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AN OVERVIEW ON NANOPARTICLES

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ABSTRACT

Nanoparticles are the building blocks of the new emerging field of nanotechnology that in broadest terms signifies the understanding and controlling of properties of matter at dimensions of roughly 1–100nm. The use of nanotechnology in medicine & more specifically drug delivery is set to spread rapidly. Currently many substances are under investigation for drug delivery, more specifically for cancer therapy. Interestingly pharmaceutical sciences are using nanoparticles to reduce toxicity and side effects of drugs and did not realize that carrier systems themselves may impose risks to the patient. The kind of hazards that are introduced by using nanoparticles for drug delivery are beyond that posed by conventional hazards imposed by chemicals in classical delivery matrices. For nanoparticles the knowledge on particle toxicity as obtained in inhalation toxicity shows the way how to investigate the potential hazards of nanoparticles. The toxicology of particulate matter differs from that of substances as the composing chemical may or may not be soluble in biological matrices, thus influencing greatly the potential exposure of various internal organs. The situation is different as their size opens the potential for crossing the various biological barriers within the body. From a positive viewpoint, especially the potential to cross the blood brain barrier may open new ways for drug delivery into the brain. This paper provides an overview on some of the currently used systems for drug delivery. Besides the potential beneficial use also attention is drawn to the questions which techniques are in recent usage within the industry.

KEYWORDS: Drug Delivery, Cancer Therapy, Nanoparticles, Toxicology, Pharmaceuticals.

Pharmaceutical nanotechnology offers new tools, opportunities And scope, which are expected to have a great impact on many areas in disease Diagnostics and therapeutics. Pharmaceutical nanotechnology is now well-established as specialized area for drug delivery, diagnostics, prognostic and treatment of diseases through its nano-engineered tools. Pharmaceutical nanotechnology provides opportunities to improve materials, medical devices and help to develop new technologies where existing and more conventional technologies may be reaching their limits.

INTRODUCTION

The prefix "nano" has found in last decade an ever-increasing application to different fields of the knowledge. The prefix comes from the ancient Greek "νᾶνος" through the Latin "nanus" meaning literally dwarf and, by extension, very small. Within the convention of International System of Units (SI) it is used to indicate a reduction factor of 109 times. The nanosize world is typically measured in nanometers (1nm corresponding to 10-9 m) and it encompasses systems whose size is above molecular dimensions and below macroscopic ones (generally > 1 nm and < 100 nm). Nanotechnology focuses on the very small and it is uniquely suited to creating systems that can better deliver drugs to tiny areas within the body. Nano-enabled drug delivery also makes it possible for drugs to permeate through cell walls, which is of critical importance to the expected growth of genetic medicine over the next few years. In further part of this review, we would focus on every small detail of nanoparticles and also the most widely used methods to produce them.

CHARACTERISTICS OF NANOPARTICLES

Nanoparticles are generally characterized by their size, morphology and surface charge. The average particle diameter, their size distribution and charge affect the physical stability and the in vivo distribution of the nanoparticles. Electron microscopy techniques are very useful in ascertaining the overall shape of polymeric nanoparticles, which may determine their toxicity. The surface charge of the nanoparticles affects the physical stability and redispersibility of the polymer dispersion as well as their in vivo performance. This is done using such advanced microscopic techniques as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM).

Particle size: Particle size distribution and morphology are the most important parameters of characterization of nanoparticles. Morphology and size are measured by electron microscopy. It has been found that particle size affects the drug release. Smaller particles offer larger

surface area. As a result, most of the drug loaded onto them will be exposed to the particle surface leading to fast drug release. On the contrary, drugs slowly diffuse inside larger particles. As a drawback, smaller particles tend to aggregate during storage and transportation of nanoparticle dispersion. Hence, there is a compromise between a small size and maximum stability of nanoparticles. Polymer degradation can also be affected by the particle size. For instance, the degradation rate of poly (lactic-co-glycolic acid) was found to increase with increasing particle size in vitro.

Surface Charge: The nature and intensity of the surface charge of nanoparticles is very important as it determines their interaction with the biological environment as well as their electrostatic interaction with bioactive compounds. The colloidal stability is analyzed through zeta potential of nanoparticles. This potential is an indirect measure of the surface charge. It corresponds to potential difference between the outer Helmholtz plane and the surface of shear. The measurement of the zeta potential allows for predictions about the storage stability of colloidal dispersion. High zeta potential values, either positive or negative, should be achieved in order to ensure stability and avoid aggregation of the particles. The extent of surface hydrophobicity can then be predicted from the values of zeta potential. The zeta potential can also provide information regarding the nature of material encapsulated within the nanocapsules or coated onto the surface.

Surface hydrophobicity/wettability: Among numerous properties (e.g., size, shape, surface charge, and coating), surface hydrophobicity or hydrophilicity has a pivotal impact on their stability, fate, transport, and interfacial interactions such as inter-particle repulsion or attraction. It can be determined by several techniques such as hydrophobic interaction chromatography, biphasic partitioning, adsorption of probes, contact angle measurements etc.

Drug Release: A central reason for pursuing nanotechnology is to deliver drugs, hence understanding the manner and extent to which the drug molecules are released is important. The drug loading of the nanoparticles is generally defined as the amount of drug bound per mass of polymer. It could also be given as percentage relative to the polymer. The technique used for this analysis is classical analytical methods like UV spectroscopy or high performance liquid chromatography (HPLC) after Ultra centrifugation, ultra filtration, gel filtration, or centrifugal ultrafiltration. Drug release assays are also similar to drug loading assay which is assessed for a period of time to analyze the mechanism of drug release.

CLASSIFICATION OF NANOPARTICLES

There are various approaches for classification of nanomaterials. Nanoparticles are generally classified based on one, two and three dimensions.

One dimension nanoparticles: One dimensional system, such as thin film or manufactured surfaces, has been used for decades in electronics, chemistry & engineering. Production of thin films (sizes1-100 nm) or monolayer is now common place in the field of solar cells or catalysis. These thin films are using in different technological applications, including information storage systems, chemical and biological sensors, and fiber-optic systems, magneto-optic and optical device.

Two dimension nanoparticles: Carbon nanotubes are hexagonal network of carbon atoms, 1 nm in diameter and 100 nm in length, as a layer of graphite rolled up into cylinder. CNTs are of two types, singlewalled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). The small dimensions of carbon nanotubes, combined with their remarkable physical, mechanical and electrical properties, make them unique materials. They display metallic or semi conductive properties, depending on how the carbon leaf is wound on itself.

Three dimension nanoparticles: Fullerenes (Carbon 60) are spherical cages containing from 28 to more than 100 carbon atoms, contain C60. This is a hollow ball composed of interconnected carbon pentagons and hexagons, resembling a soccer ball.

KEY FACTORS IMPACTING DRUG DELIVERY

In order to achieve effective drug delivery, nano-carriers must have suitable circulation time to prevent the elimination of drugs before reaching their target. Based on previous investigations, size, shape and surface characteristics are key factors that impact the efficiency of drug delivery systems.

Size & Shape: Particle size plays a key role in particle functions, such as degradation, vascular dynamics, targeting, clearance and uptake mechanisms. Particles have been shown to have different velocities, diffusion characteristics and adhesion properties, depending on their size, resulting in different uptake efficiencies. The size should be large enough to prevent rapid leakage in blood capillaries, but, at the same time, small enough to escape the capture of macrophages in the endothelial system. Some of the main outcomes reported in these studies are that the size limits for internalization of NPs through endocytosis are clearly

cell dependent. Particles less than 200 nm in size will mainly follow endocytic pathways. Particles above this size can be either engulfed through endocytosis or not internalized at all and microsized particles have to be internalized by phagocytic pathways. Apart from size, recent studies have shown that the shape of particles can also have an intriguing effect on particle functions, especially in biological processes, including internalization, transport through the blood vessels and targeting diseased sites. Varying toxicities of materials having identical chemical composition (silica), but different shape, was also reported. Recent advances with particular focus on the importance of particle shape, as well as the challenges yet to be overcome, are reviewed elsewhere.

Surface Characteristics: Besides size and shape, surface characteristics also determine their lifespan during circulation in the blood stream. It was noted in a study that particles coated with hydrophilic polymer molecules, such as PEG, can resist serum protein adsorption, prolonging the systemic circulation of the particle. The surface charge on the particle also affects other functions, such as internalization by macrophages. Positively charged particles have been shown to exhibit higher internalization by macrophages and dendritic cells compared with neutral or negatively charged particles, although surface charge effect could also be cell-type dependent.

PREPARATION OF NANOPARTICLES

The selection of appropriate method for the preparation of nanoparticles depends on the physicochemical character of the polymer and the drug to be loaded. The primary manufacturing methods of nanoparticles from preformed polymer includes:

1. Emulsion-Solvent Evaporation Method: This is one of the most frequently used methods for the preparation of nanoparticles.

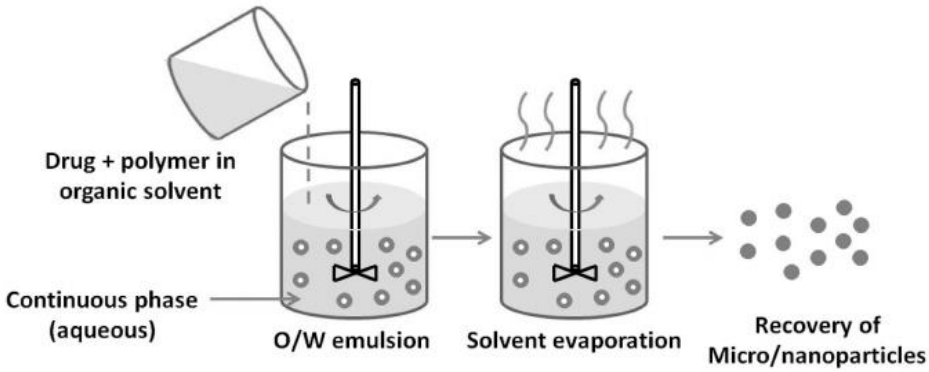

Figure 1: Emulsion Solvent Evaporation Method.

It involves two steps. The first step requires emulsification of the polymer solution into an aqueous phase. During the second step polymer solvent is evaporated, inducing polymer precipitation as nanospheres. The nano particles are collected by ultracentrifugation and washed with distilled water to remove stabilizer residue or any free drug and lyophilized for storage. Modification of this method is known as High pressure emulsification and solvent evaporation method. This method involves preparation of an emulsion which is then subjected to homogenization under high pressure followed by overall stirring to remove organic solvent. The size can be controlled by adjusting the stirring rate, type and amount of dispersing agent, viscosity of organic and aqueous phases and temperature. Polymers used in this method are PLA (Polylactic Acid), PLGA (Poly Lactic-co-glycolic Acid), EC (Ethyl cellulose), CAP/INN (Cellulose acetate phthalate), PCL (Poly e- caprolactone), and PHB (Poly-βhydroxybutyrate).

2. Double Emulsion and Evaporation Method

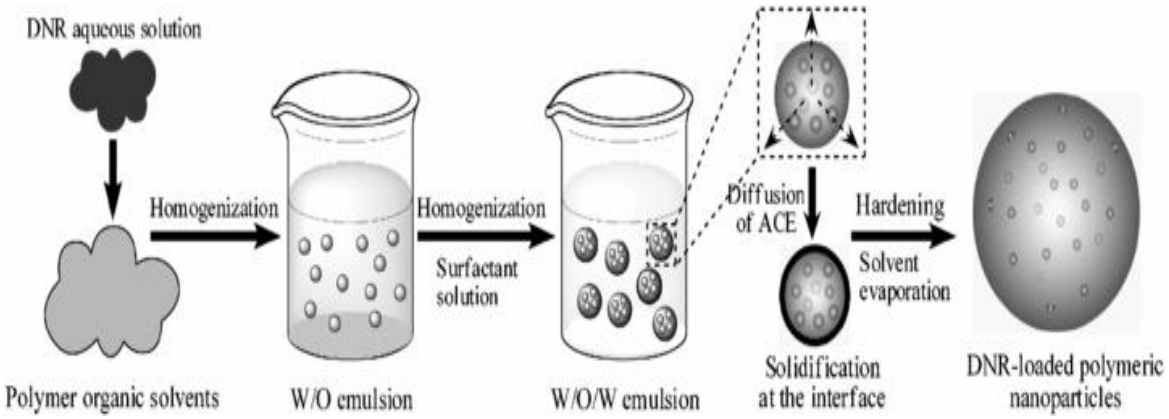

Figure 2: Double Emulsion and Evaporation Method.

The emulsion and evaporation method suffer from the limitation of poor entrapment of hydrophilic drugs. To encapsulate hydrophilic drug the double emulsion technique is employed, which involves the addition of aqueous drug solutions to organic polymer solution under vigorous stirring to form w/o emulsions. This w/o emulsion is added into second aqueous phase with continuous stirring to form the w/o/w emulsion. The emulsion then subjected to solvent removal by evaporation and nano particles can be isolated by centrifugation at high speed. The formed nanoparticles must be thoroughly washed before lyophilisation. In this method the amount of hydrophilic drug to be incorporated, the concentration of stabilizer used, the polymer concentration, the volume of aqueous phase are the variables that affect the characterization of nano particles.

3. Salting Out Method

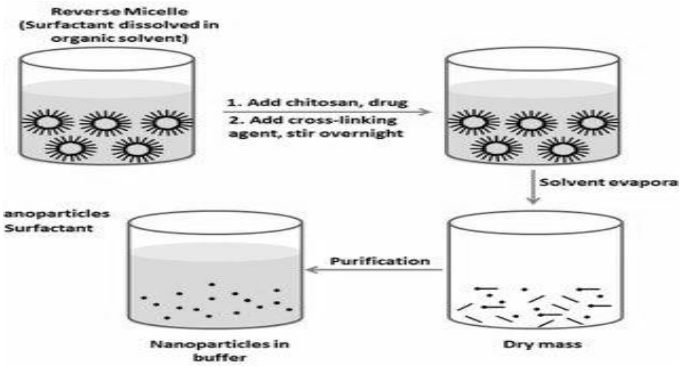

Figure 3: Salting Out Method.

Salting out based on the separation of a water-miscible solvent from aqueous solution via a salting-out effect. It is based on the separation of a water miscible solvent from aqueous solution via a salting-out effect. Polymer and drug are initially dissolved in a solvent which is subsequently emulsified into an aqueous gel containing the salting out agent (electrolytes, such as magnesium chloride and calcium chloride, or non- electrolytes such as sucrose) and a colloidal stabilizer such as polyvinylpyrrolidone or hydroxyethylcellulose. This oil/water emulsion is diluted with a sufficient volume of water or aqueous solution to enhance the diffusion of solvent into the aqueous phase, thus inducing the formation of nanospheres. Several manufacturing parameters can be varied including stirring rate, internal/external phase ratio, concentration of polymers in the organic phase, type of electrolyte concentration and type of stabilizer in the aqueous phase. This technique used in the preparation of PLA, Poly (methacrylic) acids, and Ethyl cellulose nanospheres leads to high efficiency and is easily scaled up. Salting out does not require an increase of temperature and therefore may be useful when heat sensitive substances have to be processed. The greatest disadvantages are exclusive application to lipophilic drug and the extensive nanoparticles washing steps.

4. Emulsions- Diffusion Method: This is another widely used method to prepare nanoparticles.

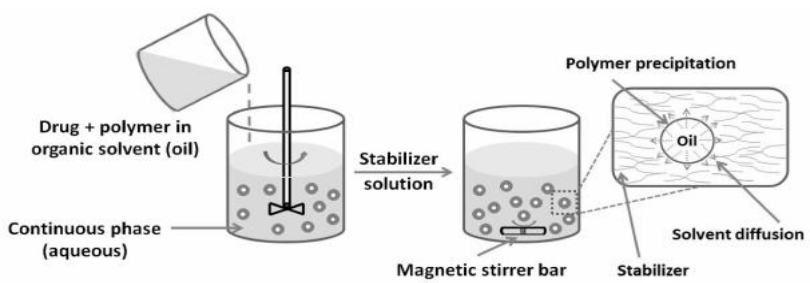

Figure 4: Emulsion Diffusion Method.

The encapsulating polymer is dissolved in a partially water-miscible solvent (such as propylene carbonate, benzyl alcohol), and saturated with water to ensure the initial thermodynamic equilibrium of both liquids. Eventually, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilize. This leads to solvent diffusion to the external phase and the formation of nanospheres occurs, according to the oil-to-polymer ratio. The solvent is eliminated by evaporation or filtration, according to its boiling point. This technique presents several advantages, such as high encapsulation efficiencies (generally 70%), no need for homogenization, high batch-to-batch reproducibility, ease of scale up, simplicity, and narrow size distribution. Several drug-loaded nanoparticles were produced by the technique, including mesotetra (hydroxyphenyl) porphyrin-loaded PLGA (p-THPP) nano particles, doxorubicin-loaded PLGA nano particles, and cyclosporine (cy-A-); loaded sodium glycolate nanoparticles.

5. Solvent Displacement / Precipitation method

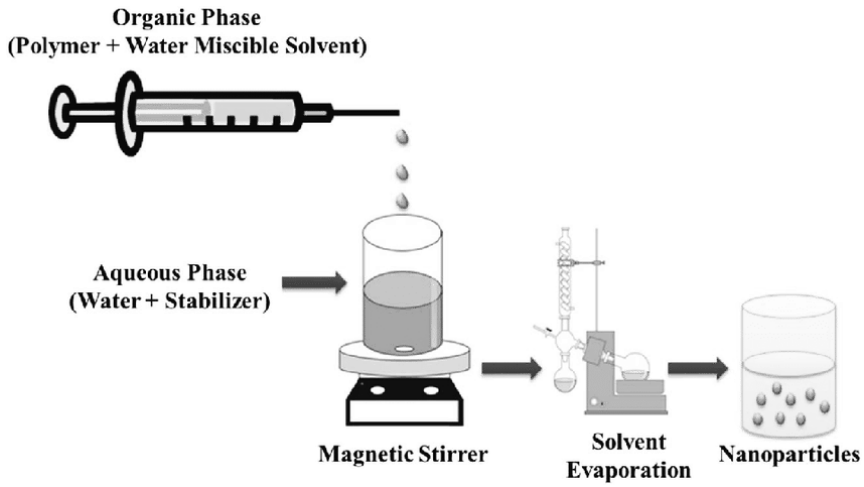

Figure 5: Solvent Displacement/Precipitation Method.

Solvent displacement involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of surfactant. Polymers, drug, and or lipophilic surfactant are dissolved in a semi polar water miscible solvent such as acetone or ethanol. The solution is then poured or injected into an aqueous solution containing stabilizer under magnetic stirring. Nano particles are formed instantaneously by the rapid solvent diffusion.

TECHNIQUES FOR ANALYSIS FOR PARTICLES

Recently, several sophisticated analytical techniques are reported in literature for surface analysis of nanoparticles. There are several tools for determining nanoparticle size as

discussed below Dynamic light scattering (DLS): Currently, the fastest and most popular method of determining particle size is photon-correlation spectroscopy (PCS) or dynamic light scattering (DLS). DLS is widely used to determine the size of Brownian nanoparticles in colloidal suspensions in the nano and submicron ranges. Shining monochromatic light (laser) onto a solution of spherical particles in Brownian motion causes a Doppler shift when the light hits the moving particle, changing the wavelength of the incoming light. This change is related to the size of the particle. It is possible to extract the size distribution and give a description of the particle's motion in the medium, measuring the diffusion coefficient of the particle and using the autocorrelation function. The photon correlation spectroscopy (PCS) represent the most frequently used technique for accurate estimation of the particle size and size distribution based on DLS.

Scanning Electron microscopy: Scanning electron microscopy (SEM) is giving morphological examination with direct visualization. The techniques based on electron microscopy offer several advantages in morphological and sizing analysis; however, they provide limited information about the size distribution and true population average. For SEM characterization, nanoparticles solution should be first converted into a dry powder, which is then mounted on a sample holder followed by coating with a conductive metal, such as gold, using a sputter coater. The sample is then scanned with a focused fine beam of electrons. The surface characteristics of the sample are obtained from the secondary electrons emitted from the sample surface. The nanoparticles must be able to withstand vacuum, and the electron beam can damage the polymer. The mean size obtained by SEM is comparable with results obtained by dynamic light scattering. Moreover, these techniques are time consuming, costly and frequently need complementary information about sizing distribution.

Transmission electron microscope: TEM operates on different principle than SEM, yet it often brings same type of data. The sample preparation for TEM is complex and time consuming because of its requirement to be ultra thin for the electron transmittance. The nanoparticles dispersion is deposited onto support grids or films. To make nanoparticles withstand the instrument vacuum and facilitate handling, they are fixed using either a negative staining material, such as phosphotungstic acid or derivatives, uranyl acetate, etc, or by plastic embedding. Alternate method is to expose the sample to liquid nitrogen temperatures after embedding in vitreous ice. The surface characteristics of the sample are

obtained when a beam of electrons is transmitted through an ultra thin sample, interacting with the sample as it passes through.

Atomic force microscopy: Atomic force microscopy (AFM) offers ultra-high resolution in particle size measurement and is based on a physical scanning of samples at sub-micron level using a probe tip of atomic scale. Instrument provides a topographical map of sample based on forces between the tip and the sample surface. Samples are usually scanned in contact or noncontact mode depending on their properties. In contact mode, the topographical map is generated by tapping the probe on to the surface across the sample and probe hovers over the conducting surface in non-contact mode. The prime advantage of AFM is its ability to image non-conducting samples without any specific treatment, thus allowing imaging of delicate biological and polymeric nano and microstructures (Shi & Farber, 2003). AFM provides the most accurate description of size and size distribution and requires no mathematical treatment. Moreover, particle size obtained by AFM technique provides real picture which helps understand the effect of various biological conditions.

APPLICATION OF NANOPARTICLES

Application of nanotechnology in the different field are as listed below:

Route of Administration: Nanoparticles can nter the human body in several ways: (i) via the lungs where a rapid translocation through the blood stream to vital organ is possible, including crossing the BBB and absorptions by (ii) the intestinal tract (iii) the skin.

In gene therapy Liposomes measuring less than 100 nm can be used for delivery of genetic material into cells. Liposomes incorporated with polyethylene glycol and galactose target liver cells effectively due to their rapid uptake by liver Kupffer cells. Thus gene therapy may be tried with such liposomal nanoparticles for various liver disorders such as Wilson's disease and hereditary hemochromatosis. Moreover, polymeric nanoparticles have been applied in gene therapy to breast cancer cells, resulting in antiproliferative effects.

Molecular Diagnostics The combination of nanoparticles with other nanotechnology-based materials has the potential to address this emerging challenge and provide technologies that enable diagnoses at the level of single cells and single molecules.

Stem cell therapy

Nanoparticles may prove effective tools for improving stem cell therapy, new research suggests. In stem cell therapy magnetic nanoparticles coupled to antibodies are added to a blood or bone marrow sample that contains the target adult stem cells. The magnetic particles bind the target cells, which then can be recovered using a magnet. This technique is used in cell therapies to isolate adult stem cells that are then retransplanted in the patient e.g. to treat blood disorders or cardiac diseases.

In Cancer treatment

Colloidal drug delivery modalities such as liposomes, micelles or nanoparticles have been intensively investigated for their use in cancer therapy. The effectiveness of drug delivery systems can be attributed to their small size, reduced drug toxicity, controlled time release of the drug and modification of drug pharmacokinetics and biological distribution.

Artificial organs and implants

Another field where the achievements of nanotechnology can be practically applied is creation of artificial cells, tissues and organs. Artificial cells are being actively investigated for use in the replacement of defective or incorrectly functioning cells and organs, especially related to metabolic functions.

Drug discovery

Pharmaceutical Nanotechnology helps in identification and validation of target by identifying the protein present on the surface or target surface. Nanotech will enhance drug delivery process, through miniaturization, automation, aped and reliability of assays. Single walled nanotubes are successfully used to identify surface protein of pathogen. Quantum dots- track individual lysine receptors and to analyze their dynamics in the neuronal membrane of living cells, for periods ranging from milliseconds to minutes. Gold nano particles, anybodies (smallest, available, intact antigen-antibody fragments) produced by ablynx are some commonly used nanomaterials in diagnosis.

ADVANTAGES OF NANOPARTICLES

Significant advantages of nanoparticles are as follows:

- Increased bioavailability through Dose proportionality.
- Smaller dose form.

- Increased surface area results in a faster dissolution of the active agent in an aqueous environment.
- Faster dissolution generally equates greater absorption and bioavailability.
- Smaller drug doses less toxicity.
- Reduction in fed/ fasted variability.

FUTURE OPPORTUNITIES AND CHALLENGES

Nanoparticles have already been applied as drug delivery systems with great success. Nanoparticles provide massive advantages regarding drug targeting, delivery and with their potential for combine diagnosis and therapy and one of the major tools in Nanomedicine Drug delivery techniques were established to deliver or control the amount & rate. Most major and established internal research programmes on drug delivery that are formulations and dispersion containing components down to nano sizes.

Currently there are many technical, challenges in developing the few techniques as enlisted below: Architecting of biomimetic polymers, control of sensitive drugs, functions of active drug targeting, bioresponsive triggered systems, systems interacting with me body smart delivery, nanochips for nanoparticle release, carriers for advanced polymers for the delivery of therapeutic peptide / proteins.

CONCLUSION

Nanotechnology-enabled drug delivery is opening prospective future in pharmaceutics. The emergence of nanotechnology is likely to have a significant impact on drug delivery sector, affecting just about every route of administration from oral to injectable. The present pharmaceutics is often characterized by poor bio-availability which far too often results in higher patient costs and inefficient treatment but also, more importantly, increased risks of toxicity or even death. Nanotechnology is now widely regarded as the enabling technology of the 21st century. Today, nanostructured materials and nanotechnology techniques are being used to produce better composite materials, materials with enhanced catalytic activity, hardness and scratch resistance, and a wide range of consumer products that improve human life. Pharmaceutical nanotechnology has emerged as a discipline having enormous potential as a carrier for spatial and temporal delivery of bioactives and diagnostics and provides smart materials for tissue engineering. It offers new tools, opportunities and scope, which are expected to have a great impact on many areas in disease, diagnostics, prognostic and treatment of diseases through its nano-engineered tools. Pharmaceutical nanotechnology

provides opportunities to improve materials, medical devices and help to develop new technologies where existing and more conventional technologies may be reaching their limits. It raises new hope to industries by providing new patentive technologies in view of revenue loss caused due to off-patent drugs. In future it will provide us the new nanotechnology such as smart medicine and nano-robots to make significant contributions to disease detection, diagnosis, therapy, and prevention.

The payoff for doctors and patients from nanotechnology-enabled drug delivery should be lower drug toxicity, reduced cost of treatments, improved bioavailability and an extension of the economic life of proprietary drugs.

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