FORMULATION AND CHARECTERIZATION OF ARTEMISININ NANOPARTICLES

A Dissertation Submitted to

The Tamil Nadu DR. M.G.R. Medical University

Chennai-32

In partial fulfillment for the award of degree of

MASTER OF PHARMACY

IN

BRANCH-I -> PHARMACEUTICS

Submitted by

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THIRUNINRAVUR-602024

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NOVEMBER - 2019

CERTIFICATE

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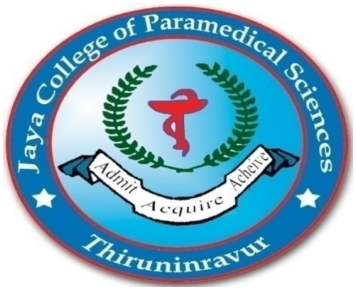

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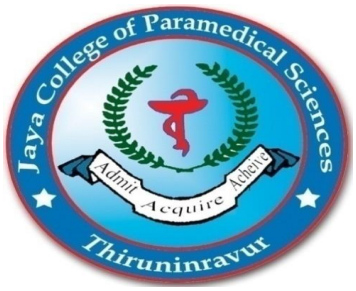

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DECLARATION BY THE CANDIDATE

I hereby declare that the matter embodied in the dissertation entitled "**FORMULATION AND CHARACTERIZATION OF ARTEMISININ NANOPARTICLES**"is a genuine research work carried out by me under the guidance of **Dr.B.SENTHILNATHAN.,M.Pharm.,Ph.D**. HOD, Department of Pharmacy Practice, Jaya College of Paramedical Sciences, College of Pharmacy. The work embodied in the thesis is original and has not been submitted on the basis for the award of diploma, degree associate ship(or) fellowship of any other Institution (or) University.

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EVALUATION CERTIFICATE

This is to certify that the dissertation entitled "**FORMULATION AND CHARACTERIZATION OF ARTEMISININ NANOPARTICLES**" submitted to The Tamilnadu Dr.M.G.R Medical University, Chennai is a bonafide project work of **Ms.V.SHRUTHI(Reg.No-261711363)**carried out in the Department of Pharmaceutics,Jaya college of Paramedical Sciences,College of Pharmacy,Thiruninravur, Chennai-24 in partial fulfillment for the degree of MASTER OF PHARMACY (PHARMACEUTICS) under the guidance of **Dr.B.SENTHILNATHAN,M.Pharm.,Ph.D.,**HOD,Department of Pharmacy Practice, Jaya College of Paramedical Sciences, College of Pharmacy,Thiruninravur Chennai-24

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CONTENTS


Introduction

INTRODUCTION

NANO DRUG DELIVERY SYSTEMS

Nano drug delivery systems represent a promising drug delivery system of controlled and targeted drug release. They are specially designed to release the drug in the vicinity of target tissue. They exist as particulates or globular dispersions with a size in the range of 10-1000nm. In nano drug delivery systems, the drug or active ingredient is dissolved, entrapped, encapsulated, adsorbed or attached to the matrix system.

Nano sized materials have more advantages over other dosage forms with larger particle size, as it can provide more surface area and increased solubility. It also provides the facility to control the drug release along with the ability to deliver the entrapped therapeutic agents to the desired site of action. Nano drug delivery systems are widely investigated for the oral delivery of drugs. Oral delivery is the most preferred route of drug administration due to convenience, patient compliance and cost-effectiveness. This system has been also used to increase bioavailability of drugs having poor bioavailability. However, due to the small size, extensive research was carried out on administration of these systems by various parenteral routes like intravenous, intra muscular and subcutaneous routes. The drugs loaded in nano drug delivery system were also reported to exhibit improved shelf life and stability.[1]

The wide choice of methods of preparation available to formulate these dosage forms facilitates utility of various polymers, inorganic materials, proteins and even lipids. But, the components used for the preparation should be non toxic, biodegradable, non immunogenic and non-inflammatory. The nano drug delivery systems has been reported for incorporation of anticancer, anti-Alzheimeric, Antiparkinson, analgesic, anti-inflammatory, antiviral, anti-infective agents etc.

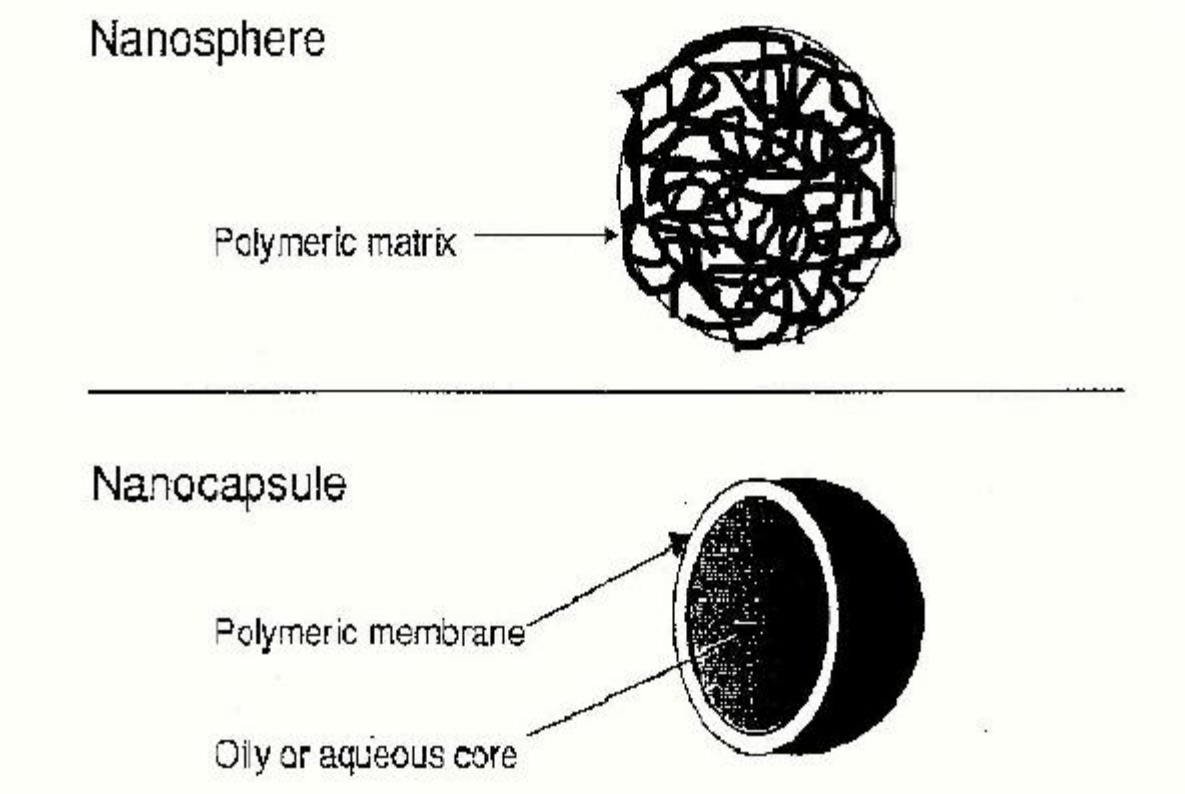


Fig . 1 : Nanosphere and Nanocapsules

MERITS OF NANO DRUG DELIVERY SYSTEMS:

1. They can be enabled with site specific delivery of drugs.

2. Site specific delivery of drugs facilitates enhanced therapeutic response and reduction in adverse effects.

3. Drug degradation by enzymatic action can be prevented.

4. Active and passive targeting of drug can be achieved by manipulating the particle size and surface characteristics.

5. Suitable for incorporation of wide range of therapeutic agents ranging from inorganic, organic, synthetic, semi-synthetic compounds, lipids and proteins.

6. They can be used for administration by oral, nasal, parentral and ocular routes.

7. Controlled release of drug can be achieved

8. Surface modification using ligands can be made to improve the specificity of drug targeting.

DEMERITS :

1. Aggregation of particles due to their small size and large surface area, however, this problem can be overcome by adjustment of surface charge (zeta potential) of the nanoparticles by addition of suitable surface active agents.

2. Difficult to handle in liquid forms (nanosuspensions). Freeze drying (lyophyllisation) had facilitated the maintenance of nanoparticles in dry form with enhanced stability.

3. Due to their ultra small size they have limited drug loading capacity.

4. These are susceptible to bursting and leakage of drugs.

5. Scaling up problems due to the equipments used in the preparation and need for maintenance of uniform ultrafine size.[2]

Polymers used in preparation of Nanoparticles:

The polymers should be compatible with the body in the terms of adaptability (non-toxicity) and (non-antigenicity) and should be biodegradable and biocompatible.

Natural polymers:

- Chitosan
- Gelatin
- Sodium alginate
- Albumin

Synthetic Polymers :

- Polylactides(PLA)
- Polyglycolides(PGA)
- Poly(lactide co-glycolides) (PLGA)
- Poly(N-vinyl pyrrolidone)
- Poly(methyl methacrylate)
- Poly(vinyl alcohol)
- Poly(acrylic acid)
- Poly acrylamide
- Poly(ethylene glycol)
- Poly (methacrylic acid)[3]

TYPES:

Nano drug delivery systems are classified based on the morphological characters, structure, composition as well as the arrangement of drug and polymer in the formulation:

Polymer Nanoparticle

Polymer Nanoparticles are defined as solid, colloidal particles in the range of 10-1000 nm. Polymer Nanoparticle is known as nanosphere and nanocapsules. Different methods like solvent evaporation, salting out, dialysis, supercritical fluid evaporation and rapid expansion of supercritical solution are being used .

Ideal properties of polymeric-based NPs:

1.Particle diameter < 100 nanometers.

2.Stable in blood

3.BBB-targeted (ie, use of cell surface ligands, receptor mediated endocytocis).

4.No activation of neutrophills.

5.No platelet aggregation.

6.Avoidance of the reticuloendothelial system.

7.Non-inflammatory.

8.Prolonged circulation time.

9.Scalable and cost effective with regard to manufacturing process.

10.Amenable to small molecules, peptides, proteins or nucleic acids.[4]

Metallic Nanoparticles

Metal nanoparticle is used to describe nanosized metals with dimension (length, width and thickness) range between 1-100 nm. Nobel metal Nanoparticles with spherical shaped and size, were produced continuously by the chemical reduction methods .

Magnetic Nanoparticles

Magnetic Nanoparticles have been synthesized with number of different compositions and phases including pure metals like CO, Fe and Ni, metal alloys such as FePt, CoPt . Using magnetic nanoparticles particle size of approximately 3 nm can be obtained. The size of particles will be hundreds of atoms which enable us to make recording media which can be achieved by correctly organizing the particles. Various methods are been reported few among them are coprecipitation, sonochemistry, colloidal method, solvothermal, combustion synthesis, hydrothermal method,microemulsion and thermal decomposition methods [5].

Nano suspension and Nano crystal

Nano suspensions refer as the colloidal dispersions of drug particles.A Pharmaceutical nano suspension is delivered as very finely colloid,biphasic,dispersed,solid drug particles in an aqueous vehicle.They have particle size below 1 micron without any matrix material,stabilized by surfactants and polymers,prepared by suitable methods for drug delivery. They were administered via various routes like oral,topical,parenteral.

Nano crystals are defined has the nanoscopic crystals have the particle size less than 1000nm.Nano crystal dispersion contains dispersion media,active ingredients ,surfactants and polymers required for stabilization,The dispersion media can be aqueous phase and non aqueous phase.

Nanoshells

Nanoshells are the new modified forms of targeted therapy, having core of silica and a metallic outer layer . These thin coated core particles of different material have gained considerable attention now days. Nanoshells are synthesized to create novel structures with different morphologies, since not possible to synthesize all the materials in desired morphologies.

Liposomes

Liposomes are lipid based vesicles that are extensively explored and most developed nanocarriers for novel and targeted drug delivery. Drugs that can deliver through liposomal delivery system are highlighted . These vesicles are synthesized by hydration of dry phospholipids. Depending upon on their size and number of bilayers they are classified into three basic types.

Ceramic Nanoparticles

The development of ceramic nanoparticles for biomedical application grows rapidly. Nanoscale ceramic such as hydroxyapatite,zirconia,silica,titanium oxide and aluminum were made from the new synthetic method to improve physical,chemical properties to reduce the cytotoxicities in biological system.

Nanobubbles

Nanobubbles (NBs) are nanoscaled bubble like structures that are generated in the interface of hydrophobic surfaces in liquids. These nanobubbles remain stable at room temperature and when heated to physiological temperature within the body coalesce to form microbubbles. The mechanism of NB formation is based on the nucleation of gas at the

hydrophobic surface from a supersaturated solution, leading to trap atmospheric gases. these nanobubbles can be easily visualized in tumor by means of various ultrasound methods .[6]

LOCALISATION OF NANOPARTICLES: BY PASSIVE OR ACTIVE TARGETING :

a. Passive targeting

The majority of these nanoparticles exhibit prolonged circulation times in vivo and thus accumulate at particular sites simply due to blood hemodynamic forces and diffusive mechanisms. The advantages of utilizing passive targeting of nanoparticles in the field of oncology due to its widely reported ‘‘enhanced permeation and retention’’ (EPR) effect. However, in the case of cytotoxic drugs have longer elimination half-lives in tumors than the normal tissue. Therefore, the delivery of higher amounts of drugs to tumors can lead to longer durations of drug exposure at higher concentrations and enhanced efficacy.

b. Active targeting

Active targeting is based on the affinity of the ligands to direct the binding of nanoparticles to antigens, differently over expressed on the plasma membrane of diseased cells or to the extra-cellular matrix proteins that are differentially over expressed in the diseased tissue. Targeting molecules can be either antibody or non-antibody ligands.

The advantages of the non-antibody ligands, like peptides, sugars or vitamins, they are readily available, inexpensive to manufacture and easy to handle. Actively targeted nanoparticles can be utilized in applications where drug release is either extracellular or intracellular. The act on intracellular sites of action are most effectively delivered with targeted nanoparticles.

Antibodies, have a higher specificity and a wide range of binding affinities as compared to the non –antibody ligands. [7]

Solvents

The selection of organic solvent is critical in developing a successful biodegradable nanoparticulate formulation. The miscibility of solvent with water and its ability to dissolve the polymer and the drug has an impact on particle size and encapsulation efficiency. In general, the organic solvent should have low solubility in water to yield a more stable emulsion that eventually leads to high-quality nanoparticles.

The effect of solvent on the particle size and drug encapsulation efficiency and their effect on release kinetics of PLGA nanoparticles and acetone as initial solvents. The size of PLGA nanoparticles prepared from DMA, DMF, and DMSO as an initial solvent were about 200–400 nm, smaller than those obtained by the use of acetone. [8.]

Stabilizers

A stabilizer is required to avoid coalescence and formation of agglomerates during and after the emulsification process of nanoparticles. Adsorption of stabilizers at the interface prevents this coalescence by lowering the interfacial tension and the energy of the system. Both particle size and potential are important physicochemical properties because they determine the physical stability and biopharmaceutical properties of nanoparticles, influencing drug release rate, biodistribution, mucoadhesion, and cellular uptake.[9]

TYPES OF COATINGS OF NANOPARTICLES:

SILVER

Silver nanoparticles have proved to be most effective because of its good anti microbial efficacy against bacteria,viruses and other eukaryotic micro organisms. They are the most used nano materials among all therby being used as anti microbial agents in textile materials,water treatment,sunscreen lotions.

GOLD

Gold nanoparticles are used in immuno chemical studies for identification of protein interactions .They are used as a lab tracer in a DNA fingerprinting to detect the presence of DNA in a sample.[10] They are also used for detection of amino glycoside antibiotics like streptomycin ,gentamycin and neomycin .Gold nanorods are being used to detect cancer stem cells, beneficial for cancer diagnosis and for identification of different classes of bacteria.

ALLOY

Alloy nanoparticles exhibit structural properties that are different from their bulk samples. Since Ag has the highest electrical conductivity among metal fillers and other metals.Ag flakes are mostly used .Bimetallic alloy nanoparticles properties are influenced by both metals.

MAGNETIC

Magnetic nanoparticles like fe_3o_4 and fe_2o_3 are biocompatible .They have been actively investigated for targeted cancer treatment ,stem cell sorting,manipulation,guided drug delivery,gene therapy ,DNA analysis and magnetic resonance and imaging.[11]

Classification of Nanoparticles :

1.Polymeric Nanoparticles:

The polymeric nanoparticles can be further classified into two types:

Nanospheres, which are polymeric matrix systems which consist of the drug that is dispersed physically and uniformly. The polymeric nano sized spherical matrix system provides effective control of the loaded drugs.

Nanocapsules are those systems containing vescicles in which the drug is confined to a cavity surrounded by a polymer membrane. In nanocapsules, the polymeric membrane encapsulates the core material, usually a drug.[12]

METHODS OF PREPARATION:

1) Polymerization of Monomers:

Two different approaches are generally adopted for the preparation of nanospheres using in-situ polymerization techniques:

- Method in which monomer to be polymerized is emulsified in non-solvent phase (emulsion polymerization) or,
- Method in which monomer is dissolved in a solvent that is non-solvent for the resulting polymer(dispersion polymerization).

i) Emulsion Polymerization:

Emulsion polymerization is one of the fastest methods for nanoparticle preparation and is readily scalable.[13] The method is classified into two categories, based on the use of an organic or aqueous continuous phase.

a) Micellar Nucleation and Polymerization:

The micellar nucleation and polymerization involve the swollen monomer micelles as the site of nucleation and polymerization. The monomer is emulsified in the non-solvent phase with the help of surfactant molecules. The process lead to the formation of monomer swollen micelles and stabilized monomer droplets. Swollen micelles exhibit size in the nanometric range and thus have a large surface area in comparison to monomer droplets.

The polymerization reaction generally process through two steps, nucleation and propagation in the presence of chemicals or physical initiator. The energy provided by the initiator creates free reactive monomers in the continuous phase, which then collides with surrounding unreactive monomers and initiates the polymerization chain reaction which lead to the formation of nanoparticles.

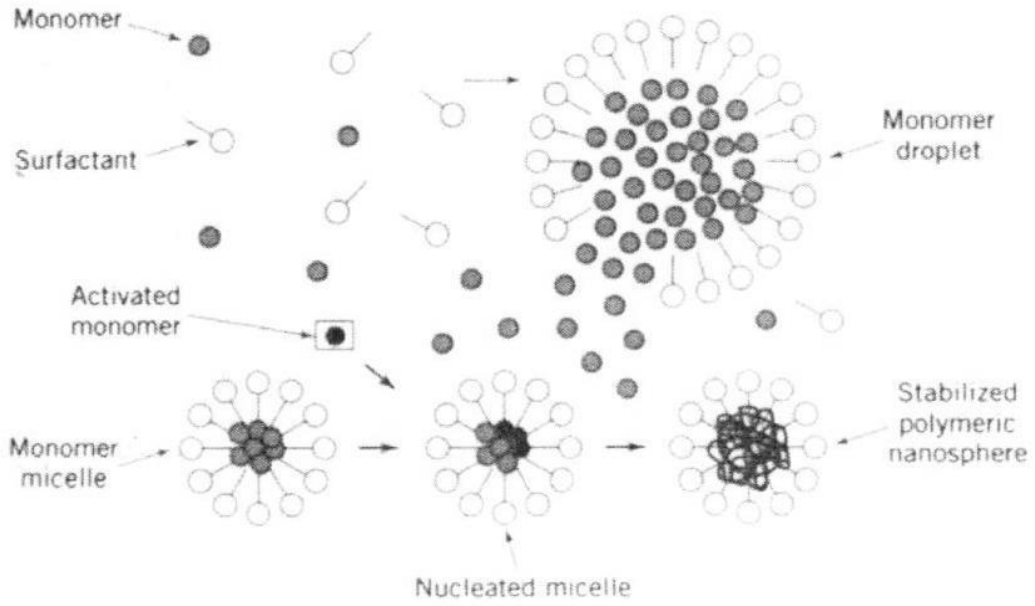


Fig . 2: Miscellar Polymerization

b) Homogeneous Nucleation and Polymerization:

This method is applied in cases where monomer is sufficiently soluble in the continuous outer phase. The nucleation and polymerization stages can directly occur in this phase leading to the formation of primary chains called oligomers.[14] In this situation, both the micelles and the droplets play the role of monomer reservoirs throughout the polymer chain length. When oligomers have reached certain length, they precipitate and form primary particles which are stabilized by the surfactant molecules provided by the micelles and droplets.

Depending on the bulk conditions and system stability, the end product nanospheres are formed either by additional monomer input into the primary particles or by fusion of the primary particles.

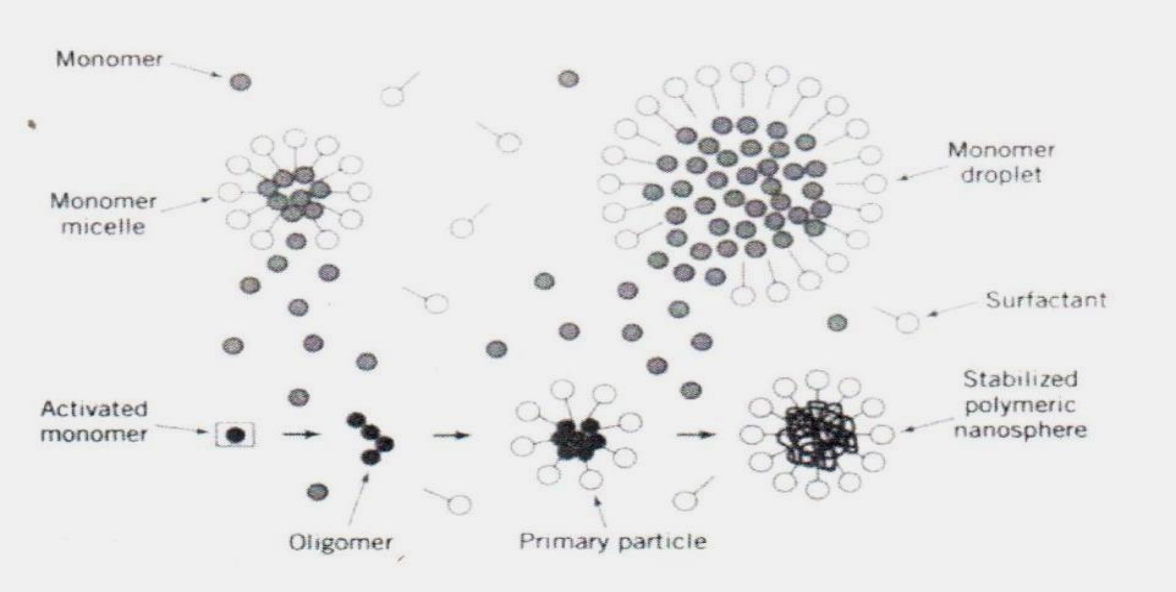


Fig. 3: Homogeneous nucleation

ii) Dispersion Polymerization:

The method in which the monomer is emulsified in an immiscible (non-solvent) phase by means of surfactants is termed as emulsion polymerization where as in the dispersion polymerization method, the monomer is dissolved in an aqueous medium instead of being emulsified, which acts as a precipitant for subsequently formed polymers.

Polymerization based methods essentially involves in-situ controlled polymerization of appropriate monomers where drugs may be added to monomeric phase or may be added to the formed polymeric nanoparticulates dispersion for adsorptive loading.

The monomer is introduced in to the dispersion medium of an emulsion or an inversed emulsion into non-solvent based polymeric solution. The polymerization is initiated by adding a catalyst and proceeds with a nucleation phase followed by a growth phase (propagation). On the other hand in the case of dispersion polymerization the nucleation is directly induced in the aqueous monomer solution and the presence of stabilizer or surfactant is not absolutely necessary for the formation of stable nanospheres[15].

iii) Interfacial Polymerization:

In this method the preformed polymer phase which is finally transformed in to an embryonic sheath. A polymer that eventually becomes core of nanoparticle and drug molecules to be loaded are dissolved in a volatile solvent. The solution is then poured in to a non-solvent for both polymer and core phase. The polymer phase is separated as a coaservate phase at O/W interphase.

The resultant mixture instantaneously turns milky owing to the formation of nanocapsules. The size of nanocapsules formed by this method usually ranges from 30-300 nm and the drug loading efficiency critically depends on drug solubility in core phase. Optimal concentration of surfactants can be added to stabilize the dispersion.[16] Interfacial polymerization method can be successfully adopted for encapsulation of proteins, enzymes, antibodies and cells.

.**iv) Interfacial Complexation:**

This method utilizes the basic principle of micro-encapsulation. In the case of nanoparticles preparation, aqueous polyelectrolyte solution is carefully dissolved in reverse micelles in a polar bulk phase with the help of an appropriate surfactant. Subsequently, competing polyelectrolyte is added to the bulk, which allows a layer of insoluble polyelectrolyte complex to coacervate at the interface.

2) Preparation Methods of Nanoparticles :

i) Emulsification-Solvent Evaporation:

Emulsification-solvent evaporation method involves two steps. The first step requires emulsification of polymeric solution into an aqueous phase. During the second step the solvent used for dissolving the polymer is evaporated, inducing polymer precipitation as nanospheres.

A polymeric solution made of an organic solvent in which the drug is dissolved is dispersed into nanodroplets, using a dispersing agent and high energy homogenization, in a non-solvent or suspension medium such as chloroform or ethyl acetate.[17] The polymer precipitates in the form of nanospheres in which the drug is finely dispersed in the polymer matrix network.

The solvent is subsequently evaporated by increasing the temperature under pressure or by continuous stirring. The size can be controlled by adjusting the stir rate, type and the amount of dispersing agent, viscosity of organic and aqueous phases, and temperature. Even though many different types of emulsions may be used, O/W emulsions are favoured by its simplicity, reduced preparation cost and risk of agglomeration. Frequently used polymers are PLA, PLGA, ethyl cellulose(EC), cellulose acetate phthalate, poly(E-caprolactone).

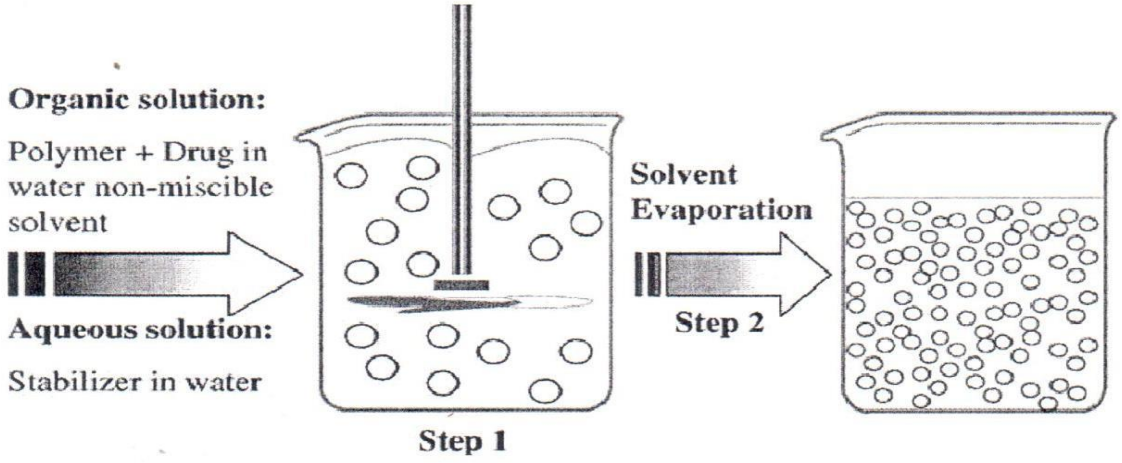


Fig.4:Schematic representation of the emulsification–evaporation technique

ii) Solvent Displacement and Interfacial Deposition:

The solvent displacement and interfacial deposition are similar methods based on spontaneous emulsification of the organic internal phase containing the dissolved polymer into the aqueous external phase. The solvent displacement method can be used for the preparation of nanospheres as well as nanocapsules, where as interfacial deposition can yield only nanocapsules.[18]

The polymer generally PLA, is dissolved in a water miscible solvent of intermediate polarity, leading to the precipitation of nanospheres. This phase is injected into a stirred aqueous solution containing a stabilizer as a surfactant.

Polymer deposition on the interface between the water and the organic solvent, caused by the fast diffusion of the solvent, leads to the instantaneous formation of colloidal suspension.

To facilitate the formation of colloidal polymer particles during the first step of the procedure, phase separation is performed with a totally miscible solvent that is also a non-solvent of the polymer.

iii) Emulsification/Solvent Diffusion:

The polymer is dissolved in a partially water soluble solvent such as propylene carbonate and saturated with water to ensure the initial thermodynamic equilibrium of both liquids.

In fact to produce the precipitation of the polymer and the consequent formation of nanoparticles, it is necessary to promote the diffusion of the solvent of the dispersed phase by dilution with excess of water when the organic solution is partially miscible with water or with another organic solvent in the opposite case.

Subsequently the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion in the external phase and the formation of nanospheres or nanocapsules, according to the oil-polymer ratio.[19] Finally, the solvent is eliminated by evaporation or filtration, according to its boiling point.

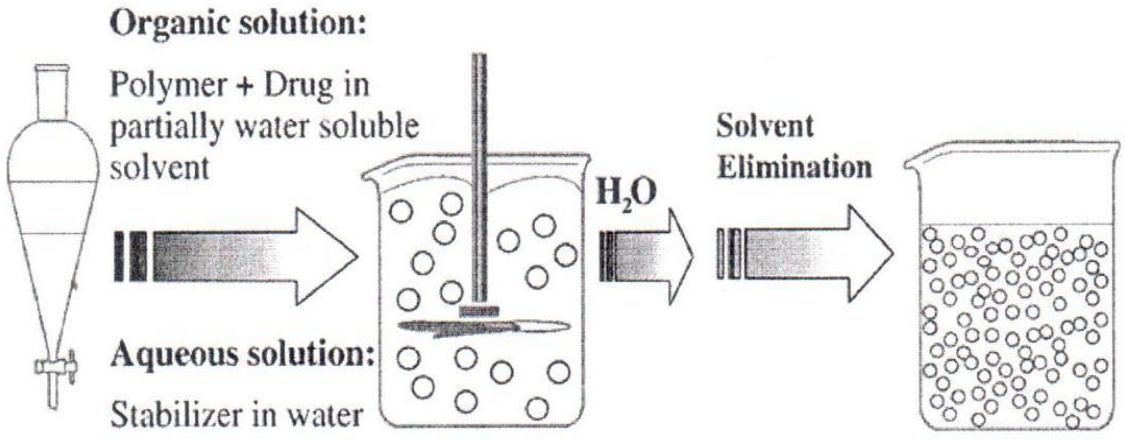


Fig.5: Schematic representation of the ESD technique

iv) Salting out with Synthetic Polymers:

Salting out is based on the principle of separation of a water miscible solvent from aqueous solution via a salting-out effect. The salting out procedure can be considered as a modification of the emulsification/solvent diffusion.

Polymer and the drug are initially dissolved in a solvent such as acetone, which is subsequently emulsified into an aqueous gel containing the salting-out agent (electrolytes, such as magnesium chloride, calcium chloride, and magnesium acetate, or non-electrolytes such as sucrose) and a colloidal stabilizer such as polyvinyl pyrrolidone or hydroxyl ethyl cellulose. This oil/water emulsion is diluted with sufficient volume of water or aqueous solution to enhance the diffusion of acetone to the aqueous phase, thus inducing the formation of nanospheres.

The selection of the salting-out agent is important, because it plays an important role in the encapsulation efficiency of the drug.[20] Both the solvent and the salting-out agent are then eliminated by cross-flow filtration. Salting out does not require an increase of temperature and therefore, may be useful when heat sensitive substances have to be processed.

v) Nanoprecipitation Method:

Nanoparticles can be synthesized by the nanoprecipitation method. In this method, polymer and drug are dissolved in acetone, ethanol, or methanol and incorporated under magnetic stirring into an aqueous solution of the surfactant. The organic solvent diffuses instantaneously to the external aqueous phase, followed by precipitation of the polymer and drug. After formation of the nanoparticles, the solvent is eliminated and the suspension concentrated under reduced pressure.

Improved bioavailability of proteins and peptides was demonstrated using PLGA nanoparticles by the nanoprecipitation method.

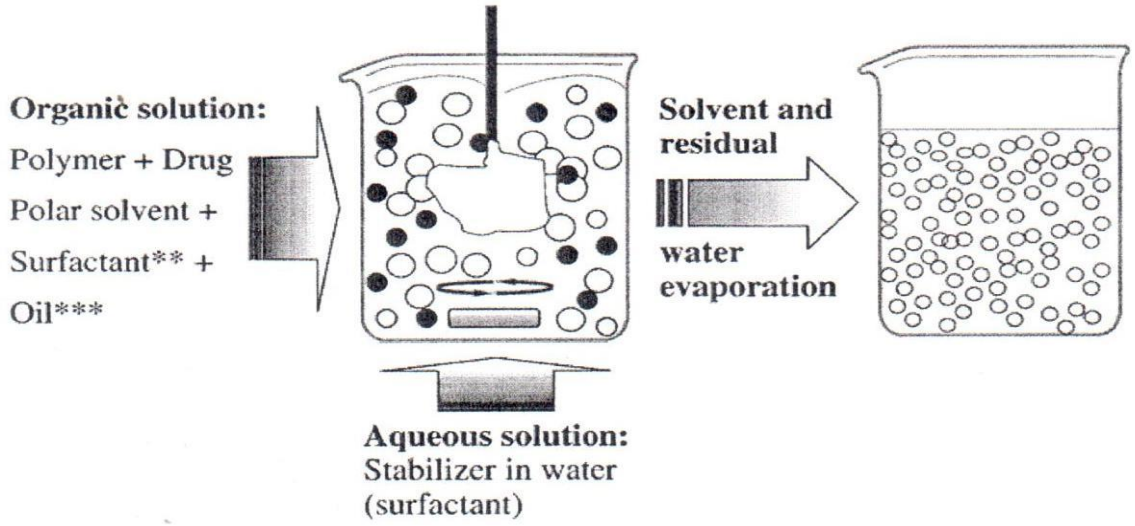


Fig.6: Schematic representation of the precipitation technique

vi) Solvent Evaporation Method:

In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate, which is also used as the solvent for dissolving the hydrophobic drug.

The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Small particle size can be achieved by the application of high-speed homogenization or ultrasonication.[21]

vii) Coacervation or Ionic Gelation Method:

This method is an ideal method for the preparation of nanoparticles using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic Chitosan nanoparticles by ionic gelation. The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanions sodium tripolyphosphate.

In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction.

viii) Supercriticial Fluid Technology:

Conventional methods such a solvent extraction-evaporation, solvent diffusion and organic phase separation methods require the use of organic solvents which are hazardous to the environment as well as to physiological systems.[22] Therefore, the superficial fluid technology has been investigated as an alternative to prepare biodegradable micro and nanoparticles because supercritical fluids are environmentally safe.

A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure.

Supercritical CO_2 (SC CO_2) is the most widely used supercritical fluid because of its mild critical conditions (Tc = 31.1^0C, Pc = 73.8 bars), non-toxicity, non-inflammability, and low price. The most common techniques involving supercritical fluids are supercritical anti-solvent (SAS) and rapid expansion of critical solution.

The process of SAS employs a liquid solvent, e.g. methanol, which is completely miscible with the supercritical fluid to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting in the formation of nanoparticles.

ix) Desolvation:

In this method for fabrication of nanoparticles is carried out by the slow addition of a desolvation agent, such as natural salts or alcohol, to the protein solution. The desolvation factor changes the tertiary structure of protein. On reaching the critical level of desolvation, protein clump will be formed which on crosslinking with a chemical substance (e.g. glutaraldehyde) will result in the formation of nanoparticles.[23]

2. Nanoemulsions:

Nanoemulsions are referred as submicron, ultrafine or fine dispersed emulsions with a characteristic droplet size of 20-200nm. They are thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules. Based on the composition the nanoemulsions can be classified as oil in water, water in oil and bi-continuous nanoemulsions wherein microdomains of oil and water are interdispersed within the system.

In all three types of nanoemulsions, the interface is stabilized by an appropriate combination of surfactants and/or co-surfactants. The nanoemulsions differ for the conventional emulsions in appearance, i.e it appears clear, transparent or slightly turbid, whereas the macroemulsions are opaque and white. Further, the nanoemulsions do not require high energy input for preparation, whereas the normal emulsion mostly utilizes high energy inputs for the

dispersion of one phase in the other. Nanoemulsions can be administered by parenteral, oral, topical, ocular and pulmonary routes.

Advantages of Nanoemulsion :

- Increased bioavailability and faster rate of absorption of the drugs incorporated.
- The incorporated drug is protected from hydrolysis and oxidation as drug in oil phase in O/W nanoemulsions
- Eliminates variability in absorption.
- Solublization of lipophilic drug.
- An ideal tool for development of liquid dosage form of water insoluble drugs.
- Wide choices of routes of administration like parenteral, oral, topical, ocular and pulmonary routes
- Enhanced drug penetration.
- Taste masking of bitter and nauseous drugs.
- Improved stability
- Capability of carrying both lipophilic and hydrophilic drugs.

Disadvantages of Nanoemulsion :

- Use of a large concentration of surfactant and co-surfactant are necessary for stabilizing the nanodroplets.
- Limited solubilizing capacity for high-melting substances.
- The surfactant must be nontoxic for using pharmaceutical applications.
- Nanoemulsion stability is influenced by environmental parameters such as temperature and pH. [24]

Preparation of Nanoemulsions:

Factors to be considered during preparation of nanoemulsion

- Selection of surfactants:
    - Surfactants must be carefully chosen so that an ultra low interfacial tension (< 10-3 mN/m) can be attained at the oil / water interface which is a prime requirement to produce nanoemulsions.
- Concentration of surfactant :
    - Concentration of surfactant must be high enough to provide the number of surfactant molecules needed to stabilize the microdroplets to be produced by an ultra low interfacial tension.
- Nature of the interface:
    - The interface must be flexible or fluid enough to promote the formation of nanoemulsions.

The drug is usually dissolved in the lipophilic part (oil phase) and the surfactant and cosurfactant are added to the aqueous part (water phase) and added at a slow rate with gradual stirring until the system is transparent. The amount of surfactant and cosurfactant to be added and the percentage of oil phase that can be incorporated shall be determined with the help of pseudo-ternary phase diagram.[25]

As nanoemulsions are non-equilibrium systems, many cases require high energy inputs from mechanical devices or from the chemical potential of the components is required, nanoemulsion formation by the so called dispersion or high energy emulsification method is generally achieved using high shear stirring, high pressure homogenizers and ultrasound generators.

METHODS OF PREPARATION OF NANOEMULSIONS:

i) Ultrasonication:

In ultrasonic emulsification, the energy input is supplied in the form of ultrasonic sound from the probes. The sonicator probe contains piezoelectric quartz crystals that expand and contract with response to the alternating electrical current supplied to it. As the tip of sonicator probe is kept in contact with the liquid, it generates ultrasonic vibrations(0-200kHz) due to which the cavitations occur.

Cavitation is referred as the formation and collapse of vapour cavities in a flowing liquid. Such a vapour cavity forms when the local pressure is reduced to that of at the temperature of the flowing liquid because of local velocity changes. The collapse of these cavities causes powerful shock waves that radiates throughout the solution and breaking the dispersed droplets into smaller droplets. The droplet size in the nanoemuslsion varies with respect to the intensity of the vibration and the period of the exposure.

ii) Microfluidisation:

In the microfluidization technique, is a jet stream homogenizer of two fluid streams collide with high velocity (up to 1000m/sec) under pressures up to 4000 bar. The turbulent flow and the high shear forces of the stream of fluids leads to particle size diminution to nanometer range. The high pressure applied and the high streaming velocity of the lipid can also lead to cavitation additionally, contributing to size diminution.

The microfluidzer uses high pressure collusion of pre-emulsion streaming from two nozzles located in opposite direction inside an interaction chamber. Compressed air is used for streaming the emulsion at a pressure of about 750 Mpa.[26] High shearing action caused by the high pressure streaming through microchannels in the impingement area contributes nanoemulsion.

iii) Jet Dispersion :

In this method the fine globule size is mainly achieved by forcing the pre-emulsion through jet nozzles of fine bore size with high pressure. Modified jet dispersers with two or more jets of crude emulsion each from opposing bores of 0.3-0.5mm diameter are made to collide each other are also available. Orifice plate is the simplest construction form for a homogenizing nozzle. The diameter of orifice bore is of same order of magnitude as the jet dispersers and inlet head diameter of orifice plate is typically 10-60nm.

In jet dispersers and orifice plates, droplets are disrupted predominantly due to laminar elongational flow ahead of the bores. Unlike radial diffusers, the nozzle is microfluidizers; jet dispersers and orifice plate contain no moving parts, so they can be used at high pressures up to 300-400 Mpa.

3. Solid Lipid Nanoparticles:

Solid lipid nanoparticles (SLN) are lipid based submicron sized colloidal drug delivery system introduced in 1991 as an alternative drug carrier for other colloidal carriers, such as emulsions, liposomes and polymeric micro- and nanoparticles. Generally, they are made of solid hydrophobic core having a monolayer of phospholipids coating. The solid core contains the drug dissolved or dispersed in the solid high melting fat matrix.[27] The hydrophobic chains of phospholipids are embedded in the fat matrix. They have potential to carry lipophilic or hydrophilic drugs. SLNs combine the advantages of the other colloidal carrier systems but devoid of some of their major disadvantages.

Advantages of SLN:

• SLN can be used for the controlled or targeted release of drugs.

• They exhibit excellent biocompatibility as most of the components are made up of lipids.

• Improved stability can be acheived.

• Enhancement of drug loading capacity, when compared with other lipid based drug delivery systems.

• The methods of preparation facilitates scale up process for large scale manufacturing.

• Sterilization of SLN is possible.

• Better control over release kinetics of encapsulated compounds.

• Enhanced bioavailability of entrapped bioactive compounds.

• The lipids used in the preparation provide chemical protection to the chemo-labile drugs incorporated.

• Methods of preparation are simple than that of the biopolymeric nanoparticles.

• No organic solvents are required for the preparation of SLN's.

• Conventional emulsion manufacturing methods applicable.

• SLN can be freeze dried and can be stored for longer period.

Disadvantages:

• The SLN tend to aggregate and form larger particles..

• Unpredictable tendency for gelation.

• Unexpected dynamics of polymeric transitions.[28]

METHODS OF PREPARATION OF SOLID LIPID NANOPARTICLES:

i) High pressure homogenization (HPH) technique:

This technique is the widely used for the preparation of SLNs in which the high pressure homogenizer pushes a liquid with high pressure (100-2000 bar) thorough a micro gap. Previously this technique was used for manufacturing of nano-emulsions. Hot homogenization and cold homogenization are the two basic production methods for SLNs. For both techniques the drug needs to be dispersed or solubilized in the lipids above their melting points.

In *hot homogenization technique* lipid components are first melted by heating above their melting points. Therefore it can be regarded as the homogenization of an emulsion. Drug is either dispersed or dissolved in the molten lipids and the aqueous surfactant is added at the same temperature. This pre-emulsion of the drug loaded lipid melt and aqueous surfactant phase, is obtained with the help of high shearing devices. High pressure homogenization of the pre-emulsion is to be carried out at the temperature higher than the melting point of the lipid. The increased temperature lowers viscosity of the inner phase.

The process is continued till desired particle size. In most cases, 3-5 homogenization cycles are sufficient for the requisite particle size. However unnecessary increase of cycles results in increase in particle size due to particle coalescence. After the homogenization process, the nano-emulsion is formed due to liquid nature of the lipid which on cooling gives rise to solid lipid nanoparticles. This technique has the advantage, as it is suitable for scale up.[29] However possible concerns regarding hot homogenization is the stability of the drug molecule to the elevated temperatures of the lipid melt. So to avoid heat-accelerated drug degradation, the length of time for drug exposure should be shortened.

Cold homogenization has been developed to overcome the following problems of the hot homogenization technique: (a) drug distribution into the aqueous phase during homogenization, (b) temperature induced drug degradation, (c) complexity of the crystallization step of the nano-emulsion leading to several modifications or supercooled melts.

In *cold homogenization technique* the drug containing lipid melt is cooled by means of liquid nitrogen or dry ice to obtain drug loaded lipid. Rapid cooling leads to formation of solid solution (homogeneous distribution) of drug in lipid matrix and then the solid lipid is ground to lipid microparticles (approximately 50-100 mm) and these lipid microparticles are dispersed in a cold surfactant solution yielding a pre-suspension.

Then this pre-suspension is homogenized at or below room temperature, to obtain the required particle size. Since high shear homogenization process involved here is assumed that it does not elevate the temperature of the dispersion, this method provides protection to heat sensitive drug entities.

This method has the potential of minimizing the partitioning of less hydrophobic drug out of lipid to the aqueous phase during homogenization.

ii) Ultrasonication or High Speed Homogenization:

The mechanism involved in the ultrasonication technique was discussed in detail under preparation methods of nanoemulsions. SLN can also developed by high speed stirring or sonication. The problem in this method is the wide particle size distribution range. This leads to physical instability likes particle growth on storage. Titanium contamination due to ultrasonication is also one of the problems in ultrasonication method.

iii) Solvent Emulsification/Evaporation:

For the preparation of nanoparticle dispersions by precipitation in o/w emulsions, the lipophilic material is dissolved in water-immiscible organic solvent (eg. cyclohexane) and this is emulsified in an aqueous phase. Upon evaporation of the solvent nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium.[30]

iv) Micro Emulsion Based SLN Preparations

SLN preparation techniques which are based on the dilution of microemulsions are made by stirring an optically transparent mixture at 65-70^{o}C which is typically composed of a low melting fatty acid (stearic acid), an emulsifier (polysorbate 20, polysorbate 60, soy phosphatidylcholine, and sodium taurodeoxycholate), co-emulsifiers (sodium monooctylphosphate) and water.

The hot microemulsion is dispersed in cold water (2-3^{o}C) under stirring. Typical volume ratios of the hot microemulsion to cold water are in the range of 1:25 to 1:50. The dilution process is critically determined by the composition of the microemulsion. According to the literature, the droplet structure is already contained in the microemulsion and therefore, no energy is required to achieve submicron particle sizes. With respect to the similarities of the production procedure of polymer nanoparticles described by French scientists, different mechanisms might be considered.

v) Supercritical Fluid Technology:

This method is similar to that of the preparation of the polymeric nanoparticles, which was described earlier.[31] It is a relatively new technique for SLN production and has the advantage of solvent-less processing. There are several variations in this platform technology for powder and nanoparticle preparation. SLN can be prepared by the rapid expansion of supercritical carbon dioxide solutions (RESS) method. Carbon dioxide (99.99%) was the good choice as a solvent for this method.

vi) Double Emulsion Method:

For the preparation of SLN loaded hydrophilic drugs, a novel method based on solvent emulsification-evaporation is used. In this method, the drug is encapsulated with a stabilizer to prevent drug partitioning to external water phase during solvent evaporation in the external water phase of w/o/w double emulsion.

4. Carbon Nanotubes:

The carbon nanotubes exhibit many unique intrinsic physical, chemical, mechanical and thermal properties and have been intensively used as drug delivery systems. Structurally they are the long hollow seamless cylinders made of graphene. The diameter of these tubes is in the range of 1-100 nm. These tubes are normally capped with about half of fullerence molecules at both the ends.

Carbon nanotubes can be classified into two type's namely single walled carbon Nanotubes and multiwalled Nanotubes. A single-walled carbon nanotube (SWCNT) consists of a single graphene sheet wrapped around to form a cylinder whereas a multi-walled carbon nanotube (MWCNT) is concentrically nested cylinders of graphene sheets.

Carbon nanotubes have the capability to carry therapeutic agents to various target sites including the infectious organisms. The hollow and smaller structure permits loading of more volume of therapeutic agents when compared to other nano drug delivery systems.

They are also capable of carrying DNA and protein molecules to the inside and outside of the nanotube walls. Both low as well as high molecular weight drugs can be load into the CNT

because of the large inner volume.[32] CNT are suitable for delivery of hydrophilic and lipophillic drugs. CNTs can also be used in the multi-drug therapy as it can be loaded with more than one drug. Recently, Liu and group demonstrated that the carbon nanotubes are capable of loading different chemical moieties like poly ethylene glycol- a surfactant, Doxirubicin-an anticancer drug and fluorescent tags together. CNTs are also reported for controlled release of drugs over a predetermined period of time.

Functionalization of CNTs:

To use CNT as a drug delivery carrier, it needs to be functionalized as they are basically insoluble in water, whereas aqueous solubility is highly essential for g.i.t absorption, blood transportation, secretion, and biocompatibility. The non-covalent functionalization of CNT can be carried out by exposing CNTs to vapors containing functionalization species that non-covalently bonds to the nanotube surface while providing chemically functional groups at the nanotube surface.

A stable functionalized nanotube surface can be obtained by exposing it to vapor stabilization species that reacts with the functionalization layer to form a stabilization layer against desorption from the nanotube surface while depositing chemically functional groups at the nanotube surface. The stabilized nanotube surface can be exposed further to at least another material layer precursor species that can deposit as a new layer of materials.

Covalent functionalization of CNTs can be carried out by treatment of side walls of CNTs to strong acid solution and the carboxylic acid groups are generated at the defect point, predominantly on the open ends. Thus this method provides more secure conjunction of functional molecules.

METHODS OF PREPARATION OF CARBON NANOTUBES:

i) Arc Discharge Method:

It is the most common and the easiest way to produce carbon nanotubes. In this method two carbon rods placed end to end, separated by approximately 1mm, in an enclosure filled with inert gas (helium of argon) at low pressures i.e. between 50 to 700 mbar. A direct current of 50 to 100A driven by approximately 20V creates a high temperature discharge between two electrodes.

This discharge causes vaporization of one of the carbon rods and forms deposition of carbon nanotubes on the other rod. Depending on the modifications in the technique it is possible to selectively grow SWCNTs or MWCNTs and the typical yield is up to 30 to 90%. This method is used for preparations of bulk quantities of nanotubes.

ii) Laser Ablation Method:

This method was reported in 1995, by Smalley's group at Rice University, in which a pulsed or continuous laser is used to vaporize a graphite target in an oven at 1200^{o}C. The pulsed laser utilizes high light intensity (100KW/cm2) when compared with continuous laser (12KW/cm2). The oven is filled with helium or argon gas in order to maintain the pressure at 500 Tor. A very hot vapour plume formed, that expands and cools rapidly.

As the vaporized species cool, small carbon molecules and atoms quickly condense to form larger clusters, possibly including fullerences. The catalysts also begin to condense and attach to carbon clusters and prevent their closing into cage structure, yielding bundles of single walled carbon Nanotubes.

iii) Chemical Vapour Deposition (CVD):

Carbon vapours are achieved by using any of the carbon sources like methane, carbon monoxide and acetylene by using an energy source, such as plasma or a resistively heated coil to maintain the temperature between 650 to 900^{o}C.

The energy source is used to crack the molecule into reactive atomic carbon and then, the carbon diffuses towards the substrate, which is heated and coated with a catalyst (Ni, Fe & CO) where it will bind.CVD synthesis is a two step process consisting of a catalyst preparation followed by the actual synthesis of CNT.

The catalyst is generally prepared by sputtering a transition metal onto a substrate and then chemical etching or thermal annealing is used to induce catalyst particle nucleation. Thermal annealing results in cluster formation on the substrate, from which the nantoube will grow. The typical yield of carbon nanotubes by this method varies from 20 to 100%.

4. NANOCRYSTALS:

The term nanocrystals are used for nanosized particles made up of drug alone, without any added matrix substances like polymers or other excipients. The particles with less than 100 nanometers exhibit an entirely new set of properties. The decreased size of particles will increase the solubility of drugs hence, this technology is extensively applied to enhance the oral bioavailability of sparingly water soluble drugs.

The technique of preparation of nanocrystals do not utilize toxic solvents and surfactants encourages the application of this technology to develop injectable solutions of sparingly water soluble drugs. Nanocrystals can also be used to develop formulations like ophthalmics and topical preparations, in which size is the critical factor for administration through various routes.

Advantages of nanocrystals:

- Improved dissolution rate due to increase in surface area
- Increased rate of absorption
- Increased oral bioavailability
- Rapid effect
- Improved dose proportionality
- Reduction in required dose
- Wide choice for routes of administration
- Reduction in fed/fasted variability

- Rapid, simple and cheap formulation development
- Possibility of high amounts (30-40 %) of drug loading
- Lesser side effects, as they are proportional to drug concentration and the nanocrystal enables decrease in the concentration of the drug administration.

METHODS FOR PREPARATION OF NANOCRYSTALS:

The common methods adopted for preparation of drug nanocrystals were milling, high-pressure homogenization and precipitation. However the capability of the nanocrystals to improve the bioavailability of poorly soluble drugs demanded the researchers to carry out intensive research for development of new technologies lead to the outcome of many other novel approaches for the production of drug nanocrystals.

Ssupercritical fluid techniques like Rapid expansion of supercritical solution (RESS), rapid expansion from supercritical to aqueous solution (RESAS), solution-enhanced dispersion by the supercritical fluids (SEDS), spray freezing into liquid (SFL) evaporative precipitation into aqueous solution (EPAS) and aerosol solvent extraction (ASES) were also applied for preparation of nanocrystals.[33]

Media milling process:

Nanocrystalline dispersions can be prepared by media miling process in which, a milling chamber is charged with milling media, dispersion medium (normally water), stabilizer, and the drug. The drug particles are reduced in size by shear forces and forces of impaction generated by a movement of the milling media. Small milling pearls or larger milling balls are used as milling media.

With a reduction in the size of grinding media in a media mill, the number of contact points is increased exponentially, resulting in improved grinding and dispersing action (i.e., leading to smaller particles). The pearls or balls consist of ceramics (cerium- or yttrium-stabilized zirconium dioxide), stainless steel, glass, or highly cross-linked polystyrene resin-coated beads. As in the case any milling process, the erosion from the milling material during the milling process is also common with pearl media milling process.

Precipitation Methods:

It is a patented hydrosol technology of Sandoz (Novartis) in which a poor water-soluble drug is dissolved in a water- miscible organic medium. Pouring of this solution into a non solvent, such as water, will cause a precipitation of finely dispersed drug nanocrystals. The problems associated with this technology are that the formed nanoparticles need to be stabilized to avoid aggregation of larger sized crystals and the drug needs to be soluble at least in one solvent. Lyophilization of the prepared nanocrystal is also recommended to preserve the particle size.

HOMOGENIZATION METHODS:

i) Microfluidization:

The details of the microfluidiser are described under the methods of preparation of nanoemulsions. To preserve the particle size, stabilization with phospholipids or other surfactants and stabilizers may be added.[34] Extensive production time particularly passing the medium 50-100 cycles to reach the required size, is considered as a major disadvantage of this process. Commercially this technology is used by SkyePharma Canada, Inc. (RTP, Inc.) for its IDD-P™ technology to produce submicron particles of poorly soluble drugs.

ii) Piston-gap homogenization:

Drug nanocrystals can also be produced by high-pressure homogenization using piston gap homogenizers. Depending on the homogenization temperature and the dispersion media, there is a difference between the Dissocubes® technology and the Nanopure® technology. Dispersion medium of the suspensions was water. A piston in a large bore cylinder creates pressure up to 2000 bar.

The suspension is pressed through a very narrow ring gap. The gap width is typically in the range of 3-15 micrometer at pressures between 1500-150 bars. There is a high streaming velocity in the gap according to the Bernouli equation. Due to the reduction in diameter from the large bore cylinder (e.g. 3 cm) to the homogenization gap, the dynamic pressure (streaming velocity) increases and simultaneously decreases the static pressure on the liquid.

The liquid starts boiling, and gas bubbles occur which subsequently implode, when the suspension leaves the gap and is again under normal pressure (cavitation). Gas bubble formation and implosion lead to shock waves which cause particle diminution.

iii) Nanopure Technology:

In 1999, Müller et al. found that a similar effective particle diminution can also be obtained in nonaqueous or water-reduced media. An elegant method to obtain a final formulation directly is the production of nanocrystals in non-aqueous homogenization media. Drug nanocrystals dispersed in liquid polyethylene glycol (PEG) or oils can be directly filled as drug suspensions into gelatine or HPMC capsules.

The non-aqueous homogenization technology was established against the teaching that cavitation is the major diminution force in high pressure homogenization. Efficient particle diminution could also be obtained in non- aqueous media.

iv) Combination Technologies:

The Nanoedge technology by the company Baxter covers a combination of precipitation and subsequent application of high energy shear forces, preferentially high pressure homogenization with piston-gap homogenizers. As mentioned above the precipitated particles have a tendency to grow. According to the patent obtained by Kipp et al, treatment of a precipitated suspension with energy (e.g. high shear forces) avoids particle growth in precipitated suspensions (annealing process).

The relative complex patent description can be summarized in a particle size by precipitation. Precipitated particles can be amorphous or partially amorphous. This implies the risk to the product that during the storage, the amorphous particles can recrystalize, leading subsequently to a reduction in oral bioavailability or a exhibit a change in pharmacokinetics. The *NANOEDGE* process is particularly suitable for drugs that are soluble in non aqueous media possessing low toxicity, such as N- methyl-2-pyrrolidinone.

Nanopure® XP technology provides an advantage of scaling up ability and the possibility to produce on large scale, applying "normal" production conditions.

Nanopure XP technology is a pre-treatment step with subsequent homogenization to produce particles well below 100 nm. Drug nanocrystals with a size of about 50 nm and below are distinctly smaller than the wavelength of the visible light, and so the nanosuspensions are translucent.[35]

v) Rapid expansion from a liquefied-gas solution (RESS) :

RESS process uses the high solvating power of supercritical fluids. After loading the supercritical fluid with the solute, an extremely fast phase change from the supercritical to the gas like state takes place during the expansion in the supersonic free jet.

This phase change leads to high supersaturation and subsequently to particle formation. Since the solvent is a dilute gas after expansion, the RESS process offers a solvent free final product. The improvement of the bioavailability of the RESS-produced griseofulvin has been verified by dissolution experiments. The dissolution rate of griseofulvin produced by RESS is about 2-fold higher than the common micronized material.

vi) Spray Freezing into Liquid (SFL):

SFL technology was developed by the University of Texas at Austin (Austin, TX) and commercialized by Dow Chemical Company (Midland, MI).In this is a particle engineering technology, a feed solution containing drug and dissolution enhancing excipient(s) which is atomized directly into a cryogenic liquid, such as nitrogen.

The resulting dried powder is composed of discrete microparticles where the molecular dispersion of drug in polymeric matrix is obtained. The danazol nanoparticles produced by the SFL process exhibited significantly enhanced dissolution rates when compared with the micronized bulk danazol.

PROCESSING OF NANOPARTICLES:

The prepared nano drug delivery system needs to be further processed so as to render

them suitable for usage as a standardized dosage form. The purification and sterilization is very important steps to eliminate from any adverse effects or removal of toxic impurities like organic solvents initiators and stabilizers. Various methods are developed for the purification of nano drug delivery systems, i.e, ultra-centrifugation technique, gel filtration technique and dialysis instruments.[36] The sterilization of nanoparticles can be performed by membrane filtration method using 0.22 µm filter in a aseptic area with aseptic techniques. Sterilization of thermostable systems can also be performed using autoclave.

DRUG LOADING IN NANOPATRICLE:

Nano drug delivery systems are exhibited with high drug loading capacity. This ability reduces the amount of the excipients like polymers, lipids etc. required for drug delivery in the body. The incorporation of the drug into nanoparticles can be carried out by two methods

a) By incorporating the drug at the time of preparation of nanoparticles

b) After the formation of nanoparticles the drug is adsorbed which is achieved by incubating nanoparticles in a concentrated drug solution.

Various factors like chemical structure of drugs and polymer and condition of drug loading influences the amount of drug bound to nano drug delivery systems.

MECHANISM OF DRUG RELEASE:

Sustained/controlled release of drug depends on release mechanism of the system. The drug release rate depends on various parameters like solubility of drug, desorption of the surface bound adsorbed drug, drug diffusion through matrix system of polymer. To manipulate the drug release a thorough understanding of the mechanism of drug release is required which includes knowledge of solubility, diffusion and biodegradation of the carrier system.

On polymer based nano drug delivery systems such as polymeric nanoparticles the release rate of drug can be modified by varying the concentration of polymer in the matrix system. Larger particles have initially smaller burst release than small particles.

CHARACTERISATION OF NANOPARTICLES:

i) Measurement of Particle Size and Zeta Potential:

Photon correlation spectroscopy (PCS) and laser diffraction (LD) are most powerful techniques for the routine measurement of particle size. The Coulter counter method is rarely used to measure nanoparticle size because of difficulties in the assessment of small nanoparticles and the need for electrolytes which may destabilize colloidal dispersions.

PCS (also known as dynamic light scattering) measures the fluctuation of the intensity of the scattered light which is caused by the particle movement. This method covers a size range from a few nanometers to about 3 mm. This means PCS is a good tool to characterize nanoparticles. They can be visualized by means of LD measurements. This method is based on the dependency of the diffraction angle on the particle radius (Fraunhofer spectra). Smaller particles cause more intense scattering at high angles compared to the larger ones. A clear advantage of LD is the coverage of a broad size range from the nanometer to the lower millimeter range.

Almost all particulate or macroscopic materials in contact with a liquid acquire an electronic charge on their surfaces. Zeta potential is an important and useful indicator of this charge which can be used to predict and control the stability of colloidal suspensions or

emulsions. The greater the zeta potential the more likely the suspension is to be stable because the charged particles repel one another and thus overcome the natural tendency to aggregate. The measurement of zeta potential is often the key to understanding dispersion and aggregation processes.[37]

The measurement of the zeta potential by a zetasizer allows prediction about the storage stability of the colloidal dispersion. In general particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion. However, this rule cannot strictly apply for systems which contain steric stabilizers, because the adsorption of steric stabilizer will decrease the zeta potential due to the shift in the shear plane of the particle.

ii) Particle Morphology:

In some cases the interest is in the shape of the particle and the nature of its surface. In other cases interest focuses on the particle disposition, such as aggregation state or location within a matrix. Particularly when the objects to analyzed have a high aspect ratio, such as the case with nanotubes, an actual picture is a valuable complement to instrumental methods.

a) Scanning Electron Microscopy (SEM): The nanoparticle size is measured by scanning electron microscopy, either in transmission or scanning mode, are the primary tools of the analyst. The magnifications are much higher in the former mode (SEM), but the 2-D appearance of images and the extensive sample preparation serve as drawbacks. Freeze-fracture sample preparation, where a cast is taken of the sample of interest and then examined in turn is valuable in those instances where the sample is fragile, but it is a laborious procedure that requires a high level of skill to properly utilize.

b) Atomic Force Microscopy (AFM): Recently there has been a great interest in applying atomic force microscopy to the analysis of pharmaceutically relevant nanoparticles. Very high resolution images of particles in their native environment can be obtained by using AFM, even though at the expense of time since it is a rastering method.[38] Depending on the interaction established between the probe tip and the sample, a wide range of material characteristics can be mapped on the nanoscale, e.g., electrostatic potential or hardness.

The particles must be located on a solid support like mica, so understanding the effect that later (AFM) has on the former (SEM), flattening for example, is important, and constitutes one reason that experience of the analyst with AFM is necessary.

iii) Drug Loading:

Ideally, a successful nanoparticulate system should have a high drug-loading capacity thereby reduces 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 the formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption or absorption technique). Drug loading and entrapment efficiency very much depends 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 end functional groups.

iv) Nanoparticle Drug Release:

To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. In general drug release rate depends on:

- ❖ Solubility of drug;
- ❖ Desorption of the surface bound/ adsorbed drug;
- ❖ Drug diffusion through the nanoparticle matrix;
- ❖ Nanoparticle matrix erosion/degradation; and
- ❖ Combination of erosion/diffusion process.

Thus solubility, diffusion and biodegradation of the matrix materials govern the release process. In the case of nanospheres where the drug is uniformly distributed, the release occurs by diffusion or erosion of the matrix under sink conditions.[39] If the diffusion of the drug is faster than matrix erosion, the mechanism of release is largely controlled by a diffusion process. The rapid initial release or burst is mainly attributed to the weakly bound or adsorbed drug to the large surface of nanoparticles. If the nanoparticle is coated by polymer, the release is then controlled by diffusion of the drug from the core across the polymeric membrane.

The membrane coating acts as a barrier to release, therefore, the solubility and the diffusivity of the drug in polymer membrane becomes determining factor in drug release. Furthermore release rate can also be affected by ionic interaction between the drug and addition of auxillary ingredients.

When the drug is involved in interaction with auxillary ingredients to form a less water soluble complex, then the drug release can be very slow with almost no burst release effect. Various methods which can be used to study the in vitro release of the drug are:

- Side-by-side diffusion cells with artificial or biological membranes;
- Dialysis bag diffusion technique;
- Reverse dialysis bag technique;
- Agitation followed by ultracentrifugation/centrifugation;
- Ultra-filtration or centrifugal ultra-filtration technique.

b) Molecular Weight:

The molecular weight of the nanoparticulate polymer can be determined by

a) Gel permeation chromatography
b) Atomic force microscopy
c) By using a refractive indexdetector.

c) Particulate Morphology:

The morphology of the nanoparticle suspension was observed under transmission electron microscopy by negative staining which was observed by dropping nanoparticle suspension on to a copper grid and dyed with 2% phosphotungstic acid.

e) Density:

The density distribution across the matrix of the nanoparticles can be obtained from the structural imperfections, which can be determined by

a) Gas pycnometer by using either air or helium
b) Scanning electron microscopy
c) Transmission electron microscopy.

j) *In vivo* distribution studies:

Animal testing:

The invivo animal studies of nano drug delivery systems need to be carried out to explain the following:

a. Toxicology of the developed nano drug delivery system
b. Activity of the drug loaded in the system
c. Pharmacokinetic parameters of the drug in the newly developed nano drug delivery system.

Nanotoxicology is one of the latest concerns to be attended by the researchers, as there are various controversies existing about the toxic effects of these ultrafine invisible structures. The researchers should pay a special attention in this regard if the materials either polymers or lipids or inorganic substances they use to prepare the nano drug delivery has not been studied for its toxicity, a nanotoxicological study becomes inevitable. The importance of the nanotxicological study is further stressed as notable deviations are reported in behavioral pattern of the materials on conversion to nano structures.

The improvement in the activity of the loaded drug can be studied in healthy animals or disease/ disorder induced animals depending upon the nature of the drug. It is recommended to include a group of animals treated with regular dose of the plain drug, which enables the researcher to compare the improvement in the activity of nano drug delivery system with that of the plain drug.

The improvement in the bioavailability of the drug in the newly developed dosage form needs to be assessed by studying the pharmacokinetic parameters in detail. A comparative study of the nano drug delivery system with either the plain drug itself or a marketed dosage of the same drug is very common. To conclude the pre-clinical studies an in vitro-in vivo (IVIV) correlation studies can be done to evaluate the reliability of the developed nanodrug delivery system.

APPLICATIONS OF NANOPARTICLES:

a) Cancer treatment:.It can target tumor, sense the pathological changes in tumor cells and delivers the neurotherapeutic agents on tumors. Nanoparticles are nanosized but still these are bigger than many anticancer drugs which makes it difficult to evade many organs .Indeed, recently that researchers have begun to design nanoparticles which involves attaching molecules known as ligands which can actively target tumor cells.

b) Treatment of tuberculosis: Nanoparticles are used for directing antitubercular drugs in to the target cells as the causative microorganisms are found inside the cells. Nanoparticles provide sustained release of antitubercular drugs and increased their efficacy after oral administration.

c) Colon targeting: Colon specific drug delivery to target colon can help to improve bioavailability of drugs and helps in treating colon diseases like ulcerative colitis, chronous disease etc.

d) Brain targeting: The preclinical studies using nanoparticles are reported to cross blood brain barrier and delivers the neurotherapeutic agents to treat several central nervous system ailments like Parkinson's disease, Alzheimer's disease, schizophrenia and other neurodegenerative disorders. They are also being investigated as possible agents in imaging brain disorders.

e) To target anti inflammatory drugs to inflamed tissues, that helps to reduce the associated adverse effects like gastric irritation, nausea and vomiting.

f) Nanoparticle suspensions have been used to deliver drugs like pilocarpine in to eye.

g) These have promising effects in oral delivery of proteins and peptide drugs.

h) Nanoparticles containing radionucleotides are used as diagnostic agents

i) The oily core of solid lipid nanoparticles containing cosmetics oils and other materials are formulated as skin and hair care products

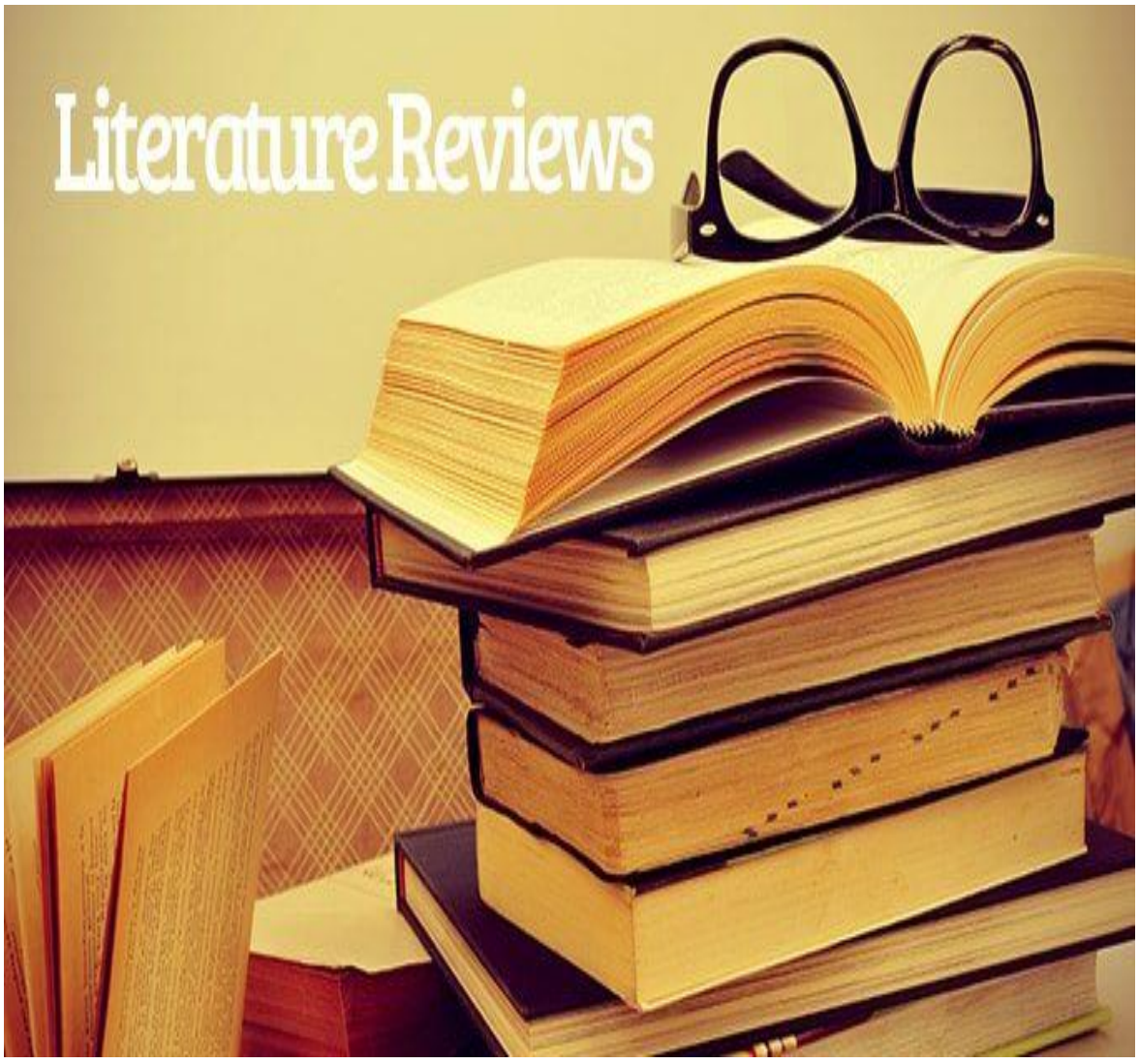
j) Nanoparticles are used for delivering genes and the application for the gene therapy is confirmed.[40]

PERSPECTIVES:

Significant progresses recorded in nanoparticle design to enable more precise delivery of the drugs to the target site. The development of new polymers and excipients had largely contributed to the application of the nano drug carriers to reach more targets. But, still the long term effects of nanoparticles *in vivio* are in dark and the researchers need to focus their investigation to eliminate the mystery behind the nanotoxicty. The utility of nano systems for the drug delivery can be reinforced by

a) Development of basic theories of drug loading and drug release from nanocarriers.
b) Sorting out the toxic nanomaterials, if any, and avoiding their usage.
c) Development of tailor made nano drug delivery platforms for reaching specific sites.
d) Development and standardization of simple and faster manufacturing procedures suitable for scaling up.
e) Identification of cheaper and more reliable materials for preparation of nano drug delivery systems.

The research reports made on nano drug delivery systems in last two decades are indicative of the potential of this system to reach new targets safely, effectively and more economically.


Literature Reviews

2.LITERATURE REVIEW

Shanmugasundaram Sangeetha *et al* (2007)[41] developed formulation of sodium alginate nanospheres containing amphotericin B for the treatment of systemic candidiasis by using sodium alginate 0.1% w/v to induce gellification. It provided in-vitro release characteristic and in-vivo anti-fungal activity.

Anindita Mukherjee *et al* (2009)[42] developed formulation characterisation and evaluation of curcumin loaded PLGA nanospheres for cancer therapy by solvent evaporation technique. It concluded that in-vitro release profiles on cell viability in prostate cancer cell lines.

BENEDETTA ISACCHI *et al* (2011) [43] reported a detailed development and optimization of artemisinin-loaded liposomes having proper physical characteristics as drug carriers for parenteral administration, in terms of particle size, PDI, encapsulation efficacy, and zeta - potential. The in vivo pharmacokinetic studies led to conclude that encapsulation of artemisinin into liposomes is a reasonable method to prolong its circulating time in the blood.

Khariya et al (2012)[44] developed formulation and evaluation of polymeric nanoparticle of anti-viral drug for gastro retention. Acyclovir was selected due to its narrow absorption window in stomach and upper GIT by nano-precipitation method using hydrophilic polymer such as bovine serum albumin, chitosan in 1:1 ratio. Study revealed no major interaction between the drug and polymer.

Naik J B *et al* (2012)[45]developed formulation and evaluation of repaglinide loaded nanoparticles as sustained release carriers by using Eudragit RL 100 polymer through high pressure homogenisation emulsification method. Results showed no interaction between drug and polymer and good sustained release characteristics in- vitro studies.

Afifa Bathol *et al* (2012)[46] developed characterisation of atorvastatin calcium loaded chitosan nanoparticles for sustain drug delivery prepared by solvent evaporation method for sustained

release using Polytron PT 1600,concluded that in vitro release study showed the release was sustained upto seven days , hence prepared nanoparticles were proved to be promising dosage form for sustained drug delivery of atorvastatin.

P Srinivas *et al* (2012)[47] developed formulation and evaluation of monifloxacin hydrochloride ocular nanoparticles by solvent displacement method using eudragit RL 100 as polymer. Formulation possess good antibiotic activity against *Bacillus subtilus, E.coli, and Staphylococus aureus* microorganisms proved no eye irritation on in vivo testing.

Adlin Jino Nesalin *et al* (2012)[48] developed preparation and evaluation of chitosan nanoparticles containing zidovudine loaded that is prepared by ionic gelation of chitosan with tripolyphosphate anions concluded that all the drug batches were found to follow first order and produced release over period of 24 hours.

D.Karthikeyan *et al* (2013)[49] developed formulation and evaluation of stavudine nanoparticles by using chitosan 0.1% gel prepared by ionotropic pre-gelation method. It showed better entrapment efficiency and cumulative percentage of drug release comparing to other formulations.

A Dinda *et al* (2013)[50] was developed formulation evaluation of paclitaxel loaded solid-lipid nanoparticles which are important for their size and stability prepared by solvent emulsification evaporation method, in-vitro release studies showed initial burst release followed by controlled release for 48 hours. Drug diffuses from SLN's at comparatively slow rate as the distance for diffusion increases.

Sutar PS *et al* (2013)[51] developed gemcitabine loaded PLGA nanoparticle and as polymer in the ratio of 50:50, prepared by modified emulsification solvent evaporation technique. This novel dosage form help to overcome side effects and is used to target the colonic region.

Vikram S Shenoy *et al* (2013)[52] developed in-vitro anti-cancer evaluation of 5-flurouracil lipid nanoparticle using B16F10 melanoma cell lines and this method was prepared by GMS & Cetyl palmitate by hot homogenisation method. They concluded that B16F10 cell lines revealed CPSLN had better cytotoxicity than 5-FU solution. GMS SLN's at 48 hours of incubation

resulted that encapsulating 5-FU in CP would be a promising drug delivery system.

.**Amol Kumar Lokhande *et al* (2013)**[53] developed formulation and evaluation of glipicide loaded nanoparticles prepared by emulsification solvent evaporation technique sustained release nanoparticles of glipicide could be able to manage type-2 DM with reduced dose frequency and decreased side effects with improved patient compliance.

Sagar S Jadhav *et al* (2013)[54] developed formulation and characterisation of chitosan nanoparticles loaded with rizatriptan benzoate by ionic gelation of chitosan with biphosphate anions study concluded that percentage muco adhesion on nasal mucosa of goat was found to be 29.4% it revealed that rizatriptan benzoate loaded chitosan nanoparticles is most suitable for intranasal drug delivery.

DADGAR NEDA *et al* (2013) [55] reported the efficacy of artemisin in nano liposome, artemisin in nano liposome polyethyleneglycol on breast cancer cell line (MCF -7 cell line). In this Liposomes were prepared by reverse phase evaporation method. Phophatidylcholine, cholesterol and artemisinin were combined together at certain concentrations in this method. The stability of the prepared formulation was increased by pegylattion (Polyethyleneglycol 2000). The diameter of the nano liposomes were instrumentally determined by Zetasizer. The results showed that encapsulation and release of artemisinin from only liposomated formulation was more than pegylated form. This study also revealed that the cytotoxicity effect of artemisinin liposome polyethyleneglycol was more than that of artemisinin liposomes

Aenugu Saritha Reddy *et al* (2014)[56] developed preparation characterisation of aspirin loaded ethyl cellulose nanoparticles by solvent evaporation technique in presence of Tween 20 the average particle size of best formulation was found to be 444.5nm and zeta potential of 41.5mV release sustained up to 12 hours.

Bhaskar Daravath1 and Rama Rao Tadikonda (2014)[57] carried out a study on Formulation and in vitro evaluation of flurbiprofen-polyethylene glycol 20000 solid dispersions.In this study n the present study, flurbiprofen solid dispersions were prepared using solvent evaporation method by incorporating polyethylene glycol 20000 and evaluated for solubility studies, drug-carrier compatibility studies and in vitro dissolution studies. From the solubility studies, formulations F4 were selected to prepare in the form of tablets and compared with control tablets (conventional tablets using pure drug). From the results of in vitro dissolution study, tablets

containing polyethylene glycol 20000 showed almost complete drug release within the 15 min. From the above results, they concluded that the formulation of solid dispersions using polyethylene glycol 20000 is a suitable approach to improve the solubility and dissolution rate of flurbiprofen.

OMAR H. EL-GARHY *et al* (2015)[58] carried out a study on formulation and evaluation of flurbiprofen sustained release matrix tablets using an alternative technique as potential economic approach. In this the FP tablets prepared by the alternative technique displayed the best physical characteristics. All FP prepared tablets displayed good sustained-release patterns. FP tablets prepared by the traditional method showed a progress decrease in drug dissolution by increasing matrix concentration and hence, more matrix agent or multiple granulations was needed which makes granulation process to be difficult and cost. While, FP tablets prepared by the alternative technique displayed dissolution profiles with minimal differences in-between reflecting the low

labor cost of granulation process where good sustained patterns could be obtained by a minor of the matrix agent. Histologically, the ulcerogenic effects of FP on the rats were highly reduced by FP tablets prepared by the alternative technique rather than others. The release kinetics of different prepared FP tablets displayed a coupled release pattern between diffusion and dissolution.This work proved the potential of the alternative technique as an effective economic approach for formulating FP sustained released tablets with better characteristics and low labor cost.

YITONG J.ZHANG et al (2016)[59] preparation of artemisinin nanoparticles and lipid formulation inhibit growth of breast cancer cells in vitro and induce down regulation of breast cancer cells.The new artemisinin dimer piperzine derivatives remained tightly associated with liposomal nanoparticles at neutral ph but were efficiently released at acidic ph that are known to exist within solid tumors and organelles such as endosomes and lysosomes.The new artemisinin derivatives showed improved cell proliferation inhibition effects compared to known dimer derivatives.

AMIR GHARIB et al (2017)[60] preparation and characterization and invitro efficacy of magnetic nanoliposomes containing the artemisinin and transferrin.in this study was to prepare the artemisinin and transferrin loaded magnetic nanoliposomes in thermosensitive and non-

thermosensitive forms and evaluate the antiproliferative activity against MCF-7 AND MDA – MB-231 cells for better tumour therapy.the results showed that the artemisinin and transferrin loaded magnetic nanoliposomes would be an effective choice for tumor –targeted therapy due to its suitable stability and high effectiveness.

.**SUBRAMANIAN NATESAN et al (2017)**[61]prepared artemisinin loaded chitosan magnetic nanoparticles for the efficient targeting to the breast cancer.It is an natural anti-malarial agent also possesses anti proliferative and anti-angiogenic activity in cancer cells with very low toxicity to normal healthy cells.The developed magnetic nanoparticles of artemisinin were smooth and spherical in nature and their size was in the range of 349 to 445nm.Around 62% to 78% of artemisinin was released from the artemisinin magnetic nanoparticles over the period of 48hrs.

YUNGUIN ZHANG et al (2018)[62] reported a detailed explanation about antitumor research on Artemisinin and its bioactive derivatives such as dihydroartemisin (DHA), artemether (ATM),arteether, artemisone and artesunate(AS) appear to be more potent than artemisinin.

.**YOUFANG CHEN et al (2018)** [63] studied artemisinin nanocapsules as anticancer drug delivery systems.artemisinin crystals were encapsulated with chitosan, gelatin and alginate for the purpose of controlled release of this anticancer drug.

JABER EMAMI et al (2018)[64] described artemisinin loaded nanostructured lipid carriers were prepared using solvent evaporation method and the impact of various formulation were assessed .optimised artemisinin nanostructural lipid carriers was then coupled with transferrin as targeting ligand and its invitro cytotoxicity was investigated against U-87MG brain cancer cell line.Mean particles size ,zeta potential, polydispersity index,entrapment efficiency ,Mean release time of adopted formulation done respectively.

HAMED NOSRATI et al (2019)[65] described artemisinin is used as an antimalarial and anticancer agent with minimal toxic effects on the host body.Biotin PEG-PCL polymers have been used for targeted dug delivery to cancer as well as to improve the pharmacokinetics of the drug and reduce its effects. The toxicity of artemisinin and its nanoparticles have been

investigated on MCF-7 and normal HFF2 cells.The results showed that the encapsulation efficacy of artemisinin in nanoparticles was 45.5± 0.41%.

DEEPIKA KANNAN et al (2019)[66] studied preclinical studies of iron oxide nanoparticles fortified artesunate for efficient targeting of malarial parasite.


OBJECTIVES

3. AIM & OBJECTIVE

AIM OF THE WORK:

The main aim of present study is to prepare and characterize polymeric nanoparticles for the selected drug Artemisinin.

OBJECTIVE OF THE WORK:

- To achieve controlled release of Artemisinin
- To reduce the frequency of dosing
- To improve the patient compliance
- To reduce the dose size
- To enhance the bioavailability


PLAN


WORK

4. PLAN OF WORK

1. Preformulation studies of selected Artemisinin drug
2. Preparation of calibration graph of Artemisinin by UV
3. Characterization of Artemisinin
4. Drug-Polymer interaction by DSC
5. Preparation of Artemisinin nanoparticles
6. Optimization of polymer concentration
7. Characterization of Artemisinin polymeric nanoparticles
    - Particle size
    - Zeta potential
    - Drug content
    - Entrapment efficiency
    - In vitro drug release study

DRUG PROFILE

5.DRUG PROFILE

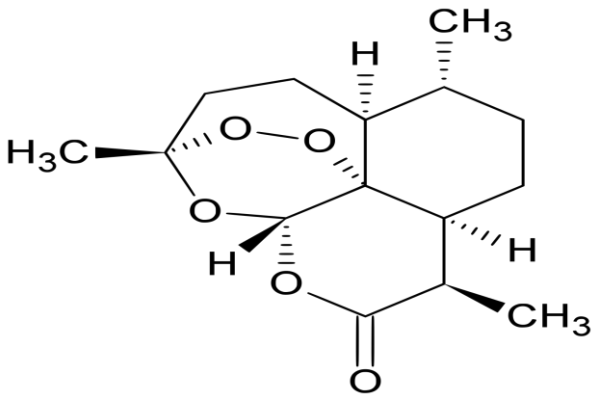
ARTEMISININ

SYNONMYS:

Artemisinine, qinghaosu

Artemisinin is a sesquiterpene lactone obtained from sweet wormwood, Artemisia annua, which is used as an antimalarial for the treatment of multi-drug resistant strains of falciparum malaria.

CHEMICAL STRUCTURE:



Formula: $C_{15}H_{22}O_5$

Molar mass: 282.332 g/mol

ATC code: P01BE01 (WHO)

Melting point: 152 to 157 °C (306 to 315 °F)

IUPAC NAME: (3R,5aS,6R,8aS,9R,12S,12aR)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10(3H)-one[67].

CLASS: antimalarial

CHEMISTRY :

An unusual component of the artemisinin molecules is an endoperoxide 1,2,4-trioxane ring. This is the main antimalarial centre of the molecule. Modifications at carbon 10 (C10) position give rise to a variety of derivatives which are more powerful than the original compound. Because the physical properties of artemisinin itself, such as poor bioavailability, limit its effectiveness, semisynthetic derivatives of artemisinin have been developed.

Derivatives of dihydro artemisinin were made since 1976. Artesunate, arteether and artemether were synthesised in 1986. Many derivatives have been produced of which artelinic acid, artemotil, artemisone, SM735, SM905, SM933, SM934, and SM1044 are among the most powerful compounds. There are also simplified analogs in preclinical research. Over 120 other derivatives have been prepared, but clinical testing has not been possible due to lack of financial support.

Artemisinin belongs to a class of sesquiterpene lactones. The solid is poorly soluble in oils and water. Therefore, it is mostly applied through the digestive tract, either by oral or rectal administration. Some chemical modification are suitable for administration by injection. Artesunate is the only artemisinin compound available for all types of administration procedure.A synthetic compound with a similar trioxolane structure (a ring containing three oxygen atoms) named RBx-11160 showed promise in in vitro testing.[68] Phase II testing in patients with malaria was not as successful as hoped, but the manufacturer decided to start Phase III testing anyway.

Artemisinin and derivatives:

Artemisinin is a Chinese herb (qinghaosu) that has been used in the treatment of fevers for over 1,000 years,[69] thus predating the use of Quinine in the western world. It is derived from the plant Artemisia annua with the first documentation as a successful therapeutic agent in the treatment of malaria is in 340 AD by Ge Hong in his book Zhou Hou Bei Ji Fang(A Handbook of Prescriptions for Emergencies).[70] Ge Hong extracted the artemesinin using a simple macerate and this method is still in use today.

The active compound was isolated first in 1971 and named artemisinin. It is a sesquiterpene lactonem with a chemically rare peroxide bridge linkage.

It is thought to be responsible for the majority of its anti-malarial action, although the target within the parasite remains controversial.[71] At present it is strictly controlled under WHO guidelines as it has proven to be effective against all forms of multi-drug resistant P. falciparum, thus every care is taken to ensure compliance and adherence together with other behaviors associated with the development of resistance. It is also only given in combination with other anti-malarials.

- **Artemisinin** has a very rapid action and the vast majority of acute patients treated show significant improvement within 1–3 days of receiving treatment. It has demonstrated the fastest clearance of all anti-malarials .currently used and acts primarily on the trophozite phase, thus preventing progression of the disease.[72] Semi-synthetic artemisinin derivatives (e.g. artesunate, artemether) are easier to use than the parent compound and are converted rapidly once in the body to the active compound dihydroartemesinin.

  On the first day of treatment 20 mg/kg should be given, this dose is then reduced to 10 mg/kg per day for the 6 following days. Few side effects are associated with artemesinin use. However, headaches, nausea, vomiting, abnormal bleeding, dark urine, itching and some drug fever have been reported by a small number of patients. Some cardiac changes were reported during a clinical trial, notably non specific ST changes and a first degree atrioventricular block (these disappeared when the patients recovered from the malarial fever)[73].

- **Artemether** is a methyl ether derivative of dihydro artemesinin. It is similar to artemesinin in mode of action but demonstrates a reduced ability as a hypnozoiticidal compound, instead acting more significantly to decrease gametocyte carriage. Similar restrictions are in place, as with artemesinin, to prevent the development of resistance, therefore it is only used in combination therapy for severe acute cases of drug-resistant *P.* falciparum.

  It should be administered in a 7-day course with 4 mg/kg given per day for 3 days, followed by 1.6 mg/kg for 3 days.[74]

Side effects of the drug are few but include potential neurotoxicity developing if high doses are given.

- **Artesunate** is a hemisuccinate derivative of the active metabolite dihydroartemisin. Currently it is the most frequently used of all the artemesinin-type drugs. Its only effect is mediated through a reduction in the gametocyte transmission. It is used in combination therapy and is effective in cases of uncomplicated P. falciparum.[75]

  The dosage recommended by the WHO is a 5 or 7 day course (depending on the predicted adherence level) of 4 mg/kg for 3 days (usually given in combination with mefloquine) followed by 2 mg/kg for the remaining 2 or 4 days. In large studies carried out on over 10,000 patients in Thailand no adverse effects have been shown.

- **Dihydroartemisinin** is the active metabolite to which artemesinin is reduced. It is the most effective artemesinin compound and the least stable. It has a strong blood schizonticidal action and reduces gametocyte transmission. It is used for therapeutic treatment of cases of resistant and uncomplicated P. falciparum. 4 mg/kg doses are recommended on the first day of therapy followed by 2 mg/kg for 6 days. As with artesunate, no side effects to treatment have thus far been recorded.[76]
  - **Arteether** is an ethyl ether derivative of dihydroartemisinin. It is used in combination therapy for cases of uncomplicated resistant P. falciparum. The recommended dosage is 150 mg/kg per day for 3 days given by IM injections. With the exception of a small number of cases demonstrating neurotoxicity following parenteral administration no side effects have been recorded.

MEDICAL USES:

- Uncomplicated malaria
- Severe malaria
- Cancer

UNCOMPLICATED MALARIA:

Artemisinins can be used alone, but this leads to a high rate of recrudescence (return of parasites) and other drugs are required to clear the body of all parasites and prevent recurrence. The World Health Organization (WHO) is pressuring manufacturers to stop making the uncompounded drug available to the medical community at large, aware of the catastrophe that would result if the malaria parasite developed resistance to artemisinins.

The WHO has recommended artemisinin combination therapies (ACT) be the first-line therapy for P. falciparum malaria worldwide. As short-acting drugs, artemisinin compounds are given with one or two long-acting drugs like amodiaquine, mefloquine, sulfadoxine/pyrimethamine or lumefantrine. Combinations are effective because the artemisinin component kills the majority of parasites at the start of the treatment, while the more slowly eliminated partner drug clears the remaining parasites.

Several fixed-dose ACTs are now available containing an artemisinin component and a partner drug which as a long half –life,such as mefloquine (ASMQ), Lumefantrine (coartem), Amodiaquine (ASAQ),Piperaquine(Duo–cotecxin) and pyronaridine(Pyramax).Increasingly these combinations are being made to GMP standard.

Artemisinins are not used for malaria prevention because of the extremely short activity (half-life)of the drug. To be effective, it would have to be administered multiple times each day.

SEVERE MALARIA:

Artesunate administered by intravenous or intramuscular injection has proven superior to quinine in large, randomised controlled trials in both adults and children. Combining all trials comparing these two drugs, artesunate is associated with a mortality rate that is approximately 30% lower than that of quinine. Reasons for this difference include reduced incidence of hypoglycaemia, easier administration and more rapid action against circulating and sequestered parasites.

Artesunate is now recommended by the WHO for treatment of all cases of severe malaria.[77] Effective treatment with ACT (artemisinin combination therapy) has proven to lower the morbidity and mortality from malaria within two years by around 70%.

CANCER:

To determine the efficiency of the drug against cancer cells, several clinical trials have been conducted with neoplastic cell cultures, animals, and humans. The National Cancer Institute conducted a study with 55 different cancer cell lines to evaluate their response to in vitro treatment with artesunate[78]. In this study, several cancer cells demonstrated susceptibility to the compound, including breast, prostate, ovary, colon, kidney, central nervous system, and melanoma cellS.

Zhao and colleagues tested artemether in a study with diffuse large B-cell lymphoma cells. There were no previous reports on artemisinin or any derivative with those types of cells. They concluded that artemether inhibited the proliferation of the cancer cells, arrested them in the G0/G1 phase, and with an increased concentration of the drug, they managed to induce apoptosis .[79]

In a similar study, Cheng and colleagues also found that artemisinin and the derivative SM1044 effectively induced apoptosis and degraded the survivin protein in diffuse large B-cell lymphoma cells.[80]

Morrissey and colleagues also found that treatment with 2Py, a synthetic dimer they produced, resulted in loss of the survivin protein in prostate cancer cell lines.[82]

The reported clinical trials in humans include patients with breast cancer, cervical cancer, hepatocellular carcinoma, non-small cell lung carcinoma, and squamous cell laryngeal carcinoma who were treated with an artemisinin derivative .[83]

Singh and Verma reported a case of a patient with squamous cell laryngeal carcinoma.[84] The patient was treated for fifteen days with a daily 60 mg intramuscular dose of artesunate followed by 50 mg of oral artemisinin for nine months. After two months of treatment, they reported a 70% reduction of the tumor, as well as a drastic improvement of the patient’s dysphagia and dysphonia .[85]

PHARMACOLOGY AND MODE OF ACTION:

As of 2018, the exact mechanism of action of artemisinins was not known because of the complex chemical interactions involved. Artemisinins do not directly attack malarial parasites or cells. They have to undergo chemical changes in the blood. Their functional group endoperoxide ring has to be activated first. Activation is done by cleavage of the endoperoxide ring.

As the drug molecules come in contact with the haem (inside the haemoglobin of the red blood cells), the iron(II) oxide breaks the endoperoxide ring.This process produces free radicals that in turn damage susceptible proteins, resulting in the death of the parasite. In 2016 artemisinin was shown to bind to a large number of targets suggesting that it acts in a promiscuous manner.[86] Unlike other antimalarials which are active only on a particular stage of malarial parasite, artemisinin is able to kill all the life cycle stages.

PHARMACOKINETICS:

METABOLISM AND PHARMACOKINETICS:

Once absorbed, the artemisinin derivatives are converted primarily to dihydroartemisinin (DHA) and thence to inactive metabolites via hepatic cytochrome P-450 and other enzyme systems. DHA is itself a potent antimalarial with an elimination half life of about 45 minutes.

The extent of conversion to DHA differs between derivatives. Artemisinin itself is not metabolised to DHA but acts as the primary antimalarial, while artesunate is rapidly (within minutes) hydrolysed to DHA and its antimalarial activity is largely mediated by DHA. Artemether and arteether contribute to antimalarial activity, probably to a similar extent as DHA, to which they are converted more slowly.

DHA is mostly (90%) bound to plasma proteins.[87]Pharmacokinetic studies on artemisinins have been limited by difficulties of assay; several techniques with differing accuracies have been used by various groups.

Furthermore, studies must necessarily take into account active metabolites (mostly DHA). Bioassay techniques measuring total antimalarial activity account for this and, along with

advances in assay methods, have allowed clearer pharmacokinetic profiling to emerge for drug formulations that have often been used empirically for many years. These studies are improving our understanding pharmacodynamic and toxicological aspects of this group of compounds.

In uncomplicated malaria, when artemisinins are used orally, most pharmacokinetic information is now available for artesunate followed by artemether. The absolute bioavailability of antimalarial activity after a single dose of oral artesunate in uncomplicated adult malaria is about 60% although there is greater interpatient variation than in healthy volunteers.Time to maximum DHA concentration is typically one to two hours. Studies suggest that clearance after artesunate is reduced during acute infection compared with recovery, either via disease effects on pharmacokinetics or enzyme autoinduction.

Although absolute bioavailability studies for artemether, artemisinin, and DHA are not possible given lack of intravenous formulations, pharmacodynamic activity (parasite clearance) after oral dosing of these derivatives is satisfactory. When studied, oral formulations show appropriately reliable and rapid absorption in the treatment of uncomplicated malaria. As for artesunate, studies of oral artemether and artemisinin show increasing clearance with multiple dosing and during recovery from acute infection.

In severe malaria, the delayed and variable absorption of the oil soluble derivatives artemether and arteether when given by the intramuscular route is of great potential clinical relevance.[88]

Metabolism:

In the liver artemisinin is converted to different inactive metabolites such as deoxyartemisinin, deoxydihydroartemisinin, crystal 7, and 9,10-dihydrodeoxyartemisinin. The metabolites have lost the endoperoxide group and become ineffective. The reaction is catalysed by an enzyme CYP2B6, while another enzyme CYP3A4 acts as a secondary catalyst.

In the absence of CYP2B6, CYP3A4 becomes the primary enzyme. These enzymes belong to cytochrome P450 group present in the smooth endoplasmic reticulum. Artemisinin

derivatives are metabolised differently. They are first converted to dihydroartemisinin (DHA). DHA itself is a strong antimalarial molecule and is active in the blood circulation for two to three hours. The antimalarial activity of artesunate is actually only through DHA. (Artemisinin, arteether, artemether, etc. are directly antimalarials.) Artesunate is converted to DHA within a minute of its absorption.

About 90% of the total DHA normally binds to blood plasma. In the liver, cytochrome P450 enzyme system (including CYP2A6, CYP3A4, and CYP3A5) convert DHA into inactive metabolites. All the metabolites undergo glucuronidation after which they are excreted through the urine or faeces. UDP-glucuronosyltransferases, in particular UGT1A9 and UGT2B7, are responsible for the process. DHA is also removed through bile as minor glucuronides, such as tetrahydrofurano acetate. Due to fast metabolism, artemisinins are relatively safe drugs.

LIMITATIONS OF ARTEMISININS:

Putting aside questions of cost, which may be the most important for users of antimalarials but have been comprehensively reviewed in a recent authoritative report from the Institute of Medicine, there are certain inherent problems with current artemisinins that require discussion.

Poor cure rate of monotherapy:

Artemisinins reliably reduce initial malaria parasitaemia by a factor of 104 per 48 hour asexual cycle and modelling studies therefore suggest that six days of treatment should cure parasite burdens of up to 1012 parasites.[89] This model is difficult to reconcile with the high recrudescence rates (10%–15%) seen with artemisinin monotherapy.

This poor efficacy of cure (which is not due to resistance) is usually attributed to the intrinsically short half life of artemisinins, which is further shortened by the increased drug clearance that develops during repeat dosing and/or convalescence with various oral artemisinin derivatives Blaming pharmacokinetic factors alone for the poor efficacy of artemisinin monotherapy may not be justified because constant drug levels are not necessary for potent pharmacodynamic effects (at least in the initial, visible phase of parasitaemia).

Furthermore, if pharmacokinetic behaviour were a problem, prolongation of treatment course may be predicted to compensate, but this is not generally observed in practice; seven days of monotherapy with artemisinin still only cures 80%–90% of uncomplicated falciparum infections. Parasite reduction ratio models for artesunate derived on data obtained at the start of treatment may not be applicable to the process of eradication of small numbers of residual parasites, which determines eventual cure rates.

Other phenomena may exist that permit escape from artemisinin therapy, necessitating a second (albeit less visibly effective) antimalarial. Although it has been strongly argued that, in any case, combination therapy has long term benefit in preventing resistance, the poor efficacy of monotherapy with the current generation of artemisinins remains a troubling and poorly explained phenomenon.

Neurotoxicity:

Despite pre-clinical evidence of brainstem toxicity in animals, millions of doses in various formulations have been given to humans without significant evidence of major toxicity, even when particular attention is given to monitoring for neurotoxicity both clinically and pathologically.[90] This discrepancy between animal and human toxicity has been attributed to the comparatively high and prolonged dosing regimens used in certain animal studies.

In addition, pharmacokinetic studies of parenteral artemether and arteether have showed the slow release and consequently long exposure times seen with oil based formulations of these drugs in both animals and humans.

It is probably the duration of exposure to artemisinins that determines neurotoxicity rather than the maximum concentrations reached. Prolonged high concentrations of artemisinins are certainly not seen in oral regimens, which constitute the vast majority of artemisinin courses given, and there is no pathological evidence of neurotoxicity in patients exposed to an average of 76.5 hours of intramuscular artemether.

A recent claim that artemether-lumefantrine induces mild but significant hearing loss seems to contradict this view but needs to be reproduced independently and the mechanism dissected, particularly in terms of the time course of hearing loss. Concern with regard to neurotoxicity should also be maintained in the context of children who have more vulnerable neurological systems and where therapeutic experience is more limited.[91] Even taking into account these concerns, artemisinin derivatives have less major toxicity than other available antimalarial drugs.

POLYMER PROFILE

CHITOSAN- Poly-(D)glucosamine

SYNONYMS:

Chitosan is a linear nontoxic polycationic polysaccharide consisting of 6-(1-4) linked 2-amino -2-deoxy-d-glucose (d-glucosamine)and 2-acetamido -2-deoxy-d-glucose(N-acetyl-d-glucosamine)units.

DESCRIPTION:

Chitosan is an amino polysaccharide, produced from the deacetylation of chitin obtained from crustaceans and insects. The diverse biological activity of this polymer has been demonstrated by a vast number of assays conducted in animals and a few clinical studies in humans.

Therapeutic applications have been proposed for chitosan because of its properties such as antioxidant activity, cholesterol, and triglyceride trapping, and antibacterial and hypoglycemic effects for the prevention and treatment of chronic diseases.

Chitosan possesses antibacterial and antifungal properties and has been extensively studied as a potential natural antimicrobial agent in the pharmaceutical, cosmetic, agricultural, and food industries. The antimicrobial properties of chitosan are affected by molecular weight, degree of acetylation and pH.[92]

Application in foods usually requires higher concentrations of chitosan than those in laboratory media because of interactions with complex food constituents that can alter chitosan activity.

CHARACTERISTICS :

Biocompatible, biodegradable, non-toxic, anti-microbial and soluble in wide range of solvents.

Chitosan occurs as odorless, white or creamy-white powder or flakes. Fiber formation is quite common during precipitation and the chitosan may look 'cottonlike'.

SOLUBILITY

- Soluble in organic acids-acetic, lactic, succinic.
- Insoluble in phosphoric and sulphuric acid
- Chitosan is insoluble in neutral or alkaline media, hence it should be first dissolved in aqueous solution.[93]

FUNCTIONAL CATEGORY: Coating agent; disintegrant; film-forming agent; tablet binder; viscosity-increasing agent.

STRUCTURE

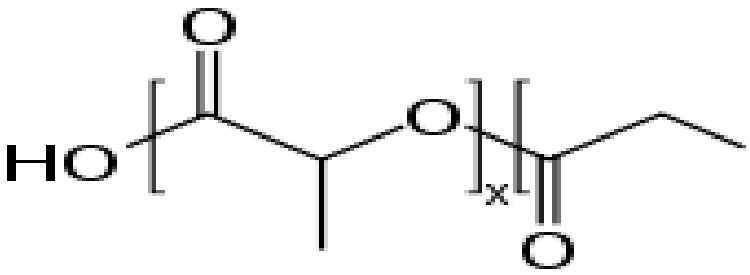


Fig.7: STRUCTURE OF CHITOSAN

PROPERTIES:

- Molecular weight : 1526.454g/mol
- Inherent viscosity: 200-800 Cp
- Acidity/alkalinity: pH = 4.0–6.0 (1% w/v aqueous solution)
- Density: 1.35–1.40 g/cm3
- Glass transition temperature: 203°C
- Moisture content: Chitosan adsorbs moisture from the atmosphere, the amount of water adsorbed depending upon the initial moisture content and the temperature and relative humidity of the surrounding air.

STABILITY AND STORAGE CONDITIONS:

Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool, dry place.

APPLICATIONS IN PHARMACEUTICAL FORMULATION:

Chitosan is used in cosmetics and is under investigation for use in a number of pharmaceutical formulations. These include controlled drug delivery applications, use as a component of mucoadhesive dosage forms, rapid release dosage forms, improved peptide delivery, colonic drug delivery systems,anduse for gene delivery.

Chitosan has been processed into several pharmaceutical forms including gels, films, beads, microspheres, tablets, and coatings for liposomes. Furthermore, chitosan may be processed into drug delivery systems using several techniques including spray-drying, coacervation, direct compression, and conventional granulation processes.[94]

DISEASE PROFILE-MALARIA

MALARIA :

Malaria is caused by the plasmodium parasite. This parasite is spread via the female Anopheles mosquito. Approximately 1,500 to 2,000 cases are diagnosed in the every year. The disease is present in both rural and urban environments. Anywhere with stagnant water is at risk of harbouring a breeding population of mosquitoes.

SYMPTOMS:

Initially, malaria feels like the flu with high fever, fatigue, and body aches, with hot and cold stages. They can also include vomiting, diarrhoea and muscle pains Symptoms typically appear within a week to eighteen days after infection, which can occur after even a single bite from a parasite-carrying mosquito[95]. Anemia is common in patients with malaria, in part due to the effects of the Plasmodium parasite on the red cells. If bites occur and these symptoms are apparent, medical attention should be sought out immediately.

CAUSES:

Malaria is caused by protozoa of the genus Plasmodium and is transmitted to humans by mosquitoes. The history of malaria shows that it was difficult to determine the disease's mode of transmission. When some cultures reviewed the facts available to them, they concluded that malaria was caused by bad air without realizing that the same swamps that created foul-smelling air also were excellent breeding grounds for mosquitoes. In 1880, the parasite was identified in an infected patient's blood.

STAGES:

There are several stages in the life cycle of *Plasmodium*, including sporozoites, merozoites, and gametocytes. The bite of an infected mosquito transmits the sporozoite stage of the organism to humans.[96] The parasite travels into the bloodstream and eventually makes its way to the liver, where it begins to multiply by producing merozoites. The merozoites leave the

liver and enter red blood cells to reproduce. Soon, young parasites burst out in search of new red blood cells to infect.

Sometimes, the reproducing *Plasmodia* will create a form known as a gametocyte in the human bloodstream. If a mosquito takes a blood meal when gametocytes are present, the parasite begins to reproduce in the insect and create sporozoites that are infectious to people, completing the life cycle.

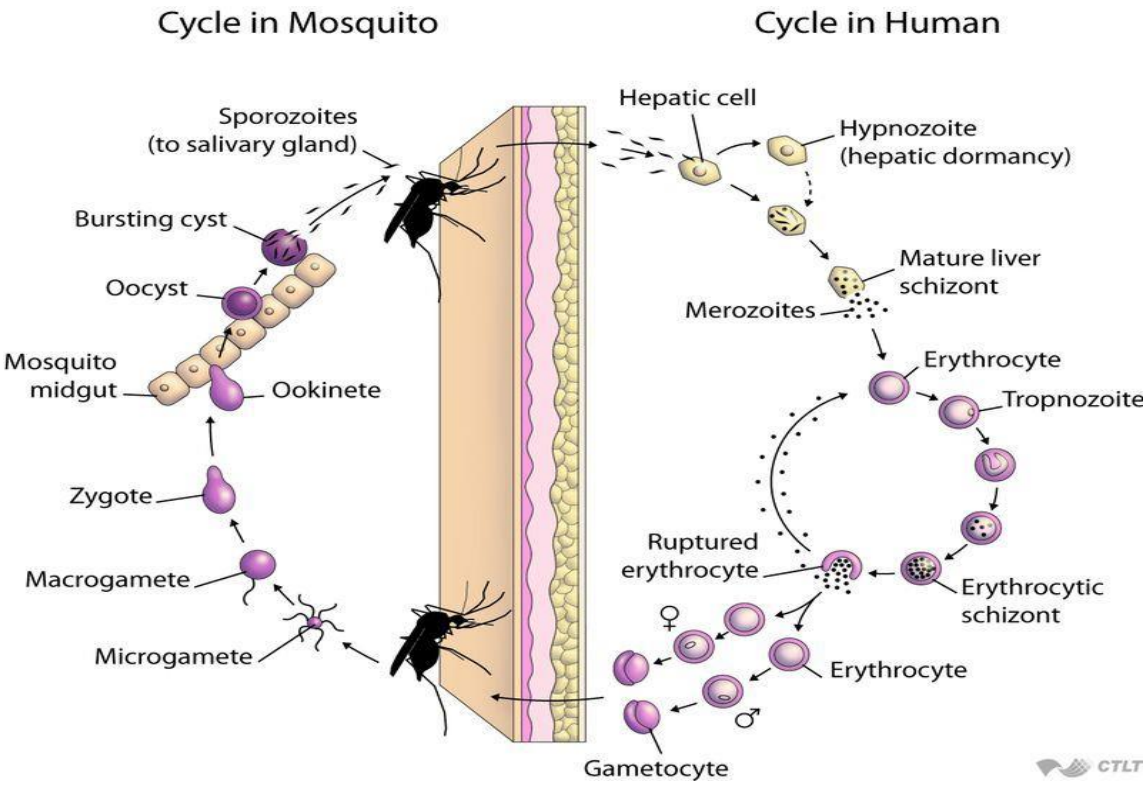


Fig.8: Life cycle

SPECIES OF PLASMODIUM:

There are five species of *Plasmodium* that infect humans:

- *P. vivax*: This species is most commonly found in Asia, Latin America, and parts of Africa.[97] Infections can sometimes lead to life-threatening rupture of the spleen.

This type of malaria can hide in the liver (this is called the "hepatic phase" of the life cycle). It may then return later to cause a relapse years after the first infection. Special medications are used to eradicate *P. vivax* from the liver.

- *P. ovale*: This species is rarely found outside Africa or the western Pacific islands. Symptoms are similar to those of *P. vivax*. Like *P. vivax*, *P. ovale* can hide in the liver for years before bursting out again and causing symptoms[98].

- *P. malariae*: It is found worldwide but is less common than the other forms. This form of malaria is hard to diagnose because there are usually very few parasites in the blood. If untreated, the infection can last many years.

- *P. falciparum*: This is the most life-threatening species of malaria. Although present throughout much of the tropical and subtropical world, it is particularly common in sub-Saharan Africa[99]. *P. falciparum* is resistant to many of the older drugs used to treat or prevent malaria. Unlike *P. vivax* and *P. ovale*, this species does not hide in the liver.

- *P. knowlesi*: Found predominantly in Malaysia, the Philippines, and Southeast Asia, this species can also cause high levels of parasites in the blood, leading to organ failure or death.[100]

PREVENTION:

The female *Anopheles mosquito* tends to feed at night. This fact makes it important that mosquito nets preferably treated with insecticide are used when sleeping. This prevention strategy alone has been shown to reduce disease incidence considerably.

Covering the skin to minimise the chances of being bitten, or spraying anti-mosquito sprays onto the skin may also reduce the risk of contracting the disease.

Antimalarials come in many varieties, with the type used depending on the stage of malaria, as well as the type of malaria parasite.[101]

Medicines such as Chloroquine are typically used as a frontline treatment, though antimalarial resistance is becoming a more common issue. This will require other medications.

DIAGNOSIS:

Malaria is diagnosed from a blood smear and the parasite is seen under the microscope. Other tests are available, but microscopy remains the cornerstone of diagnosis.

MALARIA TREATMENT:

Most patients recover completely after being treated. However, infection with *P. vivax*or *P. ovale* may be associated with organisms that hide in the liver for months or years, resisting treatment. Special medications are used to help eradicate these organisms, so it is important to closely follow medical directions when taking preventive medicines. Never stop the medication early if instructed to continue taking it for a few weeks after leaving the area where malaria is present.[102]

Two types of tests are available for diagnosis of malaria.

- Rapid tests can detect proteins called antigens that are present in *Plasmodium*. These tests take less than 30 minutes to perform. However, the reliability of rapid tests varies significantly from product to product. Thus, it is recommended that rapid tests be used in conjunction with microscopy.
- The second type of test is the polymerase chain reaction (PCR), which detects malaria DNA.[103] Because this test is not widely available, it is important not to delay treatment while waiting for results.

Treatment Options and Medications for Malaria:

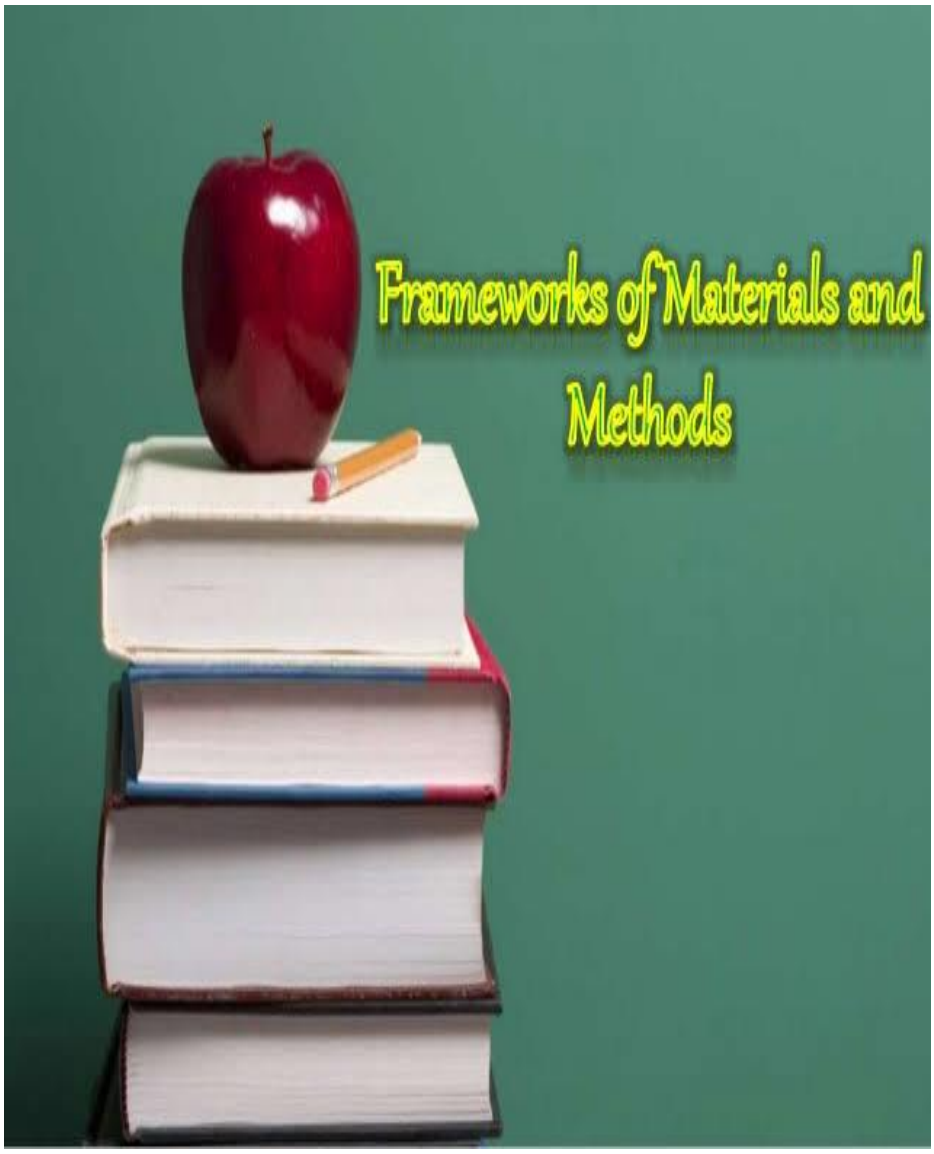
There are several medications available to treat malaria, including

- Chloroquine (Aralen);
- Artemether-lumefantrine (Coartem)
- Artesunate-amodiaquine (Amonate)
- Artesunate-mefloquine
- Dihydroartemisinin-piperaquine
- Artesunate-sulfadoxine-pyrimethamine (SP), in areas with known SP sensitivity[104]
- atovaquone-proguanil (Malarone) (Mepron is the trade name for atovaquone alone; it is not used by itself to treat malaria but only in combination with proguanil as Malarone.);
- mefloquine (Lariam);
- quinine (Qualaquin);
- quinidine (Quinaglute Dura-Tabs, Quinidex Extentabs, Quin-Release);
- Doxycycline (Adoxa, Avidoxy,Acticlate, Doryx, Monodox, Oraxyl, Vibramycin, Vibramycin Calcium, Vibramycin Monohydrate, Vibra-Tabs, used in combination with quinine);
- clindamycin (Cleocin HCl, Cleocin Pediatric, used in combination with quinine);
- Artesunate (available only through the CDC).

Most medications are available only as tablets or pills. Intravenous treatment with quinidine may be needed in severe malaria or when the patient cannot take oral medications.

INCUBATION PERIOD:

The incubation period between infection with malaria by a mosquito bite and initial symptoms may range from one week to one year. Generally, the incubation period ranges from nine to 14 days for *P. falciparum*, 12-18 days for *P. vivax,* and 18-40 days for *P. ovale*.


Frameworks of Materials and Methods

MATERIALS AND METHODS

List of Materials

Table 6.1. Materials used

Materials	Supplier
Artemisinin	Sigma aldrich pvt.ltd
Chitosan	Sigma aldrich pvt.ltd
Poloxamer	Sigma aldrich pvt.ltd
Ethanol	Sigma aldrich pvt.ltd
Potassium di hydrogen phosphate	M/S SD Fine Chemicals, Mumbai, India
Ortho phosphoric acid	M/S SD Fine Chemicals, Mumbai, India

Table 6.2. Equipment list

S.NO	EQUIPMENTS	MODEL
1.	Electronic balance	Metler Toledo AG 135.
2.	Ultra centrifuge	Remi instruments, Mumbai.
3.	Mechanical stirrer	Remi instrument.
4.	DSC	Schimadzu DSC-60.
5.	Particle size analyzer	Malveran master sizer.
6.	UV spectrophotometer	Schimadzu 1710, Mumbai.
7.	USP dissolution apparatus	Lab india, DS8000.

METHODS: Preformulation Studies:

Preparation of Calibration Graph For Artemisinin:

Preparation of Calibration Curve in PH 1.2, PH 7.4 and PH 6.8 buffer solutions:

An accurately weighed amount of Artemisinin 100mg was dissolved in small volume of buffer solutions in each of three 100 ml volumetric flask and the volume was adjusted to 100 ml with 1.2 pH buffer in first volumetric flask, 7.4 pH buffer in second volumetric flask and the third one was adjusted to 100 ml with 6.8 pH buffer. A series of standard solution containing in the concentration range from 10 to 50 µg/ml of Artemisinin were prepared for 1.2 pH buffer solution, 7.4 pH buffer solution and 6.8 pH buffer solution separately, absorbance was measured at 195 nm and calibration graph was plotted using concentration versus absorbance.

Drug-Excipient Compatibility Study By DSC:

Differential Scanning Calorimetry (DSC)

Samples of individual components as well as each drug-excipient were weighed (Mettler Electronic balance) directly in pierced aluminum crucible pans (5-10 mg) and scanned in the 50-300°C temperature range under static air, with heating rate of 10 ºC /min, using shimadzu DSC-60 equipment.

METHOD OF PREPARATION

Table 6.3. Formula Used For The Preparation of Artemesinin Nanoparticles:

S.NO	FORMULATION	DRUG (mg)	CHITOSAN (%W/V)	TWEEN (%W/V)
1.	**ANP-1**	100mg	0.10	2
2.	**ANP -2**	100mg	0.15	2
3.	**ANP -3**	100mg	0.20	2
4.	**ANP -4**	100mg	0.25	2
5.	**ANP -5**	100mg	0.30	2

METHOD:

PREPARATION OF ARTEMESININ NANOPARTICLES :

Chitosan was dissolved in 1% acetic acid and 100 mg of Artemesinin in phosphate buffered saline. This solution was added to 10 ml of liquid paraffin containing 2% w/v tween 20. This mixture was stirred using a homogenizer 3 minutes to form water in oil (w/o) emulsion.

- The resultant Artemesinin nanoparticles were centrifuged at 3000 rpm for 60 mts and washed using ethanol and water, consecutively to remove the remaining surfactant and liquid paraffin.
- Later they were dried in air for 3 hour followed by hot air oven at 50° for 4 hour and stored in a dessicator.[105]
- Several batches namely **(ANP1, ANP2, ANP3, ANP4 and ANP5)** were formulated by changing the drug and chitosan concentration and the effect of chitosan concentration on the encapsulation efficiency and the drug loading capacity was studied.

CHARACTERIZATION STUDIES:

- Drug content
- Particle size and zeta potential
- Drug content
- Encapsulation efficiency
- *In vitro* drug release

Particle Size and Surface Charge:

Surface charge is important in adhesion and interaction of particle with cells. The zeta- potential is used to measure the cell surface charge density. It can be measured using Malvern-Zeta sizer. The prepared Artemesinin nanoparticles were evaluated for their particle size and surface charge by photon correlation spectroscopy (PCS) using zeta sizer. The formulations were diluted to 1:1000 with the aqueous phase of the formulation to get a suitable kilo counts per second (kcps). Analysis was carried out at 25°C with an angle of detection of 90°. In this experiment six

replicates were taken for the measurement. The results were given in results and discussion section.

Drug Content:

1gm of Artemesinin nanoparticles were accurately weighed and transferred into a 25ml volumetric standard flask. The sample was dissolved with methanol .1ml of this solution was diluted to 25ml with the purified water. The standard Artemesinin was dissolved and diluted with same methanol and water respectively.

Then the standard and sample absorbance was measured at 195 nm using UV-Visible spectrophotometer. The percentage of drug content was calculated.

The results were given in results and discussion section.

Entrapment Efficiency:

The drug loaded Artemesinin nanoparticles in buffer solutions were subjected to centrifugation at 15000 rpm for 30 min.The supernatant liquid was separated and 1ml of this solution was diluted with buffer solution and the absorbance was measured at 195 nm. The amount of Artemesinin unentrapped in the supernatant was calculated.[105] The amount of Artemesinin entrapped was determined by subtracting amount of free unentrapped Artemesinin from the total amount of Artemesinin taken for the preparation.

The formula used to calculate entrapment efficiency was given below

$$\text{Drug entrapment}(\%) = \frac{\text{mass of drug in nanoparticles x100}}{\text{mass of drug used in formulation}}$$

The results were given in results and discussion section.

***In vitro* release:**

The release of Artemesinin nanoparticles were carried out using USP Type II dissolution apparatus at a rotation speed of 50 rpm, and a temperature of 37±0.5°C. The drug release studies were carried out in 7.2 pH phosphate buffer. An aliquot of 5 ml was collected at predetermined time intervals and replaced with fresh dissolution medium.[106] The samples were filtered, by filtering through 0.45 μm membrane filters and analyzed spectrophotometrically at 195 nm. From the absorbance values the cumulative percentage drug release was calculated[107]. The results were given in results and discussion section.


Results

7. RESULTS AND DISCUSSION

Preformulation studies:

Preparation of calibration graph for Artemisinin:

Standard calibration data of Artemisinin in pH 1.2, 7.4 and 6.8 buffers at 195 nm

Table 7.1. Absorbance of Artemisinin in Buffer solutions

S.No	Concentration (µg/ml)	Absorbance		
		PH 1.2	PH 7.4	PH 6.8
1.	10	0.042	0.052	0.066
2.	20	0.085	0.105	0.133
3.	30	0.128	0.155	0.199
4.	40	0.179	0.206	0.264
5.	50	0.211	0.253	0.329

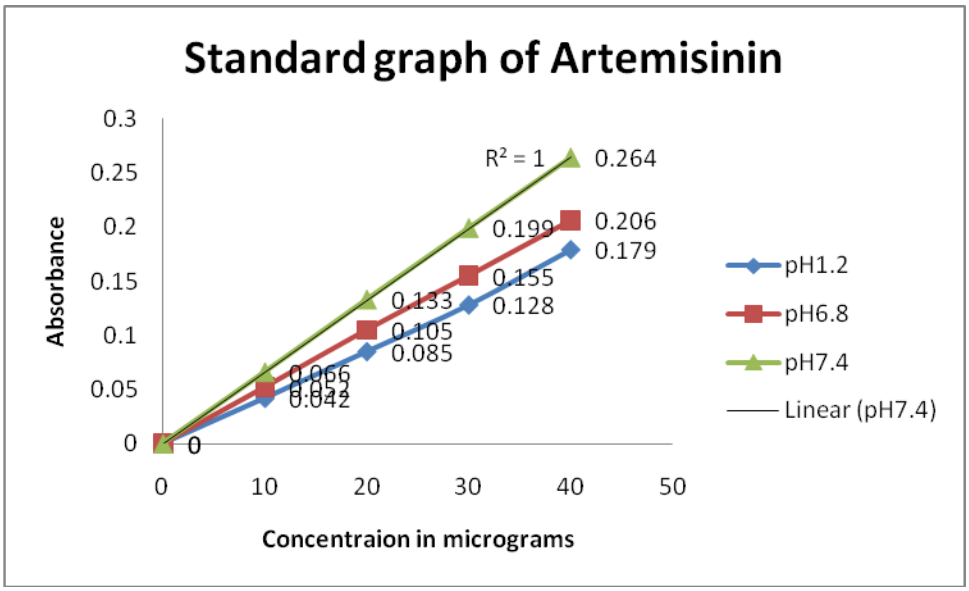


Fig. 7.1.Calibration curve of Artemisinin in pH 1.2,7.4 and 6.8 buffers

Standard calibration curve of Artemisinin was carried out in 1.2 pH, 7.4 pH and 6.8 pH buffer at 195 nm. The r^2 value in the entire medium shows nearly 1, which signifies linearity.

DSC Analysis:

DSC of Artemisinin showed a sharp endothermic peak at about 206.26^{0}C (melting point). The physical mixture of Artemisinin with other excipients also showed the same thermal behavior (206.28^{0}C) as the individual component. DSC results also revealed that the physical mixture of Artemisinin with excipients showed superimposition of the thermogram. There was no significant change observed in melting endotherm of physical mixture of Artemisinin and excipients.

Hence from the DSC study, it was found that there was no interaction between Artemisinin and other excipients used in the formulation.

The DSC thermogram were given in the **Fig.7.2 and 7.3**

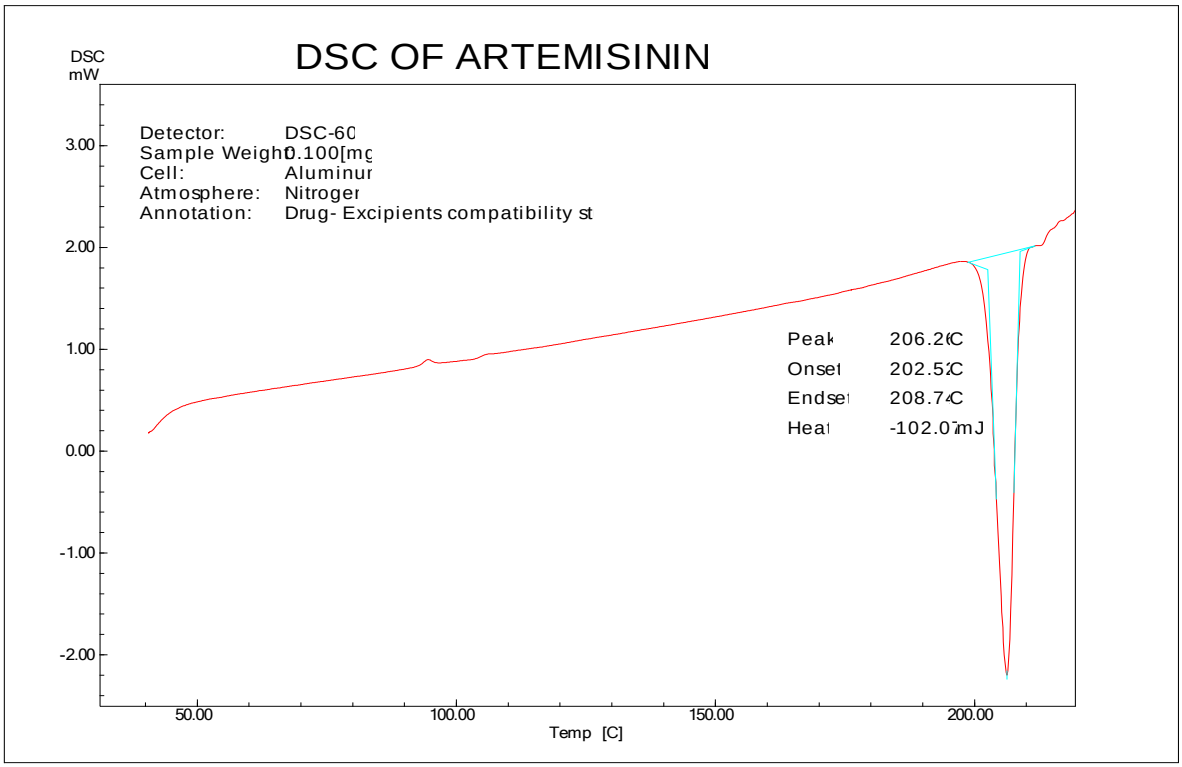


Fig.7.2

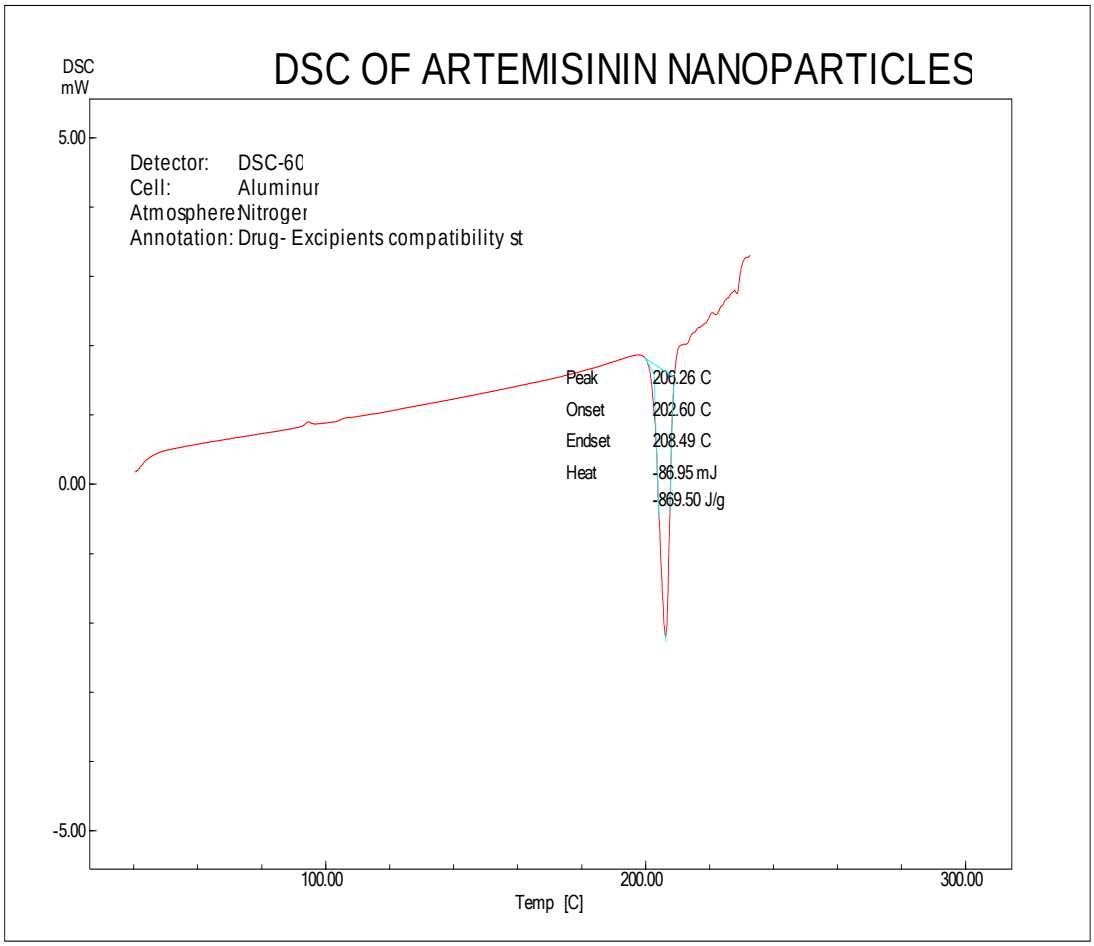


Fig.7.3

Fig.7.2 and 7.3. DSC Thermogram of Artemisinin and Artemisinin nanoparticles

Drug –Excipients Accelerated Compatibility Study - Physical Observation and Assay:

Upon analysis of the drug excipient mixture for their physical characteristics no colour change was observed. Based on the chemical evaluation it was found that there was no significant change observed indicating that the drug is compatible with the added ingredients. The results of this study were given in Table 7.2

Table 7.2. Physical Characteristics of Artemisinin:

S.No	Physical parameters	Results
1.	Description	Off white powder
2.	Melting point	196°C
3.	Loss on drying	0.35%
4.	Assay	98.58%

Table 7.3 Physical Characteristics of Individual Drug and Excipients:

S.No	Sample ID	Initial Description	Final Description
1.	Artemesinin	Off white powder	No change
2.	Chitosan	Off white powder	No change

Table 7.4 Physical Characteristics of Drug-Excipient Mixture:

S.No	Sample ID	Initial Description	Final Description
1.	Artemesinin	Off white powder	No change
2.	Artemesinin + Chitosan	Off white powder	No change

Table 7.5 Chemical Characteristics of Drug-Excipient Mixture:

S.No	Sample ID	Initial Assay (%)	Final Assay (%)
1.	Artemesinin	98.55±0.35	98.54±0.75
2.	Artemesinin+ Chitosan	98.58±0.46	98.56±0.56

n = 3; Mean ± S.E.M.

Table.7.6 Drug Content and Entrapment Efficiency Particle Size and Zeta Potential of Artemisinin Nanoparticles

Trials	Zeta potential (mV)	Particle size (nm)	Entrapment Efficiency (%)	Drug Content (%)
ANP 1	23.8	605.4	45.65	98.46
ANP 2	24.4	618.8	52.76	98.51
ANP 3	26.7	625.7	60.29	98.52
ANP 4	**27.3**	**637.2**	**86.85**	**98.53**
ANP 5	31.9	645.8	86.71	98.54

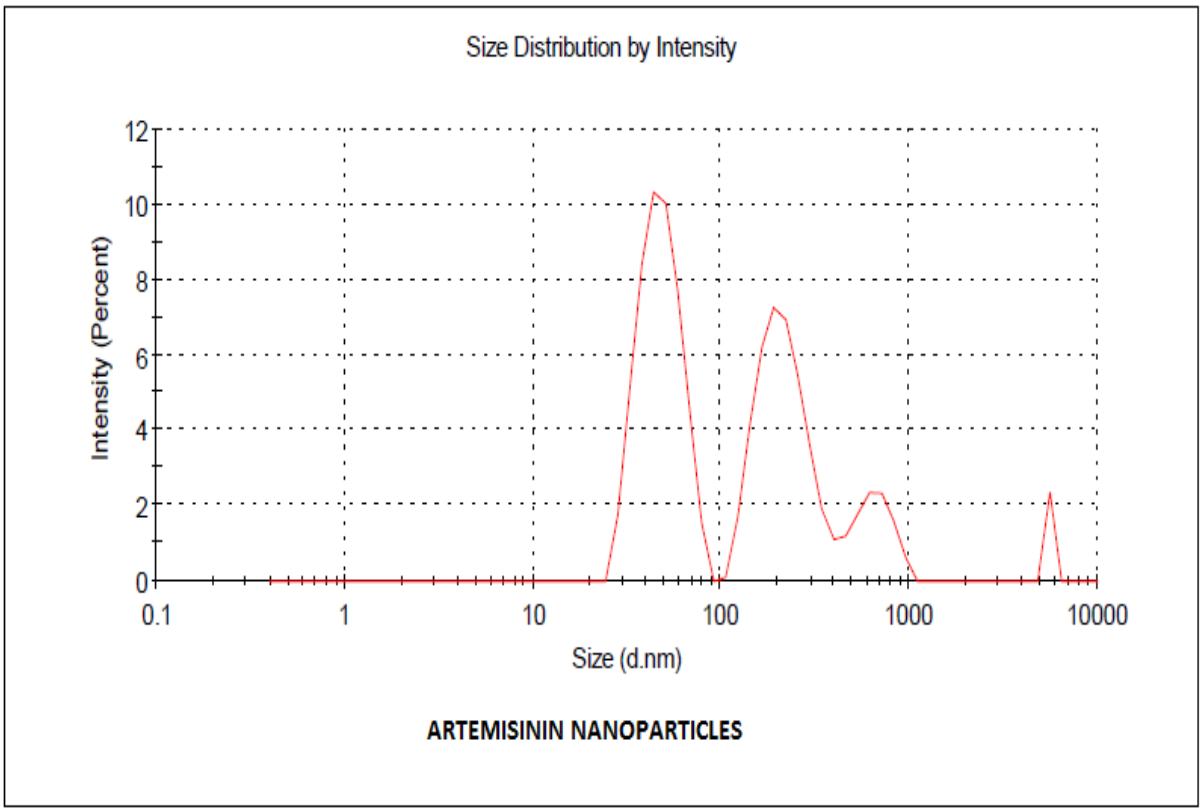


Fig.7.3 Particle Size of Optimized Artemisinin Nanoparticles (ANP 5)

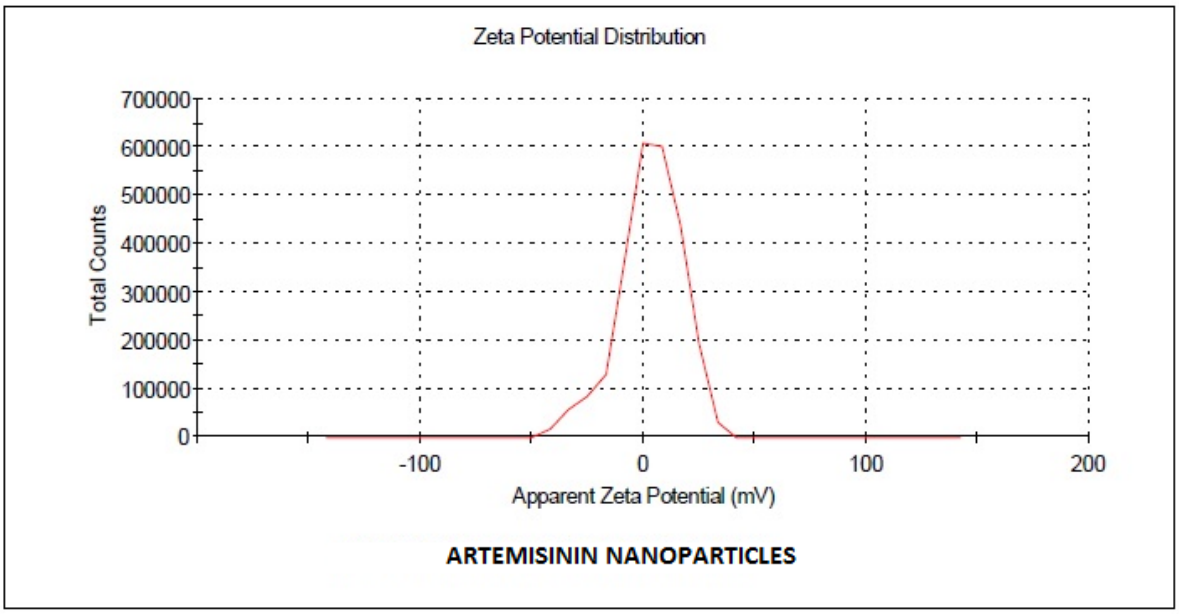


Fig.7.4 Zeta potential of Optimized Artemisinin Nanoparticles (ANP 5)

- Particle size and entrapment efficiency of the **Artemisinin nanoparticles (ANP 1 - ANP 5)** were increased with increasing **Chitosan** concentration.
- This may be due to high amount of availability of **Chitosan** to encapsulate the drug, upon increasing the **Chitosan** concentration, number of layers coated the drug was increased, this resulted in increased particle size and entrapment efficiency.
- Further increase in the **Chitosan** concentration **(ANP 1- ANP 5)**, there is no much increase in the entrapment efficiency due to the availability of the drug to be incorporated is low which is not enough for further encapsulation of drug by **Chitosan**.
- Based on the results of Particle size and entrapment efficiency of the **Artemisinin nanoparticles (ANP 1- ANP 5)**, the trial **ANP 4** which contains **0.25%w/v of Chitosan** concentration was selected as the best formulation.

***In- vitro* Drug Release:**

Table 7.7 *In vitro* Release Studies of Artemisinin Nanoparticles

S.NO	Time (Hrs)	%CUMULATIVE DRUG RELEASE				
		ANP 1	ANP 2	ANP 3	ANP 4	ANP 5
1	0.5	38.68	36.82	25.72	19.77	11.73
2	1	79.32	70.35	48.55	45.29	28.49
3	6	**98.55**	88.21	65.21	58.74	41.22
4	12	98.52	**98.56**	80.49	69.81	50.29
5	16	98.54	98.51	**98.53**	78.43	61.05
6	20	98.56	98.52	98.51	87.58	72.67
7	24	98.51	98.54	98.54	**98.57**	**85.77**

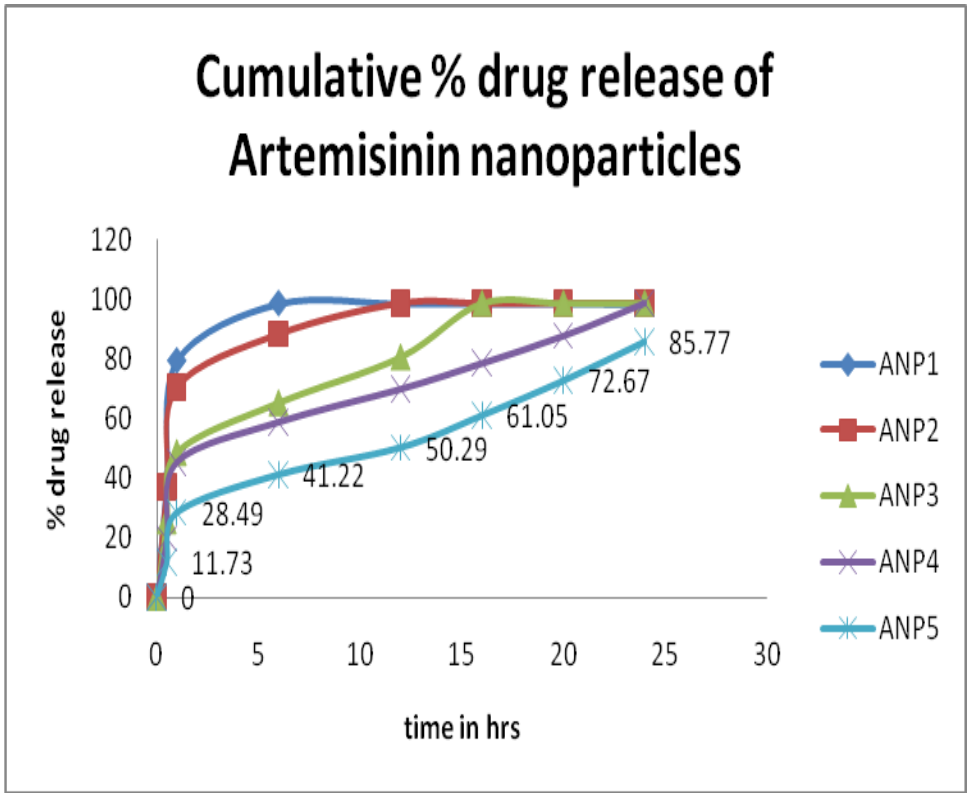


FIG.7.5:CUMULATIVE% DRUG RELEASE OF NANOPARTICLES

Effect of Chitosan concentration on Invitro drug release of Artemisinin nanoparticles

From the *in vitro* drug release study results, the maximum percentage drug release **98.57** at the end of 24h was observed with trial **ANP4** which contains **0.1% w/v of drug** and **0.25% w/v of Chitosan** and **2% w/v of tween**

Chitosan concentration as in the case of trial **ANP5** the maximum percentage drug release **85.77%** was obtained at the end of 24h which was found to be not suitable for controlled release of Artemisinin

Chitosan concentration as in trials **ANP1, ANP2 and ANP3,** the maximum % drug release were 98.55%, 98.56% and 98.53% respectively. The maximum percentage of drugs were released within 6h,12h and 16h for the formulations ANP1,ANP2 and ANP3 respectively**.**

At higher **Chitosan** concentration, slow drug release (**ANP5)** and at low concentration rapid drug release (**ANP1-ANP3)** were observed.

From the *in vitro* drug release data for **ANP1- ANP5,** it was observed that increase in Chitosan concentration delays the drug release due to increased particle size and reduced surface area of the prepared nanoparticles.

From all the formulations, **ANP4** was selected as best formulation due to its ideal particle size (**637.2** nm), Zeta potential (27.3), high entrapment efficiency (**86.85%**) and desirable drug release 98.57% at the end of 24 h.

8. SUMMARY AND CONCLUSIONS

The active pharmaceutical ingredient Artemisinin was evaluated for its Organoleptic properties and solubility. The results obtained were satisfactory.

Artemisinin nanoparticles were prepared by emulsion-droplet coalescence method and the polymer concentrations were optimized by various trials

In the present study Chitosan nanoparticles containing Artemisinin was prepared. The effect of increase in Chitosan concentration on various parameters like particle size, zeta potential,Entrapment Efficiency and *invitro* release profile were studied.

The results showed that the in vitro drug release for **ANP1, ANP2, ANP3, ANP4** and **ANP5** were found to be 98.51%**,** 98.54%, 98.54%, **98.57%** and 85.77% respectively at the end of 24hrs.

Based on the drug content,entrapment efficiency,particle size,zeta potential and *in vitro* drug release profile of Artemisinin nanoparticles formulations **(ANP1-ANP5)** formulation **ANP4**was selected as the best formulation in which the particle size was **637.2 nm**.

The *in vitro* % drug release of **ANP4** formulation was **98.57%** and it was found to be suitable formulation to treat Malaria. Hence it can be concluded that the newly formulated controlled release nanoparticulate drug delivery systems of Artemisinin may be ideal and effective in the treatment of Malaria by allowing the drug to release continuously for 24 hrs

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