   - *In vitro* drug release of formulated nanoparticles.

5. Surface morphology of formulation (SEM) of the optimized formulation.

6. Zeta potential analysis of the optimized formulation.

7. Stability studies for the best formulation at different temperature.

8. Drug release kinetics.

4. METHODOLOGY

4.1. INSTRUMENTS AND MATERIALS USED

TABLE.NO.1 MATERIALS USED

MATERIALS	SOURCE
Irbesartan	Hetero Laboratory Ltd.(Chennai)
Poly vinyl alcohol	Micro labs, Hosur.(Karnataka)
β cyclodextrine	Micro labs, Hosur.(Karnataka)
Potassium dihydrogen phosphate	S.D.Finechemicals, Boisar.(Maharashtra)
Disodium hydrogen phosphate	S.D.Finechemicals, Boisar.(Maharashtra)
Sodium Hydroxide	S.D.Finechemicals, Boisar.(Maharashtra)
Ethanol	S.D.Finechemicals, Boisar.(Maharashtra)

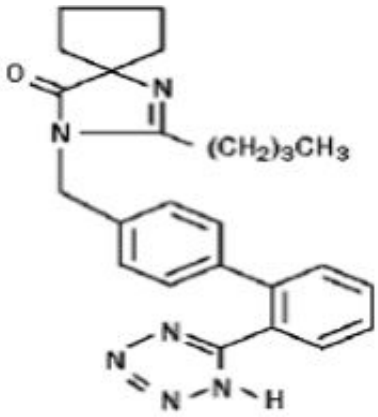
TABLE.NO.2 INSTRUMENTS USED

EQUIPMENTS	SOURCE
Vortex mixer	Remi motors Ltd, Mumbai.(SC-1275)
Electronic balance	Shimadzu Corporation, Japan.(ELB300)
Stability chamber	Osworld, Mumbai.(F-2749)
Ultra centrifuge	Remi motors Ltd, Mumbai.(L-3452)
pH – meter	ElicoPvtLtd, Chennai.(SC-1352)
FTIR spectroscopy	Perkin Elmer, Germany.(D-7864)
Double beam UV spectrophotometer	Perkin Elmer, Germany(S-8965)
Hot air oven	Biochemicals, Mumbai.(S-501)
Membrane filter	Gotting Ltd, Germany.(G-678)

4.2. DRUG PROFILE[89]

IRBESARTAN

Chemical structure



Molecular formula : C22H28N6O

Molecular weight : 428.53

Chemical Name : 2-butyl-3-[P-(0-1H-tetrazol-5-ylphenyl)benzyl]-1,3-diazapiro[4,4]non-1-en-4-one.

Solubility : irbesartan is insoluble in water,slightly soluble in alcohol and dicholoromethane.

Pharmacology:

May be used as a first line agent to treat uncomplicated hypertension, isolated systolic hypertension and left ventricular hypertrophy. May be used as a first line agent to delay progression of diabetic nephropathy. Losartan may be also used as a second line agent in the treatment of congestive heart failure, systolic dysfunction, myocardial infarction and coronary artery disease in those intolerant of ACE inhibitors.

Pharmacodynamics:

Angiotensin II, the principal pressor agent of the renin-angiotensin system, is responsible for effects such as vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal reabsorption of sodium. Irbesartan is a specific competitive antagonist of AT_1 receptors with a much greater affinity (more than 8500-fold) for the AT_1 receptor than for the AT_2 receptor and no agonist activity. Irbesartan's inhibition of angiotensin II binding to the AT1 receptor leads to multiple effects including vasodilation, a reduction in the secretion of vasopressin, and reduction in the production and secretion of aldosterone. The resulting effect is a decrease in blood pressure

Mechanism of action

Irbesartan is a nonpeptide tetrazole derivative and an angiotensin II antagonist that selectively blocks the binding of angiotensin II to the AT_1 receptor. In the renin-angiotensin system, angiotensin I is converted by angiotensin-converting enzyme (ACE) to form angiotensin II. Angiotensin II stimulates the adrenal cortex to synthesize and secrete aldosterone, which decreases the excretion of sodium and increases the excretion of potassium. Angiotensin II also acts as a vasoconstrictor in vascular smooth muscle. Irbesartan, by blocking the binding of angiotensin II to the AT_1 receptor, promotes vasodilation and decreases the effects of aldosterone. The negative feedback regulation of angiotensin II on renin secretion is also inhibited, but the resulting rise in plasma renin concentrations and consequent rise in angiotensin II plasma concentrations do not counteract the blood pressure–lowering effect that occurs. The action of ARBs is different from ACE inhibitors, which block the conversion of angiotensin I to angiotensin II, meaning that the production of angiotensin II is not completely inhibited, as the hormone can be formed via other enzymes. Also, unlike ACE inhibitors, irbesartan and other ARBs do not interfere with response to bradykinins and substance P, which allows for the absence of adverse effects that are present in ACE inhibitors.

Pharmacokinetics:

Absorption

Rapid and complete with an average absolute bioavailability of 60-80%. Food has no affect on bioavailability**.**

Distribution

53- 93 L. 90% bound to serum proteins.

Metabolism

Hepatic Irbesartan is metabolized via glucuronide conjugation and oxidation. *In vitro* studies of irbesartan oxidation by cytochrome P450 isoenzymes indicated irbesartan was oxidized primarily by 2C9; metabolism by 3A4 was negligible.

Elimination

Half-life is about 11-15 hrs. Irbesartan is metabolized via glucuronide conjugation and oxidation. Irbesartan and its metabolites are excreted by both biliary and renal routes. Irbesartan is excreted in the milk of lactating rats.clearence value 157-176 mL/min.

Side effects

- Most commonly, headache and dizziness
- Chills
- Cold sweats
- confusion

Dosage and Administration

Hypertension
Adults

Initial dosage: 150 mg once daily.

Maximum dosage: 300 mg once daily.

Diabetic Nephropathy

Adult

Dose: 300mg daily treatment of diabetic nephropathy with an elevated serum creatinine and proteinuria in patient with type2 diabetes and hypertension

Renal dose adjustments - No adjustments

Liver dose adjustments - No adjustments

Storage/Stability

Store at 59°to86°F in tightly closed container. Protect from moisture.

4.3. POLYMER PROFILE

POLY VINYL ALCOHOL

Synonym

Poly ethanol, homo polymer, Polyviol, Vinol, Alvyl, Alcotex.

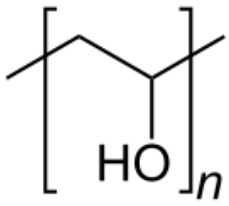
Empirical formula

$(C_2H_4O)X$

Molecular Weigh

13000-23000

Structural formula:



Application

Paper adhesive with boric acid in spiral tube winding and solid board production

Thickener, modifier, in polyvinyl acetate glues

Textile sizing agent

Paper coatings, release liner

As a water-soluble film useful for packaging. An example is the envelope containing laundry detergent in "liqui-tabs".

Feminine hygiene and adult incontinence products as a biodegradable plastic backing sheet.

Carbon dioxide barrier in polyethylene terephthalate (PET) bottles

Typical properties

Melting Point : 200 °C

Boiling Point : 228 °C (Predicted)

Density : 1.19-1.31 g/cm^3

Solubility

Soluble in water

Stability and Storage

Store at room temperature

β -CYCLODEXTRIN

Synonym

Cycloheptaamylose, Cyclomaltoheptaose; Betadex

Empirical formula

$C_{42}H_{70}O_{35}$

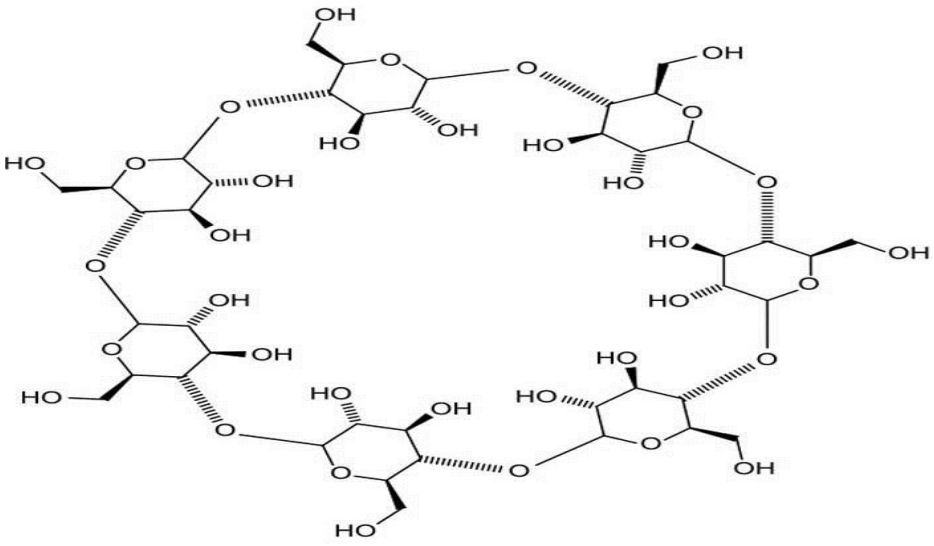
Molecular Weight

1134.98

Description

β-Cyclodextrin is made of homogeneous cyclic α1,4-linked D-glucopyranose units in a seven member ring. Forms clathrates and suitable for use with dansyl chloride to form water-soluble complexes for fluorescent labeling of proteins.

Structural formula



Application

Useful for forming water-soluble complexes for fluorescent labeling of proteins

Typical properties

Melting Point : 290-300 °C (lit.)(dec.)

Boiling Point : 1541.18 °C at 760 mmHg (Predicted)

Density : 1.44 g/cm^3 at 20

Solubility

Soluble in water (10 mg/ml), and 1 M NH4OH (50 mg/ml)

Stability and Storage

Store at room temperature

5. EXPERIMENTAL INVESTIGATIONS

5.1 CONSTRUCTION OF STANDARD CURVE FOR IRBESARTAN

UV Spectroscopy Method

Irbesartan is estimated spectrophotometrically at 220 nm and it obey Beer-Lambert` s Law in the range of 5-50 mcg /ml.

Detrminatiion of Absorbance maximum (λ_{max})

Irbesartan was dissolved in phosphate buffer saline pH 7.4 Solution with 50 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 220 nm. The drug was later quantified by measuring the absorbance at 220 nm in phosphate buffer saline pH 7.4

Preparation of release media

1.38gm of disodium hydrogen phosphate, 0.19gm of potassium dihydrogen phosphate and 8gm of sodium chloride was dissolved in sufficient amount of distilled water and produced 1000ml. pH was adjusted to 7.4

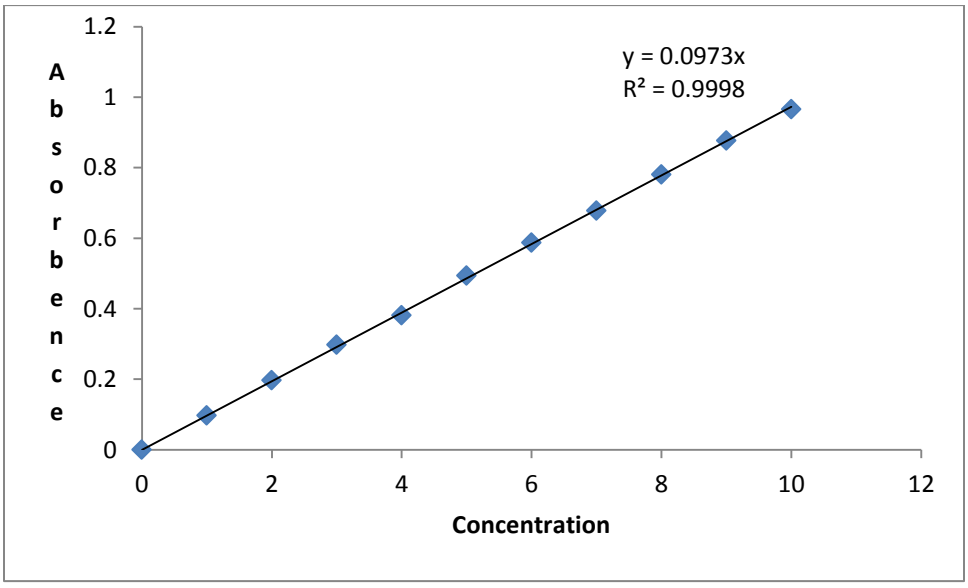
4.5.2. Standard curve for Irbesartan (By UV method) [90]

A stock solution of Irbesartan was prepared by dissolving 50mg of pure drug in pH 7.4 phosphate buffer saline in a 100ml volumetric flask. From the above stock solution, 10ml of solution was pipetted out into a 100ml volumetric flask and made upto the mark. From the secondary stock solution, 1ml, 2ml, 3ml, 4ml upto 10ml were taken and diluted to 10ml to obtain the concentrationof 5 to 50 µg/ml.The absorbance of the solutions were measured against the blank in a UV spectrophotometer. A calibration curve was obtained at 220 nm for a series of concentration in the range of 5 to 50 µg/ml.

TABLE.3 CALIBRATION CURVE OF IRBESARTAN

Concentration (µg/ml)	Absorbance at 220nm
5	0.097
10	0.197
15	0.298
20	0.381
25	0.494
30	0.587
35	0.678
40	0.781
45	0.877
50	0.966

Fig: 1 STANDARD CURVE FOR IRBESARTAN



5.2. PREFORMULATION STUDY

IR studies:

Identification of the pure drug was performed using IR spectroscopy. IR spectroscopy (using Perkin Elmer) by KBr pellet method carried out on drug. They are compressed under 15 tones pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000-400cm^{-1} in a spectrophometer and peaks obtained were identified.

5.3. METHOD OF PREPARATION OF IRBESARTAN NANOPARTICLE

NANOPRECIPITATION METHOD:

All batches of nanoparticles were prepared by nanoprecipitation method. The required quantity of polymer dissolved in 3ml ethanol, and drug was dissolved in 3ml of ethanol, added finally both were mixed together and added β-cyclodextrin. The mixer was homogenized in vortex mixture for 1 min and then the Final volume of the preparation was to 10ml. Then this preparation was centrifuged at 15000rpm at 4^0c for half an hour. The supernatant was discarded and precipitate was washed 3times with distilled water. The nanoparticles thus obtained were dried overnight in oven at 60^0c and stored in desiccators.

The prepared formulation were characterized for loading efficiency, entrapment efficiency, particle size, particle size distribution , zeta potential and drug polymer compatability studies.

Table: 6 Various Composition of Nanoparticles Formulation

FORMULATION CODE	DRUG (Irbesartan) in mg	PAV in mg	β CYCLODEXTRIN
F1	50	25	5
F2	50	50	5
F3	50	75	5
F4	50	100	5
F5	50	25	10
F6	50	50	10
F7	50	75	10
F8	50	100	10
F9	50	25	15
F10	50	50	15

5.4 .EVALUATIONOF NANOPARTICLES

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 using ultra centrifuge) The absorbance was measured at 220 nm by UV spectrophotometrically.

INVITRO DRUG RELESE STUDIES

BY UV Spectrophotometric Method:

The *invitro* 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 a beaker containing 200ml of diffusion medium (phosphate buffer saline pH 7.4) the medium was maintained at 37^{o} C under magnetic stirred at constant speed. At fixed time interval of 1ml sample was taken from the diffusion medium for every 1 hrs and it was replaced by 1 ml fresh medium. This process was carried out for 24 hrs. The sample was measured UV spectrometrically at 220nm. The percentage of drug released at various time intervals was calculated from calibration graph.

SCANNING ELECTRON MICROSCOPY

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

SURFACE CHARGE (ZETA POTENTIAL DETERMINATION)

Zeta potential is an important parameter to evaluate and establish an optimum condition for stability of colloidal or dispersed systems .The prepared nanoparticle suspension 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 polyvinyl alcohol and β-cyclodextrine on the surface characteristics of the nanoparticle was studied.

pH AND PHYSICAL APPERANCE:

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.

STABILITY STUDIES OF NANOPARTICLES

The Stability studies of nanoparticles involves observing the formulation at 45^{o}C /70% RH which constitutes accelerated condition and (4^{o}C) on refrigerator and room temperature. The formulations were kept in both the temperature for 3 months and sufficient amount of sample 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

DRUG RELEASE KINETICS STUDIES

The optimized formulation was subjected to graphical treatment to assess the kinetics of drug release

ZERO ORDER PLOT

The zero order plot obtained by plotting cumulative % drug release versus time.

HIGUCHI PLOT

The Higuchi plot was made by plotting cumulative percentage (%) drug release versus Square root of time.

KORESMEYER PLOT

The graph was obtained by log cumulative percenetage(%) drug release versus log time.

FIRST ORDER KINETIC RELEASE STUDY

The first order plots were obtained by plotting log remaining cumulative percentage drug release versus time.

RESULTS AND DISCUSSION

DEVELOPMENT OF IRBESARTAN NANOPARTICLES[37, 44]

All batches of nanoparticles were prepared by nanoprecipitation method. The required quantity of polymer dissolved in 3ml ethanol, and drug was dissolved in 3ml of ethanol, added finally both were mixed together and added β-cyclodextrin. The mixer was homogenized in vortex mixture for 1 min and then the Final volume of the preparation was to 10ml. Then this preparation was centrifuged at 15000rpm at 4^0C for half an hour. The supernatant was discarded and precipitate was washed 3 times with distilled water. The nanoparticles thus obtained were dried overnight in oven at 60^0C and stored in desiccators.

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

DRUG AND POLYMER COMPATABILITY STUDIES BY FTIR[27, 76]

Identification of the pure drug was performed using IR spectroscopy. IR spectroscopy (using Perkin Elmer) by KBr pellet method carried out on drug. They are compressed under 15 tones pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000-400cm^{-1} in a spectrophometer and peaks obtained were identified.

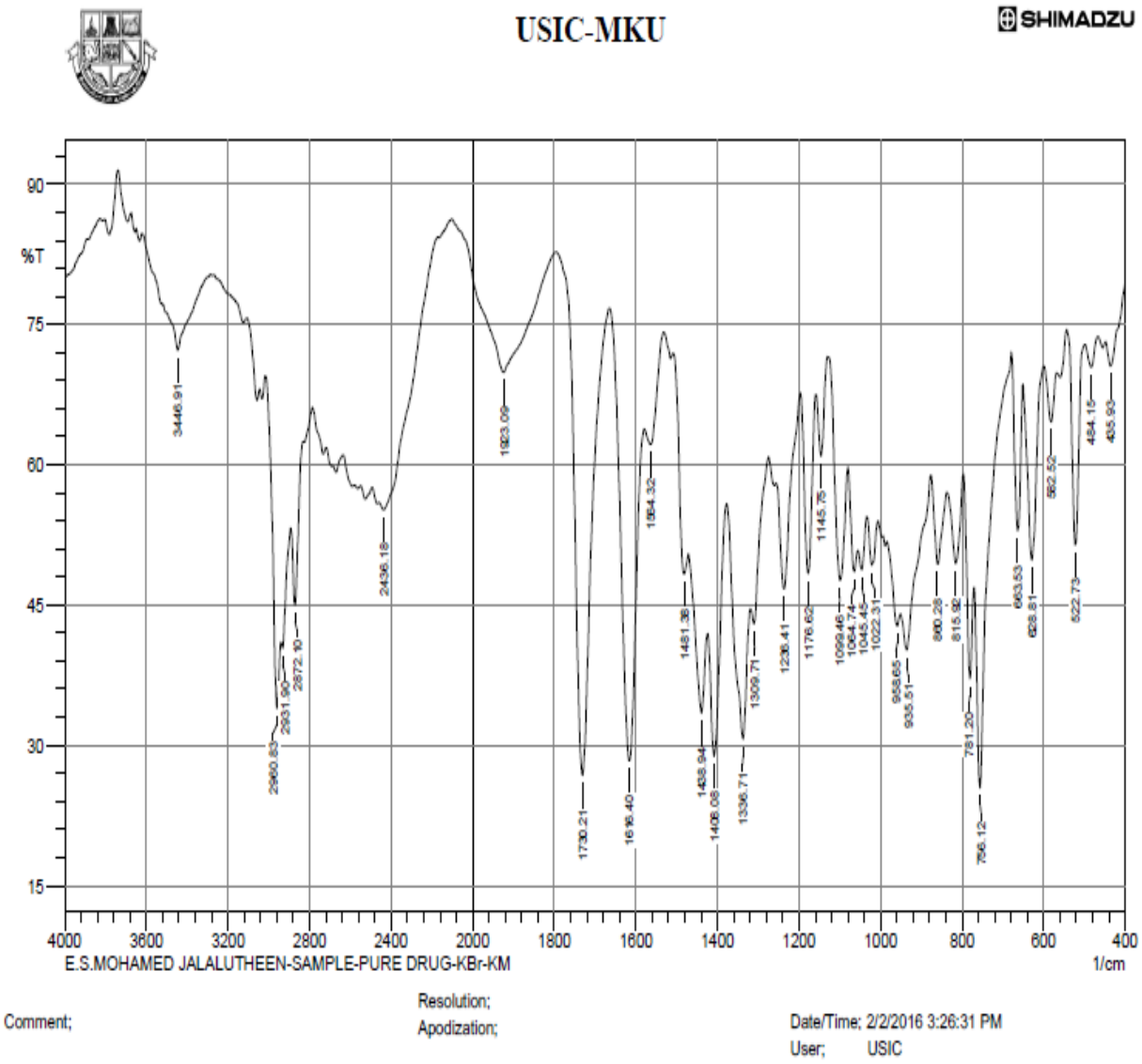


TABLE 4: I.R SPECTRA DATA FOR PURE IRBESARTAN

Wave no. (cm^{-1})	Group Assigned
1730.21	C=O – Stretching
1408.06	C=C – Stretching
1099.46	C-N - Stretching

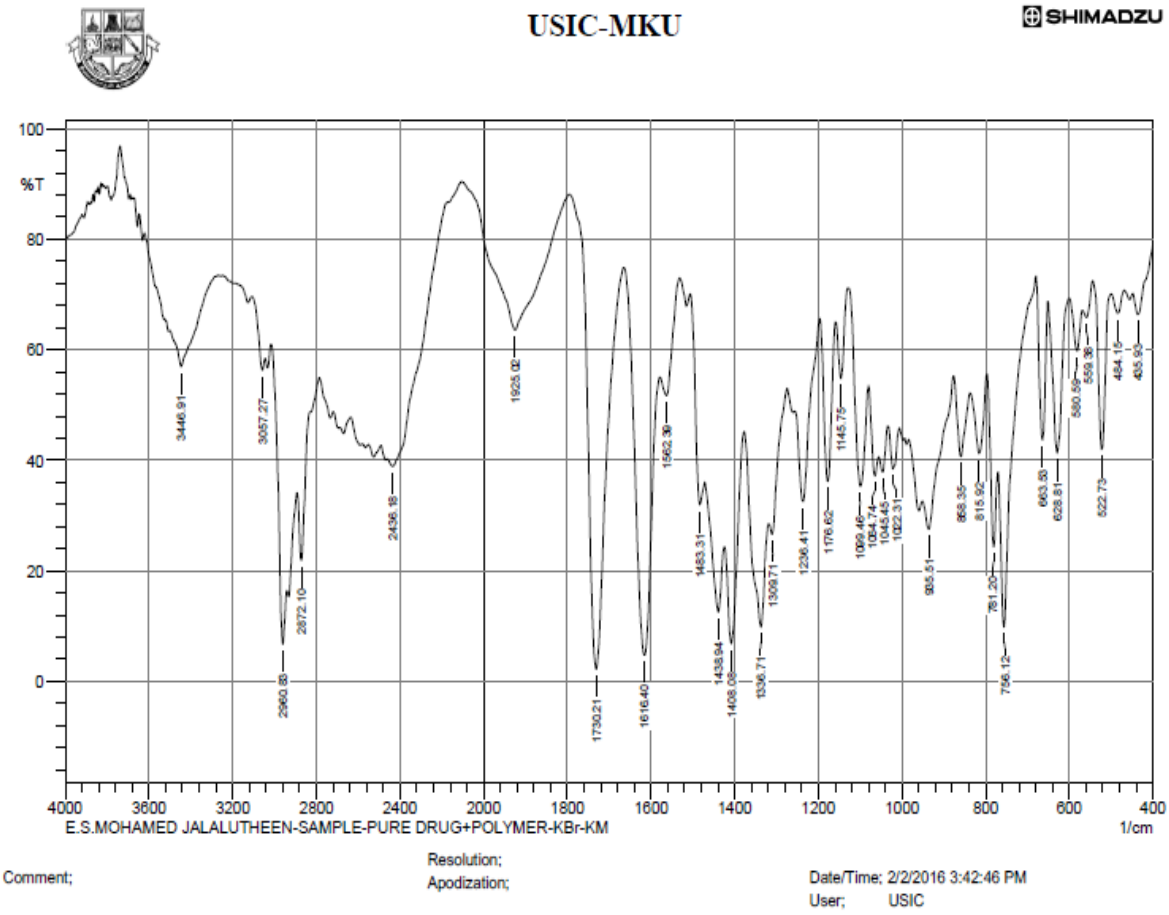


TABLE 5: I.R SPCTERA DATA FOR PHYSICAL MIXTURE

Wave no.(cm^{-1})	Group Assigned
1734.01	C=O – Stretching
1409.06	C=C – Stretching
1099.43	C-N - Stretching

REPORT:

In FTIR spectra the peaks of physical mixture was compared with the original spectra. Same peaks were observed, indicates no possible molecular interaction between the drug and the polymer.

ENTRAPMENT EFFICIENCY OF NANOPARTICLES[31, 72]

The entrapment efficiency of Irbesartan nanoparticles was prepared by nano precipitation method. The formulation F1(Irbesartan 50 mg with 25 mg of Polyvinyl alcohol and β -cyclodextrin) shows less entrapment value of 60.16% this may be the due to repulsive force between drug and the polymer.

Table No: 7 Entrapment efficiency of Irbesartan nanoparticles

Formulation code	Drug (mg)	Poly vinyl alohol (mg)	β cyclodextrin (mg)	Ethanol	Entrapment Efficiency (%)
F1	50	25	5	2%	60.16±0.14
F2	50	50	5	2%	64.15±0.17
F3	50	75	5	2%	68.28±0.15
F4	50	100	5	2%	71.12±0.09
F5	50	25	10	2%	88.23±0.12
F6	50	50	10	2%	94.26±0.18
F7	50	75	10	2%	99.38±0.08
F8	50	100	10	2%	87.42±0.09
F9	50	25	15	2%	85.35±0.06
F10	50	50	15	2%	82.25±0.04

In formulation F2 Polymer concentration was increased (Irbesartan 50 mg with 50mg of Polyvinyl alcohol and 5 mg β -cyclodextrin) the entrapment efficiency was to 64.15%. Further increase in polymer concentration in formulation F3 (Irbesartan 50 mg with 75 mg of Polyvinyl alcohol and 5mg β -cyclodextrin)entrapment efficiency was 68.28%. Further increase in polymer concentration in formulation F4 (Irbesartan 50 mg with 100 mg of Polyvinyl alcohol and5 mg β -cyclodextrin) entrapment efficiency was 71.12%. Formulation F5, F6, was carried out by same process as like previous formulation but changes in polymer concentration 25mg, 50mg, 50 mg of Irbesartan and10 mg β -cyclodextrin was taken. The entrapment efficiency was found to be F5,88.23% for F6,94 .26% .

Formulation F7 was carried out by increasing the polymer concentration same (Irbesartan 50 mg with 75 mg of Polyvinyl alcohol and 10 mg β -cyclodextrin) the entrapment efficiency was increased to 99.38%.

Formulation F8 was carried out by increasing the concentration (Irbesartan 50 mg with 100 mg of Polyvinyl alcohol and 10 mg β -cyclodextrin) which give the percentage of entrapment efficiency was 87.42% but In F8 the *invitro* release of drug shows less than F7 formulation. So F7 formulation is optimized and further study was carried out.

Further formulation F9 and F10 was carried out in same process, drug and polymer concentration (Irbesartan 50 mg with 25 and 50mg of Polyvinyl alcohol and 15 mg β -cyclodextrin) the Entrapment efficiency is F9 85.35%, F10 82.25% From the above result formulation F7 shows highest percentage of entrapment efficiency of 99.38%. So hence this formulation was optimized and further study was carried out.

In F1,F2,F3, F4 formulations, when increasing the polymer concentration the entrapment efficiency is not satisfactory limit. Nanoparticle using 5 mg β -cyclodextrin showed lower entrapment.

So further increasing the concentration of β –cyclodextrin in F5, F6 and F7 formulations. (Irbesartan 50 mg with 25 mg 50mg and 75 mg of Polyvinyl alcohol and 10 mg β -cyclodextrin). In this formulations the entrapment efficiency was F5 for 88.23% ,F6 for 94.26% and F7 for 99.38%.In this the optimum entrapment efficiency obtained in F7.

Further increase the concentration of β –cyclodextrin in formulation F8,F9 and F10. The entrapment efficiency also decreased.

IN VITRO DRUG RELEASE PROFILE OF NANOPARTICLES

- The *in-vitro* drug release of Irbesartan nanoparticles can be carried out by membrane diffusion method for 24 hrs.
- The *in-vitro* drug release of Irbesartan nanoparticles with Polyvinyl alcohol and β –cyclodextrin.
- The *in-vitro* drug release of formulation F1 (Irbesartan 50 mg with 25mg of Polyvinyl alcohol and 5 mg β -cyclodextrin) The percentage of *in vitro* drug release was 97% in 9 hrs.
- The formulation F2 was carried out by the increasing the polymer concentration (Irbesartan 50 mg with 50mg of Polyvinyl alcohol and 5 mg β -cyclodextrin) The percentage of *in vitro* drug release was found to be 96.40% in 11 hrs.
- The formulation F3 was carried out by further increasing in polymer concentration (Irbesartan 50 mg with 75 mg of Polyvinyl alcohol and 5 mg β -cyclodextrin) The percentage of drug release was found to be 98.44% in 13 hrs.
- The formulation F4 was carried out by further increasing in polymer concentration (Irbesartan 50 mg with 100mg of Polyvinyl alcohol and 5 mg β -cyclodextrin). The percentage drug release found to be 96.2% in 16 hrs.
- The formulation F5 was carried out by further increasing in polymer concentration (Irbesartan 50 mg with 25 mg of Polyvinyl alcohol and 10 mg β -cyclodextrin). The percentage drug release was found to be 98.0% in 19 hrs.
- The formulation F6 was carried out by further increased in polymer concentration (Irbesartan 50 mg with 50mg of Polyvinyl alcohol and 10 mg β -cyclodextrin).The percentage drug release was found to be 94.42% in 24 hrs.
- The formulation F7 was carried out by combination of (Irbesartan 50 mg with 75mg of Polyvinyl alcohol and 10 mg β -cyclodextrin). The percentage of drug release was found to be 98.46% in 24 hrs.
- The formulation F8 was carried out by the combination of increasing the polymer concentration of (Irbesartan 50 mg with 100mg of Polyvinyl alcohol and 5 mg β -cyclodextrin) percentage drug release was found to be 88% in 24 hrs.
- The formulation F9 was carried out by the combination of increased polymer concentration (Irbesartan 50 mg with 25 mg of Polyvinyl alcohol and 15 mg β -cyclodextrin) percentage of drug release was found to be 95% 14 hrs.

- The formulation F10 was carried out by the combination of increased polymer concentration (Irbesartan 50 mg with 50mg of Polyvinyl alcohol and 15 mg β - cyclodextrin) percentage of drug release was found to be 96.4% 17 hrs.
- From the above formulation (F1-F10) confirms that the percentage of drug release was satisfactory in formulation F7 and it shows higher percentage of drug release of 98% for 24 hrs. So it was selected as a optimized formulation.

When increasing the polymer concentration the *in vitro* drug release also increased to a certain extent in the drug and polymer ratio up to 1:1.5

Further the polymer concentration is increased in F8 formulation the *in vitro* drug release increased but not extend upto 24hrs. So F7 was selected as a optimized formulation.

Table: 9. *In vitro* drug release for formulation F-I

Time(in Hrs)	Cumulative amount of drug release	Cumulative % amount of drug release.
1	9.2	18.41
2	12.4	24.8
3	18.5	37
4	22.3	44.6
5	28.2	56.4
6	34.1	68.2
7	38.3	76.6
8	42.5	85
9	48.5	97

INVITRO DRUG RELEASE FOR FORMULATION F-I

Figure: 12

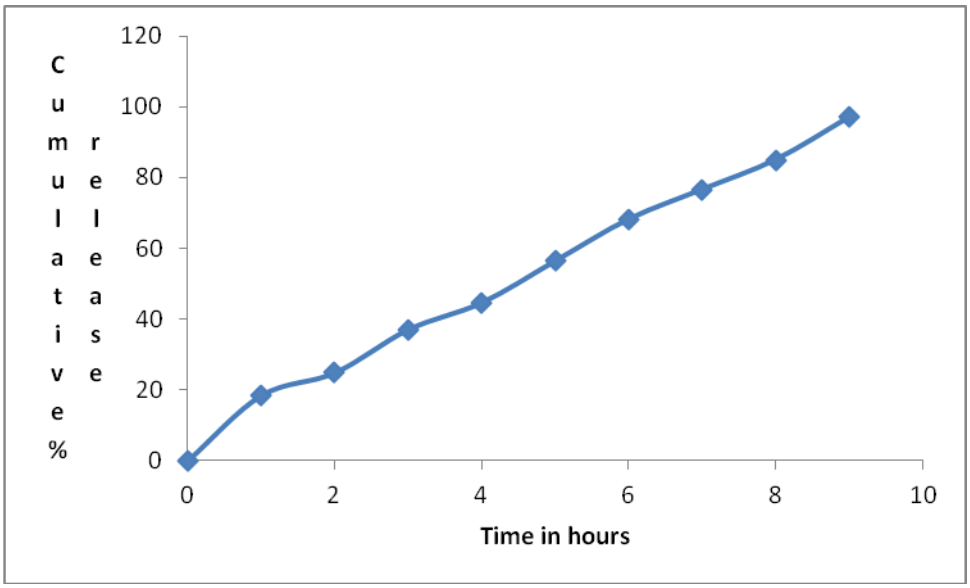


Table: 10. *In vitro* drug release for formulation F-II

Time(in Hrs)	Cumulative amount of drug release	Cumulative % amount of drug release.
1	16	32
2	19.08	38.16
3	20.09	40.18
4	23.10	46.20
5	26.11	52.22
6	33.13	66.26
7	36.16	72.33
8	40.18	80.36
9	45.02	90.04
10	47.2	94.5
11	48.22	96.40

INVITRO DRUG RELEASE FOR FORMULATION F-II

Figure: 13

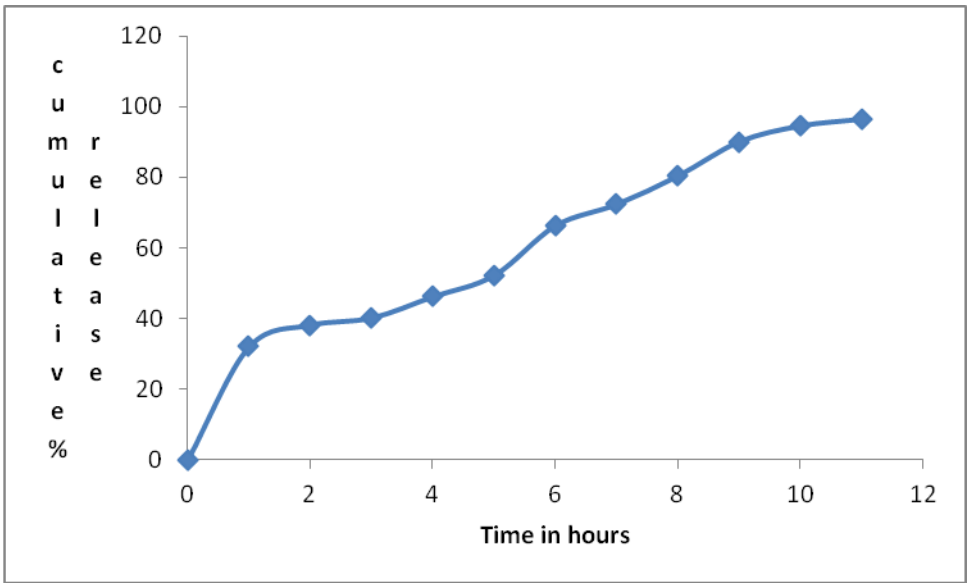


Table: 11. ***In vitro*** **drug release for formulation F-III**

Time (in Hrs)	Cumulative Amount of drug release	Cumulative % amount of drug release
1	10.03	20.6
2	13.05	26.1
3	15.06	30.12
4	18.07	36.14
5	19.09	38.18
6	23.09	46.18
7	28.11	56.22
8	29.14	58.28
9	33.14	66.28
10	36.16	72.32
11	40.18	80.36
12	44.2	88.4
13	49.22	98.44

INVITRO DRUG RELEASE FOR FORMULATION –F-III

Figure: 14

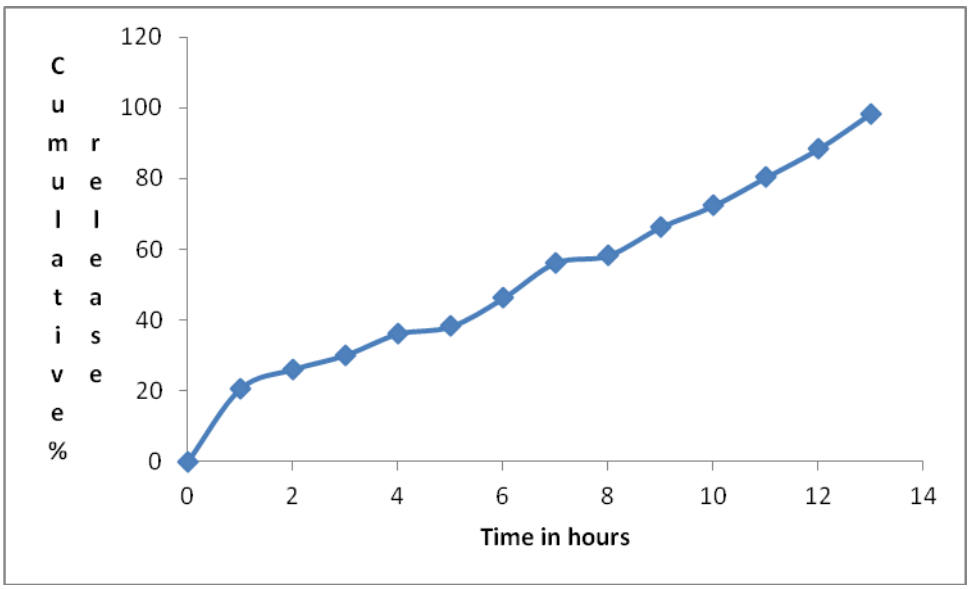


Table: 12. *In vitro* drug release for formulation F-IV

Time (in Hrs)	Cumulative amount of drug release	Cumulative % amount of drug release.
1	7.2	14.4
2	8.5	17.0
3	11.2	22.4
4	15.5	31.0
5	16.8	33.6
6	17.2	34.4
7	19.8	39.6
8	21.3	42.6
9	27.2	54.4
10	30.6	61.2
11	33.5	67.0
12	36.4	72.8
13	38.2	76.4
14	41.3	82.6
15	44.5	89.0
16	48.1	96.2

INVITRO DRUG RELEASE FOR FORMULATION F-IV

Figure: 15

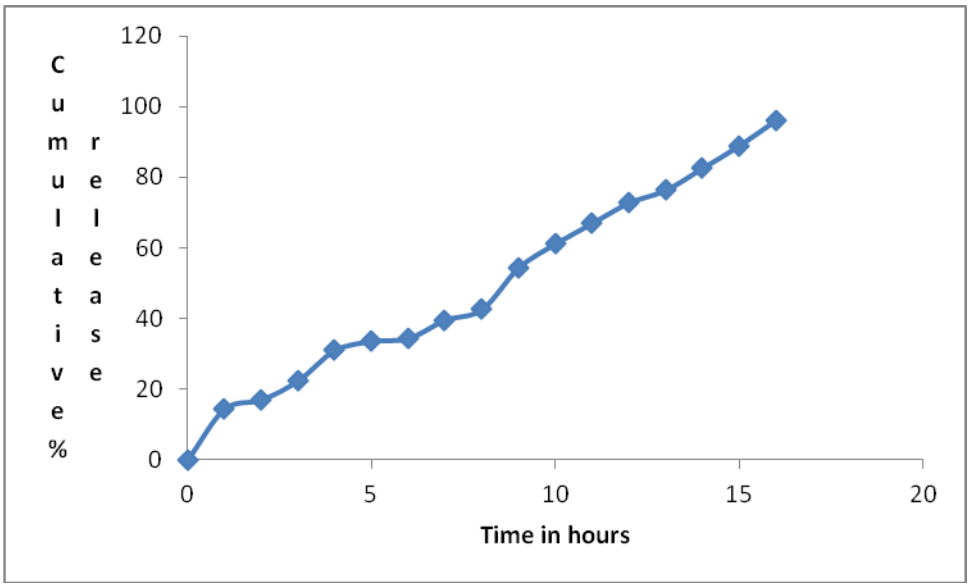


Table.13. *In vitro* drug release for formulation F-V

Time (in Hrs)	Cumulative amount of drug release	Cumulative % amount of drug release.
1	9	18
2	10.04	20.08
3	12.05	24.10
4	14.06	28.12
5	17.07	34.14
6	18.08	36.16
7	20.09	40.20
8	22.10	44.22
9	25.11	50.25
10	26.12	52.26
11	28.13	56.26
12	31.14	62.28
13	32.15	64.32
14	34.16	68.32
15	37.17	74.36
16	40.18	80.36
17	44.19	88.38
18	47.20	94.40
19	49.0	98.0

INVITRO DRUG RELEASE FOR FORMULATION F-V

Figure: 16

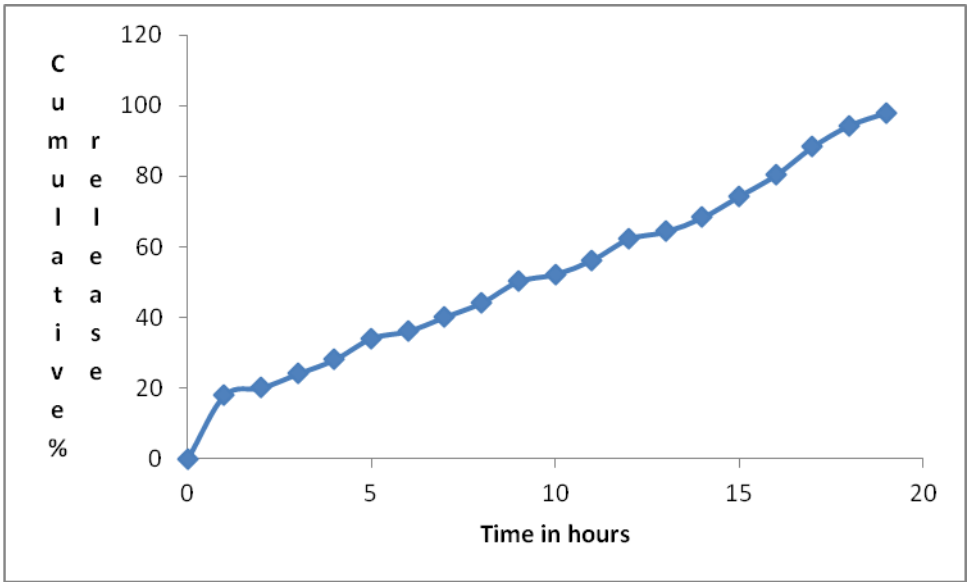


Table: **14.** ***In vitro*** **drug release for formulation F-VI**

Time (in Hrs)	Cumulative amount of drug release	Cumulative %amount of drug release
1	4.8	8.16
2	5.7	10.14
3	7.2	14.4
4	8.8	17.6
5	10.2	20.4
6	11.6	23.2
7	13.4	26.8
8	14.8	29.6
9	16.3	32.6
10	17.9	35.8
11	19.4	38.8
12	21.2	42.4
13	23.4	46.8
14	25.2	50.4
15	26.8	53.6
16	29.2	58.4
17	32.1	64.2
18	34.4	68.8
19	36.6	73.2
20	38.9	77.8
21	42.1	84.2
22	44.5	89
23	46.22	92.44
24	47.21	94.42

INVITRO DRUG RELEASE FOR FORMULATION –F-VI

Figure-17

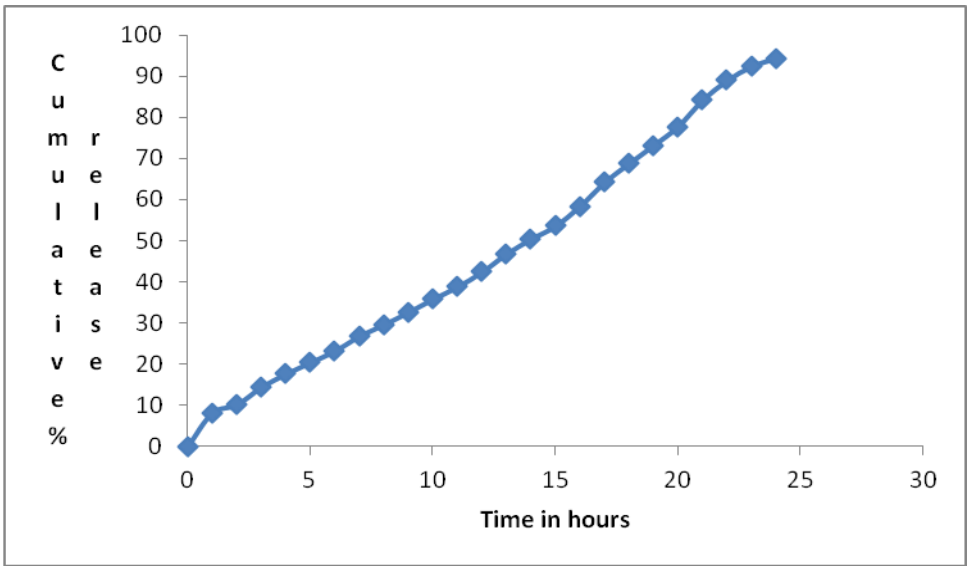


Table: 15. *In vitro* drug release for formulation F-VII

Time (in Hrs)	Cumulative amount of drug release	Cumulative % amount of drug release
1	2	4
2	4.01	8.02
3	6.02	12.04
4	8.03	16.06
5	10.04	20.08
6	12.05	24.1
7	14.06	28.12
8	16.07	32.14
9	18.08	36.16
10	20.09	40.18
11	22.1	44.2
12	24.11	48.22
13	26.12	52.4
14	29.13	58.26
15	31.14	62.29
16	33.15	66.31
17	35.16	70.33
18	37.17	74.35
19	39.18	78.37
20	41.19	82.39
21	43.20	86.41
22	45.21	90.43
23	47.22	94.45
24	49.23	98.46

INVITRO DRUG RELEASE FOR FORMULATION F-VII

Figure: 18

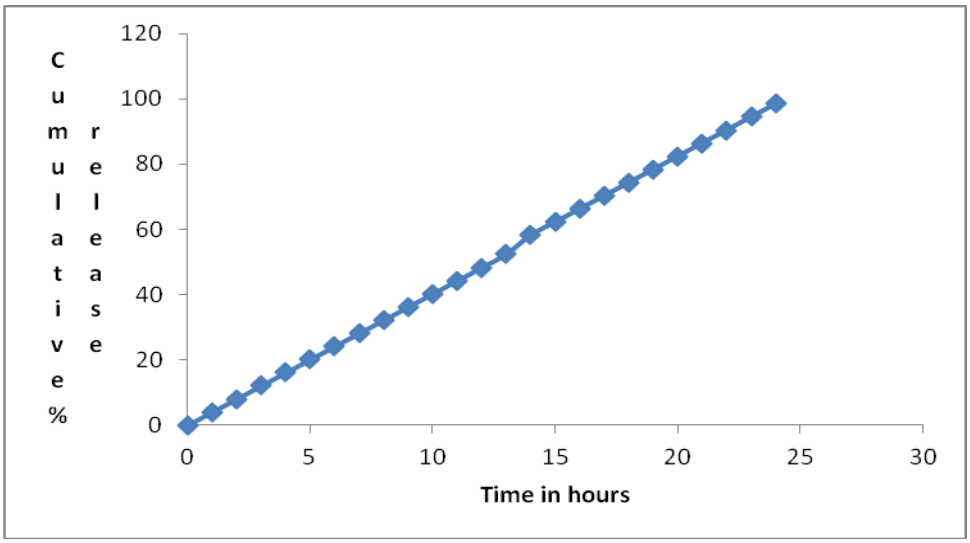


Table: 16. *In vitro* drug release for formulation F-VIII

Time (in Hrs)	Cumulative amount of drug release	Cumulative %amount of drug release
1	8.9	17.08
2	9.8	19.06
3	12.0	24
4	13.5	27
5	15.9	31.8
6	16.8	33.6
7	18.4	36.8
8	20.3	40.6
9	22.2	44.4
10	24.4	48.8
11	25.2	50.4
12	26.7	53.4
13	28.1	56.2
14	29.7	59.4
15	31.0	62
16	32.2	64.4
17	33.5	67
18	35.1	70.2
19	36.6	73.2
20	38.2	76.4
21	39.4	78.8
22	41.1	82.2
23	42.5	85
24	44	88

INVITRO DRUG RELEASE FOR FORMULATION F-VIII

Figure: 19

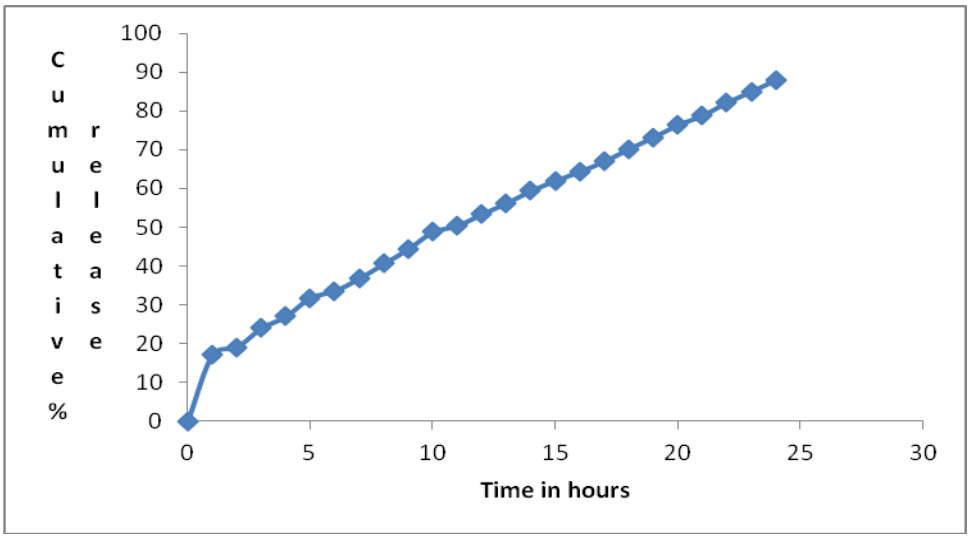


Table: 17. ***In vitro*** **drug release for formulation F-IX**

Time (in Hrs)	Cumulative amount of drug release	Cumulative % amount of drug release
1	8.6	17.2
2	11.2	22.4
3	13.3	23.6
4	15.8	31.6
5	17.2	34.4
6	21.1	42.2
7	23.9	47.8
8	27.3	54.6
9	30.8	61.6
10	34.2	68.4
11	37.1	74.2
12	41.8	83.6
13	44.2	88.4
14	47.5	95

INVITRO DRUG RELEASE FOR FORMULATION F-IX

Figure: 20

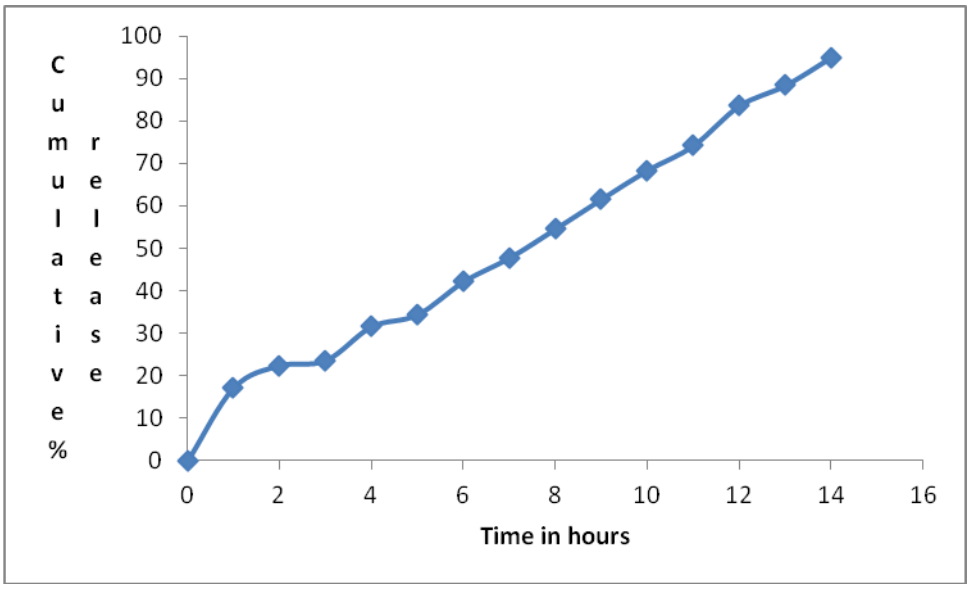
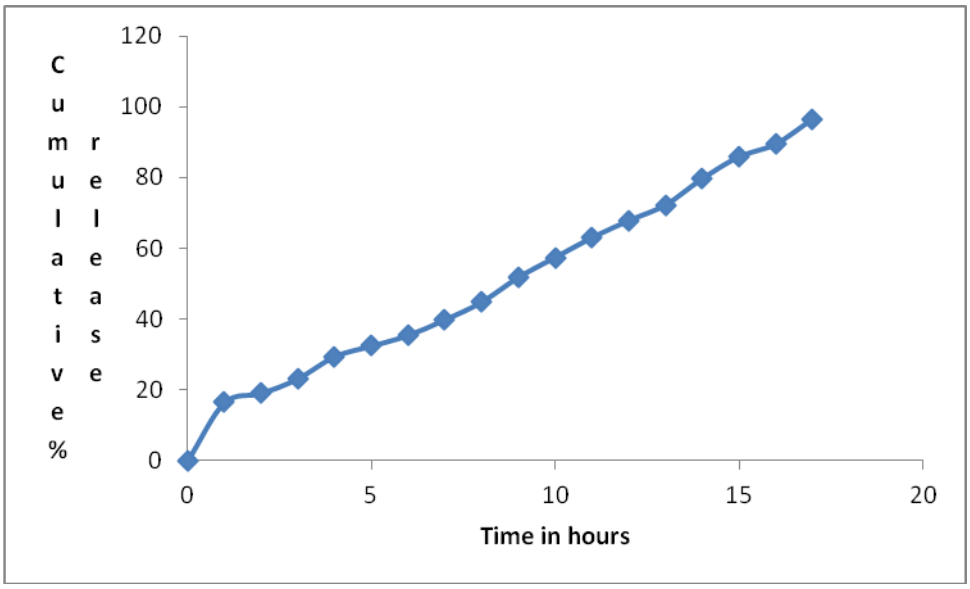


Table: 18. *In vitro* drug release for formulation F-X

Time(in Hrs)	Cumulative amount of drug release	Cumulative %amount of drug release
1	8.2	16.4
2	9.5	19
3	11.6	23.2
4	14.7	29.4
5	16.2	32.4
6	17.8	35.4
7	19.9	39.8
8	22.4	44.8
9	25.9	51.8
10	28.8	57.4
11	31.5	63
12	33.9	67.8
13	36.1	72.2
14	39.8	79.6
15	42.9	85.8
16	44.8	89.4
17	48.2	96.4

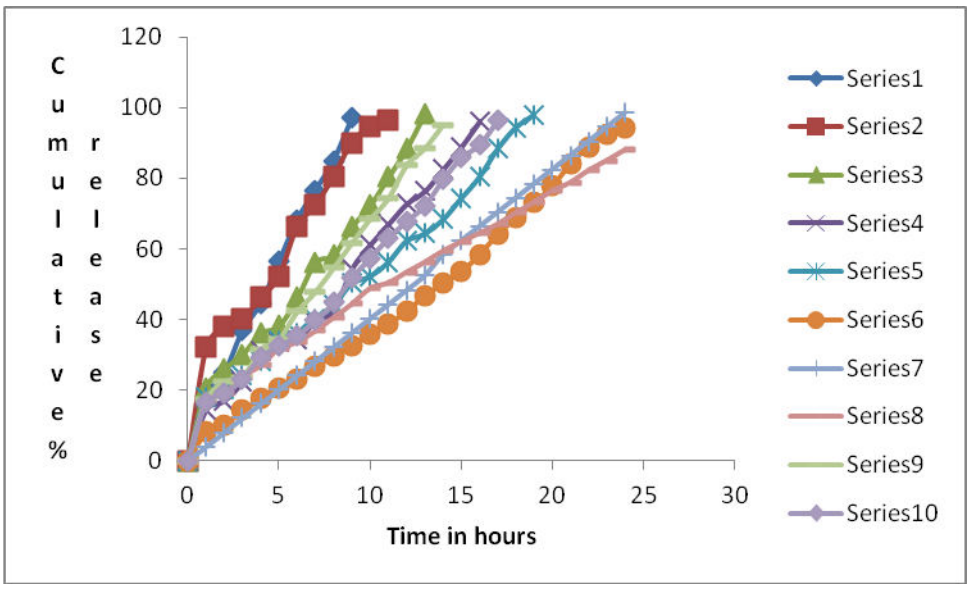
INVITRO DRUG RELEASE FOR FORMULATION F-X

Figure: 21



COMPARITIVE INVITRO RELEASE STUDY OF IRBESARTAN NANOPARTICLE FORMULATIONS F1 TO F10

Figure: 22



6.5. SCANNING ELECTRON MICROSCOPY [11, 26]

The surface characteristics of optimized formulation (F7) particle size were studied by scanning electron microscope.SEM image of prepared nanoparticle formulation shows the coating of polymer mixture on drug particles. The appearance of nanoparticles in scanning electron microscope is in granule form, which indicates a thin and uniform coating over the drug. SEM image revealed that the Irbesartan nanoparticles were in nano size range,and smooth spherical in shape in this F7 Formulation.

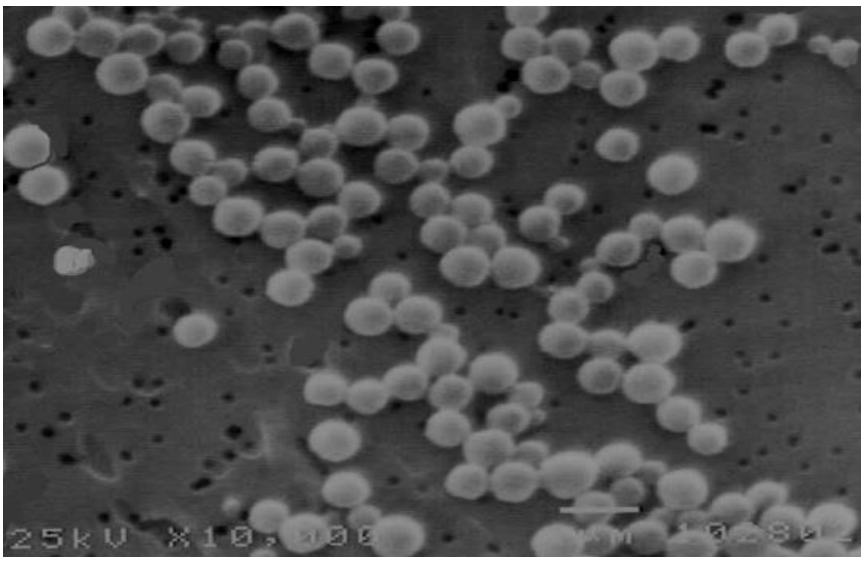


Fig .No:12 SEM IMAGE OF F7

6.6.SURFACE CHARGE (ZETA POTENTIAL)[19,42,25]

The zeta potential of a nanoparticles is commonly used to characterize the surface charges property of nanoparticles. It reflects the electrical potential of particles influenced by the composition of the particles band the medium in which it is dispersed. When nanoparticles formulations are administered through intravenous route they are easily identified and detected by the phagocytes. The particle size and the hydrophobicity surface of the nanoparticles determine the adsorption of blood components (proteins) called as opsonins. The opsonin in turn decides the fate of the nanoparticles. Binding of these opsonins on to the surface is known as Opsoniazation. Non modified nanoparticles were rapidly opsoniazed and gets easily eliminated from the body. Hence, to increased minimize the opsoniazation and to prolong the circulation of nanoparticles *invivo*.

The zeta potential of the nanoparticle formulation with poly vinyl alcohol (formulation F7) particles which present in the formulation are de-aggregated and remain same and more stable in the substance and zeta potential (mV) is 38 mv and zeta Deviation (mV) is 9.16 and conductivity (mS/cm) is 0.899.So this polymer is more suitable for nanoparticles preparation and the result shows smooth surface character and efficient repelled action and it decreases the opsoniazation.

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Sample Details

Sample Name: Aj F4 1
SOP Name: mersettings.nano
General Notes:

File Name: tri.dts
Record Number: 3422
Material RI: 1.59
Material Absorption: 0.010
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Monday, November 30, 2015 ...

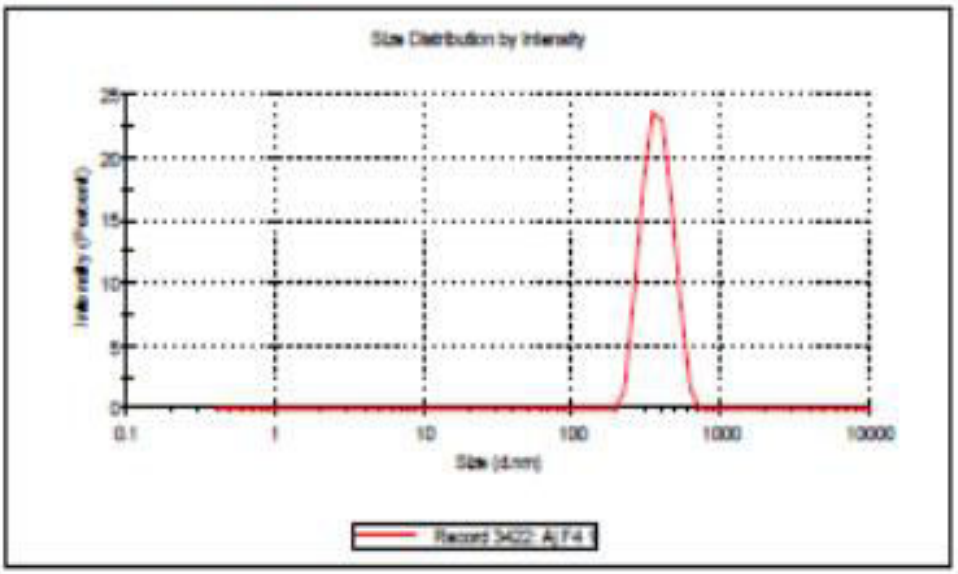
System

Temperature (°C): 25.0
Count Rate (kcps): 98.2
Cell Description: Disposable sizing cuvette
Duration Used (s): 100
Measurement Position (mm): 4.65
Attenuator: 11

Results

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 358.4	Peak 1:	375.1	100.0	83.80
PdI: 0.013	Peak 2:	0.000	0.0	0.000
Intercept: 0.777	Peak 3:	0.000	0.0	0.000

Result quality : Good



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Sample Details

Sample Name: Aj F4 Z 1
SOP Name: mansettings.nano
General Notes:

File Name: tril.dts
Record Number: 3423
Date and Time: Monday, November 30, 2015 4:5...
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

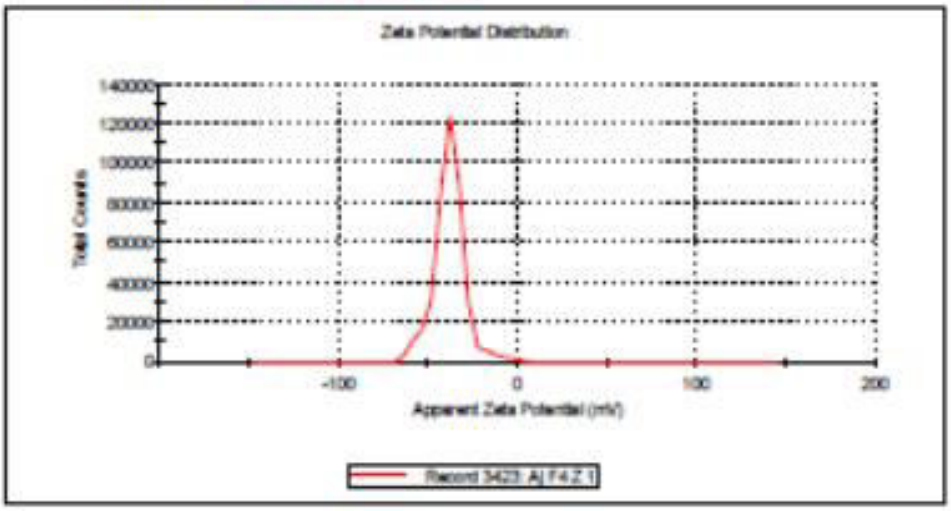
System

Temperature (°C): 25.0
Count Rate (kcps): 17.2
Cell Description: Zeta dip cell
Zeta Runs: 12
Measurement Position (mm): 4.50
Attenuator: 11

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -38.0	Peak 1:	-38.0	100.0	9.16
Zeta Deviation (mV): 9.16	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.899	Peak 3:	0.00	0.0	0.00

Result quality : See result quality report



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Zetasizer Ver. 7.11
Serial Number : MAL1180482

File name: tril
Record Number: 3423
© Dec 2015 4:55:30 PM

6.7. STABILITY STUDIES OF IRBESARTAN NANOPARTICLES[75]:

The stability studies of optimized nanoparticle formulation F7 was carried our for 3 months. The test was performed in three conditions 4° C, Room temperature and 45°C/70% RH. At the time interval of one month the nanoparticle formulation were evaluated for entrapment efficiency. The stability of nanoparticles formulation was more stable in refrigerator (4°C) when compared to room temperature and at (45°C/70% RH)

Table: 18 Stability studies for Irbesartan nanoparticle

S.NO	Storage Condition	Test parameters	1st month	2th month	3rd month
1	4°C	pH	7.5	7.5	7.5
		colour	Clear& colour less	Clear& colour less	Clear & colour less
		stability	97.46	96.47	95.46
2	Room Temperature	pH	7.4	7.4	7.3
		colour	Clear & colour less	Clear & colour less	Clear & colour less
		stability	93.41	92.42	91.42
3	Acceleration condition at 45°C/70% RH	pH	7.4	7.3	7.3
		Colour	Clear & colourless	Clear& colourless	Clear& colourless
		Stability	90.42	88.24	86.41

Table: 19 In vitro release for optimized formulation (F7) stability study at 4°C

Time(in Hrs)	Cumulative % amount of drug release		
	1st Month	2nd Month	3rd Month
1	4	3	3
2	7.02	7.03	6.01
3	11.03	11.05	10.03
4	14.05	14.07	13.05
5	19.07	19.09	18.07
6	23.09	22.09	20.09
7	26.11	25.11	24.11
8	31.13	30.13	29.14
9	35.15	34.16	34.17
10	39.17	38.18	37.18
11	43.18	43.21	41.20
12	47.21	46.22	45.22
13	52.23	51.25	50.25
14	56.26	55.27	55.27
15	61.28	60.28	58.29
16	65.31	64.31	64.31
17	69.32	68.33	67.33
18	73.34	72.36	71.35
19	78.36	77.38	77.36
20	81.38	80.41	79.39
21	85.40	84.42	83.41
22	88.41	88.44	87.43
23	93.45	93.45	92.44
24	97.46	96.47	95.46

Fig.No.13: STABILITY STUDY RELEASE DATA FOR FORMULATION (F7) AT $4^{O}C$

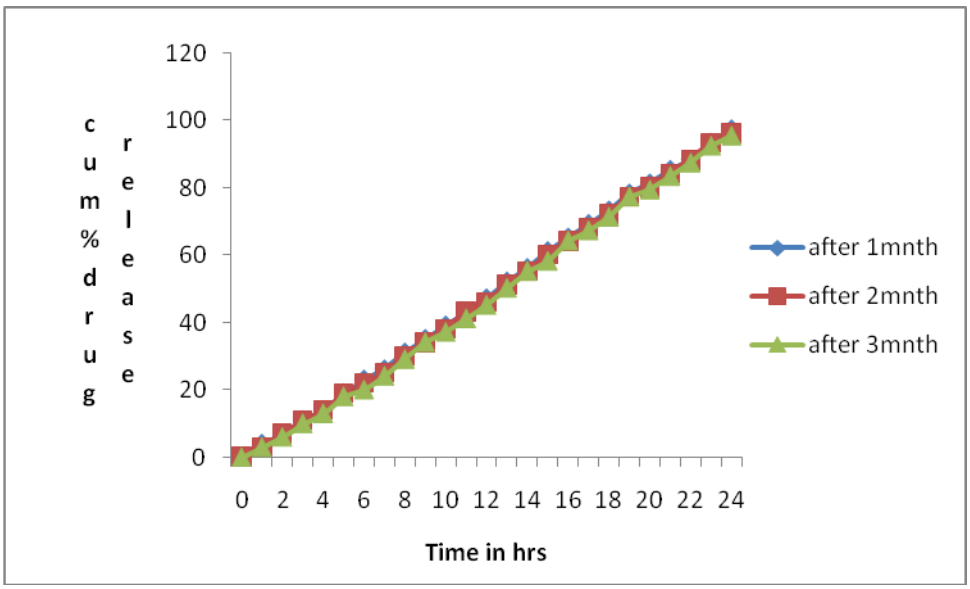


Table: 20 In vitro data for optimized formulation (F7) stability study at room temperature

Time(in Hrs)	Cumulative % amount of drug release		
	1st Month	2nd Month	3rd Month
1	3	3	3
2	6.02	6.02	6.04
3	10.04	10.04	11.55
4	13.05	12.05	13.07
5	17.06	16.07	15.1
6	20.07	19.10	18.11
7	23.09	23.51	22.63
8	29.11	28.32	26.15
9	34.13	33.15	32.17
10	36.15	36.17	34.18
11	41.17	41.18	38.20
12	44.19	44.20	42.23
13	50.21	50.23	46.24
14	54.23	54.24	50.26
15	58.25	58.26	53.28
16	64.27	64.28	57.3
17	67.29	67.30	63.82
18	70.31	70.32	68.34
19	76.33	76.33	72.86
20	77.34	77.86	76.36
21	82.35	82.37	81.37
22	84.37	85.37	84.41
23	88.38	89.41	87.24
24	93.41	92.42	91.42

Fig.No.14: STABILITY STUDY RELEASE DATA FOR FORMULATION (F7) AT ROOM TEMPERATURE

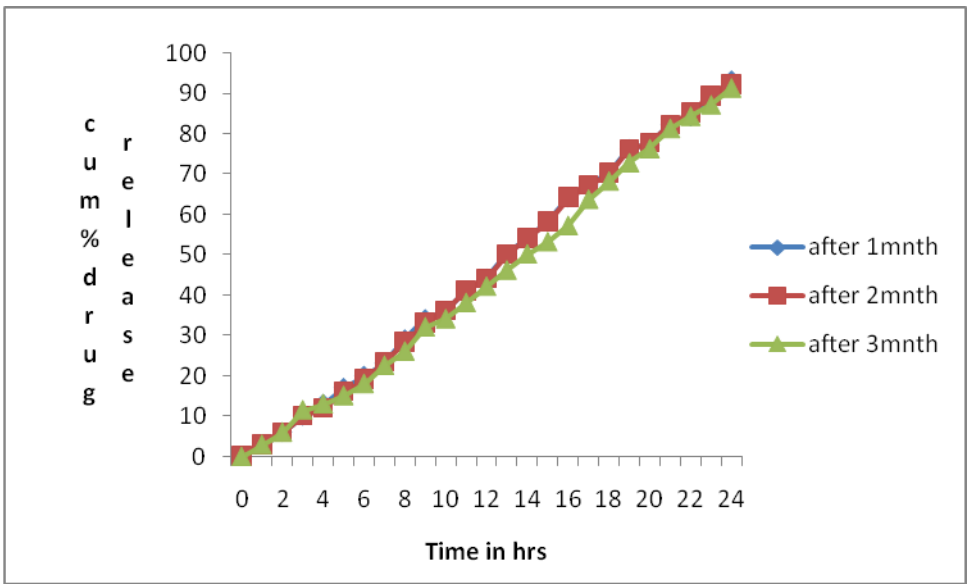
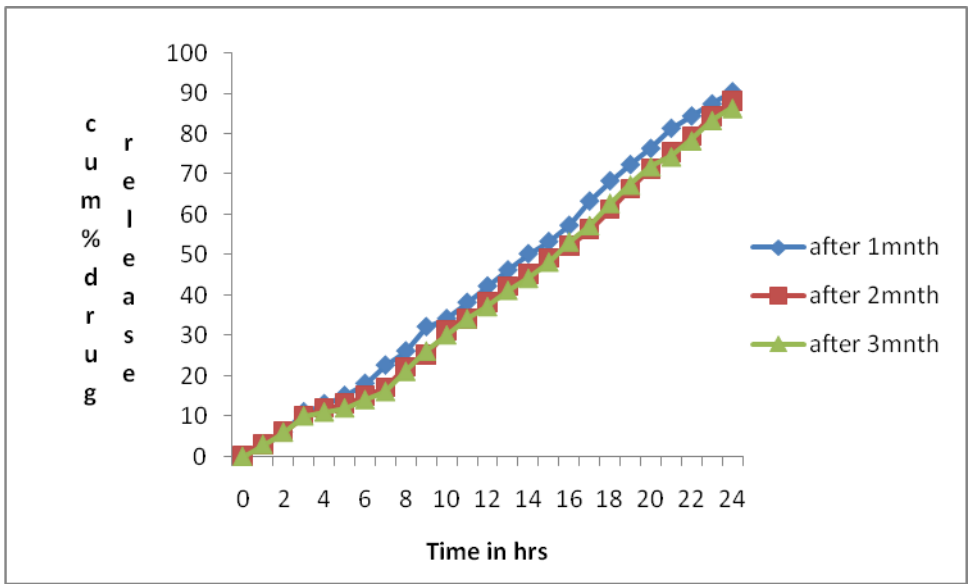


Table: 21 In vitro data for optimized formulation (F7) stability study at 45°c/75%RH

Time(in Hrs)	Cumulative % amount of drug release		
	1st Month	2nd Month	3rd Month
1	3	3	3
2	6.04	6.04	6.04
3	11.05	10.05	10.05
4	13.07	12.06	11.06
5	15.1	13.07	12.07
6	18.11	15.1	14.1
7	22.63	17.11	16.11
8	26.15	22.13	21.13
9	32.17	25.15	26.15
10	34.18	31.17	30.17
11	38.20	34.18	34.18
12	42.23	38.20	37.20
13	46.24	42.23	41.23
14	50.26	45.24	44.24
15	53.28	49.26	48.26
16	57.3	52.28	53.28
17	63.32	56.3	57.30
18	68.34	61.32	62.82
19	72.36	66.34	67.34
20	76.36	71.36	71.86
21	81.37	75.36	74.36
22	84.41	79.37	78.37
23	87.41	84.41	83.40
24	90.42	88.24	86.41

Fig.No.15: STABILITY STUDY RELEASE DATA FOR FORMULATION (F7) AT 45°C/75% RH



Kinetics of drug release for optimized formulation F7

The optimized formulation F7 was introduced in to graphical treatment for kinetic of drug release.

IN VITRO DRUG RELEASE FORMULATION F7

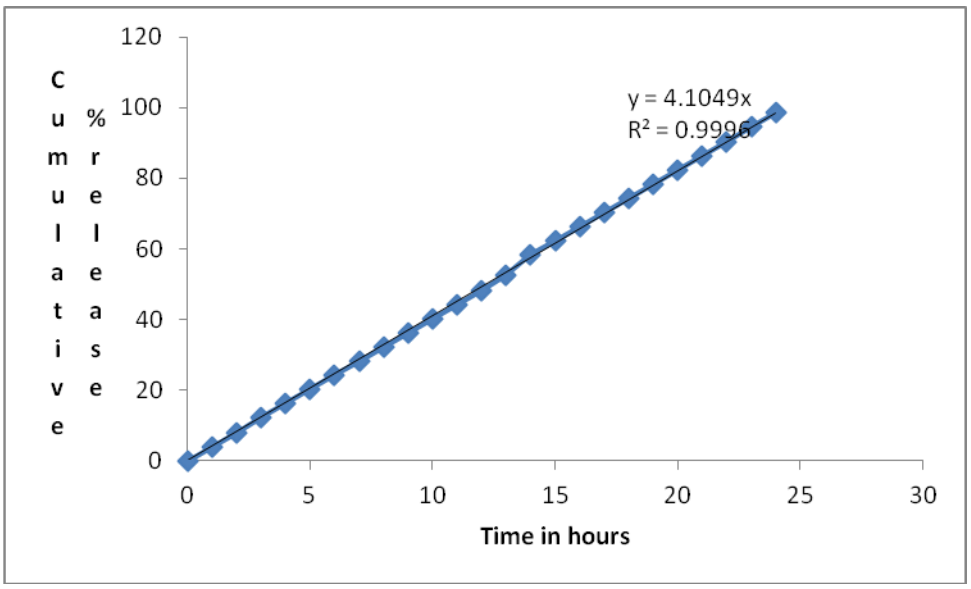


Fig.No.16: Zero order plot for formulation F7

Regression=0.999

The optimized formulation F7 of nanoparticles is more suitable for parentral administration it shows good *invitro* release kinetic study. The zero order plots were obtained by plotting cumulative percentage drug release versus time. The regression value is 0.999.

HIGUCHI 'S PLOT:

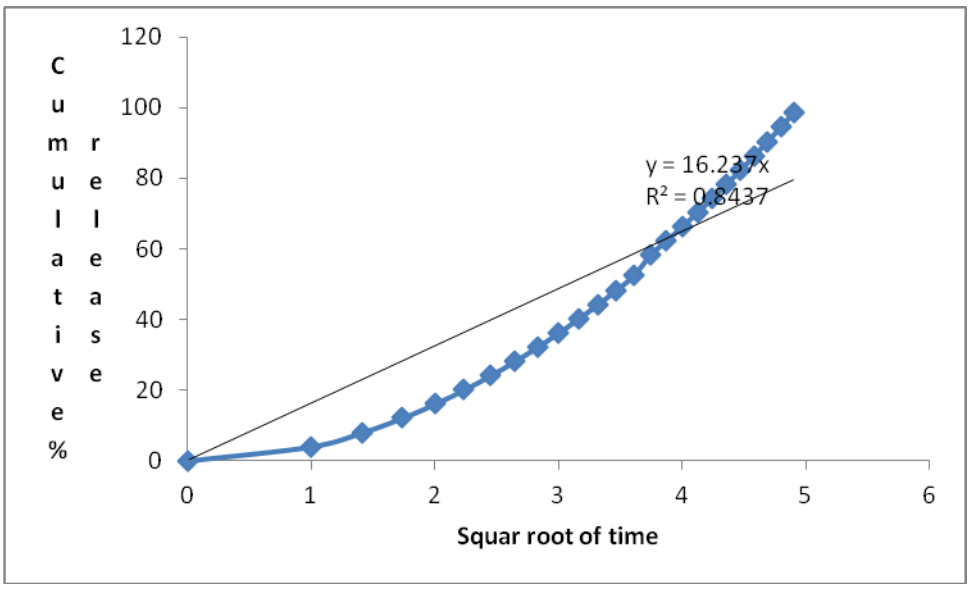


Fig.No.17: HIGUCHI' S PLOT FOR FORMULATION F7

Regression=0.843

Higuchi plot was made by plotting cumulative percentage % drug release against square root of time. The regression value was found to be 0.843.This indicates that diffusion is one of the mechanism of drug release.

KORSEMEYER PLOT:

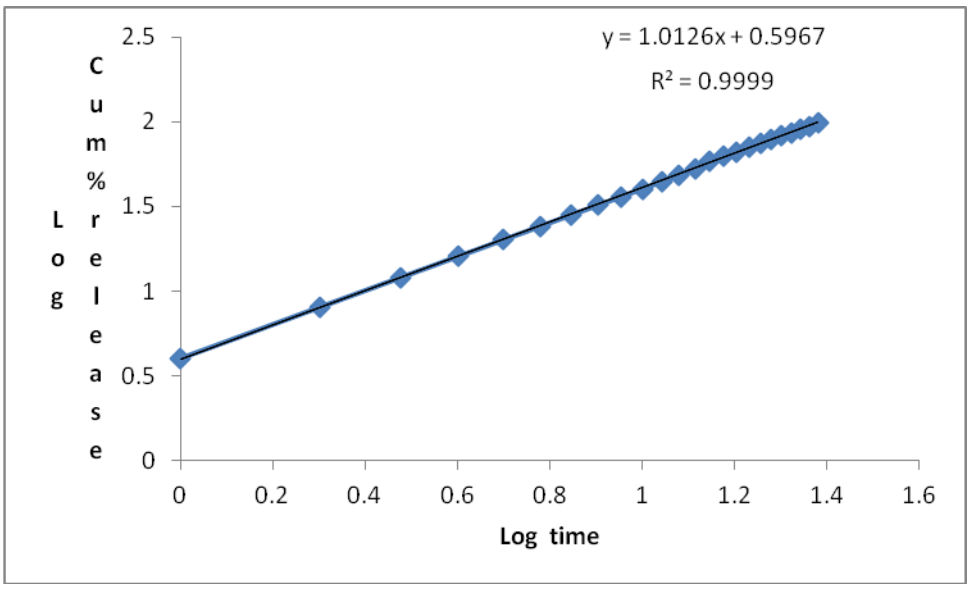


Fig.No.18: KORSEMEYER'S PLOT FORMULATION F7

The graph was plotted between log cumulative % of drug release and log time. The n' value was found to be 1.0126 indicated may nonfickian diffusion mediated.

Kinetic of drug release of first order for optimized formulation F7

The optimized formulation F7 was introduced into graphical treatment for kinetics of drug release.

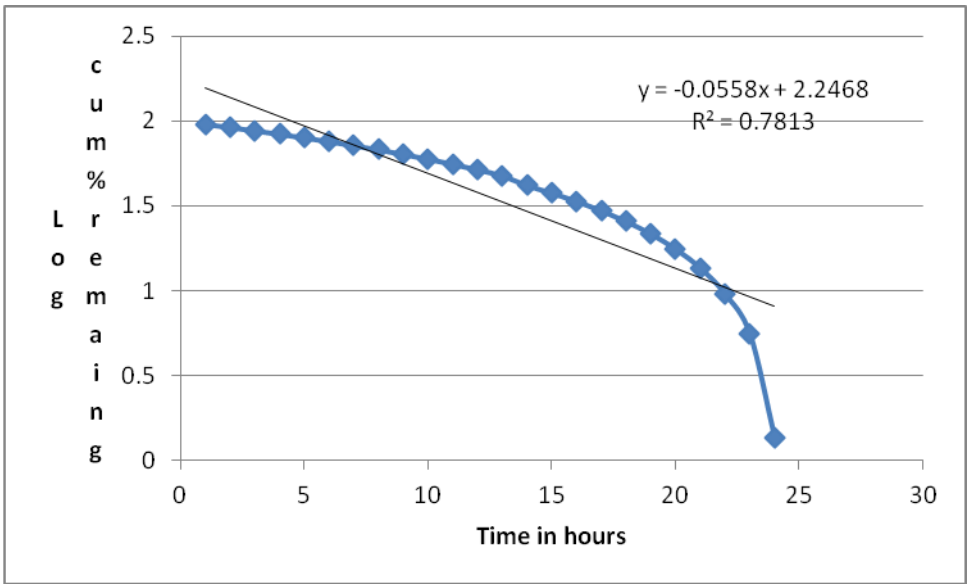


Fig.No.19: First order plot for formulation F7

Regression = 0.7813

The optimized formulation F7 of nanoparticles is more suitable for parentral administration it shows the *invitro* release kinetic study. The first order plots were obtained by plotting log remaining cumulative percentage drug release versus time. The regression value is 0.7813.

7. SUMMARY AND CONCLUSION

The present study was aimed to develop a nanoparticulate drug delivery system of antihypertensive drug irbesartan using polymer (poly vinyl alcohol).The polymer enhances the binding of irbesartan nanoparticles in specific or targeted site with sustained release of drug increasing therapeutic efficacy. These nanoparticles may also reduce the dose frequency with desired therapeutic response.

All batches of nanoparticles (F1-F10) were prepared by nano precipitation method.

The entrapment efficiency of the optimized formulation F7 (drug 50mg, polyvinyl alcohol 75mg, β –cyclodextin 10 mg) was 99.38 ±0.08 and *invitro* drug release was 98.46% after 24 hours. It also obey the zero order, follows diffusion and erosion mechanism of release.

Surface morphology of optimized formulation (F7) indicated that lrbesartan nanoparticles were found to be in average nanometer range(358.4nm) and showed ideal surface morphology.

The stability test performed revealed that the formulation (F7) showed no change in its characters. The optimized formulation (F7) was also examined for zeta potential determinations.

The formulation(F7) showed maximum deviation of 9.16 mV which demonstrated that the particles are separate and highly repelling property found to be more useful in decreasing opsonization and favors target specificity.

The developed irbesartan nanoparticle formulation increases water solubility, reduces the dose frequency and improves the bioavailability of drug.

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