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Nanoparticles: Fundamental and Prospectives.

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ABSTRACT

This review explores the recent therapeutic work on drug delivery using nanoparticles as a carrier for small and large molecules. As compared to direct delivery of the drug, delivery through a carrier increase the efficacy of a drug as well as decrease the side effects by enhancing permeability and retention effect. Nanoparticles are solid colloidal particles in size from 10nm-1000nm. They consist of macromolecular particle which entrapped, dissolved or encapsulate pharmacologically active agent and release in a controlled manner to achieve site- specific action at an optimum rate and dose regimen. They also alter and improve pharmacokinetic and pharmacodynamic properties of less efficacious drugs. The main approach in designing nanoparticles is to control particle size, surface properties and release pattern. The Current review reveals the methods of preparation, characterization and application of several nanoparticles drug delivery system.

Keywords: Introduction, types, advantages, preparation methods, evaluation parameters, applications.

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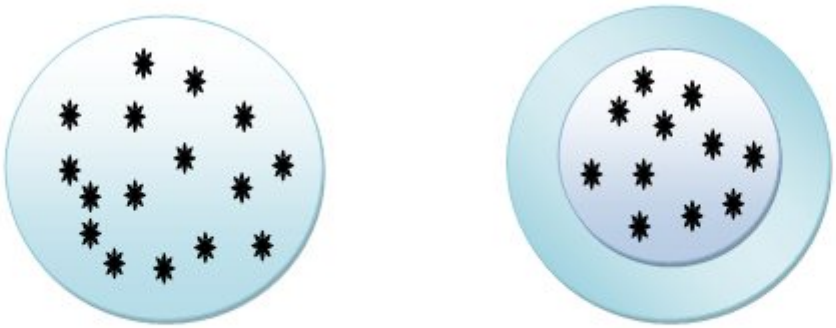
INTRODUCTION

The word "Nano" is a household name today. Nanotechnology embraces the study and application of nanomaterials of diverse kinds in diverse fields. The nanotechnology is being explored to develop newer nanostructures. The nanotechnology-enabled solutions have lead to development at atomic, molecular or macromolecular level, in length of approximately 1-100 nm range, not only to provide essential understanding of phenomena and materials at the nanoscale, but also to create and use structures, devices and systems with novel properties and functions. Considering the current state of art, nevertheless, the advantages of this cutting-edge nanotechnology could also be availed even at much higher size, say in order of 500nm. Nanostructured drug delivery has been successfully employed to deliver the therapeutically active drug molecules only to the desired site of action, without affecting the healthy organs and tissues. Targeted nanotechnology-based methods have been endeavored for treating various disease conditions like cancer, diabetes, and infection disorders, including bacterial, fungal and viral. Currently, there is no cure and preventive measures for HIV/AIDS, but with the help of advancement of nanotechnology, several treatment options have now been emerging.

Nanoparticles are one of the novel potential carrier systems which comes under the category of colloids, they replace the use of polymers because synthetic polymers cause toxicity inside the body but nanoparticles are made up of lipids as same as that phospholipids membranes present in our body and they do not cause any type of harm reactions that's why nanotechnology in the field of science plays a dominant role. Nanotechnique deals with production, manipulation and use of material ranging in nanometres. Nanoparticles are solid colloidal particles in size from 10nm-1000nm. Because of this nanosize, nanoparticles easily cross all the membranes and reach the specific site and gives controlled release on target site. They increase permeability as well as retension factor of pharmacologically active drug. Nanoparticles increase the solubility of poorly soluble drug by encapsulating the active ingredient inside the cavity of lipid membrane. Nanoparticles increase efficacy of low efficacious drugs as well as improve the safety parameters. [1-4]



Nanosphere Nanocapsule

- **Nanospheres** are matrix system in which drug is entrapped inside the cavity as a core material.
- **Nanocapsules** are a system where core material is encapsulated inside the cavity but surrounded by a unique polymer membrane.

ADVANTAGES OF NANOPARTICLES [5-7]

a) Nanoparticles Control and target drug release at the specific site.
b) Reduction in fed/ fasted variability.
c) Dose proportionality.
d) Smaller dose form.
e) They have greater compatibility.
f) High and enhanced drug content.

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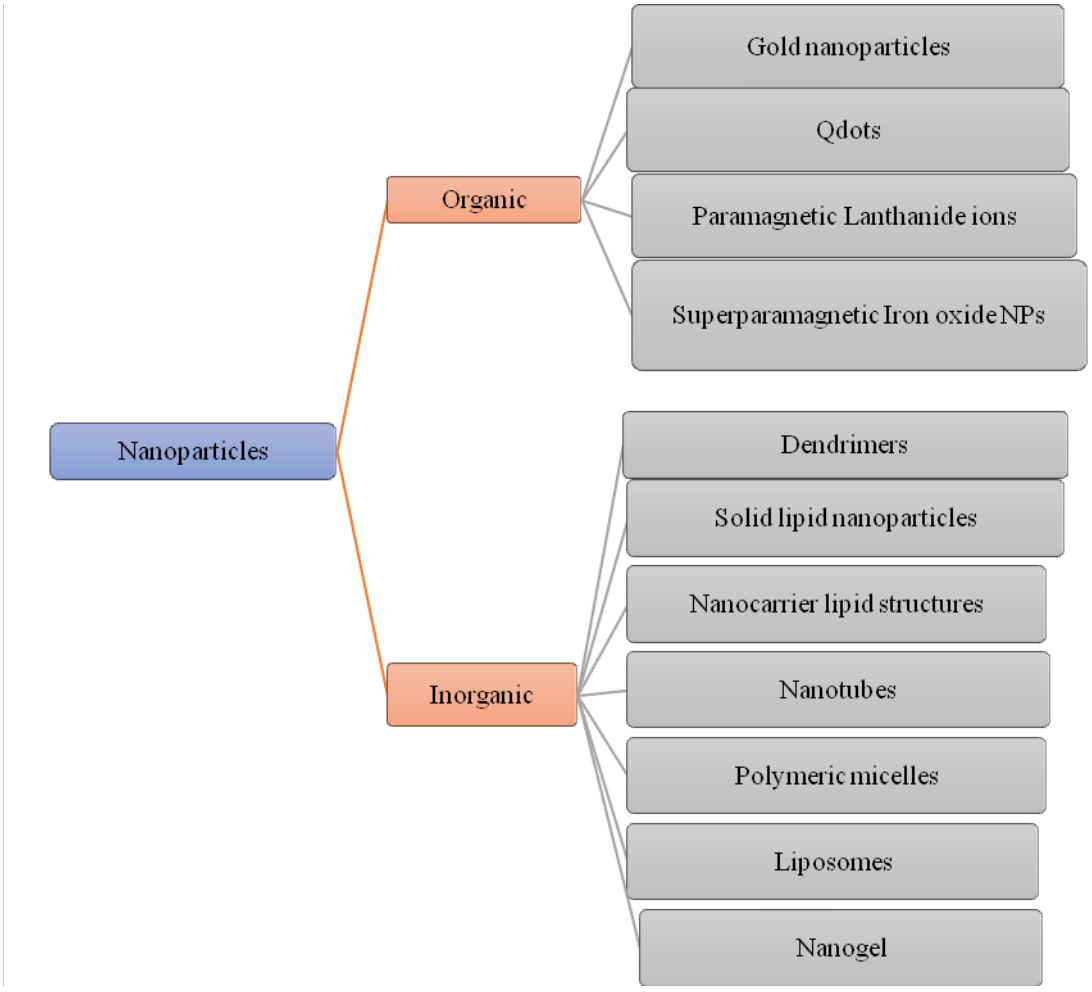
g) Easy to scale up and sterilize.
h) They are biodegradable, non-toxic, site specific and they are suitable under storage for one year.
i) Nanoparticles enhance the aqueous solubility of poorly soluble drug, which improve bioavailability of drug.
j) Due to small particle size of nanoparticles overcome resistance by physiological barriers in the body and easily penetrate to cell walls, blood vessels, stomach epithelium and blood brain barrier.
k) Useful to diagnose various diseases.
l) Drug loading is high and drugs can be incorporated into the system without any chemical reactions.
m) The system can be used for various route of administration.
n) To achieve drug targeting [Active as well as Passive], critical parameters of nanoparticles like particle size and surface properties can be easily controlled.

IDEAL CHARACTERISTICS

- Nanoparticles are biochemically inert and non-immunogenic.
- They have controllable and predictable rate of drug release and minimal drug leakage during transit.
- Carrier used must be biodegradable and readily eliminated from the body without causing any type of problem.
- These restrict drug distribution to target cells or tissues or organs and should have uniform capillary distribution.
- These are physically and chemically stable under *in -vivo* and *in-vitro* conditions.
- Nanoparticles release therapeutic amount of drug .
- Preparation of the nanoparticles should be easy, simple, reproductive and cost effective.

TYPES OF NANOPARTICLES [8, 9]



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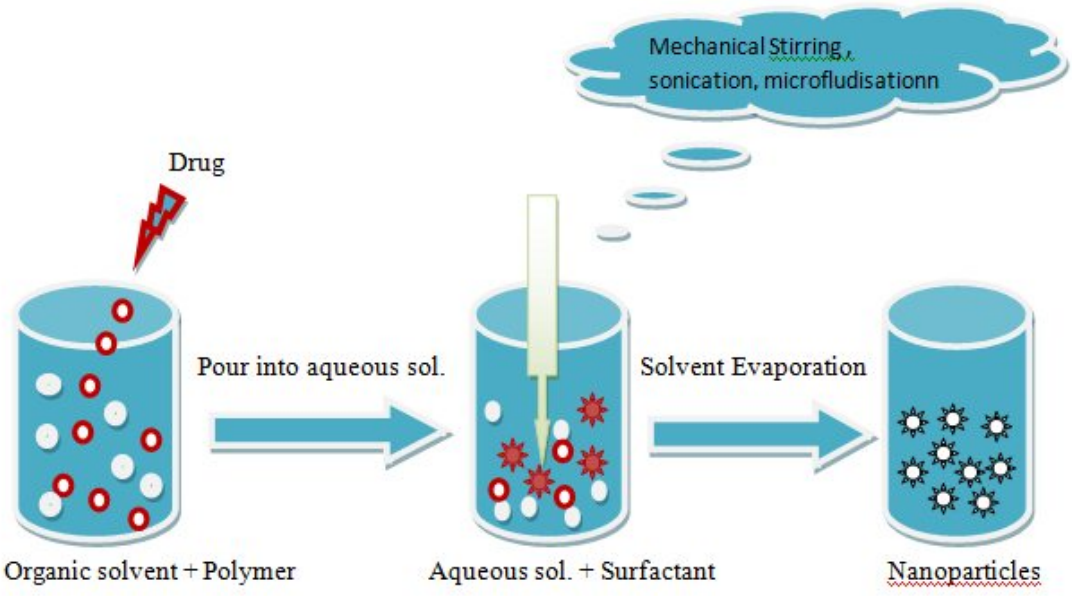
METHODS OF PREPARATION

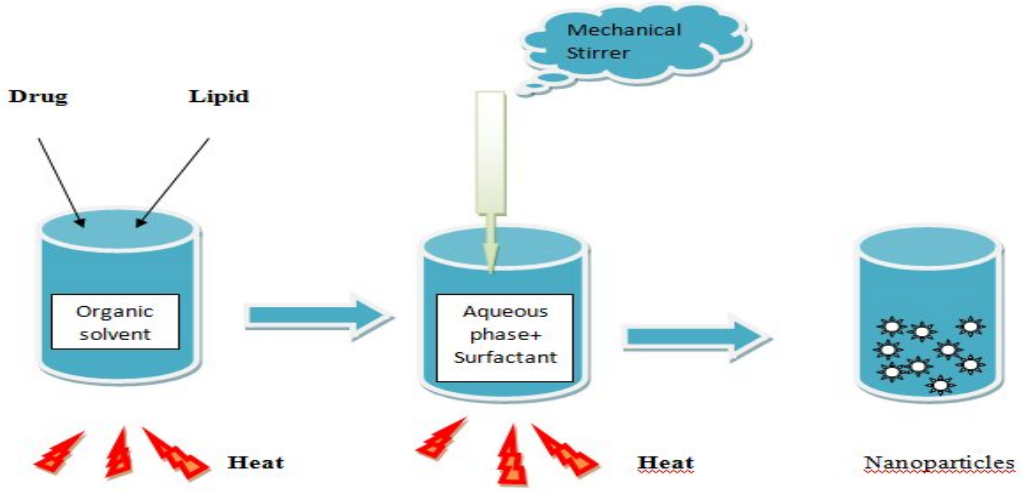
Solvent Evaporation Method [10-15]

In this method, polymer is dissolved in organic solvent (dichloromethane, chloroform or ethyl acetate) and then the drug is dispersed in this solution. Then this mixture is emulsified in an aqueous phase containing surfactant (polysorbates, polaxomers, poly vinyl alcohol) to make an oil in water (O/W) emulsion by using mechanical stirring, sonication or micro fluidization. After the formation of an emulsion the organic solvent is evaporated by increasing the temperature and using reduced pressure with continuous stirring.



Solvent Diffusion method [16-19]

In this method, the drug and lipid are dissolved in organic, water miscible solvents at elevated temperature and the resultant solution are rapidly injected into an aqueous phase containing surfactant, under mechanical stirring. As the temperature lowers (cools), the lipid droplets solidify and nanoparticles suspension of the drug are formed. This method has attracted great interest due to its simplicity and ease of handling.



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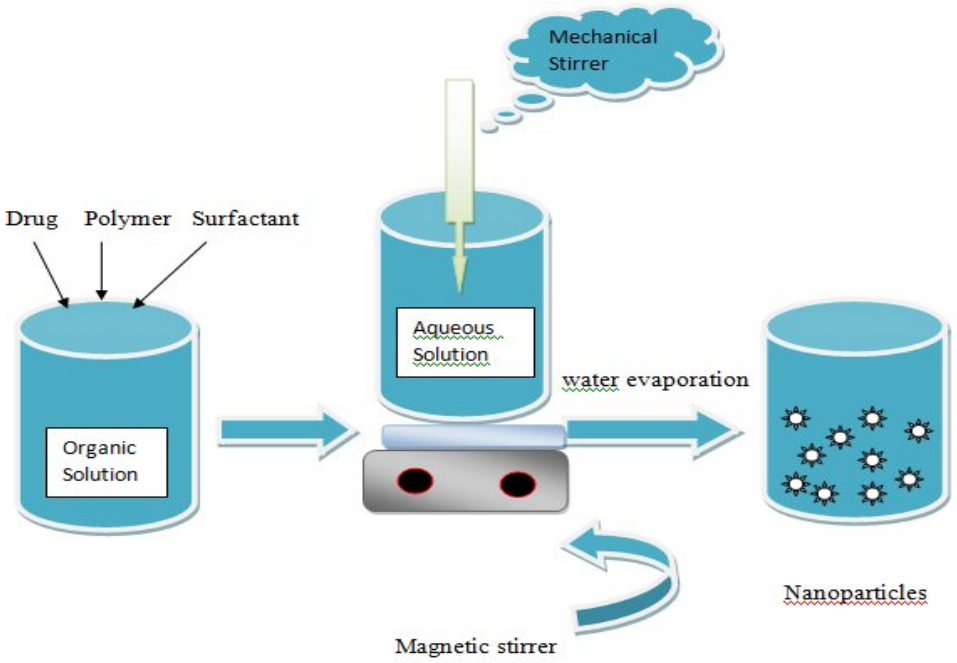
Nano precipitation method without surfactant [20-22]

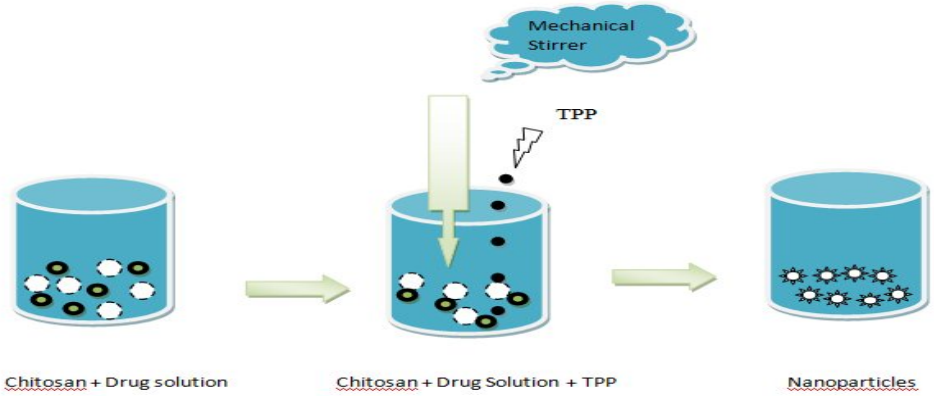
This method is best suited for water insoluble drugs. Drug solution (lyophobic) is mixed with anti-solvent under magnetic stirring. Precipitates are formed quickly and after that nanoparticles were filtered and then product is lyophilized.

The size and drug release is controlled by adjusting preparation parameters. Factors which effect size of nanoparticles are:- Addition of organic phase into aqueous phase and adjusting concentration of polymer.



Ionic Gelation Method or Coacervation Method [23-26]

In this method, hydrophilic polymers are used which are having biodegradable nature such as Chitosan, gelatin and Sodium alginate. In this method we need a mixture of two aqueous phases and coacervates are termed as a result of electrostatic interaction between two aqueous phases, where as ionic gelation happens due to the conversion of material from liquid phase to gel phase. Calvo and Co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation.



In Ionic gelation, material undergoing transition from liquid to gel at room temperature.

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Supercritical fluid technology [27]

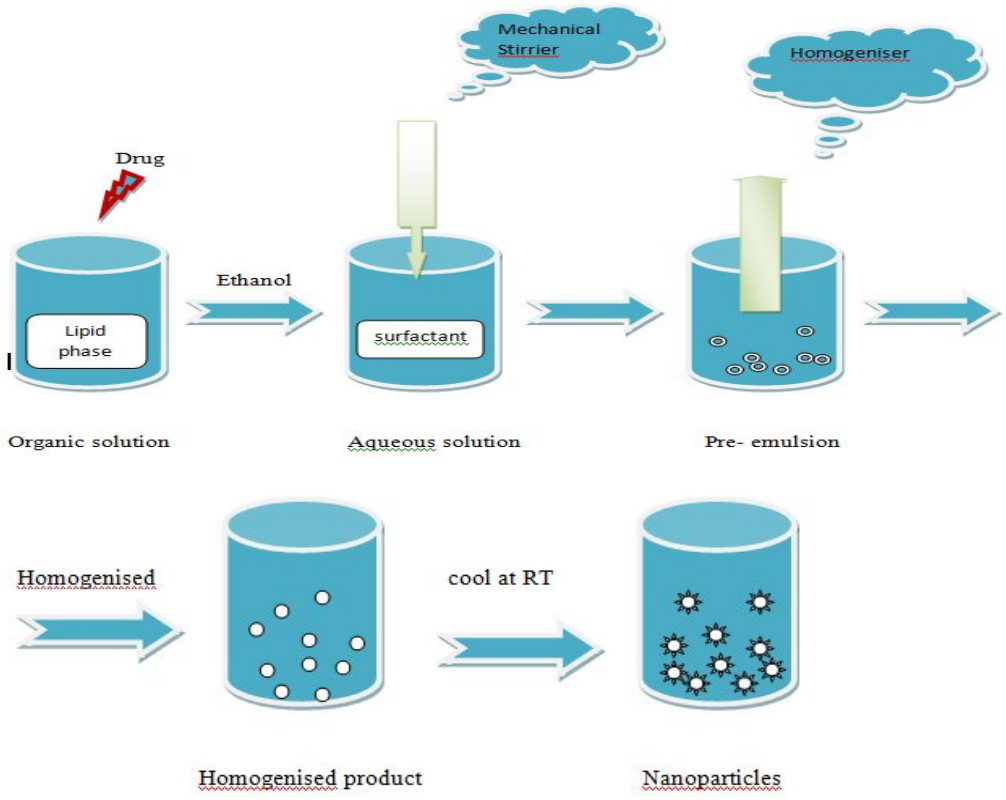
Supercritical fluids are environmentally safe so they have no hazardous effects which occur in case of other methods because in other methods organic solvents are involved. *Supercritical fluid is defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure.* They are widely used because of non-toxicity, non-flammability and low price. In this method CO_2 is mostly preferred as a supercritical fluid because of its mild conditions. This supercritical fluid is used in two main techniques and the first one is **Supercritical anti- Solvent** and other one is **Rapid expansion of critical solution (RESS).**

In Supercritical Anti-Solvent process solvents are used which are liquid in nature and which should completely solubilize with supercriticle fluid. Eg methanol is used as solvent which is miscible with the supercritical fluid, the extract of the solvent by supercritical fluid leads to the instantaneous precipitation of the solute which results formation of particles in nano range.

Rapid expansion of critical solution (RESS) This technique is suitable for controlled drug delivery applications. In RESS, Solute (Drug) is dissolved in a supercritical fluid to form a solution. Then this solution is rapidly expanded across an orifice or a capillary nozzle into ambient air by the rapid pressure reduction in the expansion. This results in homogenous nucleation and thereby formation of well dispersed nanoparticles. It is best technique because it is solvent free.

High Pressure Homogenisation [28-30]

In this method the drug is premilled by using low pressure homogeniser and then add aqueous solution of surfactant at different level of concentrations. Drug is dissolved in lipid phase (melted) and this solution was then dissolved in ethanol. The organic solution, is then transferred to the aqueous surfactant solution under stirring at 500 rpm for 20 min using a mechanical stirrer. This results into formation of a pre-emulsion which is then subsequently homogenised to reduce the size using high pressure homogenizer. Finally ,the mixture is cooled to room temperature yielding drug-nanoparticles.



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CHARACTERIZATION [31-34]

Measurement of Zeta potential

Zetapotential gives information about the charge on the surface of nanoparticles and evaluate the stability of colloidal dispersion. It depends upon the composition of the particles and the dispersed medium. Zeta potential is above (± 30mv), more the charge on the surface less will be aggregation. It is measured by Zeta meter. It is also used to determine whether the product is encapsulated or adsorbed. Zeta potential values can also be utilized in evaluating surface hydrophobicity.

DSC analysis

It determines habit of the powder sample (crystalline, amorphous) and the nature of crystallinity within the nanoparticles. It gives sharp endothermic peak which determine the crystalline nature, melting point and glass transition temperature.

Transmission Electron Microscope (TEM)

TEM is a microscopic technique to determine the morphology and 3-dimensional structure of the product with high contrast comparison to SEM. In this technique, the beam of electrons in straight line transmitted through thin specimen and after interaction with specimen it forms an image on the imaging device and that can be detected by photographic film. It is used to determine physical characteristics of nanoparticles.

***In-vitro* drug release**

These profiles are used to determine the controlled release potential and the release of drug from lipid nanoparticles. These studies are carried out in USP Type II dissolution apparatus (50 rpm at 37±0.2ºC).

Release behavior depends upon the;

- Concentration of the aqueous phase
- Surfactant
- Lipid phase

Surface Lipophillicity

Surface lipophillicity determines the concentration of absorbed blood components and it may be a proteins (Opsonins). Surface lipophilicity helps to binding opsonins on the surface of nanoparticles called opsonization. Opsonins makes link between nanoparticles and phagocytes. Surface lipophilicity on nanoparticles can be determined by hydrophobic interaction chromatography, biphasic partitioning, adsorption of probes, contact angle measurements etc. The x-ray photon correlation spectroscopy helps to detect specific chemical groups on the surface of nanoparticles.

Drug entrapment efficacy determination

Drug entrapment efficiency is used to determine the concentration of drug entrapped in a lipid matrix. After centrifugation process, supernatant will be collected and analysed on UV and find the concentration of drug in supernatant liquid if the concentration is zero it means all the drug is entrapped within the lipid matrix and then formula for entrapment efficiency is used to calculate the concentration entrapped in percentage that is given below :-

Drug entrapment Efficiency (% w/w) = Total amount of drug – Amount of drug in supernatant × 100/ Total amount of drug

Drug Loading (% w/w) = Initial drug – free drug / Mixed lipid × 100

Stability of nanoparticles

The nanoparticles are determined by storing optimized formulation at 5ºC ± 1ºC and 25ºC ± 2ºC, 40ºC ± 2ºC, 75± 5% RH in stability chamber for 6 months. The sample were analysed after a time period for their drug content, drug release rate (t50%) as well as any changes in their physical appearance (ICH Q1A)

APPLICATIONS

Target at a tumor site as a drug carrier [35, 36]

Nanoparticles were target at a tumor site because of small size they easily cross all the membranes which are made up of lipids, drug itself cannot cross the lipiodal membranes and target at the deceased cell but by using nanoparticles as a carrier which is made up of lipids help to prevent further growth of tumor. Incorporate anti-cancerous drugs in these formulation are very beneficial because nanoparticles increase permeability and retension effect.

Because of site specific action, no any kind of toxicity occur because they prevent accumulation of the free drug and no effect on healthy tissues only act on the deceased cells. This can also be achieved by active targeting by ligands on the surface of nanoparticles.

Long circulating and site specific nanoparticles [37-39]

Nanoparticles gives a auspicious effect via delivering drug at desirable site. These colloidal system are easily identified by kupffer cells through a blood because they are already located in liver and are also known as a stellate macrophage they invade the colloidal carriers and circulate for a long time in a body. They posses a major impact on vascular drug delivery, passive as well as active targeting, release and the transfusion of drugs. These type of nanoparticles are also known as PEGylated nanoparticles and “stealth” particles because so much efforts has been done to develop these type of nanoparticles which are not identified by macrophages or phagocytes and no any kind of antibodies are devolped against colloidal carriers. These type of nanoparticles are only beneficial in one case when the drug is present in the blood, and not whenever it is located in the cytoplasm or lysosomes after endocytosis.

Nanoparticles as Antituberculosis drug carrier [40]

ATDs (rifampicin, isoniazid, pyrizinamide and ethambutol). According to research, TB is induced in a mice by M.tuberculosis bacteria and then evalauate the therapeutic efficacy of nanoparticle encapsulated drug by three oral doses of the formulation. The relative bioavailability was increased. After 15 days there was a complete elimination of the bacteria from the body or organ as compared to 45 conventional formulations.

Nanoparticles for delivery into the brain [41-45]

The blood-brain barrier (BBB) is the main limiting step to deliver the drug in the central nervous system. BBB is made up tight endothelial cells junctions which is impermeable for high size molecules and hydrophilic drugs. It restricts movement of water-soluble molecules from blood circulation to the CNS, and can also reduce the concentration of lipid-soluble molecules in brain by the function of enzymes or efflux pumps. Consequently, the BBB provides a selective transport of molecules that are essential for brain function.

Oral delivery of peptides and proteins in the form of nanoparticles [46-48]

A significant advances in the field of biochemistry and biotechnology leads to discovery of numerous new molecules which is bioactive and made up of proteins and peptides. But, because of their large size they cannot cross the epithelial barriers of the gastrointestinal tract and there is a chance of degradation by the digestive enzymes. So nanoparticles play a very crucial role to encapsulate the drug and protect it from the environmental degradation and cross the epithelial barriers easily and increase the bioavailability of the drug efficiently.

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Nanoparticles for gene delivery [49-52]

Delivery of therapeutic gene into the target cell is done by the help of nanoparticles .These genes are used to correct the defective genes responsible for disease development. It is also known as gene therapy in this therapy introduction of genes into existing cells to prevent or cure a wide range of diseases.

There are several advancements to gene therapy, including:

- Replacing a mutated gene that causes disease with a healthy copy of the gene.
- Removal or " Switching off " the mutated gene.
- Introducing a new gene into the body to help fight a disease.

Numerous diseases are treated by gene therapy such as (including inherited disorders, some types of cancer, and certain viral infections), the technique remains risky and work is being carried out to make this technique more safe and effective. Gene therapy is being used to treat the diseases which have no other therapeutic option. Nanoparticles are also used as a vector to encapsulate a gene inside the cavity and delivered at the target site.

CHALLENGES [53, 54]

Recently, Nanoparticles has tremendous growth and advancements. With many efforts by both academia and biobharmaceutical industry only a few nanoformulations containing drugs has been approved for clinical use. Because there is no any doubt that nanosystem are still at its early stage with success stories few and far apart. During the development of nanoparticles there are number of challenges.

1. Biological Barriers to drug delivery
2. Delivery of hydrophobic drugs
3. Nanoparticles components and Charaterstics
4. Manufacturing Challenges
5. Regulatory Challenges
6. Safety challenges

So, a new nanoparticle system needs to successfully overcome several hurdles before it approved for marketing.

Biological Barriers to drug delivery

Drugs which are in circulation need to croos the barriers to reach at the target site for effective therapeutic use. BBB (Blood-Brain-Barrier) restricts the diffusion of hydrophilic drugs or large molecule inside the cerebrospinal fluid and it is the major obstacle in the treatment of various type of brain diseases such as brain tumor. So, it is a major delivery challenge. Nanomedicine which gives high permeability and retention effect in the tumor environment helps to overcome the risk of toxicity because during tumor, permeability of the vasculature is enhanced and lymphatic drainage results interstitial fluid pressure which is an another hurdle in the delivery of nanomedicines.

Delivery of hydrophobic drugs

Drugs which are hydrophobic in nature is not a easy task to deliver at the target site so there is a need of various kind of surfactants as well as solvents such as cremophor, tween and polysorbate which effects on the drug distribution but they cause various harmful side effects. For example, Taxol®, it is the conventional formulation of paclitaxel in which high concentration of Cremophor- EL® is used it is a solvent which is associated with toxicity like lethal hypersensitivity, anaphylactic reactions and prolonged peripheral neuropathy.

Nanoparticles components and Characteristics

To choose a components for the formulation of nanomedicines is a very big deal. Firstly choice a drug or targeting moiety which could play important role for efficacy and safety. Because these targeting moieties could alter various factors which are essential for high bioavailability such as Partition coefficient, Biodistribution and cellular uptake. The *in- vitro* and *in –vivo* properties of nanoparticles are dependent upon size, size distribution, surface properties, surface charge, Stearic hinderance, drug loading capacity, drug release kinetics and heamodynamic factors. To deliver different kinds of drugs differ type of formulations are prepared such as liposome, dendrimers, niosomes, quantum dots, nanoshells, nanocrystals and carbon nanotubes. These are differently designed to achieve different approaches.

Manufacturing Challenges

Nanoparticles are a 3- dimensional structure which are made up of multiple components with spatial arrangements with their functions. If there is a subtle change in the process or the components it will adversely effects on the formulation. The major challenge during manufacturing is reproducibility. Formulation should be highly reproducible.

Regulatory Challenges

In the field of nanocarriers, it seem apparent that the regulatory pathway for nanomedicines may face several obstacles and hurdles. Presently, there are various agencies available such as FDA, EMA which examine a new nanoparticle system on a product via product basis. Because of complex structure it is hard to compare with standard drugs.

Safety challenges

Nanoparticles posses a specific physicochemical properties for efficacy and safety so it is necessary to recognize all the parameters and study the histopathology before clinical trial. Because particles having less than 100 nm size range cause various type of lungs disorder as well as formation of free radicals inside the body which may be very harmful or lethal. But the consensus is that each and every product may have its own case by case issues so they can be handle and test product by product. Preclinical and additional testings should be essential to overcome safety related issues.

FUTURE PROSPECTIVES [54]

Worldwide Scientists have a great interest towards nanoscience and nanotechnology because nanotechnology is a promising and very prospective research field. Few decades ago, Chances of toxicity was high because small size nanoparticles can easily cross Blood Brain Barrier and remain for long time in cerebrospinal fluid and cause various types of CNS disorders. Moreover, Body recognized nanoparticles as a foreign particles and engulfed them and showed various types of hypersensitivity reactions. At that time, FDA has taken lot of time to take a decision for the clinical trials and FDA form new guidelines for additional preclinical testings.

But now-a-days, Nanoparticles have a greatest potential both at social and economic level. Improvement and modifications in the characteristics and physicochemical properties offers a new shape/ idea to the nanoparticles which are more effective, beneficial and more safe.

Conventional formulations regarding problems can be overcome by novel applications of drug delivery such as

- Carbon Nanotubes
- Nanoshells
- Nanosponges

- Quantum dots
- Fullerenes
- Metal Nanoparticles

They posses unique physical and chemical properties due to their small size and large surface area. These nanoparticles help to detect better and treat diseases like tumor, cancer. Because attachment of targeting ligands on the surface of nanoparticles help to recognize the deceased site and they also help to localize actions of the medicine and prevent random circulation of drug inside the body which may cause toxicity. So we can say that nanotechnology is a path to a rational future.

GROWTH RATE IN THE FIELD OF NANOTECHNOLOGY [55]

In the field of nanotechnology, global growth is non-uniform and has been sustained at about 15%. The growth rate (Number and quality of scientific publications) increased remarkably in P.R China and South Korea while US, EU27 and Japan have maintained lead in the discovery.

PATENTS AVAILABLE IN NANOTECHNOLOGY

1. A Topical semisolid silver nanoparticle dispersion formulation (U.S. Patent No. 3,092,552)
2. Nanoparticle formulations for delivering multiple therapeutic agents **(**US15374176**)**
3. Tumor Targeted delivery of Taxol using nanoparticles (US Patent 6,322,817)
4. Sustained release and long residing ophthalmic formulation (US Patent 6579519)
5. Smart hydrogel nanoparticles for drug delivery applications (US Patent 5847111)

CONCLUSION

In the field of Pharma Science, nanotechnology is a boon to deliver the drug at the target site and work as a protective barrier from the harmful environment and stabilize the drug from physical, chemical and biological instabilities. The foregoing show that nanoparticles improve the bioavailability of poorly soluble drugs and poorly absorbed drugs and convert into most favorable delivery system. It gives control release, reduce side effects and minimize toxicity.

Recently nanoparticles have a big demand in cancer therapy and deliver the drugs into brain. In a nutshell, drug delivery through a nanosystem is a very big achievement for researchers. It is a promising platform for the site specific or targeted drug delivery.

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